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MATERIALS OF THE SCIENTIFIC-PRACTICAL CONFERENCE WITH INTERNATIONAL PARTICIPATION «MODERN APPROACHES AND PROSPECTS OF GENETIC RESEARCH IN YAKUTIA», SEPTEMBER 7, 2017, YAKUTSK

G.N. Akhmadeeva G.N., I.M. Khidiyatova, T.R. Nasibullin, A.R. Baitimerov, R.V. Magzhanov, E.K. Khusnutdinova

RESEARCH OF ASSOCIATION OF POLYMORPHIC VARIANTS OF GENES DOPAMINE SYSTEM'S (*DRD1, DRD2, DRD3, DRD4, TH, COMT* AND *MAO-B*) WITH IDIOPATHIC PARKINSON'S DISEASE

ABSTRACT

The dopamine metabolism disorder played a major role in the pathogenesis of Parkinson's disease (PD). We analyzed the polymorphic variants of the genes of the dopaminergic system: the *rs4532* of the *DRD1* gene, *Taq1* and *rs6275* of the *DRD2* gene, *rs6280* of the *DRD3* gene, *VNTR 120bp, VNTR 48bp* and *rs747302* of the *DRD4* gene (dopamine receptors), *(TCAT)n-repeats* of the *TH* gene (tyrosine hydroxylase), *rs4680* of the *COMT* gene (catechol-O-methyltransferase) and *rs1799836* of the *MAO-B* gene (monoamine oxidase B). The study included 264 patients with idiopathic PD and 314 healthy individuals of the Tatar ethnicity living on the territory of the Republic of Bashkortostan (RB). There is the association of the allele *rs4680*G* and the genotype *rs4680*G/G* of the *COMT* gene with PD development ($p=0,5*10^{-5}$; OR=1,73 and $p=0,36*10^{-4}$; OR=2,22, respectively), especially its akinetic-rigid-trembling form ($p=10^{-6}$; OR=2,86 and $p=0,3*10^{-5}$; OR=4,87, respectively) and its manifestation after 60 years ($p=0,12*10^{-3}$; OR=2,03 and $p=0,14*10^{-2}$; OR=2,51, respectively) in Tatar ethnicity. There is the association of allele *rs1799836*C* of the *MAO-B* gene with PD development in Tatar men ($p=0,7*10^{-3}$; OR=2,88). A complex analysis using the APSampler algorithm showed that the most significant combination associated with increased PD development was the combination of *rs4680(COMT)*G* and *(TCAT)nTH*8* alleles with *rs6311(HTR2A)*A* and *rs6296(HTR1B)*G* alleles of the genes of serotonine receptors which we investigated earlier. The only protective combination was triallelic combination of *rs4532(DRD1)**T, *rs4680(COMT)*A* and *rs1800532(TPH1)*T* alleles.

Keywords: Parkinson's disease, dopamine, polymorphic variants of the gene, dopamine receptors, monoamine oxidase B, tyrosine hydroxylase, catechol-O-methyltransferase.

INTRODUCTION

Congenital adrenal cortex dysfunction (ADHD, adrenogenital syndrome (AGS), congenital adrenal hyperplasia) is a group of diseases with an autosomal recessive type of inheritance, which is based on a defect of one of the enzymes or transport proteins that participate in the biosynthesis of cortisol in the adrenal cortex [2].

For the first time the disease was described by Phillips in 1886 as pseudohermaphroditism in a girl at the age of 19 days. In 1924 O.V. Vereshchinsky for the first time in the domestic literature cited information on 12 cases of adrenal-sexual syndrome. In the years 1950-1952. F.C.Bartter, F.Albright, A.Leaf, E. Dempsye, E. Carroll, L. Wilkins deciphered the essence of this disease, the biosynthesis of hydrocortisone. VDKN is the most common pathology of the adrenal glands in children (1 case per 5000 born).

Neonatal screening contributes to the early diagnosis of ACS, especially in boys before the development of clinical symptoms, to the early onset of substitution therapy and the safe social adaptation of children. Coverage of newborns with neonatal screening in the RS (Y) annually increases. With timely treatment of adrenogenital syndrome, the rates of physical development and puberty of the child are approaching the norm.

All patients have a lossy form of the disease. Analysis of patients identified by neonatal screening did not determine significant differences in gender, place of residence. In girls, the diagnosis was made immediately after birth due to the presence of virile syndrome. A case of an incorrect determination of the sex in a girl at birth was described on the patient's medical chart and was diagnosed with hypospadias. Substitution therapy for the majority was started up to 21 days. All patients receive replacement therapy with glucocorticoids and mineralocorticoids (Cortef, Cortineff) from the time of diagnosis in an individual dosage, depending on age. Acceleration of bone age is observed only in one child, in three, a decrease in the rate of growth and a lack of body weight.

MATERIALS AND METHODS

The order of the Ministry of Health of the Republic of Sakha (Yakutia) of March 20, 2006 was issued to organize screening, introduce new methods, organize diagnostic and therapeutic care. 01-8 / 4-134a "On the progress of the activities of the section of the national project" Health "on the examination of newborn children for hereditary diseases." The Order of the Republic of Belarus No. 1-NCM dated August 31, 2006. №01-0108 / 91 "About rendering medical aid to children with cystic fibrosis, adrenogenital syndrome, galactosemia, phenylketonuria and congenital hypothyroidism, revealed by neonatal screening" [5]. The screening procedure includes blood sampling in full-term newborns on day 4 of life, in preterm patients on day 7 and determination of 17-hydroxyprogesterone (17-ONP) levels in samples using special screening kits. The level of 17-SNP in blood samples is determined by the immunofluorescence method (test kits "Delfia 17-α-OH Progesteron», Finland, and «17-α-OH-Progesterone-Immunoskrin», Russia). The following provisions are taken into account when interpreting the indicators of 17-SNP: - the level of 17-SNP for full-term children (the gestation period is more than 37 weeks, the body weight is more than 2000 gr.) Normally up

to 30 nmol / l; At a level of 17-SNP 30-90 nmol / l - the result is regarded as questionable (false positive), re-determination of 17-SNP in the control spot is required; At a level of 17-SNP more than 90 nmol / l - the result is positive, the information is transmitted at the location of the child. For preterm infants (gestation period 33-36 weeks, body weight less than 2000 g.), The normative index of 17-OHP is up to 60 nmol / l. In cases where the premature baby has levels in 17-SNP within 60-100 nmol / L - the result is doubtful (false positive). In premature infants with a level of 17-SNP more than 100 nmol / L, the result is positive, information is given at the location of the child. In children with deep prematurity (gestation period of 23-32 weeks), the result should be considered positive at a level of 17-SNP above 150 nmol / l. In this case, it is necessary to send information to the hospital or to the children's polyclinic where the child is, and to re-take and screen-test the blood sample [4].

Data on neonatal screening for the period 2006-2016. Provided by the laboratory of the Medical Genetic Center (MHC) of the Perinatal Center, data on patients - the endocrinological department of the Pediatric Center of the State Bank of the Republic of Sakha (Yakutia) «Republican Hospital No. 1-National Center of Medicine». A retrospective study of stationary charts of children with a diagnosis of congenital adrenal cortex dysfunction was carried out. Data on patients are taken from the register of admission of patients with endocrinology department (form 001 / y).

RESULTS

According to the neonatal screening program in RS (Y), only 160 626 newborns were examined, the diagnosis of VDKN was established in 11 children, the coverage was 99.5%. The frequency of VDKN 1:14 is 602 newborns. In 2006, out of 5559 newborns that had undergone the study, no ACS was detected. In 2012 Of the 16832 newborns studied, the detectability of ACS was the highest and amounted to 4 people per year at a frequency of 1: 4208 newborns (Table 1). Thus, the prevalence of ACS in the Republic of Sakha (Yakutia) is 1 case at 14,602 (Figure 1), lower than in the Russian Federation 1: 7650 and its regions: in the Ural Federal District 1: 5781, in the Siberian Federal District 1: 9681. The most frequent occurrence of ACS is observed in Alaska residents 1: 280 newborns, the lowest in China 1:28 000 [3].

Analysis of patients identified by neonatal screening did not determine significant differences in gender - 5 boys (45%), 6 (55%) girls, sex ratio 1: 1.5, place of residence - urban 5 (45%), rural 6 (55%), nationality - 4 (36%) of the Yakut child, 5 (46%) Russians, and 2 (18%) other nationalities.

Table 1

The distribution of the frequencies of alleles and genotypes of the polymorphic loci of the genes of the dopaminergic system in patients with Parkinson's disease and in the control group

The groups	Frequencies of alleles, n (p, %)		Frequencies of genotypes, n (p, %)			N
	*C	*T	*C/C	*C/T	*T/T	
1	2	3	4	5	6	7
***DRD1* (rs4532)**						
The control group	192 (28,15)	490 (71,85)	26 (7,62)	140 (41,06)	175 (51,32)	341
Patients with Parkinson's disease	120 (27,27)	320 (72,73)	19 (8,64)	82 (37,27)	119 (54,09)	220
RT form	47 (27,98)	121 (72,02)	10 (11,9)	27 (32,14)	47 (55,95)	84
AR form	19 (31,67)	41 (68,33)	4 (13,33)	11 (36,67)	15 (50)	30
ART form	26 (26)	74 (74)	2 (4)	22 (44)	26 (52)	50
Manifestation of up to 45 years	10 (26,32)	28 (73,68)	2 (10,53)	6 (31,58)	11 (57,89)	19
Manifestation 45-60 years	34 (27,42)	90 (72,58)	6 (9,68)	22 (35,48)	34 (54,84)	62
Manifestation after 60 years	53 (26,77)	145 (73,23)	8 (8,08)	37 (37,37)	54 (54,55)	99
***DRD2* (Taq1, или 32806C>T)**						
	*A1	*A2	*A1/A1	*A1/A2	*A2/A2	
The control group	176 (22,56)	604 (77,44)	17 (4,36)	142 (36,41)	231 (59,23)	390
Patients with Parkinson's disease	107 (24,21)	335 (75,79)	14 (6,33)	79 (35,75)	128 (57,92)	221
RT form	28 (19,44)	116 (80,56)	5 (6,94)	18 (25)	49 (68,06)	72
AR form	16 (25)	48 (75)	1 (3,12)	14 (43,75)	17 (53,12)	32
ART form	32 (25,4)	94 (74,6)	4 (6,35)	24 (38,1)	35 (55,56)	63
Manifestation of up to 45 years	8 (19,05)	34 (80,95)	2 (9,52)	4 (19,05)	15 (71,43)	21
Manifestation 45-60 years	30 (24,19)	94 (75,81)	3 (4,84)	24 (38,71)	35 (56,45)	62
Manifestation after 60 years	48 (23,76)	154 (76,24)	6 (5,94)	36 (35,64)	59 (58,42)	101
***DRD2* (rs6275 или NcoI)**						
	*A	*G	*A/A	*A/G	*G/G	
The control group	331 (39,4)	509 (60,6)	70 (16,67)	191 (45,48)	159 (37,86)	420
Patients with Parkinson's disease	214 (41,47)	302 (58,53)	46 (17,83)	122 (47,29)	90 (34,88)	258
RT form	76 (41,3)	108 (58,7)	19 (20,65)	38 (41,3)	35 (38,04)	92
AR form	32 (47,06)	36 (52,94)	7 (20,59)	18 (52,94)	9 (26,47)	34
ART form	52 (39,39)	80 (60,61)	10 (15,15)	32 (48,48)	24 (36,36)	66
Manifestation of up to 45 years	15 (32,61)	31 (67,39)	3 (13,04)	9 (39,13)	11 (47,83)	23
Manifestation 45-60 years	61 (42,96)	81 (57,04)	15 (21,13)	31 (43,66)	25 (35,21)	71
Manifestation after 60 years	99 (42,67)	133 (57,33)	20 (17,24)	59 (50,86)	37 (31,9)	116
***DRD3* (rs6280 или Ser9Gly)**						
	*C	*T	*C/C	*C/T	*T/T	
The control group	179 (25,07)	535 (74,93)	27 (7,56)	125 (35,01)	205 (57,42)	357
Patients with Parkinson's disease	120 (24,39)	372 (75,61)	16 (6,51)	88 (35,77)	142 (57,72)	246
RT form	44 (23,66)	142 (76,34)	6 (6,45)	32 (34,41)	55 (59,14)	93
AR form	15 (24,19)	47 (75,81)	2 (6,45)	11 (35,48)	18 (58,06)	31
ART form	31 (26,27)	87 (73,73)	4 (6,78)	23 (38,98)	32 (54,24)	59
Manifestation of up to 45 years	9 (22,5)	31 (77,5)	2 (10)	5 (25)	13 (65)	20
Manifestation 45-60 years	33 (23,57)	107 (76,43)	2 (2,86)	29 (41,43)	39 (55,71)	70
Manifestation after 60 years	54 (24,11)	170 (75,89)	8 (7,14)	38 (33,93)	66 (58,93)	112
***DRD4* (VNTR 120bp)**						
	*S	*L	*S/S	*S/L	*L/L	
The control group	124 (15,9)	656 (84,1)	8 (2,05)	108 (27,69)	274 (70,26)	390
Patients with Parkinson's disease	68 (15,39)	374 (84,61)	9 (4,07)	50 (22,62)	162 (73,31)	221
RT form	20 (14,09)	122 (85,91)	3 (4,23)	14 (19,72)	54 (76,06)	71
AR form	7 (10,94)	57 (89,06)	0 (0)	7 (21,88)	25 (78,12)	32
ART form	22 (17,19)	106 (82,81)	4 (6,25)	14 (21,88)	46 (71,88)	64
Manifestation of up to 45 years	8 (19,05)	34 (80,95)	2 (9,52)	4 (19,05)	15 (71,43)	21
Manifestation 45-60 years	16 (12,90)	108 (87,10)	1 (1,61)	14 (22,58)	47 (75,81)	62
Manifestation after 60 years	30 (14,85)	172 (85,15)	5 (4,95)	20 (19,80)	76 (75,25)	101
***DRD4* (rs747302 или 616C>T)**						
	*C	*G	*C/C	*C/G	*G/G	
The control group	285 (37,4)	477 (62,6)	48 (12,6)	189 (49,61)	144 (37,8)	381
Patients with Parkinson's disease	168 (37)	286 (63)	30 (13,22)	108 (47,58)	89 (39,21)	227
RT form	59 (37,82)	97 (62,18)	12 (15,38)	35 (44,87)	31 (39,74)	78
AR form	23 (38,33)	37 (61,67)	5 (16,67)	13 (43,33)	12 (40)	30

End of table 1

1	2	3	4	5	6	7
AR form	44 (36,07)	78 (63,93)	6 (9,84)	32 (52,46)	23 (37,7)	61
ART form	12 (31,58)	26 (68,42)	1 (5,26)	10 (52,63)	8 (42,11)	19
Manifestation 45-60 years	53 (40,15)	79 (59,85)	11 (16,67)	31 (46,97)	24 (36,36)	66
Manifestation after 60 years	66 (33,33)	132 (66,67)	11 (11,11)	44 (44,44)	44 (44,44)	99
***MAO-B* (rs1799836) (men)**						
	*C	*T	*C/C	*C/T	*T/T	N
The control group	74 (29,13)	180 (70,87)	37 (29,13)	0	90 (70,87)	127
Patients with Parkinson's disease	76 (35,19)	140 (64,81)	38 (35,19)	0	70 (64,81)	108
RT form	24 (27,91)	62 (72,09)	12 (27,91)	0	31 (72,09)	43
AR form	10 (35,71)	18 (64,29)	5 (35,71)	0	9 (64,29)	14
ART form	26 (54,17)	22 (45,83)	13 (54,17)	0	11 (45,83)	24
Manifestation of up to 45 years	6 (33,33)	12 (66,67)	3 (33,33)	0	6 (66,67)	9
Manifestation 45-60 years	16 (40)	24 (60)	8 (40)	0	12 (60)	20
Manifestation after 60 years	38 (34,55)	72 (65,45)	19 (34,55)	0	36 (65,45)	55
***MAO-B* (rs1799836) (women)**						
The control group	171 (36,69)	295 (63,31)	33 (14,16)	105 (45,06)	95 (40,77)	233
Patients with Parkinson's disease	96 (35,56)	174 (64,44)	21 (15,56)	54 (40)	60 (44,44)	135
RT form	28 (27,45)	74 (72,55)	5 (9,8)	18 (35,29)	28 (54,91)	51
AR form	16 (50)	16 (50)	4 (25)	8 (50)	4 (25)	16
ART form	21 (30,88)	47 (69,12)	5 (14,71)	11 (32,35)	18 (52,94)	34
Manifestation of up to 45 years	7 (31,82)	15 (68,18)	1 (9,1)	5 (45,45)	5 (45,45)	11
Manifestation 45-60 years	31 (32,98)	67 (67,02)	5 (10,2)	21 (42,86)	23 (46,94)	49
Manifestation after 60 years	41 (36,61)	71 (63,39)	11 (19,64)	19 (33,93)	26 (46,43)	56
***COMT* (rs4680 или 1947G>A)**						
	*H (G)	*L (A)	*H/*H (G/G)	*L/*H (G/A)	*L/*L (A/A)	N
The control group	306 (48,73)	322 (51,27)	67 (21,34)	172 (54,78)	75 (23,89)	314
Patients with Parkinson's disease	328 (62,12)	200 (37,88)	100 (37,8)	128 (48,48)	36 (13,64)	264
RT form	100 (56,82)	76 (43,18)	30 (34,09)	40 (45,45)	18 (20,45)	88
AR form	40 (58,82)	28 (41,18)	11 (32,35)	18 (52,94)	5 (14,71)	34
ART form	95 (73,08)	35 (26,92)	37 (56,92)	21 (32,31)	7 (10,77)	65
Manifestation of up to 45 years	17 (50)	17 (50)	5 (29,41)	7 (41,18)	5 (29,41)	17
Manifestation 45-60 years	56 (50,91)	54 (49,09)	16 (29,10)	24 (43,64)	15 (27,27)	55
Manifestation after 60 years	104 (65,82)	54 (34,18)	32 (40,51)	40 (50,63)	7 (8,86)	79

Note: n - number of the chromosomes, p – the frequency (%), N - number of individuals; RT - the rigidly-trembling form; AR – the akinetic-rigid form; ART – the akinetic-rigid-trembling form.

For 10 years 376 (0.23%) children had an elevated level of 17-ONP. Retest was performed in 214 children with elevated levels of 17-SNP, among them term infants 101 (47%), premature infants 113 (53%) (Table 2). As a result of the retest, an increased level of 17-SNP in 56 children, among them preterm infants 43 (77%), full-term children - 13 (23%). ACS was established in 11 children, respectively 45 children had a transient increase in 17-ONP. The level of increase in 17-SNP in these cases at birth varied from 65 to 1158 nmol / l. Concentrations of 17-hydroxyprogesterone may be elevated, even when there is no deficiency of this enzyme. This is due to the peculiarities of adrenal steroidogenesis, the immaturity of the axis "hypothalamus - pituitary - adrenal glands". It happens in preterm; Children with birth trauma or severe physical illness; Against intravenous infusion; In newborns with high blood bilirubin levels; At birth with low body weight at normal gestation terms. False negative results can also be determined if the mother during the pregnancy took dexamethasone for the prevention (therapy) of lung fetal diseases or so treated a newborn (with a lack of surfactant). In such cases it is recommended to check the hormone index repeatedly - after 5-7 days [4].

The condition of children with ACS at birth in 9 children was noted as satisfactory, 2 children needed resuscitation. On the Apgar scale, almost all have high scores. The physical parameters of newborns correspond to normal indices.

The level of 17-SNP for neonatal screening in the identified patients averaged 235.26 ± 1.09 nmol / l, (the range of oscillations from 67.17 ± 1.09 nmol / l to 413.34 ± 1.09 nmol / l). All patients have a lossy form of the disease. The diagnosis of VDKN in 3 boys was put on the 21st day and the 1st month of life against the background of a saltwort crisis, in 2 boys, VDKN was detected for neonatal screening. In 4 girls, the diagnosis was made immediately after birth by the presence of a viril syndrome and the degree of virilization according to the Prader scale was II-III. In 1 girl with virilization of external genital organs of III-IV degree, sex at birth was incorrectly determined and with the diagnosis: hypospadias entered the II stage of treatment of the Perinatal Center. In 1 female, the ACS was confirmed at 5 months of age, during the examination in the psycho-neurological department No. 2, due to the irregular structure of the external genitalia (Fig. 2).

The purpose of substitution treatment for children with VDKN is not to simulate physiological secretion, but to restore the deficit of corticosteroids, the secretion of which is reduced as a result of an enzymatic defect with suppression of increased secretion of corticotropin releasing hormone and ACTH, in preventing virilization, optimizing patient growth, ensuring normal sexual Maturation and potential fertility [1].

All patients receive replacement therapy with glucocorticoids and mineralocorticoids (Cortef, Cortineff) from the moment of diagnosis in the individual dosage depending on the age (Fig. 3). Acceleration of bone age is observed only in 1 (9%) of the child, at the age of 8 years the bone age corresponds to 11-12 years. This child needs to reduce the dose of substitution therapy. In 3 (27%) children there is a decrease in the rate of growth and a lack of body weight. In this group of children, an increase in the dose of hormonal drugs is required. The remaining 5 (45%) children have a normal growth rate and bone maturation. That indicates adequate therapy.

CONCLUSION

1. Neonatal screening coverage from 40.8% in the first years of its introduction increased to 99.8% in the period from 2006 to 2016. The frequency of ACS was 1:14 602, which shows a low incidence rate in comparison with other regions of the Russian Federation.

2. Analysis of the anamnesis, distribution by place of residence, by gender, by nationality, by the state of health at birth, by physical parameters, the Apgar score did not reveal any specific features. In all cases, there is a salt-losing form of the disease. In girls, the diagnosis of ACS is assumed at birth, due to the virilization of NGOs, in men, the diagnosis was made on the basis of clinical symptoms and neonatal screening. In most cases, the clinic begins on the 21st day of life, i.e. it is necessary for doctors neonatologists and pediatricians to send timely to the retest of newborns with increased results, to be wary of ACS, to carefully inspect the external genitalia. The average level of 17-SNP for neonatal screening was 235.26 ± 1.09 nmol / l.

3. Properly selected and timely therapy of GCS and ISS provides normal growth rates, bone maturation, sexual develop-

Table 2

Frequency distribution of alleles and genotypes of the polymorphic loci of the genes of the dopaminergic system in patients with Parkinson's disease and in the control group

Alleles	Comparable groups		Parkinson's		Genotypes	Control		Parkinson's	
	n	p (%)	n	p (%)		n	p (%)	n	p (%)
***DRD4* (VNTR 48bp)**									
*2R	78	10,29	33	7,21	*2R/2R	17	4,49	7	3,06
					*2R/3R	3	0,79	3	1,31
					*2R/4R	41	10,82	15	6,55
*3R	29	1,19	17	3,71	*2R/5R	0	0	1	0,44
					*2R/7R	0	0	0	0
					*2R/8R	0	0	0	0
*4R	605	79,82	367	80,13	*3R/3R	4	1,06	2	0,87
					*3R/4R	16	4,22	10	4,37
					*4R/4R	261	68,87	159	69,43
*5R	21	2,77	17	3,71	*4R/5R	9	2,38	8	3,49
					*4R/7R	16	4,22	13	5,68
					*4R/8R	1	0,26	1	0,44
*7R	24	3,17	19	4,15	*5R/5R	5	1,32	3	5,68
					*5R/7R	1	0,26	1	0,44
					*7R/7R	3	0,79	2	0,88
*8R	1	0,13	3	0,66	*8R/8R	8	2,11	1	0,44
					N	379		229	
TH (repeats (TCAT)n)									
*6	159	26,68	109	25,95	*6/6	18	6,04	14	6,67
					*6/7	33	11,07	27	12,86
					*6/8	19	6,38	16	7,62
*7	99	16,61	86	20,48	*6/9	24	8,05	13	6,19
					*6/9,3	46	15,44	24	11,43
					*6/10	1	0,34	1	0,48
*8	66	11,07	48	11,43	*7/7	2	0,68	6	2,86
					*7/8	11	3,69	4	1,91
					*7/9	17	5,71	15	7,14
*9	101	16,95	71	16,91	*7/9,3	33	11,07	28	13,33
					*7/10	1	0,34	0	0
					*8/8	1	0,34	1	0,48
*9,3	165	27,69	105	25	*8/9	13	4,36	15	7,14
					*8/9,3	20	6,71	11	5,24
					*8/11	1	0,34	0	0
*10	22	3,69	16	3,81	*9/9	10	3,41	4	1,91
					*9/9,3	26	8,73	20	9,52
					*9,3/9,3	20	6,71	11	5,24
*11	2	0,34	0	0	*10/10	1	0,34	0	0
					N	298		210	

Table 3

Comparative analysis of the frequencies of alleles and genotypes of the polymorphic loci of the genes of the dopaminergic system with the development of Parkinson's disease, its clinical forms and age of manifestation

Genotipe, allele	Comparable groups	p	χ^2	OR	95% CI
***MAO-B* (rs1799836) (мужчины)**					
*C/*C	ART form / control group	0,0169* (0,051**)	5,71	2,58	1,18-6,70
*T/*T		0,0169* (0,051**)	5,71	2,58	1,18-6,70
*C		0,0007*	11,42	2,88	1,53-5,39
*T		0,0007*	11,42	0,35	0,19-0,65
***COMT* (rs4680 или 1947G>A)**					
*H/*H (G/G)	patients with Parkinson's disease / control group	0,000012* (0,000036**)	19,10	2,22	1,56-3,25
*L/*H (G/A)		0,13	2,28	0,78	0,56-1,08
*L/*L (A/A)		0,0018* (0,0054**)	9,71	0,50	0,33-0,78
*H (G)		0,000005*	20,78	1,73	1,36-2,18
*H/*H (G/G)	ART form / control group	0,000001* (0,000003**)	34,25	4,87	2,78-8,53
*L/*H (G/A)		0,00097* (0,0029**)	10,88	0,34	0,22-0,69
*L/*L (A/A)		0,02* (0,06**)	5,46	0,39	0,17-0,88
*H (G)		0,000001*	25,63	2,86	1,88-4,34
*H/*H (G/G)	manifestation after 60 years / control group	0,00045* (0,00135**)	12,31	2,51	1,49-4,24
*L/*H (G/A)		0,51	0,44	0,85	0,52-1,39
*L/*L (A/A)		0,000001* (0,000003**)	53,66	0,08	0,04-0,18
*H (G)		0,00012*	14,79	2,03	1,41-2,92

Note: $\chi 2$ - the criterion of independence; OR - odds ratio; 95% CI - 95% confidence interval; p – the significance level; * - the value of p <0.005; ** - the significance level p with Bonferroni amendment.

ment and normal reproductive function.

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INVESTIGATION OF THE ROLE OF CYTOKINE GENES POLYMORPHISMS IN THE DEVELOPMENT OF THE URTICARIA IN THE REPUBLIC OF BASHKORTOSTAN

ABSTRACT

At present, there is a steady increase in the incidence and prevalence of allergic skin diseases in the world, affecting up to 25% of the population in different countries. Urticaria is an etiologically heterogeneous group of diseases and conditions characterized by the formation of itching rashes on the skin. According to epidemiological studies, at least once during a lifetime this pathology is observed in 15-25% of the population. Urticaria is a polyethological disease. Allergic mechanisms of tissue damage are involved in the development of the allergic form of hives. Cytokines play a key role in all stages of development and maintenance of allergic inflammation. The purpose of this study was to investigate the polymorphic loci of interleukins genes IL4 (rs2243250), IL4R (rs1805010), IL10 (rs1800872), IL13 (rs20541) and tumor necrosis factor gene TNF (rs1800629) in patients with hives and in the control group of individuals. The material for the study was DNA samples of 102 unrelated individuals with urticaria, and 153 healthy individuals living in the Republic of Bashkortostan. The DNA was isolated by phenol-chloroform extraction. Genotyping of polymorphic loci was carried out by real-time PCR. As a result of the analysis, we showed that the rs1800629*G allele and the rs1800629*G/G genotype of the TNF gene polymorphism are the markers of an increased risk of developing of chronic urticaria, rs2243250*C allele of the IL4 gene – of the acute urticaria, and the rs1800629*G/A genotype of the TNF gene is a marker of the urticaria with concomitant allergic diseases development. The data obtained by us are in part consistent with the results of other authors. Thus, in patients from Japan and Canada, the SNP rs2243250 of the IL4 gene is associated with the development of atopic dermatitis. The association of the TNF gene rs1800629 polymorphism with the development of bronchial asthma and atopy is indicated in patients from the USA and Spain. As in our study, patients with allergic dermatoses from Japan did not have an association of the IL4R gene SNP rs1805010 with the development of the disease. Nevertheless, a number of other studies have shown the association with the development of various allergic diseases of all the polymorphic loci we studied. Thus, this study shows an association with the development of urticaria of polymorphic variants of the TNF and IL4 genes.

Keywords: urticaria, association analysis, cytokines, genes, polymorphic variants.

INTRODUCTION

At present, there is a steady increase in the incidence and prevalence of allergic skin diseases in the world, which affects up to 25% of the population in different countries. Allergic dermatoses account for 20% in the structure of allergic diseases, and take 50 to 66% in the structure of childhood allergopathology [1]. One of the most common allergic dermatoses, found in people of different ages, is urticaria. Urticaria is a toxic-allergic dermatosis, characterized by the rapid occurrence of multiple itchy rashes (blisters) of red color on the skin and mucous membranes. According to epidemiological studies, at least once during a lifetime this pathology is observed in 15-25% of the population [2].

Urticaria is a classic polyethological disease, i.e. its symptoms can be caused by various factors. The main risk factors for the development of acute urticaria are food products, medicines and insect bites. The situation with chronic urticaria is more complicated: many factors that cause its aggravation are known, but it is possible to reveal its cause in no more than 10% of cases. At the same time, the pathogenesis of urticaria due to allergic reactions of the immediate type has been studied quite well. Allergic mechanisms of tissue damage are involved in the development of this form of hives. The leading mechanism of development of urticaria is a reagin-dependent mechanism of damage.

Cytokines play a key role in all stages of development and maintenance of allergic inflammation. In many studies, a significant association of single nucleotide polymorphisms (SNPs) of cytokines and their receptors genes with the development of allergic diseases has been demonstrated [4, 6, 8, 9, 13]. The association of polymorphic loci of cytokine genes with the development of allergic dermatoses, in particular atopic dermatitis (AD), has been shown in numerous studies conducted in patients from Japan [12,13], Korea [7], Czech Republic [6], Canada [9] and the United States [14]. In addition, the association of polymorphic variants of cytokine genes with the development of other allergic diseases, in particular bronchial asthma, has been identified [4, 15, 18]. Nevertheless, a number of other authors do not confirm this data [8; 16].

In our study, we analyzed SNPs of *IL4* (rs2243250), *IL4R* (rs1805010), *IL10* (rs1800872), *IL13* (rs20541) and *TNF* (rs1800629) genes in urticaria and in the control group of individuals living in the Republic of Bashkortostan.

MATERIALS AND METHODS

The study of polymorphic loci of cytokine genes was carried out in 102 unrelated individuals with urticaria living in the Republic of Bashkortostan. All examined individuals were patients of the Department of allergy at Municipal Clinical Hospital No. 21 in Ufa. Diagnosis of the disease was established by qualified doctors on the basis of clinical, general laboratory and additional research methods in accordance with the criteria of the program documents for the diagnosis, treatment and prevention of diseases. The sample of patients with urticaria included individuals with different forms of the disease. The acute form (with the duration of the disease up to 6 weeks) was observed in 47 patients, chronic (with duration of the disease more than 6 weeks) – in 55 patients. In addition, the patients were divided into two groups depending on the presence or absence of concomitant allergic diseases (allergic conjunctivitis , allergic rhinitis, atopic dermatitis, bronchial asthma, angioedema, or combinations thereof). The control group consisted of 153 practically healthy individuals, comparable in sex and age with patients and not having a hereditary burden of atopic diseases. Informed consent to participate in this study was obtained from all of its participants.

The isolation of DNA from peripheral blood lymphocytes was carried out by a standard phenol-chloroform extraction method. Amplification of polymorphic loci of cytokine genes was carried out using real-time polymerase chain reaction (PCR) of DNA synthesis.

RESULTS AND DISCUSSION

Five polymorphic variants of cytokine genes (*IL4* (rs2243250, c.-590C>T), *IL4R* (rs1805010), *IL10* (rs1800872, c.-627C>A), *IL13* (rs20541, p.Arg144Gln) and *TNF* (rs1800629)) were studied in patients with urticaria and individuals of the control group living in the Republic of Bashkortostan. The frequency distribution of the genotypes of all the polymorphic loci studied in the control groups of healthy individuals corresponded to the Hardy-Weinberg equilibrium.

The study of polymorphic locus rs2243250 of *IL4* gene revealed its association with the development of acute urticaria. In this group of patients rs2243250*C allele was determined in 79.35% of individuals, whereas in the control the frequency was lower and was 67.4% (p = 0.0247, OR = 1.86 (95% CI 1.08- 3.21)). In patients with chronic disease, the differences in rs2243250*C allele frequencies with control were less pronounced: here it was detected only in 63.2% of cases (p> 0.05). Statistically significant differences in the distribution of alleles and genotypes frequencies of a given polymorphic locus between healthy individuals and patients with urticaria with or without concomitant allergic diseases have also not been identified.

Association analysis of the SNP rs1805010 of *IL4RA* gene revealed a tendency to increase in patients with chronic urticaria when compared with the control, the frequency of rs1805010*Val/Val genotype, which was 19.3% and 9.9%, respectively (p = 0.054). In the group of patients with acute disease, this genotype was detected only in 8.7% of patients. Comparative analysis of the frequency distribution of alleles and genotypes of SNP rs1805010 of the IL4RA gene in patients with urticaria with concomitant allergic diseases and without them did not reveal statistically significant differences with the control group.

In the study of the rs1800872 polymorphism of the *IL10* gene, no statistically significant differences in the distribution of frequencies of alleles and genotypes between hives patients and controls were found neither. This SNP is not associated with either acute or chronic disease. It should be noted that the association of the polymorphic locus rs1800872 of the *IL10* gene with the development of urticaria was not detected, regardless of the presence or absence of concomitant allergic diseases.

A comparative analysis of the distribution of alleles and genotypes frequencies of the *IL13* gene rs20541 polymorphism in patients with urticaria and in the control group showed that in patients with chronic urticaria there is a tendency to increase of the frequency of rs20541*Arg/Gln genotype. It was detected in 54.6% of individuals from this group, and in in 40.1% controls (p = 0.06). In patients with acute urticaria, the rs20541*Arg/Gln genotype was found in 48.9% of individuals, however, these differences did not reach the level of statistical significance. Minor differences were found when control group was compared to the group of patients with hives with concomitant allergic diseases. In this group, the rs20541*Arg/Arg genotype was detected in 45% of individuals, and the rs20541*Arg/Gln genotype – in 55%. Their frequencies in the controls were, respectively, 52.5% and 40.1% (p> 0.05). The rs20541*Arg/Arg genotype was found in 46.3% of patients with urticaria without concomitant allergic diseases, and an insignificant tendency for the rs20541*Arg/Gln genotype to show an increase in frequency compared with the control (51.3%, p=0.09).

Analysis of the polymorphic locus rs1800629 of the *TNF* gene indicated the association with the development of chronic urticaria of rs1800629*G

allele and rs1800629*G/G genotype. The frequency of rs1800629*G allele was 83.9% in patients and 90.7% in the control group of individuals (p = 0.037, OR = 0.53 (95% CI 0.29-0.97)). The rs1800629*G/G genotype was detected in 67.9% of patients with chronic urticaria and 82.4% of controls (p = 0.0164, OR = 0.45, (95% CI 0.23-0.87)). The frequency of rs1800629*G/A genotype, in contrast, was higher in patients, where it was 32.1%, than in control - 16.7% (p> 0.05). In addition, we discovered the association of the SNP rs1800629 of the TNF gene with the development of urticaria with concomitant allergic diseases. In patients, the rs1800629*G/A genotype frequency was higher than that in controls (29.1% and 16.7%, respectively, p = 0.0179, OR = 2.05 (95% CI = 1.12- 3.75)). In contrast, the rs1800629*G/G genotype and the rs1800629*G allele are more often found in the control group of individuals. The rs1800629*G/G genotype frequency was 69.6% in patients and 82.4% in controls (p = 0.017, OR = 0.49 (95% CI 0.27-0.89)), and the rs1800629*G allele frequency was 84.2% and 90.7%, respectively (p = 0.02, OR = 0.54 (95% CI 0.32-0.93)).

The data obtained by us are in part consistent with the results of other authors. Thus, in patients from Japan and Canada, the rs2243250 polymorphism of the *IL4* gene is associated with the development of AD [9, 13]. However, the authors of the study conducted in China, found no association of this polymorphic locus with the development of allergic dermatoses [16]. As in our work, patients with allergic dermatoses from Japan did not have an association of the *IL4R* gene rs1805010 with the development of the disease [14], however, a large number of studies of polymorphic variants of this gene showed its role in the development of AD and other allergic diseases [17]. A number of studies conducted in Canada, Japan and other countries showed the association of SNP rs20541 of the *IL13* gene with the development of AD [9,12], which, however, is not confirmed by others [16]. Earlier, the association of the SNP rs1800872 of the *IL10* gene with the development of AD in India [5] and with an increased IgE level in patients from Korea [11] and with the development of AD in patients from the Republic of Bashkortostan [3] was found. The association of *TNF* gene SNPs with the development of allergic dermatoses was not found in some works conducted in Macedonia and Great Britain [19]. Nevertheless, the association of the rs1800629 with the development of asthma and atopy in patients from the USA and Spain has been shown [10, 18].

CONCLUSION

In this paper, we analyzed the polymorphic variants of the cytokine genes: rs2243250 of the *IL4* gene, rs1805010 of the *IL4R* gene, rs1800872 of the *IL10* gene, rs20541 of the *IL13* gene and rs1800629 of the *TNF* gene, in patients with hives and controls. As a result of the study, it was revealed that the rs1800629*G allele and the rs1800629*G/G genotype of the *TNF* gene polymorphism are the markers for the increased risk of chronic hives, and the rs2243250*C allele of the *IL4* gene polymorphism is the marker of increased risk of acute urticaria development. The marker of an increased risk of development of urticaria with concomitant allergic diseases is the rs1800629*G/A genotype of the *TNF* gene polymorphism. The rs1800629*G/G genotype and rs1800629*G allele of this polymorphic locus are markers of a reduced risk of hives with concomitant allergic diseases.

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APPROBATION OF THE MOLECULAR GENETIC METHOD FOR THE DIAGNOSIS OF *HELICOBACTER PYLORI* INFECTION IN YAKUTIA

ABSTRACT

In this paper we presented the results of approbation of the PCR method for the diagnosis of Helicobacter pylori infection based on the amplification of the *16S rRNA* marker gene of bacterial DNA isolated from samples of gastric mucosa tissue from patients with gastroduodenal diseases in Yakutia.

Keywords: Helicobacter pylori, gastroduodenal diseases, PCR, *16S rRNA* gene, histology, Yakutia.

INTRODUCTION

Helicobacter pylori (*H. pylori*) as considered is a main cause of the development of various gastroduodenal diseases, such as chronic gastritis, erosion and stomach ulcer in humans [1, 3, 5]. In 1994, the International Agency for Research on Cancer classified *H. pylori* infection to the I group of carcinogen (obvious carcinogens), along with some of the radionuclides and radiation [23]. Due to the fact that *H. pylori* is currently associated not only with certain gastroduodenal diseases, but also with severe oncological pathologies, it becomes necessary to specifically diagnose this infection. At present, in clinical practice, there are many different methods for diagnosing *H. pylori* [7, 11-13]. The variety of methods for detection of this infection can be divided into invasive (require fibrogastroduodenoscopy) and non-invasive. The main and most frequently used methods for diagnosing *H. pylori* infection are presented in Table 1. In addition, each of the method has its advantages and disadvantages [11-13]. The disadvantage of many non-invasive methods is their inaccuracy, and the invasive methods – risks of complications, as well as their duration and labor intensity. In clinical practice, the histological examination method is widely used, which allows at the same time to

Table 1

The main detection methods of *Helicobacter pylori*

Invasive methods*	Non-invasive methods**
Histological method: examination of a tissue sample of the gastric mucosa for *H. pylori*	ELISA: a study of feces for the presence of *H. pylori* antigens (using monoclonal antibodies)
Microbiological method: cultivation of *H. pylori* on substratum from a sample of gastric mucosa tissue	ELISA: detection of IgG antibodies to *H. pylori* in serum
PCR method: investigation by polymerase chain reaction on the presence of *H. pylori* DNA from a sample of the gastric mucosa tissue	Rapid urease test (CLO-test, Campylobacter-like organism test)
	Urea breath test (13C, 14C carbamide)
	PCR method: investigation by polymerase chain reaction for the presence of *H. pylori* DNA in saliva or feces

*- Require an endoscopic examination with a targeted biopsy and further study of gastrobiopsies;
** - Not require an endoscopic examination.

detect *H. pylori* and carry out a morphological evaluation of the gastric mucosa status [17]. The histological method for the detection of *H. pylori* is considered to be the "gold standard" to diagnose of this infection [8, 17], since histological sensitivity compose from 72 to 100%, and the specificity begins from 81 to 97% [18].

At present there are new approaches to the diagnosis of *H.* pylori infection, which include molecular genetic research methods by PCR, which is based on the amplification of the marker gene *16S rRNA* [10, 21]. This method excludes the possibility of amplification of homologous regions of the *16S rRNA* gene of the closest related species and *H. pylori* strains (*Campylobacter jejuni*, *Helicobacter cinaedi*, *Helicobacter mustelae и Wolinella succinogenes*) [10]. According to some authors the diagnostic sensitivity of PCR for detecting *H. pylori* in the gastric mucosa biopsies is 88-95,4% and specificity – 100% [13, 14].

Identification of *H. pylori* infection by PCR methods was not performed earlier in Yakutia. In earlier studies detection of this infection was performed by histological and cytological methods on gastrobiopsy specimens obtained at endoscopy [1, 4, 6, 7]. The aim of this study is approbation of the PCR method for the detection of *Helicobacter pylori* in patients with gastroduodenal diseases in Yakutia.

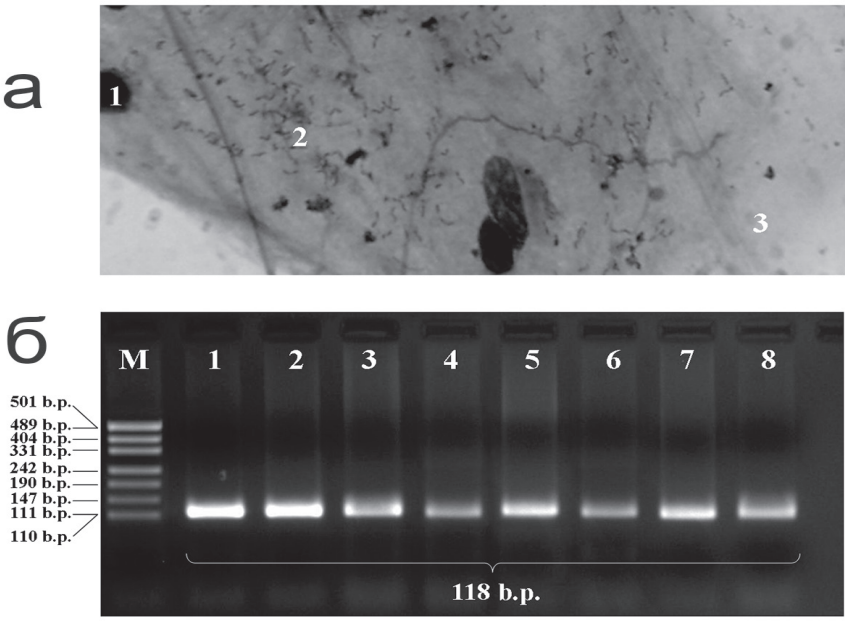

Figure 1. Examples of *H. pylori* detection using histological and molecular genetic studies.
A) Cytological micropreparation (Gram staining) of the patient's gastric mucosa with the third degree of dissemination (more than 50 microbial bodies in one visual field). 1 – cells of the epithelium of the stomach, 2 – congestion of *H. pylori*, 3 – mucous membrane;
B) Results of electrophoregram visualization after PCR analysis of *H. pylori 16S rRNA* gene. Lane 1-8 - samples of patients who gave a positive result for the presence of marker gene *16S rRNA H. pylori* (118 b.p.); M – is a mass molecular marker of pUC19/MspI

MATERIALS AND METHODS

Design of study

The sample of the study included 156 Yakut patients (from 6 to 70 years old, mean age 36.2 ± 17.5 years) with chronic gastritis. The patients observed at endoscopic department for fibrogastroduodenoscopy of the Republican Hospital No. 1 - the National Center of Medicine of the Ministry of Health of Sakha Republic (Yakutia) (RB No. 1 NCM). From 156 patients, 40 were children and adolescents (from 6 to 17 years, mean age 13.6 ± 2.6 years), the remaining 116 were adults (from 19 to 70 years, mean age 44.22 ± 13.84 years).

Endoscopic and histological examination

Fibrogastroduodenoscopy was performed in the morning, on an empty stomach. The biopsy sampling was carried out from the antral part of the stomach in an amount of 2-3 pieces during endoscopic examination with GIF-P3 fiberscope ("Olympus", Japan). The obtained biopsy samples of gastric mucosa were fixed in 10% formalin solution. Deparaffinization of shear and staining by hematoxylin and eosin performed according to standard procedures. For sighting microscopy, shears were stained by the Romanowsky-Giemsa method. The study was performed under magnification x100, x400 and x1000 on the microscope "Axioskop" ("Opton"). Morphological criteria of chronic gastritis evaluated in accordance with the visual analog scale for the modified Sydney system (Houston, USA, 1996).

Detection of 16S rRNA gene of H. pylori

Genomic DNA of *H. pylori* was isolated from frozen gastrobiopsies of the examined patients by using phenol-chloroform extraction. Amplification of the required DNA fragments that flanking *16S rRNA* gene of *H. pylori* was performed using of the oligonucleotide primers described previously (Table 2). PCR was performed on «Bio-Rad» thermocycler. Separation of amplification products was carried in the horizontal electrophoresis camera in a 3% agarose gel (Fig. 1, B). Visualization of PCR products was performed by «Bio-Rad» gel video documentary device using Image Lab ™ Software.

Informativeness of PCR analysis for detecting H. pylori

For analysis of PCR informativeness we compared the PCR results with results of histological method and performed calculations such informative parameters as sensitivity (Se) and specificity (Sp) [2].

Sensitivity was calculated by the formula:

$$Se = (TP/D^{-}) \times 100\%$$

TP – true positive samples (74 positive patients by histology and PCR);

D^{-} – infected patients (86 positive by histology).

Specificity was calculated by the formula:

$$Sp = (TN/D) \times 100\%$$

TN – true negative samples (30 negative patients by histology and PCR);

D – not infected patients (70 negative by histology).

Ethical approval

Written informed consent was obtained from all individuals. This study was approved by the local Committee on Biomedical Ethics of the Federal State Budgetary Scientific Institution of the Federal State Budgetary Scientific Institution "YNC CMP" (Yakutsk, Russian Federation, Protocol No 41, November 12, 2015. Decision №5).

Table 2

The oligonucleotide primers for detection of the of *16S rRNA* marker gene of *H. pylori*

Gene, fragment	Name of the oligonucleotide primer	Sequence from 5' → 3'	The size of the amplified fragment.
16S rRNA	*16S rRNA*	F5′-TGCGAAGTGGAGCCAATCTT-3′ R5'-GGAACGTATTCACCGCAACA-3'	118 п.н.

RESULTS

The cross method of *H. pylori* detection by PCR and histology was performed in 156 patients with gastroduodenal diseases (chronic gastritis, erosion and gastric ulcers). In 104 out of 156 examined patients (66.6%), the results of PCR completely coincided with the results of histology. 52 of 156 (33.3%) of the examined patients had mixed results (the results of PCR did not coincide with the results of histology). The results of a cross-sectional PCR and histology study are shown in Table 3.

To evaluate the informativeness of the PCR method, we analyzed the main operational characteristics such as sensitivity and specificity. The results of informativeness of the PCR analysis in relation to the histological method showed that the sensitivity of the PCR was 86.0% (p> 0.05) and specificity was 42.8% (p <0.05). The parameters of the PCR informativeness compared with the histological method are presented in Figure 2.

DISCUSSION

In this paper we presented the results of approbation of the PCR method for the diagnosis of *Helicobacter pylori* infection based on the amplification of the *16S rRNA* marker gene of bacterial DNA isolated from samples of gastric mucosa tissue from patients with gastroduodenal diseases in Yakutia. To evaluate the PCR informativeness for the detection of *H. pylori*, we compared PCR results with the histological method which has high sensitivity and specificity [18] and is considered the "golden standard" for the detection of *H. pylori* [8]. The results of histological studies conditionally were accepted by us for 100% for both sensitivity and specificity. In our study, the sensitivity of the PCR method was 86.0% and was almost inferior to the histological method (100%, p> 0.05). The specificity of the PCR method was significantly lower (42.8%) compared to the histological method (100%, p <0.05) (Fig. 2).

To evaluate the data obtained on the sensitivity and specificity of the PCR method of *H. pylori* detection, we performed a comparison analysis with the studies of other authors. Comparative analysis of the data showed that the sensitivity of the PCR method in different studies was from 55% to 100% (Table 4). The sensitivity of the PCR method in our study was 86.0% and takes an intermediate value among earlier studies (Table 4). The specificity of the PCR method among the analyzed data is from 80% to 100% (Table 4). The specificity of the PCR method of our study was significantly lower – 42.8%.

Probably, low specificity of PCR method in relation to histology is caused by a large number of false positive results (40 false positive versus 12 false negative results). This assumption is confirmed by earlier studies, in which was showed the high sensitivity of the PCR method [21]. Thus, in the work of Ramírez-Lázaro et al. [21] was studied biopsies of patients which histology was negative for *H. pylori* (n=52). In 25 of 52 patients, real-time PCR gave positive results for the presence of *H. pylori* (48%) [21]. This result shows that the histological method of study does not always reveal the presence of this infection. This phenomenon probably can be explained by the fact that the PCR method, is based on DNA detection and does not require viable bacteria and can give positive results with negative histology results.

Table 3

Cross method of *H. pylori* detection using PCR and histology

Obtained results	Cross-matches*		Mixed values**		Total
	PCR (+)/H(+)	PCR (-)/H(-)	PCR (+)/H(-)	PCR (-)/H(+)	
Number of samples, (%)	74 (47,4%)	30 (19,2%)	40 (25,6%)	12 (7,7%)	156 (100%)
In total	104 (66,6%)		52 (33,3%)		

Note: Cross-matches * – number of matches with histology; Mixed values** – number of mismatches with histology; PCR (+) – PCR positive results; PCR (-) – PCR negative results; H (+) –positive results of histology; H (-) – negative results of histology.

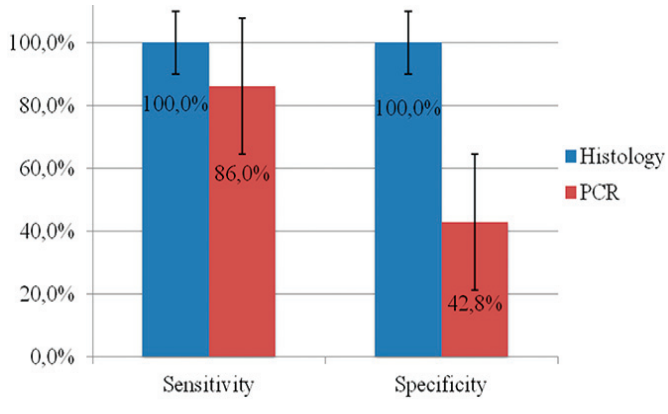

Figure 2. The parameters of the PCR informativeness compared with the histological method.

Thus, at present there is still no single method for detection of *H. pylori* with 100% sensitivity and specificity. To achieve the effectively confirm the presence or absence of this infection, it is necessary to use not one but several methods for diagnosing *H. pylori* [12]. In this regard, for the successful diagnosis of *H. pylori* in Yakutia, several cross-methods are recommended.

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Table 4

Comparative analysis of the sensitivity and specificity of the PCR method for the detection of *H. pylori*

The studied gastroduodenal diseases	Number of patients	Parameters of informativness	Molecular genetic analysis (gastrobiopsy)	Reference
Chronic gastritis, gastric ulcer, gastric adenocarcinoma	78	Sensitivity	100,0	[12]
		Specificity	90,0	
Superficial gastritis, chronic gastritis, lymphoid follicles, atrophy, metaplasia	52	Sensitivity	55,0	[18]
		Specificity	80,0	
Dyspepsia, upper gastrointestinal tract diseases	328	Sensitivity	98,	[19]
		Specificity	-	
Dyspepsia, chronic active gastritis	95	Sensitivity	94,0	[11]
		Specificity	100,0	
Chronic gastritis, erosion and stomach ulcers	156	Sensitivity	86,0	Current study
		Specificity	42,8	

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A NEW APPROACH TO GENETIC KINSHIP TESTING IN YAKUT ARCHAEOLOGY

ABSTRACT

For fifteen years, part of the work of our research team has been focused on the study of parental links between individuals living hundreds or thousands of years ago, whose remains have been found in single graves or large funerary complexes. These studies have been undertaken using methods developed by forensic genetics to identify individuals, mainly based on the genotyping of autosomal STR (Short Tandem Repeats). Issues arose from this work, namely the limits of studying small numbers of subjects, originating from groups of finite sizes where kinships cannot be inferred a priori and for which reference allelic frequencies do not exist. Although ideal human populations are rare when undertaking such studies, the Yakuts of Eastern Siberia constitute a very advantageous model, with large numbers of small pastoral communities and well-preserved archaeological material. The study of kinship in the ancient Yakuts allowed us to highlight the difficulties in analysing genetic data from small ancient human groups and to develop a strategy to improve the accuracy of statistical computations. This work describes this strategy and possible solutions to the study of populations outside of the frame of reference of global meta-populations, due either to isolation, remoteness or antiquity.

Keywords: Ancient DNA – Genetic Kinship – Population Genetics – Short Tandem Repeats

INTRODUCTION

Forensic methods have already reliably identified kinships in ancient human populations, in isolated graves (1,2), as well as funerary complexes (3,4).

Archaeological digs in Yakutia during more than ten years have exposed both single and multiple graves, either isolated or part of larger complexes. The first study applying forensic kinship methods to this data showed that some graves contained the bodies of seemingly closely related individuals (5), often parents and their children or direct siblings. In some cases, however, the occupants of a tomb appeared to be unrelated, or at least not definite close relatives. Others showed ambiguous results, where for example some tests on autosomal STR (Short Tandem Repeat) data would show clear values when studying pairs of subjects and unclear values when studying trios including the same pair. A multiple marker approach made it possible to resolve certain ambiguities, by excluding relationships based on incompatible paternal or maternal lineages, where those were relevant. Some issues however, remained undecided.

In previous studies, autosomal STR had been studied at 15 and 21 loci, while Y-chromosomal STR had been studied at 17 loci. For this study, we present the continued analysis of 15 and 21 autosomal STR loci and a pair of ancient related individuals was analysed at 83 SNPs (Single Nucleotide Polymorphisms), providing new data, independently from already obtained STR genotypes.

This work highlights the two main issues facing the study of kinship in ancient human populations from a statistics standpoint. The first question is that of the efficacy of forensic methods in remote groups, especially in resolving complex or second-degree kinship cases. Although it is advised to use closely related populations as references when a group does not belong to a larger reference population, some human groups are too remote to allow this without great approximation. The second question is the resolution power of new techniques that allow for greater numbers of markers to be analysed. Understanding the scope and reach of kinship testing methods will permit the establishment of tests and standards that supply satisfactory answers that both quantify the quality of kinship calls (going from the qualitative suggestion to the probability of specific kinship) and provide ways to study finer, second-degree kinships, while retaining statistical significance and introducing or identifying the effects of demographic events and population history.

MATERIALS AND METHODS

(1) Samples

Data was available for 128 ancient individuals (5) from four localities in Yakutia, respectively 76 individuals originating from Central Yakutia (the region of Yakutsk), 21 from the basin of the river Villuy (West of Yakutsk), 24 from the region of Verkhoyansk (North of Yakutsk) and 7 from the basin of the river Indigirka (East of Yakutsk).

(2) STR analysis

All 128 ancient individuals had been analysed with regards to 15 STR loci with the AmpFLSTR® Identifiler® Plus kit (Life Technologies™) (5). The SNP (Single Nucleotide Polymorphism) typing protocol is detailed in the Supplementary Information (Materials and Methods 1).

All STR products were run on the 3100 or 3500 genetic analyser (Life Technologies™) and analysed using GeneMapper v. 4.1 (Life Technologies™).

(3) Single Nucleotide Polymorphism (SNP) typing protocol in the Ieralaakh pair

DNA was extracted during the work realized by Keyser et al. 2015 (5).

(a) Library preparation

DNA libraries were constructed using the Ion AmpliSeq™ Library kit 2.0 (Life technologies) and the HID-Ion AmpliSeq™ Identity Panel (Life Technologies). The DNA input was 0.17ng for Ieralaakh 1, 1ng for Ieralaakh 2 and the PCR conditions were those recommended by the manufacturer (Ion AmpliSeq™ Library Preparation for Human Identification Applications Rev. A.0). After partial primer digestion, all libraries were barcoded using the Ion Xpress™ Barcode Adapters kit (Life Technologies) and purified with Agencourt AMPure XP system (Beckman Coulter). Libraries were quantified with the Ion Library Quantitation kit (Life Technologies).

(b) Template preparation and sequencing

Template preparation was done according to the manufacturer's protocol. The emulsion PCR (emPCR) was performed on the Ion OneTouch™

2 Instrument (Life Technologies) using Ion PGM™ Template OT2 200 kit (Life Technologies). Percentage of positive Ion Sphere Particles (ISP) after emPCR was measured with the IonSphere™ Quality Control Kit (Life technologies) on the Qubit® 2.0 fluorometer (Invitrogen). The emPCR products were then enriched on the Ion OneTouch™ Enrichment System (Life Technologies) using Ion PGM™ Enrichment Beads (Life Technologies) and the Ion PGM™ Template OT2 200 kit (Life Technologies). Sequencing was done on the Ion PGM™ system using the Ion PGM™ Sequencing 200 Kit v2 and the Ion 314™ v2 chip according to the manufacturer's protocol.

(c) NGS data analysis

Sequence analyses were performed with Torrent Suite™ 4.2.1 and the HID SNP Genotyper plugin v4.3 (Life Technologies). Integrative Genomics Viewer (IGV) (6) was used to examine each sequence.

(d) Results

For Ieralaakh 1 and Ieralaakh 2 DNA extracts, 72k and 56k reads were obtained respectively from the NGS run. SNP coverage varied from 25x to 1829x (with an average of 427x) for the Ieralaakh 1 DNA extract and from 9x to 4263x (with an average of 457x) for the Ieralaakh 2 DNA extract. For Ieralaakh 1, no SNP position had a sequencing depth under 20x; for Ieralaakh 2, five positions (rs729172, rs993934, rs826472, rs722290, rs12997453), were deleted for the analysis. MAF< 20% = rs1031825 deleted for Ieralaakh 1 and Ieralaakh 2. Finally, strand bias was analysed: rs430046 deleted for the 2 individuals.

Thus, 88 SNPs were successfully typed for Ieralaakh 1 and 83 for Ieralaakh 2.

(4) Computer software and test parameters

Allelic frequencies and diversities, as well as statistical tests were performed using the Genetix (7), MLrelate (8) and Arlequin (9) software.

Likelihood Ratios (LR) were computed on each pair for each relationship category against the likelihood that the individuals were unrelated, using the Familias software (10).

The three metrics that were not dependent on allelic frequencies were measured using the R language (11). Exclusions were accounted for by direct count and Relatedness was computed as a simple proportion of similarity between two genotypes. Identity-by-Descent (IBD) probability was computed using Identity-by-State (IBS) (12,13).

The relationship categories were Parent-Offspring or PO, Full-Sibling or FS, Half-Sibling or HS, Avuncular or AV (Uncle/Aunt-Nephew/Niece), Grandparent-Grandchild or GC, first Cousin or CO, and Unrelated or U. "Unrelated" was the default level of kinship in tests that required one. Thus "LR-PO" is the Ratio of the Likelihood that two individuals are a parent and his child against the Likelihood that they are unrelated.

RESULTS

CONSTITUTION OF REFERENCE POPULATIONS

The first step in constituting unrelated sets of individuals was the elimination of putative parental relationships identified by the MLrelate software, along with those examined in previous studies. Only Parent-Offspring (PO) and Full-Sibling (FS) relationships were considered accurate calls in MLrelate, due to the artefactual nature of the great majority Half-Sibling (HS) calls using this software.

At least one individual was eliminated from the reference frequency set for each putative kinship. However, many individuals were included in more than one relationship, and thus implied the existence of more distant relationships between other individuals. For the purposes of this study, the parental relationships considered grounds for eliminating subjects from the frequency set were Parent-Offspring, Full-Siblings, Half-Siblings, Avuncular, Grandparent-Grandchild and First Cousins.

Based on these criteria, 95 individuals (out of 128) from the ancient Yakut sample were considered unrelated and were included in the computation of allelic frequencies, as well as subsequent statistical tests.

Kinship results and ambiguities

(a) Kinship tests in the "Lepsei Family" using 15 autosomal STR loci

Kinship was analysed within a group of 4 ancient subjects that implied 6 parental relationships whose genealogy had been proposed in a previous study (5).

Because of the way it was built, IBD (Identity-by-Descent) called PO between two infants who showed no exclusions but could not be a parent and his child due to very young age at death. Relatedness, although it could not exclude any relationship conclusively, was only incoherent with other methods in calling a supposed PO kinship as an HS/AV/GC (Half-Sibling, Uncle/Aunt-Nephew/Niece, Grandparent-Grandchild), despite a maternal line fit and the absence of exclusions. Even though LR (Likelihood Ratio) tests gave entirely coherent results, LR values for PO and FS were very similar for the aforementioned infant pair (FS less than five times higher than PO) and distinction between LR-AV and LR-CO was also very weak for two uncle-nephew pairs (respectively less than 4 times higher and less than 2 times higher).

Although some LR values were not significantly distinct from one another and Relatedness and IBD tests each provided one incoherent call, the four methods overall concurred to confirm the proposed genealogy (5) and ambiguities were satisfactorily resolved.

(b) Kinship tests in the "Shamanic Tree Family" using 15 autosomal STR loci

The study of the "Shamanic Tree Family" (Figure 1) revealed kinship ambiguities (Table 1). Five pairs of subjects showed no exclusions, while none showed more that 4 (which is lower than any excluding value at 95% for any relationship category except PO). Relatedness proved a very unstable metric using 15 loci, with at least a third of calls not coherent with one another, as well as PO called in the presence of exclusions and FS called when paternal lineages were incompatible.

IBD called 2 relationships incorrectly (FS with paternal exclusions) and was ambiguous regarding the nature of kinship between three children ST-4, ST-5 and ST-II, identifying the three young boys as brothers, with equivocal calls of FS or HS/AV/GC for the pair ST-4/ST-II.

LR tests also gave problematic results in calling an FS relationship (ST-1/ST-4) where paternal lineages were incompatible and a CO relationship (ST-3/ST-II) where a GC relationship was implied by the study of kinship across generations (ST-3 is the mother of ST-2 who is the mother of ST-II). More significantly, it gave calls for the ST-4, ST-5, ST-II trio that were not logically consistent, making ST-5 the full brother of both ST-4 and ST-II, with ST-II the half-brother of ST-4 and the full brother of ST-5.

With the man ST-1 carrying the Y-chromosomal haplotype the most commonly found in the Yakuts (both modern and ancient) and the three boys ST-4, ST-5 and ST-II being exclusive members of a slightly different haplotype group, it seemed that direct parental or

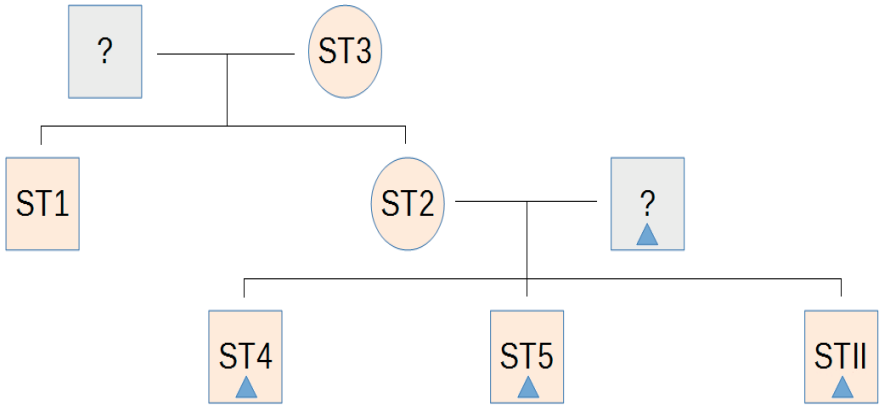

Figure 1. Shamanic Tree genealogy First Hypothesis

fraternal kinship were excluded between the adult and the three children.

(c) Kinship tests in the "Ieralaakh" pair using autosomal STR and autosomal SNP

The "Ieralaakh" pair had been classified as a possible PO relationship on the basis of 15 autosomal STR loci, using LR tests based on allelic frequencies in the ancient dataset, and corrected as an FS relationship using indel typing (5, 16). LR-FS was always higher than LR-PO, even when no exclusions were observed on 15 loci, however, the difference between the two values was less than twofold. Relatedness and IBD tests computed on 15 loci respectively called FS and PO (the present IBD test calls PO in the absence of exclusions).

The study of 21 autosomal STR loci revealed 2 exclusions that eliminated the possibility of a PO relationship (Table 2) across all metrics. Test values were however again computed on the 19 loci that showed no exclusions to identify possible discrepancies. Using 19 loci, LR-PO is higher than LR-FS and only Relatedness calls FS.

All methods analysing autosomal STR converged towards an FS relationship, without however providing clear superiority of an objective metric over another, given that LR must always be understood as a qualitative metric, not a quantitative one.

To improve on these results, 83 autosomal SNP were typed in the "Ieralaakh" pair. Only 2 exclusions were revealed, pointing once again to an FS relationship. LR was not computed in the absence of an ancient Yakut reference set of allelic frequencies but IBD pointed towards FS and more importantly Relatedness pointed towards FS, providing decisive exclusion of all levels of kinship except FS and PO. Computations of the probability of each more distant relationship (HS, AV, GC and CO) compared to the probability of FS (Figure 2) showed that the probability of FS was about 4 times that of HS using 15 STR loci, 20 times using 21 STR loci and 50 times using 83 SNP loci. Although PO is not eliminated by Relatedness (it is eliminated by allelic exclusions), when the mean value for Relatedness of all kinship levels is compared to the value observed in the case of "Ieralaakh" for 83 SNP (Figure 2) it shows that FS is 9 times more likely than PO.

DISCUSSION

(1) "Lepsei", an unambiguous result

The "Lepsei Family" is an example of forensic kinship assessment methods successfully providing a coherent genealogy for a group of ancient individuals. However, this case also highlights two of the main issues that arise from the study of 15 STR loci: an absence of exclusions is sometimes not the sign of a PO relationship but of an FS relationship and Relatedness values, that can be studied in theoretical models outside of a reference population, are poorly efficient on small numbers of markers.

Unknowing the possible level of inbreeding of the population or the validity of mutation rates, the results given by such studies as this one must be understood as indications rather than assessments.

(2) "Shamanic Tree", uncertain second-degree relationships

Many difficulties that are integral to the study of precise kinship in ancient populations arise in the analysis of parental relationships in the Shamanic Tree Family. Relatedness is once more

Table 1

Results of kinship tests in the Shamanic Tree Family using 15 autosomal STR loci

A priori relationship	Subject 1	Subject 2	Exclusions	top Relateness relationship	top IBD relationship	top LR relationship	Y-DNA fit	Mt-DNA fit	Relationship call
ПС	ST2	ST1	2	~FS	FS	FS	i	yes	FS
PP	ST2	ST4	0	FS	PO	PO	i	yes	PO
PP	ST2	ST5	0	HS/AV/GC	PO	PO	i	yes	PO
PP	ST2	STII	0	HS/AV/GC	PO	PO	i	yes	PO
PP	ST3	ST1	0	FS	PO	PO	i	yes	PO
PP	ST3	ST2	0	PO	PO	PO	i	yes	PO
ПС	ST4	ST5	1	FS	FS	FS	yes	yes	FS
ПС	STII	ST4	2	HS/AV/GC	FS/HS/AV/GC	HS/AV/GC	yes	yes	HS/FS
ПС	STII	ST5	4	~FS	FS	FS	yes	yes	HS/FS
ДТП	ST1	ST4	1	~FS	FS	FS	no	yes	AV
ДТП	ST1	ST5	2	HS/AV/GC	FS	HS/AV/GC	no	yes	AV
ДТП	ST1	STII	3	HS/AV/GC	HS/AV/GC	HS/AV/GC	no	yes	AV
ДБВ	ST3	ST4	4	HS/AV/GC	HS/AV/GC	HS/AV/GC	i	yes	GC
ДБВ	ST3	ST5	3	HS/AV/GC	HS/AV/GC	HS/AV/GC	i	yes	GC
ДБВ	ST3	STII	4	CO	HS/AV/GC	CO	i	yes	GC

Table 2

Results of kinship tests in the Ieralaakh pair

A priori relationship	Subjects	Markers	top LR relationship	top IBD relationship	top Relateness relationship	Exclusions	Relationship call	
none	Ieralaakh 1	Ieralaakh 2	15 aSTR	FS	PO	FS	0	FS
			19 aSTR	PO	PO	FS	0	PO
			21 aSTR	FS	FS	FS	2	FS
			83 aSNP	-	FS	FS	2	FS

shown to have poor discriminatory power on only 15 STR loci, while LR tests, that are dependent on allelic frequencies, do not always provide a coherent genealogy. IBD tests vary differently but with the same drawbacks of sometimes not supplying clear cut answers, or even coherent ones.

For a genealogy to be constructed, at least one kinship call must be discarded as false. The three children of ST-2 were either full brothers, or ST-II was the half-brother of the other boys. It must however be posited that, in the second solution (Figure 3), ST-II's father was a close relative of ST-4 and ST-5's father, as they shared the same exclusive paternal line (5). Moreover, to construct a genealogy, all calls between uncle ST-1 and nephew ST-4 must be considered overestimations of kinship (FS is in fact HS/AV/GC), while half the calls between grandmother ST-3 and grandchild ST-II must be considered underestimations of kinship (CO is in fact HS/AV/GC). This genealogy has anthropological implications and cultural studies of the Yakut might shed light on it.

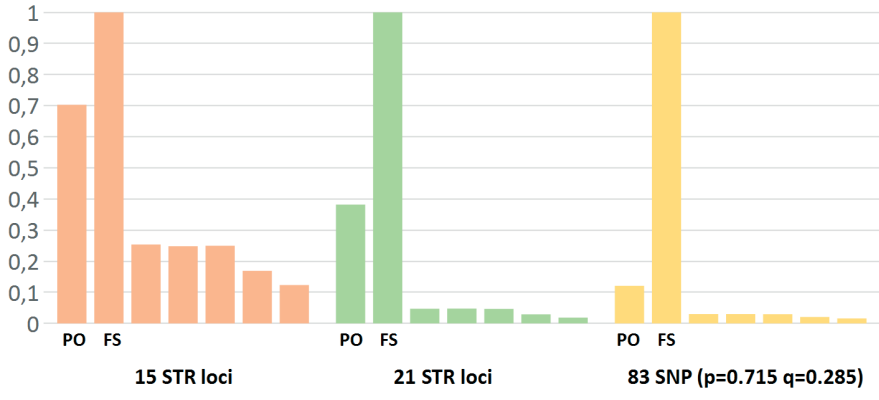

Figure 2. Leralaakh compared probabilities

(3) "Ieralaakh", towards a statistically decisive result and finer levels of kinship

The case of the "Ieralaakh" pair shows how Relatedness can in some cases eliminate possible relationship reliably (with a 5% risk of error). While LR varies according to allelic frequencies, which themselves are subject to the influence of numerous phenomena, the discriminative power of Relatedness steadily increases (given markers with comparable variability) with the augmentation of the number of markers. Moreover, LR results must be interpreted within the context of a larger framework, constituted by the large meta-population in which the subjects can be included. Objective metrics such as Relatedness, Exclusions or IBD can be used in isolation, relying on statistical models that permit the quantification of the probability that a result is accurate or otherwise.

CONCLUSION

The study of kinship using ancient DNA from a remote population meets with specific issues that arise from its isolation. Ultimately, it is not the degraded nature of genetic material that constitutes an obstacle (especially for Yakut burials, that benefit from exceptional conditions of conservation) but the unavailability of a large meta-population in which test values can be understood, and specifically, reliable allelic frequencies computed.

When interested solely in Parent-Offspring or Full-Sibling relationships, Likelihood Ratios based on STR profiles and frequencies are usually efficient, even when based on tentative reference data. However, a direct count of the number of excluding loci is sometimes as precise as those computations in calling kinship. The analysis remains a qualitative one, with similar profiles classified as "Parent-Offspring" when no exclusions can be observed, "Full-Siblings" when only a few exclusions are observed, and "other" or "Unrelated" when too many exclusions are observed (or when other data excludes PO or FS). The subtle distinction between second-degree relationships is made difficult when the approximation intrinsic to the allelic frequencies (computed on small or ill-defined populations) throws doubt on all test values that are somewhat indistinct.

The future of precise kinship studies in ancient populations resides in the multiplication of markers and the creation of models that account for cultural and social tendencies, that directly influence the genetics of the human group. It is also now possible to envisage the study of very precise levels of kinship, where

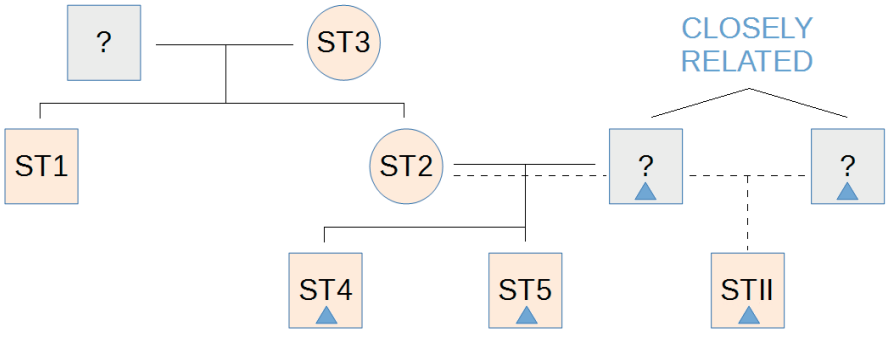

Figure 3. Shamanic Tree genealogy Alternative Hypothesis

for example the difference between siblings whose parents are unrelated and siblings whose parents are first cousins can be accurately described theoretically and observed practically. The progress to sequencing chips that gather data at millions of SNP loci, combined with the use of kinship tests based on objective similarity values, carefully calibrated for a specific social structure, will allow for finer and more accurate assessments of ancient kinship.

ACKNOWLEDGEMENTS

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ALLELIC POLYMORPHISM OF THE SEROTONIN TRANSPORTER AS A FORMATION FACTOR OF THE BEHAVIORAL COGNITIVE CONTROL IN THE YAKUTS

ABSTRACT

Allelic polymorphism of serotonin transporter 5-HTTLPR is connected with higher risk of vulnerabil-ity to the mental pathologies, which are associated with disorders in regulation of emotional behavior. However, the influence of this polymorphism on predisposition to disorders significantly differs in the different ethnic groups. The aim of our study is an exploration of the 5-HTTLPR effects on the change of theta-band spectral power in EEG in condition of recognition of the written emotional sentences in the Yakuts in comparison with the Russians. 78 healthy Yakutian students and 82 healthy Russian students participated in the study. In EEG experiment, the participants recognized a grammatical error in the Russian written sentences. The sentences contained a hidden condition, which was not clearly announced to participants: - some of sentences described unanimated objects, own anxiety of participant, anxiety of other persons, own aggression of participant or aggression of other persons. The probes of blood or buccal epithelium were taken in each participant for genetic analysis. It was revealed that the frequency of occurrence of the S-allele of the 5-HTT gene in the Yakuts was significantly higher (73%), than that in the Russians (39%). In EEG, the emotional sentences induced the higher amplitude of theta-band synchronization in comparison with the neutral sentences in all participants. The participants with the genotypes LS and SS showed essentially smaller differences in amplitude of EEG reaction after onset of sentences from different categories in comparison with the people with LL genotype. Our study supports the hypothesis that S- allele of 5-HTTLPR is associated with lower sensitivity to emotion-related stimuli, that could be connected with the risk of affective pathologies in people with such allele. Acknowledg-ment: Recording and processing of EEG was executed under financial support of the Russian Science Foundation, grant № 17-18-01019, «I and other – predictors and mechanisms of cooperative and competitive behavior». The collection of genetic probes was executive under support of the Russian Science Foundation, grant № 16-15-00128, «Structural features of gene SIRT1 as the basis for choose of personalized medicine therapy of depression».

Keywords: serotonin transporter (5-HTT), recognition of emotions, language comprehension, EEG, inter-ethnical comparison.

INTRODUCTION

Behavioral cognitive control is the complex of processes in the human brain, which is responsible for effective achievement of goals and adaptation to changeable environmental conditions [11]. According to the modern neuroscientific studies [8; 9], the behavioral control mechanism includes two contradicting to each other processes: an activation of goal-directed motions (so-called "activation control"), and an inhibition of motions, which are non-relevant to achievement of goals (i.e. "inhibitory control") [1]. Stop-signal paradigm (SSP) is the experimental method which was specially suggested for testing of balance between the activation and inhibitory processes in clinical norm and under different kinds of neurologic or mental disorders [5]. SSP experiment tests motor reactions of participants to the onset of several quickly appearance and disappearance stimuli. An examinee had to press one of two buttons quickly after the onset of different target signals. In some cases, the examinee had to suppress an movement, already begun, if after a target signal there was a stop-signal onset. The SSP technique is widely applied to testing patients with many kinds of behavioural pathologies, including Parkinson's disease, syndrome of children's hyperactivity, impulsive syndrome, etc.

Neurophysiological studies by means of EEG and fMRI techniques have shown that the activation of movements is connected with inclusion of the bottom-up processes arising in the parietal cortex. The movement inhibition is associated with top-down processes arising in the middle frontal cortex [8]. In EEG studies, the activation control is reflected in the dynamics of alpha/beta rhythm, whereas the inhibitory control is connected with the changes of delta/theta rhythm. Also, the EEG and fMRI indicators in the SSP conditions have the strong inter-personal variability depending on age, the anxiety level, and genetic features of healthy participants [7; 9].

The serotonin transporter (5-HTT) is the protein which mediates the re-uptake of serotonin from the synaptic cleft [11]. Several genetically defined modifications of this protein were detected in humans and animals. 5-HTTLPR on genetic site SLC6A4 is one of the allelic polymorphisms of the serotonin transporter. Among humans, this gene exists in two variants: "long" (L – allele) and short (S – allele) protein. Respectively, each person can have one of three genotypes: LL, LS, or SS [4]. In addition, the L- allele can be either active (La) or passive (Lg). Earlier, the numerous researches on animals and human clinical patients showed the association of an increased risk of many affective pathologies including anxiety disorder, depression, alcoholism, and predilection to suicide among people who have SS genotype [3; 4]. There was the assumption that the genetic analysis of this polymorphism can be used in preventive diagnostics of mental diseases which are related to the disturbance of self-checking of behavior. However, further researches generated some doubts about the efficiency of such an approach. In particular, it was revealed that the 5-HTTLPR polymorphism could have an opposite effect on vulnerability to mental disorder in representatives among Caucasian and Mongoloid ethnic groups. As it was shown in several independent studies, the S-allele is seldom among the population of Western Europe. This allele is associated with a higher risk of development of affective pathologies among the Europeans. On the contrary, the S- allele is widely spread among the Asian populations, including Japanese, Koreans, Mongols, and the Tuvinians. Among these Asian ethnic groups, S- allele is associated with lower vulnerability to the affective mental diseases [6; 10]. Thus, for the same mental disorder, the same genotype can appear as the factor, increasing the risk of disease and, in other ethnic groups, as the factor reducing the risk of this disease. By the present moment, the reasons of such an "invertation" of the genotype effect among different groups of people is still unclear. Is it bound to climatic conditions of life, features of education, economic factors, or to something else?

The aim of study: In this study, we compare the effect of the 5-HTTLPR allelic polymorphism on the behavioural mea-

sures and brain activity (EEG) in the experimental conditions of the Stop-signal paradigm among two ethnically different groups of healthy people (young Yakutian students who constantly live in Yakutsk, and young Russian students who constantly live in Novosibirsk). Firstly, we try to define whether the genetic differences in serotonin transporter structure influences the behavior and brain activity in the conditions of experimentally induced load to the system of behavioral cognitive control. Secondly, we do the comparative research on the 5-HTTLPR effects among two groups of healthy people differing who belong to different ethnical groups and residences.

METHODS

78 young and healthy Yakutian students of the Medical Faculty of the North-Eastern Federal University (age 20,1±2,3 years old, 30 men and 48 women) and 82 students of the Novosibirsk State University (age 22,1±3,4 years, 31 men and 51 women) participated in the study. All participants gave informed written consents prior to the experiment and filled out the questionnaire concerning their mental, or neurological diseases, or application of narcotic drugs and any other psychoactive substances. People with such diseases or those who used psychotropic drugs were excluded from the study. Besides, the level of general and emotional intelligence were estimated for all participants. IQ scores of the groups were matched as closely as possible based on their performance. The experimental protocol was approved by the ethical committee of the Institute of Physiology and Basic Medicine in accordance with the Helsinki Declaration of biomedical ethics.

The samples of blood and buccal epithelium for analysis of the 5-HTTLPR genetic polymorphism have been taken from all participants. The genetic analysis has been executed in the Institute of Cytology and Genetics of SB RAS, according to the method described in the paper of Savostyanov et al. [10].

The SSP version, earlier offered by A.N. Savostyanov with coauthors [9], was used for the EEG experiment. The participants played a computer game called “The Hunt”. The images of either a tank or a deer appeared randomly on the computer screen. In total number of 130 images with interstimulus intervals from 3 to 7 seconds were presented to each participant. Each image was demonstrated during 750 ms. The participant had to identify the image, to choose weapon (onions for a deer or anti-tank gun for a tank), and to press one of two buttons referred to a necessary weapon. If the participant managed to choose the weapon correctly before the image disappeared, their game account increased to one point (each time). If weapon was chosen incorrectly or if the choice took longer than 750 ms, one point was subtracted from the account. In 35 cases, the stop-signal (a red square in the center of the screen with the text, “STOP”) was presented after the emergence of target stimulus. Thus, two experimental conditions were suggested for each participant, “Go”, in case they needed to react quickly to a target signal, and, “Stop”, in case they needed to inhibit an already begun movement quickly.

The intervals between the emergence of target stimulus and the emergence of stop-signal were calculated individually. For this purpose, the average reaction time was defined during the first 30 presentations of target stimulus made without stop-signal. Delays between the target stimulus and the stop-signal were calculated in an equal proportion as 10%, 25%, 30% and 50% of the average reaction time. Respectively, for successful performance the participants had to level the balance of motor responses speed. They should not have reacted to a stimulus too quickly (since it increased the risk of pressing the button after stop-signal onset), and too slowly (since it increased the risk of missed pressing). Success at this game demanded difficult, balanced behavioral control taking into account several factors at once.

The average time of correct reactions in the “Go” condition, the percentage of missed pressing, the percentage of correctly and incorrectly chosen weapon, the percentage of correct inhibition after emergence of the stop-signal, the number of pressings before emergence of the stop-signal, and the number of pressings after emergence of the stop-signal were defined as the measures of behavioural indicators.

EEGs with the events markers were recorded from all the participants during the execution of linguistic task. EEGs were recorded with the help of the Brain Products amplifier, Germany, with bandpass range from 0,1 to 100 Hz, with a frequency of sampling of 1000 Hz. Among the group of Russians, the EEG records were carried out through 128 channels located according to the International 10-5% schema with the referent on Cz and grounding electrode on AFz. Among the Yakutian ethnic group, the EEG records were made through 64 channels located according to the 10-10% schema.

EEG-signals were processed by means of the EEGlab_toolbox [https://sccn.ucsd.edu/eeglab/index.php]. Under the pre-processing data, EEGs were filtered in the range from 1 to 40 Hz. Eye and motor artifacts were rejected by means of the analysis of independent components (ICA, [4]). Event-related spectral perturbations (ERSPs, [4]) were applied as the measure of brain activity connected to the tasks’ execution and for the revealing of genetic effects.

RESULTS

The significant differences between the frequencies of population distribution of the polymorphic alleles of the serotonin transporter gene were revealed among the groups of Russians and Yakuts. The frequency of La-allele was 51% among the participants of Russian group and 21% among the participants of Yakuts’ group, whereas the frequency of S-allele was 39% among Russians and 73% among the Yakuts. The frequency of Lg-allele was 6% among both groups. The significance of intergroup differences was estimated by the $\chi 2$ criteria.

The statistical significance of behavioural results was estimated independently for each of the chosen measures by means of one-way ANOVA with factors «group» (Yakuts or Russians) and «genotype» (LL, LS or SS). The main effect of «genotype» was highly significant for the percentage of correct pressings with the «Go» condition, $F(2, 150) = 5.09$; $p = 0.007$. The participants with the LL genotype have shown the reduced quality of task execution (82,7±1,3%) in comparison with the LS heterozygotes (87,4±0,9%), and with the SS genotype carriers (87,1±0,8%). Besides, the genotype had a significant influence on the indicator of general speed of reaction, $F(2, 150) = 4.31$; $p = 0.015$. The participants with LL genotype reacted to stimuli more slowly (575,2±5,9 ms) than those who carried the LS (554,4±4,2 ms) and SS (555,4±3,6 ms) genotypes. Thus, people at whom the S- allele has been revealed, both in homozygous (SS) and in heterozygote (LS) positions, have shown the higher speed of reaction and greater success at task-execution in comparison with the LL people. Significant values of the factor «group» without interaction with a factor «genotype» have been revealed for the percentage of correct pressings, $F(1, 150) = 4.88$; $p = 0.029$, (Yakuts have 88,0±1.4% and Russian have 84,3±0,9%) and also for the indicator of missed pressings, $F(1, 150) = 6.55$; $p = 0.012$, (Yakuts have 8,9±1,3% and Russians have 12,9±0,8%). Thus, the Yakutian participants have shown significant, though not too essential, advantages in comparison with Russian participants on measures of motor control, which have not been directly connected with the 5-HTTLPR polymorphism.

In general, the EEG patterns in both SSP-conditions corresponded to what was received in our earlier study [9]. The spectral power increase (synchronization) in the interval of 100-1000 ms after a target stimulus onset in the 1-7 Hz frequency range with an amplitude maximum in visual cortex was revealed in the «Go» condition. Also, during 300-1500 ms interval and at 8-12 Hz frequency range the spectral power decrease (alpha-desynchronization) was revealed in frontal and parietal cortical areas. In the central (motor) cortex, the desynchronization in the frequency range of beta-band (12-20 Hz) was obtained, which directly preceded button pressing.

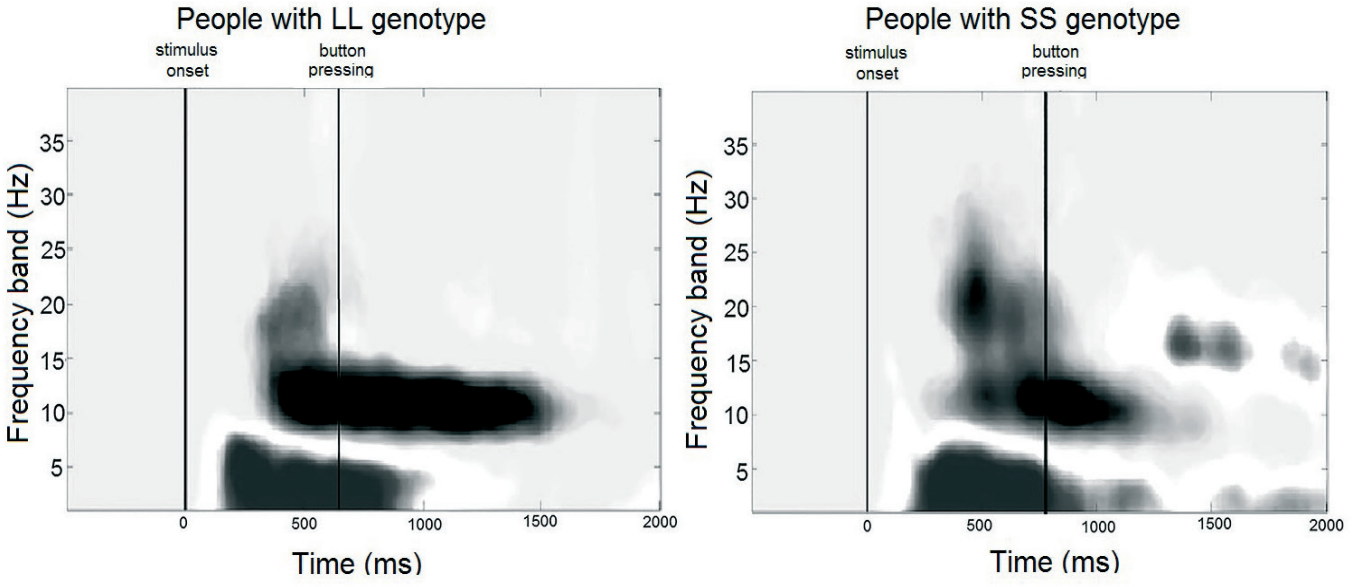

Figure 1. The differences in EEG responses in the «Go» condition of SS between the people with LL genotype (left) and SS genotype (right). A first vertical line reflects the moment of target-stimulus onset on the computer screen, second vertical line represents the moment of button pressing. The plot of event-related spectral perturba-tion (ERSP) shows values averaged among the people with LL (left panel) and SS (right panel) genotype. The duration of alpha-desynchronization is longer among the people with LL genotype than that among the SS people.

In the «Stop» condition, the amplitude of alpha and beta responses was reduced in comparison with the «Go» condition, but the additional high-amplitude synchronization in slowly-wave EEG ranges (delta and theta) was shown. Comparison of two genetically different groups of participants was carried out by means of the nonparametric bootstrap method (see the Figure 1). It was shown that the participants with LL genotype had longer (about 1500 msecs) and higher amplitude of alpha-desynchronization in comparison with the carriers of the LS and SS genotypes, both in the «Go» and «Stop» conditions of the SSP. Correlation analysis revealed that the latency of alpha-desynchronization in both experimental conditions negatively correlates with the quality of task execution ($r = -0,56$; $p = 0,045$) and the time of task execution ($r = -0,73$; $p = 0,003$), i.e. smaller latency corresponded to better and quicker task execution. Beta rebound, i.e. increase in beta-power after the task finishing, have been found in the 15-20 Hz frequency range among the carriers of the LS and SS genotypes only, but not in the LL carriers.

DISCUSSION AND CONCLUSION

Thus, we revealed that the S-allele of the 5-HTTLPR polymorphism is more widely spread among the Yakutian population in comparison with Russians and, vice versa, L- allele is spread more widely among Russians, that completely corresponds to the results which were received earlier by the comparing other Caucasian and Mongoloid populations [6; 10]. It is also revealed that among both Russians and Yakuts, people with S- allele showed significantly higher abilities to control the difficult behavior in the conditions of time deficit. In addition, Yakuts have shown better abilities to the sensory-motor task execution in the SSP conditions, in comparison with Russians, which directly are not connected with 5-HTTLPR polymorphism. Theoretically, it could be caused either by living conditions or some other genetic factors which were not researched by us. EEG shows that S- allele carriers have demonstrated shortened and lowered amplitude responses in alpha-band. It was also connected with the quality of motor control.

In the introduction to this article, we mentioned that S-allele is connected with higher vulnerability to affective disorders among the European population and with lower vulnerability to such violations among the Mongoloid populations. With the help of the results given in our study, it is possible to conclude that S-allele improves an ability to the behavioral control in the conditions of time deficit. We assumed that the high frequency of occurrence of this allele among Yakuts can be connected with the fact that it is the adaptive factor in those climatic conditions which assume the need of fast estimates of external events and executions of decisions. It is also possible to note that S-allele carriers have shown the decreased stability of attention (in case of the tasks execution that it is reflected in the alpha-band dynamics). It can be the reason of the violations arising among such people in environmental conditions, which are not connected with time deficit for decision-making, but with requiring the long and steady concentration of attention.

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ALA54THR POLYMORPHISM OF THE *FABP2* GENE AND METABOLIC SYNDROME IN THE YAKUT POPULATION

ABSTRACT
The metabolic syndrome represents complex of the metabolic risk factors connected with increase in prevalence of diabetes and cardiovascular diseases. Each component of metabolic syndrome to some extent has communication with heredity that demonstrates that genetic factors can have significant effect on pathogenesis of this syndrome. In researches among some populations the association of polymorphism Ala54Thr of gene of *FABP2* (rs1799883) with metabolic violations was shown. We analyzed association of allelic rs1799883 options (*FABP2*) with metabolic syndrome and its components at representatives of the Yakut ethnic group (on self-determination) from Berdigestyakh village of the Gorniy region of Republic Sakha (Yakutia). All surveyed people the written informed consent to participation in research. The program of research included screening by uniform technique on detection metabolic risk factors of chronic noncommunicable diseases. Polymerase chain reaction in real time was carried out in the CFX96 system of production Bio-Rad. Tests and primers were developed by means of the Beacon Designer 8 program from PREMIER Biosoft. From 228 participants of 42% had the increased level of blood pressure, 9,7% — the reduced level high density lipoproteins, 5,8% — raised triglycerides, 22,4% — fasted hyperglycemia, 56,8% — abdominal obesity, 16,8% — metabolic syndrome by IDF criteria (2005). G polymorphism of Ala54Thr *FABP2* (71%) are characteristic of the vast majority of representatives Yakut population allele carriage. Frequency of GG genotype was 42,1% (95% CI 35,9-48,6%), AG — 57,9% (95% CI 51,4-64,1%). The association of genotype AG with abdominal obesity was established at OR 1,7 (95% CI 1,01-2,99). Taking into account prevalence in this group of the population of such metabolic disturbances as obesity and the increased level of blood pressure, and also the growing incidence of diabetes type 2, it is necessary to continue search of genes of predisposition to these diseases.

Keywords: *FABP2* gene (rs1799883), Ala54Thr polymorphism of the FABP2 gene, metabolic syndrome, abdominal obesity, Yakut population.

INTRODUCTION

The metabolic syndrome is complex of the metabolic risk factors connected with increase in prevalence of diabetes and cardiovascular diseases [8, 12]. Each component of metabolic syndrome to some extent has communication with heredity that demonstrates that genetic factors can have significant effect on pathogenesis of this syndrome [9, 12, 15]. The gene of the protein connecting fatty acids in intestines (*FABP2*) participates in regulation of capture and transfer of long-chain fatty acids [5]. Polymorphic options of gene *FABP2* (rs 1799883) can exert impact on concentration of lipids in blood plasma and their intracellular transport [6]. The result of researches, conducted among northern populations was shown that lipid metabolism plays key role in effective adaptation to conditions of cold climate [1, 3]. The Republic of Sakha (Yakutia) belongs to terri-

tories with extreme climatic conditions, first of all because of low temperatures. Yakuts whose number according to census of 2010 makes 466 492 people are representatives of the Central Asian type of North Asian race. Now among this region population also as well as around the world, incidence of diabetes type 2 promptly grows. It is promoted by the high frequency of obesity and other metabolic disorders [2]. The researches devoted to studying of communication between genetic factors and health of the person can expand limits of our knowledge of influence of genes and the environment on phenotype. In this regard, studying of communication the polymorphism Ala54Thr *FABP2* gene (rs 1799883) with metabolic syndrome and its components, and also with some biochemical and anthropometrical indicators at Yakuts, representatives of the Central Asian type of North Asian race was research objective.

MATERIALS AND METHODS Groups were created during the epidemiological research among the Berdigestyakh village population of the Gorniy region Republic Sakha (Yakutia) conducted within basic unit of the State task Education and Science Ministry Russian Federation 17.6244.2917/8.9 "Clinical and genetical aspects of the diseases characteristic of Yakutia native population in modern conditions". In total 242 persons, representatives of the Yakut ethnic group participated in research (on self-determination). From them consent to genetic research was received at 228 persons. All participants of research were inspected according to the uniform program including double measurement of the blood pressure (BP), anthropometrical inspection by standard technique, the analysis of composition structure of organism on the bioimpedance Tanita analyzer (Japan) SSC 330, intake of blue blood for laboratory researches. Definition of glucose, general cholesterol (OHS), triglycerides, cholesterol of high density lipoproteins carried out on the Cardiochek PA (USA) express analyzer from the venous blood taken in the morning 10–12 hours later after meal. Concentration of cholesterol low density lipoproteins calculated by formula Fridvald at the content of triglycerides in blood less than 4,5 mmol/l. Levels of maintenance of leptin, CRP, insulin determined by the ELISA method with use of DRG sets. Existence of metabolic syndrome (MS) and its components defined by IDF criteria 2005 [8]. Control groups were created from among persons without certain metabolic disturbances.

228 DNA samples were genotyping by the PCR method. For SNP genotypings used TaqMan of test specific to the sites supporting the interesting SNP. Tests and primers were developed by means of the Beacon Designer 8 program from PREMIER Biosoft. As reporters FAM and R6G dyes, and as quencher – BHQ-1 were used. Polymerase chain reaction in real time was carried out in the CFX96 system of production Bio-Rad. The volume of reactionary mix made 25 mcl. Each reaction was carried out in three repetitions. The stage of activation was carried out at 95 °C within 3 minutes, the course of one cycle consisted of three temperature time spans – 95 °C (30 sec.), 54 °C (20 sec.) and 72 °C (20 sec.). Total quantity of cycles made 40.

Inspection of compliance of distribution of genotypes to the law of balance of equilibrium of Hardy-Weinberg was carried out with use of the online calculator on the website https://wpcalc.com/en/equilibrium-hardy-weinberg/ [7]. The statistical analysis of data was carried out in IBM SPSS STATISTICS 22. When comparing groups depending on data type used Mann-Whitney and Pearson's criteria χ^2. For measure of effect we are calculated the odds ratio (OR). Critical value of the statistical significantly was accepted equal 5%.

The research project was approved by local committee on bioethics of the Yakut scientific center of complex medical problems the Russian Academy of Medical Science (extract from the protocol No. 39 of June 26, 2014). Participation in research was completely voluntary. Obtained clinically useful information was available to participants of research.

RESULTS AND DISCUSSION

The proteins binding fatty acids *(FABPs*) concern family cytoplasmatic lipid - the binding proteins participating in intracellular transport and metabolism of lipids. *FABP2* gene expressing in epithelial cells of small intestine is located in chromosomal area 4q28-4q31, consists of 4 exons and 3 introns and codes the protein containing 131 amino acids. Polymorphism rs1799883 FABP2 gene is caused by replacement of guanine by alanine in the 54th codon that brings to replacement of alanine by threonine (Ala54Thr) in exon 2. The protein containing threonine has big affinity to long-chain fatty acids, than alanine - the containing option [5-6]. *FABP2* is considered as the candidate gene involved in pathogenesis of diabetes 2 types, metabolic syndrome and obesity in different ethnic groups [4, 10, 11, 13, 14, 16]. At the same time the analysis of literature showed ambiguity of researches results about communication between polymorphism of FABP2 gene and metabolic disturbances risk development. So, Zhao T. with coauthors in 2010 meta-analysis of results 13 researches with 13451 participants was carried out. Generalization of results showed weak communication of polymorphism of Ala54Thr of *FABP2*gene with insulin resistance degree, high level of fasted insulin and glucose in 2 hours after loading [16]. Qiu C. and coauthors meta-analysis (2014), including results of 13 researches with 2020 cases of diabetes and 2910 healthy people in control group (6 Asian and 7 European populations), showed existence of association between Ala54Thr polymorphism *FABP2* gene and risk of diabetes types 2 only for Asian populations [13]. Results of meta-analysis of Y. Liu and coauthors (2015) which combined results of 39 researches (24 —types 2 diabetes, 9 — obesity, 6 — metabolic syndrome), showed existence of statistically significant communication between polymorphism of Ala54Thr *FABP2* gene and MS (p =0,031), type 2 Diabetes (p <0,001), but did not find association with risk of obesity (p = 0,367) [11].

In present research for the analysis of association allelic rs1799883-*FABP2* variants with metabolic syndrome and its components data of 228 representatives of the Yakut population are used (on self-determination). Average age of participants made 43,9 (17,5) years. Abdominal obesity is established at 56,8% of participants, the increased level BP – at 42%, fasted hyperglycemia – at 22,4%, reduced HDL cholesterol – at 9,7%, raised triglycerides at – 5,8%, and metabolic syndrome by IDF criteria (2005) is revealed at 16,8% of the inspected population.

G polymorphism of Ala54Thr *FABP2* gene (71%) is characteristic of the vast majority of representatives' Yakut population allele carriage. According to literature the frequency of carriage of allele A in the majority of populations also makes about 30% [11]. Among inspected persons with AA genotype are not revealed. Frequency of alleles and genotypes was similar both among men and women, and in different age groups (table 1). Distribution of genotypes in subgroups will be coordinated with the equilibrium Hardy-Weinberg.

Comparative analysis of lipid and carbohydrate metabolism at the inspected persons showed that polymorphism of this gene does not exert noticeable impact on average concentration of the specified indicators (tab. 2). It should be noted that distinctions of average values of the main anthropometrical indicators (body mass index, waist circumference) depending on *FABP2* genotypes, were close, but did not reach level statistically significant that, perhaps, is caused by the small sample size.

The MS was diagnosed for 36 persons that made 16,8% of total number of inspected. Occurred among women of MS considerably more often than at men (20.9 and 7.6% respectively, p =0.016), and its frequency increased inspected with age (from 3.5% at the age of 20-39 years to 28.9% in 60 years and is more senior, p <0.001). The research of Clinical-genetic associations with use of Pearson χ^2 criteria showed that level of significance when comparing frequency of abdominal obesity in groups with different genotypes was near-critical to value (0.045). At the same time chances to have AG genotype by 1.7 times (95% CI 1.01-2.99) were higher at persons with abdominal obesity (table 3). Frequency of MS and other its components did not depend on *FABP2* genotype.

Thus, results of research showed rather high frequency of minor allele of gene of

FABP2 54Thr in Yakut population (0.29). The analysis of clinical-genetic associations showed possible communication of this polymorphism with abdominal obesity. Taking into account prevalence in this group of the population of such metabolic disorders as obesity and the increased level BP, and also the growing incidence of diabetes type 2, it is necessary to continue search of genes of predisposition to these diseases.

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Table 1

Alleles and genotypes of polymorphism ALA54THR FABP2 gene distribution in Yakut population *

Groups	n	Frequency (95% CI)		p
		Alleles		
		G	A	
Women Men	156 72	70,2 (64,9-75) 72,9 (65,1-79,5)	29,8 (25-35,1) 27 (20,5-34,8)	0,629
Both groups	228	71,1 (66,7-75)	28,9 (25-33,2)	0,548
20-39 years 40-59 years 60 years and older	91 88 49	70,9 (63,9-77) 72,2 (65,1-78,3) 69 (60-77,6)	29,1 (23-36,1) 27,8 (21,8-34,9) 30,6 (22,4-40,3)	
Genotypes				
	GG	AG		
Women Men	156 72	40,4 (33-48,2) 45,8 (34,8-57,3)	59,6 (51,8-67) 54,2 (42,7-65,2)	0,439
Both groups	228	42,1 (35,9-48,6)	57,9 (51,4-64,1)	
20-39 years 40-59 years 60 years and older	91 88 49	41,8 (32,2-52,2) 44,3 (34,4-54,7) 38,8 (26,4-52,8)	58,2 (48-67,8) 55,7 (45,3-65,6) 61,2 (47,2-73,6)	0,817

Note. * — Distribution of genotypes in all groups will be coordinated with the Hardy-Weinberg equilibrium; p — Pearson $\chi 2$ criteria statistical significantly.

Table 2

Anthropometrical and biochemical indicators in Ala54Thr *FABP2* genotypes groups

Indicator	Genotype of n (%)				p
	GG		AG		
	n	Me (Q1; Q3)	n	Me (Q1; Q3)	
Age, years	96	45,7 (25,3; 56,8)	132	47 (27; 59)	0,512
SBP, mm Hg	94	113,2 (106,3; 130,0)	132	120,0 (109,8; 132,4)	0,261
DBP, mm Hg	94	76,5 (69,6; 82,8)	132	77,3 (70,0; 86,9)	0,416
Total cholesterol, mmol/l	96	4,4 (3,5; 5,1)	130	4,4 (3,7; 5,5)	0,199
High density lipoprotein cholesterol, mmol/l	96	1,7 (1,4; 2,1)	130	1,7 (1,4; 2,2)	0,348
Triglycerides, mmol/l	96	0,9 (0,8; 1,0)	130	0,9 (0,8; 1,1)	0,295
Low density lipoprotein cholesterol, mmol/l	96	2,1 (1,4; 2,8)	130	2,2 (1,5; 3,1)	0,281
Fasting plasma glucose, mmol/l	94	5,1 (4,6; 5,5)	129	5,1 (4,7; 5,5)	0,953
Leptin, ng/ml	94	4,8 (4,3; 5,2)	130	4,7 (4,3; 5,2)	0,977
Insulin, mu/ml	94	19,5 (13; 27,5)	130	18 (12,6; 27,7)	0,730
CRP, mg/ml	94	0,2 (0,07; 1,9)	130	0,3 (0,07; 1,2)	0,720
BMI, kg/m2	94	22,6 (20,3; 27,5)	127	24,2 (21,8; 27,8)	0,066
Waist circumference, cm	92	83,8 (76,2; 95,4)	128	88,1 (79,8; 97,0)	0,082
Body fat percent	92	24,2 (16,6; 32,6)	126	29,1 (21,4; 34,6)	0,107
Fat free mass, kg	94	43,6 (40; 52,4)	127	43,1 (39,7; 49,6)	0,677

Note: p — statistical significantly Mann-Whitney criteria; Me (Q1; Q3) - median (25-75%).

Table 3

Associations of Ala54Thr *FABP2* gene polymorphism with metabolic risk factors

Risk factor *	Genotypes, n (%)		OR (95% CI)	p
	GG	AG		
Metabolic syndrome, n=214	13 (14.8)	23 (18.3)	1.57 (0.76-3.2)	0.503
Raised blood pressure, n=226	38 (40.4)	57 (43.2)	1.12 (0.65-1.9)	0.679
Reduced HDL cholesterol, n=226	10 (10.4)	12 (9.2)	0.87 (0.36-2.1)	0.766
Raised triglycerides, n=226	5 (5.2)	8 (6.2)	1.19 (0.38-3.8)	0.763
Raised fasting plasma glucose, n=223	22 (23.4)	28 (21.7)	0.91 (0.48-1.71)	0.764
Abdominal obesity, n=220	45 (48.9)	80 (62.5)	1.74 (1.01-2.99)	0.045

Note: * — by IDF criteria 2005; p — Pearson $\chi 2$ criteria statistical significantly

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GENETIC TESTING AND INFORMED CONSENT FOR SPINOCEREBELLAR ATAXIA TYPE I, THE MOST COMMON HEREDITARY DISEASE IN THE YAKUT POPULATION

ABSTRACT

The article discusses the issues of informed consent for DNA testing at type 1 spinocerebellar ataxia, the most common hereditary disease with late onset of manifestation in the Yakut population. Different stages of obtaining informed consent in medical genetic counseling and in scientific research are described. The expediency of using the bioethical principle of non-disclosure of genetic information for a participant in a scientific study on the research of hereditary diseases with late manifestation is established.

Keywords: DNA testing, hereditary diseases, informed consent, type I spinocerebellar ataxia.

INTRODUCTION

Since 70th years of last century the works about cloning of human DNA developed in high gear and accomplished with the successful Human Genome project of the full interpretation of the DNA nucleotide sequence, and it offered great opportunities for development of new fields of science and practice, including molecular genetics, ethnogenomics, molecular medicine etc.

In the world there are about 7000 nosologies of monogenic diseases, which are detected in 3-6% of newborns, and in structure of the child mortality total rate of under-fives it is 10-14% [13]. There is a conditional separation of monogenic diseases on orphan diseases with a frequency of 1:100000 (lysosomal storage disorders, etc.) and common hereditary diseases - 1:10000 (a mucoviscidosis, a phenylketonuria, etc.). Frequency of monogenic diseases in various populations of the world can differ considerably. It depends on evolutionary features of formation of a genetic pool of the people. In some populations, however, this or that mutation which is the reason of monogenic pathology owing to evolutionary and genetic features becomes frequent and can be called "ethno-specific". For example, so-called "Finnish" hereditary diseases, generally autosomal and recessive which frequency of Finns is much higher, than in any other populations are known

long ago [11,16]. The phenomenon of accumulation of monogenic diseases at Finns is bound to drift of genes, long-term isolation of population and high coefficient of an inbreeding. On the same population mechanisms there was probably also an accumulation of some hereditary diseases of Ashkenazi Jews, and the highest frequency is Tay-Sachs disease and cerebroside lipidosis (Gaucher's disease) type 1 [6,12]. In the European populations the mucoviscidosis meets with a frequency of 1:2500, whereas in the Asian populations – 1:90000.

Around the world problems of use of genetic testing of hereditary diseases in applied medicine are relevant, especially standardization and improvement of quality of molecular and genetic analyses.

In 1999 the working group on genetic testing (the Task Force on Genetic Testing) defined genetic test as: "the analysis of DNA, RNA, chromosomes and proteins to define the hereditary genotypes, mutations, phenotypes or karyotypes bound to illnesses for the clinical purposes. These purposes include clinical diagnostics and forecasts, identification of carriers of hereditary diseases, presymptomatic and prenatal diagnostics and also neonatal screening" [14].

Recently the increasing value in activity of medicogenetic consultation of the Republic of Sakha (Yakutia) gets a DNA testing as one of the main diagnostic methods of hereditary diseases. Human DNA researches are innovative for applied medicine, because modern molecular and genetic laboratory methods begin to be applied for the first time in health care of Yakutia. Genetic testing allows to detect and form risk groups of the examined patients more intensively, to hold predictive events in the preclinical stage, to use programs for the disease development risk reduction. At the same time the DNA testing has also controversial issues, and first of it are all moral aspects and psychological risks for the individuals who are exposed to genetic testing [8,9,10,17].

One of topical issues of legal adjustment of medicine is the problem of the informed consent (IC). The attention of a legislator became the instance of IC importance. Today there is a majority of the federal normative legal acts in a health care field concerning both the general, and single questions of medicine, contain regulations about the informed consent. Each person has the right to freedom of choice in many areas of the public relations. In a health care field for the patient (a person who asked for a medical care) there is a possibility of the choice of diagnostic methods and treatment. Thereby the importance of equal participation of the patient in the course of treatment of the disease is emphasized [4]. However, as practice shows, doctors and researchers don't pay sufficient attention to the informed consent giving formal character to such an important bioethical principle that can have very negative consequences for the patient who asked for the medicogenetic help.

In our article there are discussed the features of using IC in a medicogenetic consultation (MGC) and a DNA testing of the most widespread monogenic hereditary disease in the Yakut population: spinocerebellar ataxia of the I type (SCA1). In clinical researches of Platonov in 2003 and other described about the accumulation reasons, clinical and molecular and genetic characteristics of SCA1 in most details. [5].

The main way of prophylaxis of SCA1 is the prenatal diagnostics (PD) of this disease. Bioethical aspects of the DNA testing and PD of late symptomatic monogenic illness with a dynamic mutation are described in early published works [3].

Now the medicogenetic consultation seeks to achieve psychological and educational aims referred on social adaptation of a family to the genetic risk or child birth with a hereditary disease [1]. The main task of a geneticist consists not in surely to recommend molecular and genetic diagnostics, but correctly help the patient to understand sense of the informed consent and, without imposing the opinion, to help him to make the adequate decision about the DNA testing.

MATERIALS AND METHODS

Information from republican genetic register about hereditary and congenital diseases were used in the article. According to the genetic register, 252 patients with the diagnosis a spinocerebellar ataxia of the I type [7] stayed on the registry in MGC. The method of direct DNA Diagnostics by the PCR method with the specific nucleotide primers was used for the Routine DNA Diagnostics of SCA1 as described in Orr et al (1993) with further detection pathologically extended allele in 2% agarose gel [15]. Determination of number of repetitions in a gene of SCA1 was carried out by method of a capillary electrophoresis on the automatic ABIPrism3130 (AppliedBiosystems) DNA analyzer [2].

RESULTS AND DISCUSSION

SCA1 IC for clinical practice

For the first time DNA Diagnostics of SCA1 was carried out in the medicogenetic consultation of the Republican Hospital № 1 National Center of Medicine in 2000. According to the latest published materials in 14 years it was tested 1841 persons; existence of the mutation of SCA1 was confirmed at 606 people from whom 354 (58%) asymptomatic persons was agreed to carry out a predictive DNA testing. 132 individuals were tested averagely in a year. The detectability of the mutation was 33% [2].

As mentioned above, more than a half of patients (58%) of the burdened families underwent presymptomatic DNA testing. It means that by the time of the request for the medicogenetic consultation these individuals had no clinically expressed SCA1 disease symptoms. In this case there is a question: is the asymptomatic individual (SCA1 mutation carrier) a patient in full sense of this concept? On the one hand, he makes the decision about DNA testing independently, being absolutely healthy, realizing the fact that there is a risk group, and on the other hand, learned the genetic status, perhaps, the individual won't address for medicogenetic consultation during the long time.

There are several general stages of the medicogenetic consultation of the patients bound to DNA Diagnostics:

1) pre-testing MGC;

2) DNA testing;

3) post-testing MGC with psychological follow-up.

From among addressed for the medicogenetic consultation on SCA1 it is possible to allocate at least four groups:

1. group of the patients having clinical implications of SCA1 by the time of visiting a doctor;

2. group of the asymptomatic mutation carriers of SCA1 detected by the DNA testing;

3. group of healthy individuals with negative result on SCA1 mutation

carriage;

4. group of persons interested to carry out a SCA1 PD.

At the first MGC pre-testing stage the greatest importance has the bioethical "principle of the informed consent" - each individual has the right to be informed on the forthcoming diagnostic method, in particular DNA diagnostics of SCA1. It is possible to assume that in a case with the DNA testing of SCA1 the patient is already ready for DNA testing as it had time to consider this important decision, but the medical adviser-geneticist shouldn't convince the patient by all means to undergo the DNA testing in the very first day of visiting, because it can be a consequence of an emotional rush or a special psychological make-up. It is necessary to lead a quiet discussion, to disclose all possible psychological risks of receiving a positive take of a presymptomatic testing, to be convinced that the decision on DNA diagnostics is made it is weighed. During the consultation, the geneticist has to pay attention to the patient age and his educational level.

There are described the necessary stages of receiving IC for SCA1.

1) an advising geneticist opens all known information about SCA1 disease (hereditary nature, the molecular reasons, anticipation effect, clinical symptoms);

2) discloses advantages of DNA testing to the individual (to learn the genetic status for planning a family and, in general, the main priorities in life). This stage is very important for the patient that he could take measures about the important decision making, without having felt compulsoriness from the doctor;

3) discloses possible psychological risks after obtaining results of DNA testing (psychological trauma, frustration, depression, etc);

4) shows possible ways out from a difficult psychological situation (remoteness of the SCA first symptoms expression; it is also possible to explain that there is a set of other diseases which are suffered by other people; to give hope for search and development of more effective methods of stopping symptoms or SCA1 treatment);

5) opens IC alternatives (to sign the document at once, to receive time for considering, to refuse DNA - testing).

SCA1 IC for scientific research

The informed consent for scientific research differs from IC for DNA testing and the medicogenetic consultation of patients from the risk group. In scientific research the individual is an alleged participant of a research work. It is important to a researcher received IC togive emphasis to the next moments:

1) to explain that this work is scientific research and participation in it is a voluntary decision of a participant; the explanation has to be provided with understandable terms for the participant, without using difficult medical or genetic terms.

2) to explain a research object;

3) to give confidentiality guarantees at all investigation phases;

4) before signing of IC to discuss results of a scientific research as in this case there are only two options with the participant: either the participant will want to learn the genetic status or not. But SCA1 features, especially moral and psychological problems, give the reasons for recommending to the researchers to make it clear for the participant about an inexpediency of the obtaining information about the participant genetic status.

CONCLUSION

The informed consent is an important and necessary condition of providing any medical service or a scientific research with participation of a person. It is a legal condition. The advising doctors need to avoid formalistic approach in informing patients, especially when the problem is bound to DNA testing of late symptomatic hereditary diseases. Obtaining negative information about patient health status and dismal prognosis of the disease can have moral injury and the long-term mental implications. The quality and efficiency of the medicogenetic help to the patient with SCA1 are not about the convincing the patient to undergo DNA testing but helping him to make the decision independently about testing based on comprehensive informing and offering various alternatives. On the assumption of bioethical principle of hurting, genetic information about

Информированное согласие
на проведение ДНК-тестирования спиноцеребеллярной атаксии 1-го типа

Настоящее добровольное согласие составлено в соответствии Федеральным законом от 21.11.2011 N323-ФЗ (ред. от 13.07.2015, с изм. от 30.09.2015) "Об основах охраны здоровья граждан в Российской Федерации" (с изм. и доп., вступ. в силу с 24.07.2015)

Мне _____

разъяснены цели теста, манипуляции по забору материала, преимущества и риски ДНК-тестирования.

• Я полностью информирован врачом-генетиком о заболевании, его особенностях, прогнозе и течении.

• При моем желании, мне дается время подумать о необходимости прохождения ДНК – тестирования.

• Мое решение принято мною добровольно, без всякого давления со стороны врача, родственников и/или супруга(и).

• Мне предоставляются альтернативные варианты прохождения ДНК-диагностики:

• Какое бы решение Вы не приняли, это не отразится на предоставлении Вам медицинской помощи.

Варианты решения	Подпись пациента
Я прохожу ДНК-тестирование и узнаю о результатах сразу после выполнения анализа	
Я сдаю кровь в Банк ДНК и узнаю о результатах позже, когда я буду готов психологически (через месяц, год, два и т.д.)	
Я сдаю кровь в Банк ДНК и могу не узнавать о результатах ДНК-тестирования	
Я не сдаю кровь и отказываюсь от ДНК-тестирования	

• Какое бы решение Вы не приняли, это не отразится на предоставлении Вам медицинской помощи.

Подпись врача-генетика Дата

Форма информированного согласия для спиноцеребеллярной атаксии 1-го типа

existence/lack of SCA1 mutation has to be closed for the participant of the research that surely taken in an oral or written form of the informed consent.

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INDICATORS OF QUALITY OF LIFE AND COMMUNICATION OF PATIENTS WITH TYPE I SPINOCEREBELLAR ATAXIA

ABSTRACT

The quality of life (QOL) and the communication ability of 41 patients with DNA verified cases of spinocerebellar ataxia type I (SCA1) was assessed with the help of the SF-36 Questionnaire. The physical, psycho - emotional parameters of QOL and the communication ability of the patients with SCA1 with different frequencies of CAG repeats were revealed, and the relationship between the revealed parameter sand frequency of CAG repetitions was clarified. The cause-effect relationships of the established QOL data of the patients with SCA1 are discussed with the level of mutation in the SCA1 gene and the need to take into account the revealed features of psycho-emotional state of the patients with SCA1 in treatment and care at home.

Keywords: spinocerebellar ataxia type I, frequency of CAG repeats, mutation in the SCA1 gene, quality of life, physical and role functioning, emotions, physical and mental health, disability, social functioning, communication ability.

Spinocerebellar ataxia type I (SCA1), an autosomal dominant lesion of the cortex, conducting pathways, cerebellar and trunk nuclei, and other parts of the brain is rare in Russia (0.1-0.4 per 100,000) [13,14,16]. The development of the disease is caused by the expansion of tandem trinucleoid CAG repeats and is associated with a mutation in the SCA1 gene, on the short arm of the 6th chromosome pair, which codes the synthesis of an abnormal ataxin protein [5,11]. The onset of the disease depends on the rate of accumulation of this protein in the cells of the cerebellar cortex, which shows up with instability or awkwardness while fast walking or running after 30 years of age border. Gradually, the gait becomes "like a drunk's one," clear signs of impaired coordination of movement like changes in handwriting, speech (dysarthria), swallowing develop and tendon reflexes increase, pathological foot and carpal signs and clones, spasticity appear. A malignant form is characterized by early onset (under 20 years of age) and rapid progression. The late stage can lead to aphagia, aphonia, decreased sensitivity, loss of reflexes. In the final, patients with SCA1 are not able to walk, serve themselves and can suffer from atrophy of optic nerves, cachexia and pelvic disorders. Until the end of life, patients still retain intelligence. Yakutia is the largest source of accumulation of SCA1 (38 per 100 thousand) [1]. It should be noted that despite the above-mentioned high level of study of the causes of the neurological picture of the disease, there is no integral evaluation of the physical and psychological state of patients with SCA1, carried out carefully taking into account the frequency of their CAG repeats so far. In this regard, it should be noted that in the United States and Europe the Sort Form Medical Outcomes Study or SF-36 Questionnaire is used to assess the quality of life (QOL) parameters for this purpose. SF-36 Questionnaire with Instruction for Conducting Interviews (Evidence - Clinical and Pharmacological Studies) and formulas for calculating QOL parameters (http://www.sf-36.org/nbscalc/index.shtml) [4,10] have been translated into Russian and approved by Institute of Clinical and Pharmacological Research in Russia. This allows us to use it to assess QOL of patients with chronic diseases [2, 3, 6 -12, 17].

The objective of the study was to collect data on QOL of patients with a DNA verified diagnosis of SCA1 treated by ISRH, NEFU specialists and the analysis of the data. The purpose of the study was the integrated assessment of QOL of patients with SCA1 used in the work along with an assessment of their physical, psycho-emotional abilities at different frequencies of CAG repeats, which allowed to reveal the relationship between the onset of development of the main manifestations of the disease and the CAG repeats, which would allow to objectify the causes of emerging manifestations of the disease and changes in QOL parameters of SCA1. This approach to the study of patients with SCA1 is an urgent scientific task, the implementation of which is of practical interest for specialists engaged in developing measures to optimize the treatment of patients with SCA1 in the Yakutia region being a location of accumulation of this pathology.

MATERIALS AND METHODS OF RESEARCH

The questioning of the SCA1 patients of ISRH, NEFU was carried out on the standard questions of 8 sections of the SF-36 Questionnaire according to its Instruction [4] in the conditions of leaving to the communities where the patients came from. This article describes the parameters of QOL of 41 patients with SCA1 who agreed to participate in the studies using the SF-36 Questionnaire as a group with a DNA verified SCA1 (the frequency of CAG repeats ranged from 41 to 53). The questionings were conducted after another neurologic examination and analysis of the clinical course of the disease according to the data stored in the ISRH, NEFU archive and case records of the respondents. Five patients with severe speech disorders, who were unable to independently answer the SF-36 questions, were interviewed with the help of close relatives caring for the patients. According to the methodology of Oslo, the respondents were able to clarify the ability to establish social communications by the number of people close to them and their ability to attract the attention of people from outside, as well as to be helped by their neighbors. We analyzed the collected data taking into account the characteristics of the neurological symptomatology and the anamnesis of the disease of each patient. As it had been previously known, those patients suffering from SCA1 came from small communities (with a population from 300 to less than 400 people) due to the peculiarities of the lands that are unable to be reached by any means of transport and located far from the main roads and medical centers. In one of such communities with 350 residents as a control group 47 people whose family members had not been registered for hereditary neurologic disease according to the information of the neurological service of the district and the data of the district hospital that presented medical assistance to the inhabitants of this community were questioned. All the respondents in the control group agreed to participate in the surveys. Their age ranged from 18 to 68 years. All the data obtained in the studies were statistically processed using SSPS 22packages.

RESULTS AND DISCUSSION

The frequency of CAG repeats among the interviewed SCA1 patients was 46.89 ± 1.12 (the norm is under 35-36) and the age of the SCA1 patients varied from 18 to 69 years and was on average 46.67 ± 2.05 years. Analysis of QOL of the patients

on scales showed that the Physical Functioning (PF) scale, reflecting the degree to which the physical conditions of the SCA1 patients is limited,the physical exercise point(self-care, walking, climbing stairs, carrying heavy loads, etc.), calculated according to the Instruction of the SF-36 Questionnaire [5], was 25.38 ± 4.34. So among theSCA1 patients the PF index was 1/4 of that of the control group, equal to 98.78 points, and thus, the PF of the SCA1 patients was sharply low compared to the data (500) in Yakutsk residents (79.7) [7], who were not suffering from this pathology. In this case the PF of the SCA1 patients was inversely dependent on the frequencies of their CAG repeats (r = -0.612 with P = 0.001 by Pearson) and the age at which dysphagia developed (r = -42 with P = 0.05 by Pearson) and disability (R = -0.352). These data proved that the ability of the physical functioning of patients, the onset of the development of swallowing disorders (dysphagia) as well as the overall age of their disability on the whole are dependent from the level of mutation in SCA1.

The value of Role-Physical Functioning (RP) of QOL of the SCA1patients, reflecting their ability to perform usual duties, conditioned by physical condition (habitual work, performance of daily duties) was 15.28 ± 4.20 points. The revealed RP of the SCA1 patients was 1/6 of that scale in the control group (95.73 points), illustrating a 6-fold reduced level characteristic for the control group and 4.81 times less data (500) in the Yakutsk residents who were not SCA1 patients (73.6 points [7]). The analysis of the correlation of RP of the SCA1 patients showed a negative relationship with CAG repeats frequencies (r = -0.485 at P = 0.05 by Pearson). Thus, it was found that the higher the frequency of CAG repeatsamongthe SCA1patients, the lower their ability to perform usual duties. The presence of the direct, highly reliable relationship between the RP scale (r = + 0.632 at P = 0.001 according to Pearson) with the levels of their PF scales reflected the close connection of the indices of physical abilities of the SCA1 patients. The RP scale level of the SCA1 patients appeared to be directly connected with their abilities to get social assistance, i.e. to draw the attention of people from outside and to get help from neighbors (at r = + 0.480 with confidence P = 0.001 by Pearson).

The Pain Intensity scale (Bodily Pain - BP) and its impact on the ability to be occupied by daily activities, including domestic and out-of-home work, showing a limitation of pain in the ability of the SCA1 patients to be active was 70.42 ± 5.43 points. The revealed data showed that the intensity of pain in the SCA1 patients did not significantly limit their ability to do housework and beyond. Although these data differed little from the data of the control group (76.25 points), as well as the data of the urban population (Yakutsk residents 78.00 points [7]), but a detailed analysis of the pain data of theSCA1 patients showed that the proportion of theSCA1 patients suffering from pain was 1.73 times more (40.54%) than among the control group (23.4%).

The value of QOL of the SCA1 patients on the scale of General Health (GH) was 1.5 times lower (equal to 40.41 ± 2.08 points), than that (61.76 points) in the control rural and urban population groups data 61.3 points [7]). As a result, the assessment of the condition among the SCA1patients on the scale of the General Physical Health GH was equal to 65.43% of the control group index.

The Vitality scale –VT showing the feeling of full strength and energy or exhaustion of the SCA1 patients was 28.85 ± 4.50 points and indicated the presence of complaints, typical of lassitude, energy loss and weakness. The VT index in the control group was 91.67 points, and in the city residents group - 62.9 points [7]. So we established that the level of vital activity -VT of the SCA1 patients was more than 3.17 times lower than in the control group, and in comparison with the urban residents 2.18 times lower. At the same time, the VT of the SCA1 group was highly correlated with the scales of their physical (r = + 0.685 for Pearson P = 0.001) and role functioning RP (r = + 0.647 at P = 0.001 by Pearson). Thus, there is a direct connection between VT and PF and RP of the SCA1patients. Also there were direct connections between the VT scale of the SCA1patients and the number of their close relatives (r = + 0.450 Pearson P = 0.001) and assisting people (r = + 0.382 Pearson P = 0.05) and neighbors looking after them (r = + 0.496 Pearson P = 0.001). There was a direct relationship between the VT level and the scale of social functioning (communication) of the SCA1patients (with r = + 0.667 at P = 0.001 according to Pearson) (see below). So, the level of VT with SCA1 directly depends not only on physical, but affects social communications (see below).

Although the scale of Social Functioning (SF), showing the level of communication of the SCA1patients, was 49.01 ± 6.32 points and 1.5 times less than the data of the city residents (74.7) and amounted to ½ of such control (98.69 points) but with high reliability (according to Pearson P = 0.001) was associated both with PF (r = + 0.681), RP (r = + 0.647) and with VTas it was indicated. The frequency of CAG repeats in the SCA1patients had a negative effect on the onset age ("debut") of the disease (r = -0.673 at P = 0.001) and the onset of speech impairment (r = -0.680 at P = 0.05), almost as much as on the age of the beginning of the coordination disorder (unsteadiness of gait r = -0.600, awkwardness of hands r = -0.505 at P = 0.001). Thus, the clearer pronounced the mutation, the earlier developed speech disorders and coordination of fine motor functions in SCA1. In the final stage, speech impairment almost went to complete aphasia (out of 41 patients in 5 patients), combined with pronounced violations of the coordination of limb movements, ocular musculature and swallowing. So, the SF level of the SCA1patients from a significant restriction of communication with one person (contact with relatives) to the complete cessation of communication with the public environment directly influenced their ability to attract people's attention (r = + 0.481 at P = 0.001) and the possibility of assistance from neighbors (r = + 0.593 at P = 0.001). At the same time, SF of the SCA1patients is directly dependent (r = + 0.739 with a confidence of 0.001 Pearson) from the RE scale (see below).

This Role-Emotional Role scale (RE), having impact on the level of performance of work or other daily activities (including a large time expenditure, a decrease in the amount of work, a decrease in its quality, etc.) of the SCA1patients was 29.04 ± 5.76 points. The index of this control group was 1/3 (97.92 points) and was slightly less than 1/2 in the city group (70 points [7]). The value of the RE scale of the SCA1patients also directly depended on the PF and RP scales (r = + 0, 63 and + 0.531 with P = 0.001 by Pearson) and with VT as well (at r = + 0.683) and was corelated with SF, and mental health MH (r = + 0.829 at P = 0.001 by Pearson).

The scale "Mental Health - MH QOL" of the SCA1patients, which characterizes the mood background (presence or absence of depression, anxiety) and emotional state of the SCA1patients, was 1.31 ± 0.80 points. In the control group MH was 93.70 points, and among the inhabitants of Yakutsk - 67 points [7]. The revealed features of mental health of the SCA1patients reflected its poor health in the patients affected by PF and RP (at r = + 0.510 by Pearson with P = 0.05 and 0.001).

At the same time, the integral Physical Health Component (PH) of QOL of the SCA1patients was 39.0410 ± 1.42495 points. Thus, the physical component of the health of the SCA1patients was 74.22% of the number of the residents not suffering from SCA1 (52.68 points according to A.E. Mikhailova [7]). And the integral psychological component of health (Mental Health - MH) of QOL of the SCA1patients was 24.4153 ± 1.41797 points. And the mental component of the health of the patients with SCA1 was 45.37% of the value of the health component of the non-SCA1 people (53.8 points [7]).

Along with these characteristics of the lowering of QOL of the SCA1patients there were groups, established among them: those who did not have close relatives (12.9%), those with disoriented attention (12.9%) or with insufficient attention (7.7%) and 5.1% of those who " did not count on or sought the aid of neighbors who with very great difficulty or just with difficulty helped others around them" (5.4%). Moreover the number of people, who received help of neighbors, directly depended on the number of people, who payed attention on the patients (r = +0.676 at P = 0.001). The number of close people depended inversely on the number of people who paid attention (r = -0.415 at P = 0.05 according to Spirman). The last indicator was in inverse relationship (r = -0.522 at P = 0.001) with the frequency of CAG repeats.

All the above mentioned showed that the SCA1patients with strong speech disorders experienced both more difficulties in communicating and establishing communication with others. At the same time, the number of psychological health problems such as anxiety (58.5 ± 0.78%) and depressive state (53.7 ± 0.778%) corresponded to the MH index of 1.31 ± 0.80 of the SCA1 patients. This means that patients with severe communication and psychological problems need timely specialized medical care and social assistance. Although the fact is complicated by the living of the SCA1patients in remote communities with transport problems, the problems still can be solved by the authorities of the Republic.

CONCLUSION

For the first time, an integrated assessment of QOL of the SCA1patients using the SF-36 Questionnaire showed a sharp decrease in QOL due to a sharp restriction of physical, role motor functioning, combined with impaired communicative functions and mental unhappiness that occurred among more than half of the observededSCA1 patients. SCA1patients with such chracteristics should be maintained as a high-risk group.

This section of research was conducted for the first time in Yakutia as a center of accumulation of SCA1 by the method of refining the parameters of QOL of such patients. The negative nature of the established changes in QOL of the SCA1patients is related to the level of the mutations that have arisen, representing a scientific reserve, the continuation of which will have a practical solution as an opening of the social and psychological features of the SCA1clinic. The data obtained can be used in possible recommendations for the assistance and treatment of SCA1patients and will help specialists who choose the means of rehabilitation and maintenance of SCA1patients at home, as well as doctors who determine the profile of institutions where established high-risk group patients are sent.

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ANALYSIS OF ASSOCIATIONS OF GENETIC POLYMORPHISMS 481C> T, 590G> AI 857G> A GENE OF THE ENZYME N-ACETYLTRANSFERASE 2 *(NAT2)* WITH THE RISK OF LUNG CANCER IN THE YAKUTS

ABSTRACT

For the first time the search of polymorphic options of a gene of NAT2 associated with the development of lung cancer in Yakutia has been carried out. Genetic markers of the increased and lowered risk of development of lung cancerin the Yakuts have been revealed. It is established that markers of the increased risk of development of lung cancer for the Yakuts are the allele of NAT2*857A and a genotype of NAT2*857G/A, markers of the lowered risk – NAT2*857G allele, NAT2*857G/G genotype.

Keywords: lung cancer, polymorphic options, N-acetyltransferase-2.Keywords: lung cancer, polymorphic options, N-acetyltransferase-2.

INTRODUCTION

In Russia cancer of a lung takes the leading positions in structure of oncological incidence – the incidence of it for the last 20 years has increased more than twice and it is on the first place among malignant neoplasms [5]. Annually in Russia this pathology is diagnosed more, than for 63 thousand patients. The problem of cancer of lung is relevant also for Yakutia where this form of cancer for many years takes the first place in structure of an oncopathology. In Republic Sakha (Yakutia) with the population in 982,1 thousand people annually more than 300 people get cancer of a lung[2].The sharpness of a problem is caused not only by high prevalence of a disease, but also by late diagnostics as at an early stage lung cancer is possible to diagnose no more than in 15% of cases [4]. It is relevant to study all factors involved in carcinogenesis.

Lung cancer, as well as many oncological diseases, is a multiple-factor disease and in its development an important role is played as outside environment (smoking, asbestos, radon, arsenic, etc.), and genetic factors [7, 9, 11, 17]. By some authors, it is shown that polymorphic options of a gene of NAT2 make a contribution to development of oncological diseases including cancer of a lung [8, 13, 18].

The gene of NAT2 is localized on a short chromosome arm 8 (8p23.1), has about 9900 PN extension, contains 2 exons and it is expressed mainly in a liver and intestines [3, 14]. The N enzyme-acetyltransferase-2 coded by this gene represents the protein with a molecular weight of 33 cd consisting of 290 amino-acid residues. This enzyme localized in cytoplasm participates in process of biotransformation of aromatic amines, which are present at the environment. A source of aromatic amines are industrial wastes, pollution of water, air, and a number of medicines [3, 15].

MATERIAL AND METHODS

In the present article it has been done a comparative studying of polymorphism of a gene of NAT2 enzyme arylamine N-acetyltransferase among patients with cancer of a lung and among healthy people, residents of the Sakha (Yakutia) Republic. We have examined 60 patients with cancer of a lung of Yakut ethnic origin from which 43 men, 17 women received treatment in a republican oncological

clinic of the city of Yakutsk. Average age of patients was 58,86±8,72 years. The diagnosis cancer of a lung has been confirmed morphologically, endoscopically and radiologically. During checking has been investigated the group of healthy people which is corresponding to a group of patients on ethnic origin and a gender with no oncological diseases, consisting of 60 people (middle age 49,5±5,75).

For release of DNA the standard method of phenolic and chloroformic extraction was used [12]. The emitted DNA was frozen at a temperature - 400C before carrying out genotyping.

The analysis of polymorphic options 481C>T, 590>T; AU 857>A; was carried out by A of a gene of NAT2 with use of methods of polymerase chain reaction on the thermocycler of "Tertsik" of the "DNK-technology" company (Russia) and T100 of the "Bio-Rad" company (USA).

For amplification used reactionary mix volume 25 мql, which contained 2,5 мql 10 Taq-buffer (67 mMtris-HCl (pH 8,8), 16,6 mM (NH4)2 SO4, 2,5mM MgCl2, 0,01% of Tween-20), 0,1 mkg of genomic DNA, the mix dNTP (dATP, dGTP, dCTP, dTTP on 150 mkM of each), 1 unit of DNA - Thermusaquaticus polymerase (Sintol, Russia) and 5-10 pmoligonucleotidepraymer (F 5' - GCTGGGTCTGGAAGCTCCTC; R 5 '-TTGGGGTGATACATACACAAGGG). The mode of amplification was the following: preliminary denaturation (940C, 5 min.), 28 cycles of amplification: a denaturation – 940C, 45 sec.; annealing – 600C, 45 sec.; synthesis – 720C, 45 sec., the finishing synthesis (720C, 7 min.).

For definition of nucleotide replacements it was carried out hydrolysis of an amplificated fragment by the following restrictions: KpnI (481C > T), BamHI (857G >A), TaqI (590G > A) (fig. 1, 2, 3).

Products of enzymatic hydrolysis were divided by a vertical electrophoresis in 7% poliacrylamide gel with the subsequent processing bromic etidium. Visualization of bands and scanning of gel were carried out in the passing UF-light by means of the "DNAAnalyzer" video system (Moscow).

When comparing frequencies of genotypes the standard criterion $\chi 2$ with Yeats's amendment was used. Statistically significant considered distinctions at $p<0,05$. The relative risk (OR) of development of a disease at a certain genotype was calculated by a standard formula OR=a/bxd/c where a and b – the number of the patients having and not having a mutant genotype respectively and d, c – a number of people in control group, having and not having a mutant genotype. OR is specified with a 95% confidential interval.

RESULTS

The analysis of distribution of frequencies of alleles and genotypes which is carried out by us on three polymorphic locus 481C> T, 590G >A and 857G > NAT2 gene A among sick and healthy people of the Yakut ethnic origin has revealed a number of features of distribution of frequencies of alleles of the studied gene.

Frequency of alleles NAT2*481T in group of healthy individuals of the Yakut ethnic origin was 23,4%. Earlier it has been established that the prevalence of this polymorphic option in various populations varies from 2-43% [16]. In populations of Europe frequency alleles is NAT2*481T 38-43% [1, 6, 16], and in Asian populations of China and Japan with – 6% and 2%, respectively [6], that is there is a gradient of the decrease in frequency given allele from the west to the east. In comparison with literary data on occurrence frequency alleles NAT2*481T in Southeast Asia among Yakuts this value is rather high.

Frequency of alleles NAT2*857A in population of Yakuts - 21,7% that is more, than in other Asian populations. Frequency of given alleles increases from the west to the east, among Europeans it is 2-3%, in populations of Asia – 11-19% [6, 10].

According to literary data in distribution of frequencies of alleles NAT2*590A isn't revealed significant interethnic differences between populations of Asia and Europe. For alleles NAT2*590A at the population of Europe is characteristic occurrence frequency – 26-27%, among inhabitants of Asia 23-30% [6, 10]. Among Yakuts frequency alleles NAT2*590A was equal in control group to 21,7%.

For the purpose of identification of possible associations of polymorphic options of a gene of NAT2 with development of cancer of lung we have carried out the analysis of distribution of allels polymorphic option 481C > T at patients with cancer of a lung and among the people who don't have oncological diseases. We haven't found statistically significant differences in distribution of frequencies of alleles between control group and group of patients with cancer of a lung. In distribution of genotypes between control group and group of sick statistically significant distinctions were also not found (fig. 4).

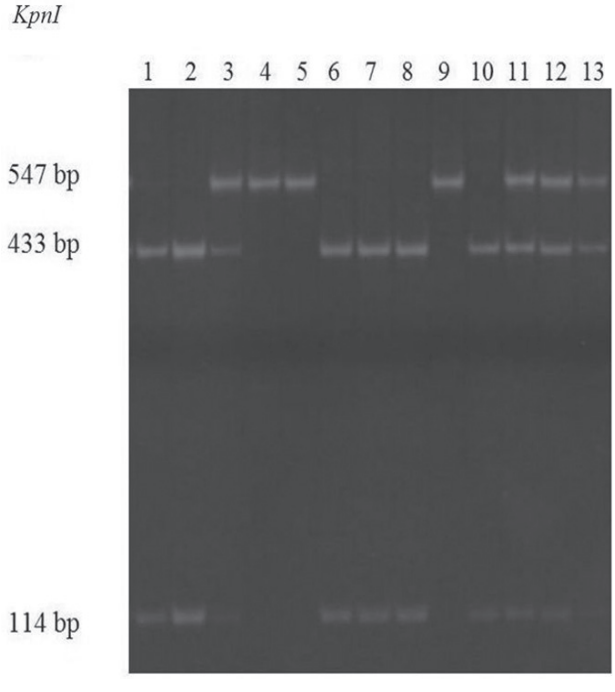

Figure 1. Detection of polymorphism 481C > gene T by NAT2 method of the RFLP-analysis (T/T – a homozygous «wild» genotype (4,5,9); T/C – a heterozygotic genotype (3,11,12,13); C/C – a homozygous mutant genotype (1,2,6,7,8,10)).

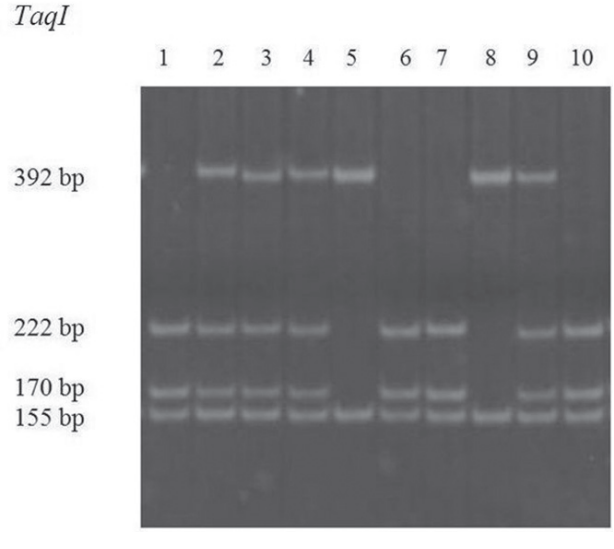

Figure 2. Detection of polymorphism 590G > gene A by NAT2 method of the RFLP-analysis (G/G – a homozygous «wild» genotype (1,6,7,10); A/G – a heterozygotic genotype (2,3,4,9); A/A– a homozygous mutant genotype (5,8)).

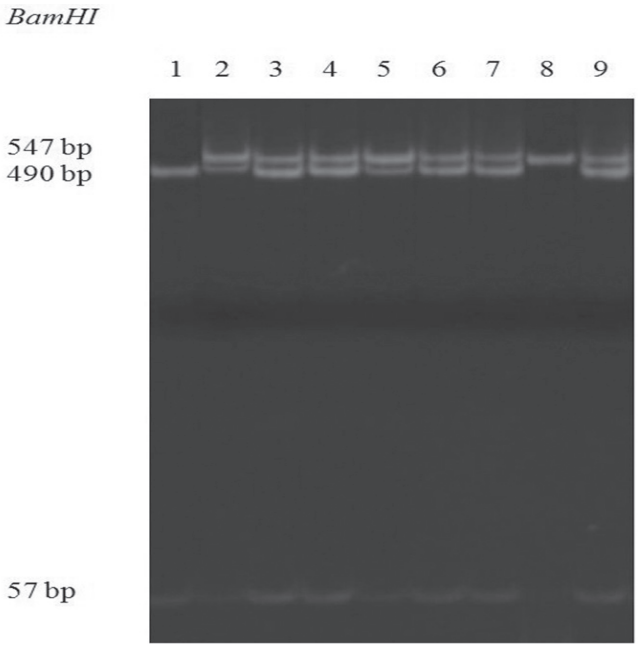

Figure 3. Detection of polymorphism 857 >gene A by NAT2 method of the RFLP-analysis (A/A – a homozygous «wild» genotype (8); A/G – a heterozygotic genotype (2-7,9); G/G – a homozygous mutant genotype (1)).

In group of patients the frequency of occurrence mutant alleles was higher than NAT2*590A in comparison with healthy (34% and 26% respectively), but statistically significant differences when comparing frequencies of alleles (NAT2*590G and NAT2*590A) at sick and healthy isn't found by us (fig. 5). In group of patients we have noted increase in frequency of occurrence of a heterozygote genotype of NAT2*590G/A by 1,7 times in comparison with control group, 40% and 23,3% respectively (χ^2=3,12; p=0,077)

The greatest statistically reliable distinctions of frequencies of alleles and genotypes were observed by us by polymorphic option 857G > A. At patients in comparison with healthy decrease in frequency of occurrence of mutant NAT2*857G alleles - 64,2% and 78,3%, respectively χ^2=42,52 is noted; p =0,000...; OR=2,02; 95% CI=1,10 – 3,78 and increase in frequency of occurrence of wild NAT2*857A alleles 35,8%, 21,7% respectively χ^2=42,52; p=0,000...; OR=6,47; 95% of CI=3,52 - 11,98 (fig. 6).

In group of patients frequency of alleles NAT2*857A increased (35,8%; χ^2=42,52; p=0,000...; OR=6,47; 95% CI=3,52-11,98) and heterozygotic genotype of NAT2*857G/A (71,6%; χ^2=13,43; p=0,000...; OR=0,23; 95% CI=0,10-0,53) and the frequency of a homozygous genotype NAT2*857G/G decreased (28,4%; χ^2=10,95; p=0,000...; OR=3,79; 95% CI=1,66-8,78) in comparison with control of 21,7%, 36,6% and 60,1% respectively.

Thus, in the analysis of associations of polymorphic options 481C > T, 590G >A and 857G >; NAT2 gene A with development by lung cancer in Yakutia have been established the allelic options and genotypes of a gene of NAT2 making a contribution to development of cancer of lung among persons of the Yakut ethnic origin. Markers of the increased risk of development of cancer of lung among Yakuts are the allele of NAT2*857A and a genotype of NAT2*857G/A, markers of the lowered risk - NAT2*857G allele, NAT2*857G/G genotype.

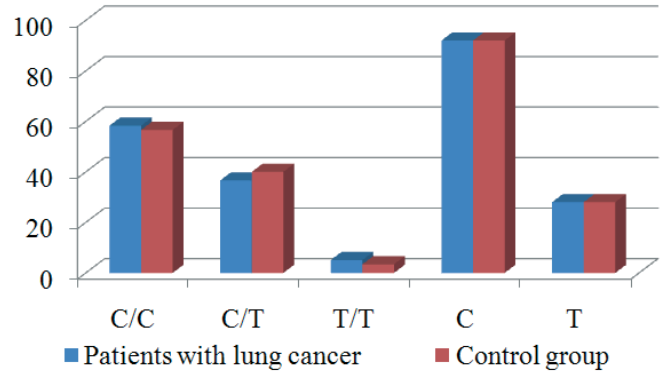

Figure 4. Distribution of frequencies of genotypes and alleles of polymorphic option (481C > T) NAT2 gene at patients with cancer of a lung and control group

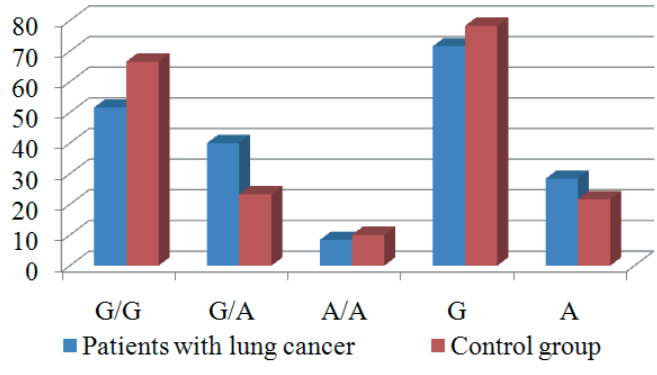

Figure 5. Distribution of frequencies of genotypes and alleles of polymorphic option (590G > A) NAT2 gene at patients with cancer of a lung and control group

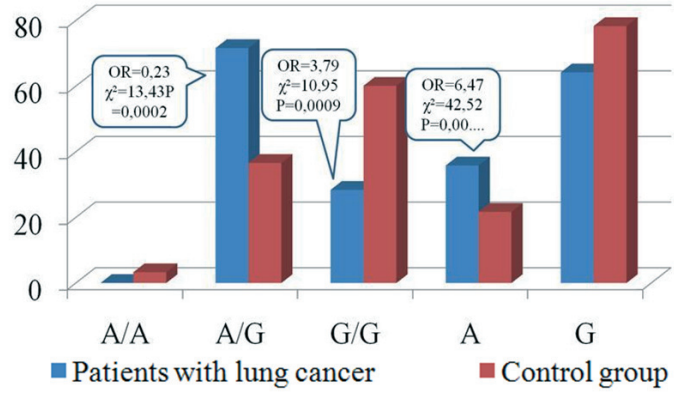

Figure 6. Distribution of frequencies of genotypes and alleles of polymorphic option (857G > A) NAT2 gene at patients with cancer of a lung and control group

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GENOME AND GENETIC HEALTH OF THE YAKUT ETHNOS

ABSTRACT

The article presents a survey of the results of studies of the ethnogenesis of the peoples of Siberia in the aspect of the genetic health of the ethnos, adapted to the extreme changes in seasonal changes in the high-latitude zone. The genome inherited from ancestors with a certain protective potential provides metabolic health and stability against stressful environmental influences. The genome of the Yakut ethnos is characterized by three main genetic components (58.5% - Central Siberian, 12.5% - European, 29% - East Asian), as well as a high level of homozygosity of the genome. In the Yakut population, the negative genetic component is manifested by a wide spectrum of age-dependent, genetically predisposed neurodegenerative diseases (multiple sclerosis, Viliuisk encephalitis, Parkinson's disease and diseases with impaired motor functions, including amyotrophic lateral sclerosis and spastic paraplegia and other). Also in recent years, the population is experiencing an increase in the burden of diseases with metabolic disorders. The main reason for the growth of the negative load of health disorders of the modern Yakut population is the consequence of genetic drift and conservatism of the genome.

Keywords: ethnogenesis, Yakut population, genome, metabolic disorders, neurodegenerative diseases

The genome of the Yakut ethnos has been the subject of much research for many years. In recent years, the scientific interest has turned to the issues of the formation of the genetic health of the ethnos. Modern genetic technologies have made it possible more in-depth research of the genome of the Yakut ethnos, but the fundamental questions of the origin of this ethnos remain unclear. Until now, there has been a sharp discussion of the question of who the modern indigenous people of Central are and North-Eastern Siberia at present. The latest genetic studies of the skeleton materials of an ancient boy found in 1928 in the village of Mal'ta in the western part of the southern extremity of Lake Baikal gave the sharpness to the discussion. The age of the find was estimated at 24 thousand years [8]. A detailed comparative analysis of the autosomal genome of humans, as well as single-parent markers (mDNA and chromosome Y) of representatives of a number of ethnoses of Siberia made it possible to develop the concept of a genetic component of an ethnos. This is a historically established set of common gene polymorphisms geographically distributed among different ethnic groups. Thus, the genetic analysis of the ancient remains of the Mal'ta camp showed that the genome of the ancient boy is made up of three ancient genetic components: the South Asian component (37%) - the migration path is traced from the plains of northern China and the Mongolian highlands, the Eastern European (34%) - is traced from the steppes of the Southern Urals and the genetic component of the modern Indians of the American continent (26%), i.e. the genome of a man who lived 24 thousand years ago near Lake Baikal does not reveal close proximity to the genome of the modern Siberian aborigines (the data will be presented below). It is assumed that as a result of the last ice age (20-18 thousand years ago) the territory of most of Siberia was depopulated, due to migration of the population to the south (the depopulation of the greater part of central Siberia occurred). At the same time, it is possible that the part of the population in some places could survive this period. The modern ethnic picture of Siberia began to form as a result of the re-settlement of Siberia about 7-6 thousand years ago with migration from the Altai-Sayan Mountains and the Amur River. It remains unclear what contribution could be made by relic (surviving the glacial period) ethnic groups to the genome of new ethnoses of Siberia.

Review of the results of studies of the genomes of modern Siberian ethnoses

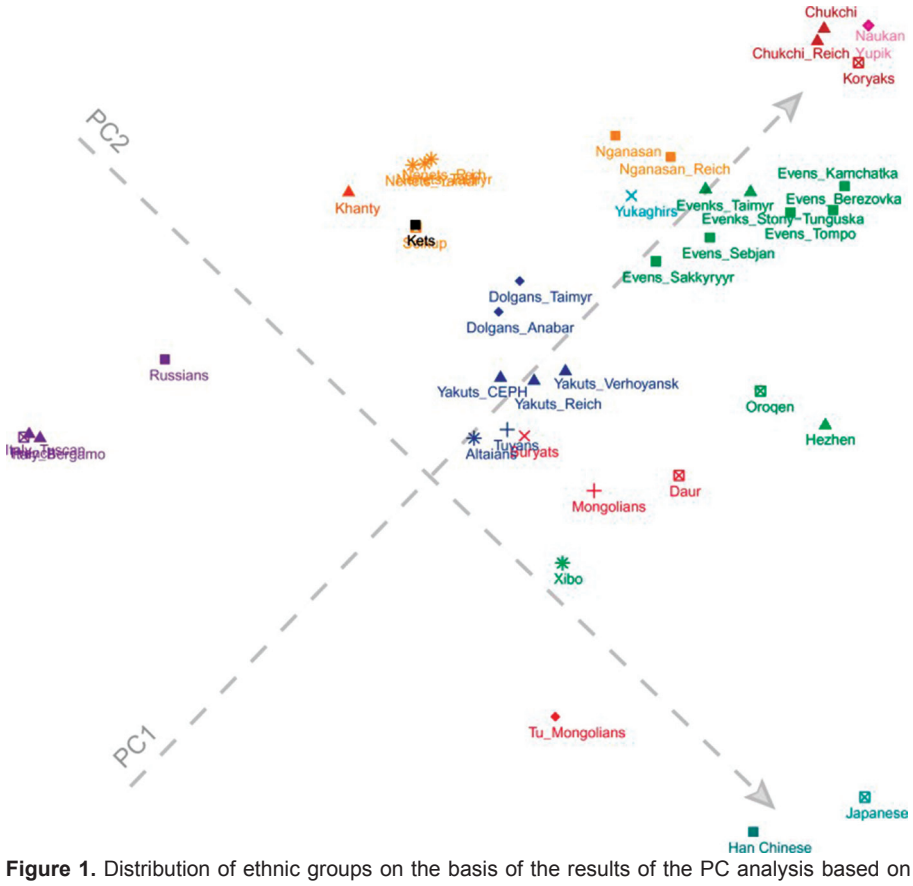

Figure 1. Distribution of ethnic groups on the basis of the results of the PC analysis based on the number of IBD blocks shared between populations. The PC axes are rotated to 45 degrees in accordance with the distribution of ethnos on the geographic map of Siberia.

Genomic analysis of the modern ethnoses of Siberia to identify inter-ethnic links was carried out using the PCA method (analysis of main components), ADMIXTURE (model based on grouping of algorithms) and IBD (fragments of the DNA molecule inherited without recombination and identical in origin). This mathematical method of processing the genetic information of DNA allows us to find the correlation of geographical coordinates with the actual place of origin of each genome [7]. The results of the analysis are shown in Fig. 1.

It can be seen from the figure that ethnic groups can be grouped into six main original ancient roots, where the percentage of mixing genomes is less than 1%: ancient European (here Italian is used), West Siberian (Khanty), Central Siberian component (Nganasan), East Asian (Chinese Ethnos khan), the Far Eastern component (Koryaks) and Yupik-Inuit.

Table 1 presents the distribution pattern of the genetic component and the contribution of their ancient roots to the genome of the modern ethnos on the geographic map of Siberia.

The European component is an integral part of the formation of the genome of the ethnoses of Siberia, with the exception of the genome of ethnic groups living in north-eastern and eastern Siberia. The Central-Siberian component was formed in the North (Nganasan), the proportion of this component in other ethnic groups is gradually falling in the direction to the South. In the Nganasan, the Yakuts, the Dolgan, the Evenks and the Evens, this component is dominant. The Even and Evenki in Fig. 1 are clearly separated by their localization, which indicates originally different roots of origin. The migration path and their expansion can be traced from the Amur region. The

The contribution of the genetic components of the ancient roots to the genomes of the modern ethnoses on the geographic map of Siberia.

Genetic component	Altaians	Tuvans	Buryats	Yakuts	Nganasan	Bone of 24,000-year-old individual (MA-1), from Mal'ta in south-central Siberia
Central Siberian	30,9	39,6	41	58,5	100	
European	21	11,6	8,3	12,5	0	34
Eastern Asian	39	41,8	50	29	0	37
Western Siberian	9,1	7	0,7	0	0	

Evenk has no European component in the structure of the genome. The ancestors of the Yakuts, the Dolgan and possibly the distant Nganasan, show genetic affinity with the South Siberian and, most of all, Altai, in spite of their territorial remoteness. This is explained by the transport availability of migration along the Yenisei River to the North and its tributary Tunguska into the territory of Central Siberia (Yakutia).

The existing component composition of the genome of the Yakut ethnos is also the result of a genetic drift in the past (1000 years ago) that determined the choice and subsequent expansion of the genome of the ethnos [4, 9].

1000 years ago there was a sharp demographic jump in the size of the population. This fact is confirmed by numerous early studies on m-DNA and genes of the Y chromosome. The demographic picture of other aboriginal ethnoses, in contrast to the Yakuts, shows a steady decline in the size of the population. Expansion of the Yakut population for such a short historical period from a small number of related ancestors affected to the structure of the Yakut genome, the level of homogeneity. When a significant part of the human genome is occupied by the share of identical pairs of genes, the risk of recessive gene manifestation increases, among which there are many rare pathogenic forms. Indeed, the conducted studies on decoding the sequence of complete DNA exome of representatives of the Yakut ethnos show a high level of homozygosity of the genome [6].

Thus, the genome of the Yakut ethnos is characterized by three main genetic components (58.5% - Central Siberian, 12.5% - European, 29% - East Asian), as well as a high proportion of homozygosity of the genome. This feature of the Yakut genome formed the basis of the genetic health of the ethnos, adapted to extreme changes in seasonal changes in the high-latitude zone. The contribution of each component in the genome structure inherited from the ancestors of the ethnos will determine the quality of genetic health of representatives of the modern ethnos, its predisposition to certain diseases and the therapeutic response.

The general state of human biological health is determined by its metabolic health, which is formed by the interaction of the genome with environmental factors. The inherited from the ancestors' genome with a certain protective potential provides metabolic health and stability from the stresses of the external environment. Reducing the protective potential of the organism with age is the cause of the development of age pathology, which is a manifestation of the negative genetic component of the genome.

A considerable number of age-dependent, genetically predisposed neurodegenerative diseases (multiple sclerosis, Vilius encephalitis, Parkinson's disease and diseases with impaired motor functions, including amyotrophic lateral sclerosis and spastic paraplegia) are registered in the Yakut population with a total number of more than 400 thousand people. A wide spectrum of rare monogenic diseases is noted (among them, with the molecular mechanism of expansion of trinucleotide repeats, which have a high prevalence among the Yakuts in comparison with the world indices - spinocerebellar ataxia type 1, myotonic dystrophy, oculopharyngeal muscular dystrophy, and several families with Friedreich's ataxia). Family cases of rare autosomal dominant and recessive diseases have been reported: Thompson's myotonia (5 families), Duchenne muscular dystrophy (11 families), and a number of other diseases [2]. Polymorphic variants of genes associated with the disease manifest themselves ambiguously in different ethnic groups.

General characteristics of the metabolic health of aboriginal Siberia are reflected in the works of Stephanie B. Levy et.al. [5]. Historically, in the representatives of populations living in the high-latitude zone, biomarkers of metabolic health were characterized by a low level (lipids, sugars in the blood) due to the high metabolic metabolism of the organism. This was associated with the traditional diet of omega-3 fats and high physical activity, which was reflected in their relatively high blood pressure. The processes of globalization of the economy and urbanization of the society radically changed the traditional way of life and human nutrition in the North, on the basis of which the phenotype of the ethnos was formed. In recent years, these populations have experienced an increase in the burden of diseases with metabolic disorders. Genetic factors predisposing to the violation of metabolic health and its complications to type 2 diabetes mellitus in the Yakut population are reflected in Osakovsky's work with co-authorship [1].

The main reason for the growth of the negative load of health disorders of the modern Yakut population is the consequence of genetic drift and conservatism of the genome. A feature of the genetic drift described above is also its sexual difference in severity. Analysis of the Y chromosome data reveals a strong effect of the male founder on the haplogroup N-TatC. This haplogroup can be a marker of the central-Siberian component. While on the maternal line the analysis of mDNA data the effect of the founder on haplogroup D5 is weakly expressed. This indicates a difference in the origin of male and female lines, as evidenced by a negative correlation between the distance matrix of SNP-based Y chromosome data and mitochondrial DNA data [3]. This feature can also have phenotypic manifestations in the clinic of diseases.

Social and economic changes in the living conditions in the North on the background of the conservatism of the evolutionarily formed human genome determine the vulnerability of the body's biological functions to metabolic disorders and an increased risk of previously not encountered in the population of diseases.

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INFORMATIVE PARAMETERS OF PREDICTIVE IN *SILICO* COMPUTER PROGRAMS IN ASSESSING CLINICAL SIGNIFICANCE OF MISSENSE VARIANTS OF GJB2 *(CX26)* GENE

ABSTRACT

Currently, in the HGMD database (The Human Gene Mutation Database) in the *GJB2* gene, coding protein connexin 26 (Cx26), 390 different nucleotide changes have been announced, most of which are associated with deafness, of which 73% are single nucleotide (missense/nonsense) variants. The pathogenetic role of most nonsense substitutions is obvious, as they lead to premature termination of translation and interruption of protein synthesis. It is more difficult to assess the mechanism of action of the missens replacement on protein, since they may have a damaging/partially damaging or neutral effect, depending on the location in the amino acid sequence of the polypeptide chain. To assess the possible effect of amino acid substitutions on the structure and/or function of the protein, in the absence of structural and functional studies, the *in silico* prognostic method is used, which is completely performed by simulation computer programs. In this study, based on the established clinical significance, 7 missense variants of the *GJB2* gene, detected as a result of the molecular genetics study of congenital deafness in Yakutia, 9 computer-*in silico* predictive programs were tested. In order to identify the program with the most accurate prediction of the clinical significance of missense variants substitutions of the *GJB2* gene, a comparative analysis of the informative parameters (accuracy, sensitivity and specificity) was carried out with the calculation of the correlation coefficient between the known clinical values of missense variants with *in silico* evaluation by the programs. In total, of the 9 analyzed programs, the most accurate *in silico* predictive estimates of the clinical significance of missense variants of the *GJB2* gene were given by two programs - SIFT and PROVEAN (R = 0,73). The results obtained can help in carrying out bioinformatic analysis, in the case of detection of missense variants substitutions of the *GJB2* gene, which were not described before in the literature.

Key words: *in silico* analysis, *GJB2* gene, connexin 26 (Cx26), missense variants, deafness, Yakutia

INTRODUCTION

Currently, methods of molecular and genetic analysis of the *GJB2* gene (Cx26) (13q12.11, MIM 121011) provide a high information content for the diagnosis of hereditary nonsyndromic hearing loss. The vast majority of patients with congenital hearing loss have recessive mutations in the *GJB2* gene in the homozygous or compound heterozygous state, which corresponds to a diagnosis of autosomal recessive deafness type 1A (DFNB1A, MIM 220290) [29]. According to the database of The Human Gene Mutation Database (HGMD), about 390 different nucleotide changes have been announced in the *GJB2* gene, of which 73% occupy single nucleotide missense/nonsense variants (http://www.hgmd.cf.ac.uk/ac/index.php). The pathogenic role of the majority of nonsense variants is obvious, as they lead to premature termination of translation and interruption of protein synthesis. Missense variants, depending on their location in the amino acid sequence of the polypeptide chain, can be neutral, damaging or partially damaging the structure of the protein, slightly affecting its function. In consequence, the pathogenetic role of many missense variants in the development of hearing impairment is difficult to assess.

To assess the possible effect of amino acid substitutions on the function and/or structure of the protein, in the absence of structural and functional studies, use the method *in silico* is used, which is fully implemented by simulation computer programs. However, existing *in silico* computer programs have a number of drawbacks, since the applied predictive algorithm can be radically different. This is due to the fact that each individual program works with different computational methods (BLAST, PSI-BLAST, PSIC) and tools (*Matrix Dirichlet*, Hidden Markov Model, Naive Bayesian classifier). In this regard, the accuracy of the predictive evaluation of *in silico* computer programs can vary widely [10, 19, 22, 23, 30].

In the previously conducted molecular genetic studies of DFNB1A in Yakutia, 12 allelic variants of the *GJB2* gene (8 pathogenic and 4 benign variants) were identified, among which 7 missense

variants led to amino acid substitution [1, 21]. The pathogenicity or non-pathogenicity of identified missense variants has been confirmed through clinical genealogical research methods, in addition to literature sources and data from databases. . Thus, the confirmed data on clinical significance of the identified *GJB2* gene missense variants , enable us to estimate the accuracy of *in silico* computer programs predictions.

The purpose of this paper is to compare the informative parameters of the most popular computer *in silico* predictive programs, to select the most accurate program for predicting the clinical significance of missense variants of the *GJB2* (Cx26) gene.

MATERIALS AND METHODS

Missense variants of the GJB2 (Cx26) gene

For *in silico* programs testing, seven missense variants of the *GJB2* (Cx26) gene were used: c.79G>A (p.Val27Ile), c.101T>C (p.Met34Thr), c.109G>A (p.Val37Ile), c.269T>C (p.Leu90Pro), c.341A>G (p.Glu114Gly), c.368C>A (p.Thr123Asn) *и* c.457G>A (p.Val153Ile), which were identified during previous molecular genetic studies of congenital deafness in Yakutia (Figure 1) [1, 21]. All of these missense variants of the *GJB2* gene were annotated in the following databases: OMIM (http://www.omim.org/); Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php); ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/); the Exome Aggregation Consortium (http://exac.broadinstitute.org); 1000 Genomes Project (http://browser.1000genomes.org/index); dbSNP (http://www.ncbi.nlm.nih.gov/snphtml).

Clinical significance of listed *GJB2* sequence variants was interpreted following the American College of Medical Genetics and Genomics (ACMG) guidance [22]. This report recommends the use of specific standard terminology: «pathogenic», «probably pathogenic», «uncertain significance», «probably benign» and «benign» to describe variants identified in Mendelian disorders [22]. In the group of pathogenic variants associated with hearing impairment (deafness/DFNB1A), three variants were classified as c.269T>C (p.Leu90Pro) - as a «pathogenic», c.101T>C (p.Met34Thr) and c.109G>A (p.Val37Ile) - as «likely pathogenic». The remaining 4 variants were classified as benign variants: c.79G>A (p.Val27Ile) and c.457G>A (p.Val153Ile) - as «benign», c.341A>G (p.Glu114Gly) - «benign/likely benign», c.368C>A (p.Thr123Asn) - as «likely benign». To assess the clinical significance of the 7 missense variants of the *GJB2* gene, we relied not only on the supervised databases, but also on the results obtained among the 580 people studied from Yakutia (393 patients with congenital hearing impairment and 187 normal hearing individuals).

In silico computer programs

Table 1 shows the *in silico* prediction programs selected for this study: SIFT (Sorting Intolerant From Tolerant), FATHMM (Functional Analysis through Hidden Markov Models), MutationAssessor, PolyPhen2 (Polymorphism Phenotyping v-2), Condel (Consensus Deleteriousness), MutationTaster, MutPred (Mutation Prediction), Align GVGD (Align Grantham Variation/Grantham Deviation) and PROVEAN (Protein Variation Effect Analyzer). Each *in silico* classification tool utilizes different parameters for variant classification, the full details of which can be found online (websites are showed in table 1).

For the query in the search windows of *in silico* programs, the genetic sequence identifiers (nucleotide, amino acid and protein) were used in FASTA format (GenBank: AHB08964.1 - gap junction beta-2 protein [Homo sapiens]) from the NCBI database - Reference Sequence (http://www.ncbi.nlm.nih.gov/protein) and in the Ensembl ID format (ENSG00000165474 - for the gene, ENSP00000372299 - for the protein, ENST00000382848 - for the transcript) from the database of The Human Protein Atlas (http://www.proteinatlas.org).

Analysis of the informative content of in silico computer programs

When obtaining predictive results of *in silico* computer programs, their analytical parameters were calculated as follows [4, 7, 23]:

Sensitivity (Se) - *part of the truly positive results (correct identification of pathogenic variants), according to formula Se=TP/TP+FN*, where *TP* - are true positive cases and *FN* - are false negative cases;

Specificity (Sp) - part of the truly negative results (the correct identification of benign variants that do not have clinical significance), according to the formula *Sp=TN/TN+FP*, where *TN* - are true negative cases and *FP* - false positive cases;

Accuracy (A) - the ratio of complete correct predictions to the total number of predictions, according to formula *A=TP+TN/TP+TN+FP+FN*;

Positive predictive values (PPV) - the proportion of positive results that were true positives, according to formula *PPV=TP/TP+FP*;

Negative predictive values (NPV) - proportion of negative results that were true negatives, according to formula *NPV=TN/TN+FN*.

To determine the relationship between the analyzed parameters (the clinical value of missense variants with predictive evaluation of programs), we calculated the correlation coefficient (in Microsoft Excel) [4, 7, 13, 23].

RESULTS

As a result of the *in silico* programs predictions, all missense variants of the *GJB2* gene (3 pathogenic, 4 benign) were assessed ambiguously, that is, they had more than one prediction (simultaneous different interpretation). Nevertheless, one variant c.269T>C (p.Leu90Pro), was evaluated by all programs as a damaging variant. The predictive estimate of missense variants of the *GJB2* gene issued by computer *in silico* programs in comparison with their established clinical significance are presented in table 2.

In table 3, informative parameters of the compared prediction programs *in silico* are presented. The accuracy of the predictions of the clinical significance of missense replacements among the analyzed programs ranged from 42.9% (FATHMM), to 85.5% (achieved by the two programs SIFT and PROVEAN). These programs also showed high sensitivity and specificity parameters - 66.7% and 100%, respectively. The programs MutationAssessor, MutationTaster, CONDEL and FATHMM had 100% sensitivity, but showed a low specificity of 50%, and CONDEL total absence. The SIFT and PROVEAN programs had the highest predictive value of the positive result (100%) and the predictivity of the negative result (80%) . The MutPred had 100% PPV, but showed a low NPV value of 57.2%.

To determine the accuracy of the predictions of computer *in silico* programs and in order to maximize the overall quality of their analytical parameters, we calculated the correlation coefficient (R). Figure 2, shows the general correlation coefficients, where the highest correlation *in silico* predictions with the clinical value of missense variants of the *GJB2* gene was detected in the SIFT and PROVEAN programs (R = 0,73), which corresponds to their analytical parameters obtained

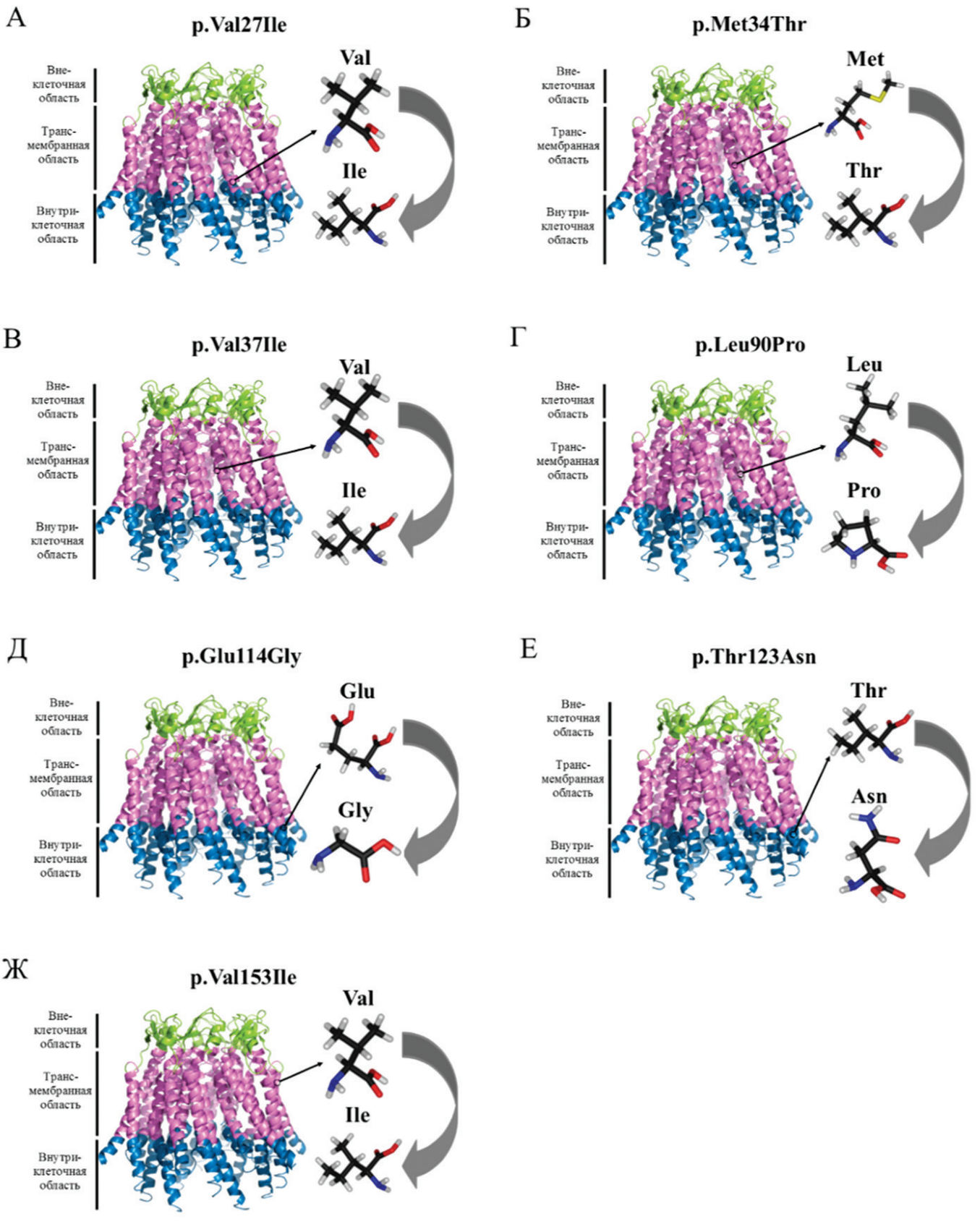

Figure 1. Localization of the revealed nonsynonymous (missense) amino acid substitutions in the protein-connexin sequence 26.
Note: 3D visualization of the structural model of the Cx26 protein was obtained using the PolyPhen-2 program (http://genetics.bwh.harvard.edu/pph2).

Computer *in silico* predictive programs

Name and web-site	The basis			Classification			References
	Algorithm	Method	Computing tools	Effect	Score	Prediction	
SIFT http://sift.jcvi.org	Evolutionary conservation	Compilation of a data set of functionally linked protein sequences using BLAST/PSI-BLAST	Matrix Dirichlet	The effect of the amino acid substitution on the structure/function of the protein	0.00 - 1	<0.05 = «Damaging»; >0.05 = «Tolerated»	[12, 16-19]
FATHMM http://fathmm.biocompute.org.uk/			Hidden Markov Model (HMM)		> -1.5 <	<-1.5 = «Damaging»; >-1.5 = «Tolerated»	[25, 26]
MutationAssessor http://mutationassessor.org/		It provides data from other databases, such as COSMIC, UniProt and the Pfam, as well as its own «functional point of influence» on the mutation	Cross-Entropy Method		-5.76 – 5.76	≤0.8 = «neutral»; 0.8≤1.9 = «low»; 1.9≤3.5 = «medium»; >3.5 = «high»	[27, 28]
PolyPhen2 http://genetics.bwh.harvard.edu/pph2/	Protein structure/function and evolutionary conservation	A statistical method of weighting and profiling sequences from subsets of identical sequences in several alignments using PSIC	Naive Bayesian classifier		Two models: HumDiv: 0.00 - 1 HumVar: 0.00 - 1	0.0 - 0.15 = «benign»; 0.15 - 1.0 = «possibly damaging»; 0.85 - 1.0 = «damaging»	[2]
Condel http://bg.upf.edu/fannsdb/		Combines SIFT, PolyPhen-2, MutationAssessor and FATHMM			0.00 - 1	0.0 = «Neutral»; 1.0 = «Deleterious»	[11]
MutationTaster http://www.mutationtaster.org/	Protein structure/function and evolutionary conservation	Integration of information from various biomedical databases (Ensembl, UniProt, ClinVar, ExAC, 1000 Genomes Project, phyloP, phastCons)	Naive Bayesian classifier	Cause of the disease	0,0 – 215 (Does not affect the forecast)	«disease causing»; «disease causing automatic»; «polymorphism»; «polymorphism automatic»	[14, 15]
MutPred http://mutpred.mutdb.org/	Protein structure/function and evolutionary conservation	Based on the established SIFT method	Support Vector Machines (SVM)	The effect of the amino acid substitution on the structure/function of the protein	g = 0.00 - 1 (g - Total score) (p - Rating 5 properties)	g>0.5, p<0.05 = «actionable hypotheses»; g>0.75, p<0.05 = «confident hypotheses»; g>0.75, p<0.01 = «very confident hypotheses»	[3]
Align GVGD http://agvgd.hci.utah.edu/agvgd_input.php	Protein structure/function and evolutionary conservation	Measurement of biochemical distances between amino acids (norm/substitution), according to MSA	Matrix of Grantham Grantham Variation (GV) Grantham Deviation (GD)	Pathogenetic effect on protein structure/ unction of the protein	GVGD = Class: C0, C15, C25, C35, C45, C55, C65	C65 - The most probable; C0 - Least probable	[8, 9]
PROVEAN http://provean.jcvi.org/index.php	Alignment and measurement of similarity between variant sequence and protein sequence homology	Compilation of a data set of functionally linked protein sequences using BLASTP, with further processing of large databases CD-HIT (ver.4.5.5)	Blocks Substitution Matrix (BLOSUM62)	Functional effect on protein	- 40 – 12.5 (threshold: -2.5)	≥- 2.5 = «deleterious»; ≤- 2.5 = «neutral»	[6, 24]

during the analysis of the informative parameters (Table 3). With an average correlation, MutationAssessor, MutationTaster and CONDEL (R = 0,54) were identified (Figure 2), which also corresponds to their analytical parameters (Table 3). A weak correlation was shown by MutPred (R = 0,47), PolyPhen2 (R = 0,41), very weak Align GVGD (R = 0,16), and the FATHMM program showed a zero value, which indicates a lack of correlation between the observed values (Figure 2).

DISCUSSION

Based on the known clinical significance of 7 missense variants of the *GJB2* gene, detected as a result of the molecular genetic study of congenital deafness in Yakutia, 9 most often used for bioinformatics analysis computer programs were tested. After the *in silico* predictions were obtained, a comparative analysis of the informative parameters (accuracy, sensitivity and specificity) of the programs was carried out, and the correlation coefficient (R) of the predicted programs with the clinical significance of the missense variants of the *GJB2* gene was calculated.

As a result, of the analysis of all the obtained parameters, of the analyzed *in silico* programs and the calculation of the correlation coefficient between the values (clinical significance with *in silico* predictions), the SIFT and PROVEAN programs were identified with the best indicators. In these programs, 85.8% of predictions are accurate which in frame of this study can be considered as the best indicator, since in the literature, where similar parameter comparisons are given, the highest accuracy of predictions reached 80% - 90% [4, 7, 14, 23, 25, 30]. The SIFT and PROVEAN programs also showed high sensitivity (66.7%) and specificity (100%) parameters, which in the context of this study is of high value, as the higher sensitivity parameters, the more effectively the pathogenic variants are predicted, and the higher the specificity, the more effectively the benign options. The middle accuracy was in the PolyPhen2 program (71.5%), and all other indicators - 66.7%. Bad results were shown by two programs FATHMM and MutPred, which produced a large number of incorrect predictions (Figure 2, Table 3).

In 2015, A. Yilmaz [31] published a study of the results of bioinformatics analysis of 211 missense mutations of the *GJB2* gene, announced in the

Table 2

Evaluation of missense variants of the *GJB2* (Cx26) gene by computer *in silico* predictive programs

Missense variant *GJB2* (Cx26)	Clinical significance	SIFT	MutationAssessor	FATHMM	Polyphen-2	MutationTaster	PROVEAN	Align GVGD	MutPred*	CONDEL
c.79G>A p.Val27Ile rs2274084	Benign	**Tolerated** score: 0.21	**Medium** FI score: 2.28 VC score: 2.16 VS score: 2.40	**Damaging** score: -5.59	**Probably damaging** HumDiv score: 1.000 HumVar score: 0.998	**Polymorphism** score: 29	**Neutral** score: -0.660	**Unclassified** Class C25 GV 0.00 GD 29.61	Probability of deleterious mutation: - general score:0.321	**Deleterious** Calculated Condel score: 0.612278613903
c.101T>C p.Met34Thr rs35887622	Pathogenic	**Damaging** score: 0.01	**Medium** FI score:2.315 VC score:2.43 VS score:2.20	**Damaging** score: -5.41	**Benign** HumDiv score: 0.038 HumVar score: 0.083	**Disease causing** score: 81	**Deleterious** score: -3.801	**Deleterious** Class C65 GV 0.00 GD 81.04	Probability of deleterious mutation: - general score:0.969	**Deleterious** Calculated Condel score: 0.58786807751
c.109G>A p.Val37Ile rs72474224	Pathogenic	**Tolerated** score: 0.34	**Medium** FI score:2.095 VC score:2.58 VS score:1.61	**Damaging** score: -5.46	**Probably damaging** HumDiv score: 1.000 HumVar score: 0.996	**Disease causing** score: 29	**Neutral** score: -0.857	**Unclassified** Class C25 GV 0.00 GD 29.61	Probability of deleterious mutation: - general score:0.902	**Deleterious** Calculated Condel score: 0.61487213316
c.269T>C p.Leu90Pro rs80338945	Pathogenic	**Damaging** score: 0	**Medium** FI score:3.33 VC score:4.26 VS score:2.40	**Damaging** score: -5.64	**Probably damaging** HumDiv score: 1.000 HumVar score: 0.996	**Disease causing** score: 98	**Deleterious** score: -6.482	**Deleterious** C65 GV 0.00 GD 97.78	Probability of deleterious mutation: **Confident Hypotheses** Gain of sheet (P = 0.039) general score:0.915	**Deleterious** Calculated Condel score: 0.676708483818
c.341A>G p.Glu114Gly rs2274083	Benign	**Tolerated** score: 0.16	**Medium** FI score:2.005 VC score:2.40 VS score:161	**Damaging** score: -4.58	**Benign** HumDiv score: 0.001 HumVar score: 0.001	**Polymorphism** score: 98	**Neutral** score: -0.123	**Deleterious** Class C65 GV 0.00 GD 97.85	Probability of deleterious mutation: - general score: 0.232	**Deleterious** Calculated Condel score: 0.556433693212
c.368C>A p.Thr123Asn rs111033188	Benign	**Tolerated** score: 0.59	**Neutral** FI score:-0.305 VC score:-0.61 VS score:- 0	**Damaging** score: -4.42	**Benign** HumDiv score: 0.000 HumVar score: 0.000	**Disease causing** score: 53	**Neutral** score: 0.797	**Deleterious** Class C55 GV 0.00 GD 64.77	Probability of deleterious mutation: - general score:0.201	**Neutral** Calculated Condel score: 0.513276654484
c.457G>A p.Val153Ile rs111033186	Benign	**Tolerated** score: 1	**Neutral** FI score:-0.305 VC score:-0.43 VS score:-0.18	**Damaging** score: -3.69	**Benign** HumDiv score: 0.003 HumVar score: 0.007	**Disease causing** score: 29	**Neutral** score: 0.138	**Unclassified** Class C25 GV 0.00 GD 29.61	Probability of deleterious mutation: - general score:0.488	**Neutral** Calculated Condel score: 0.491937780564

Note: Gray is highlighted with «truly» positive and «truly» negative results.

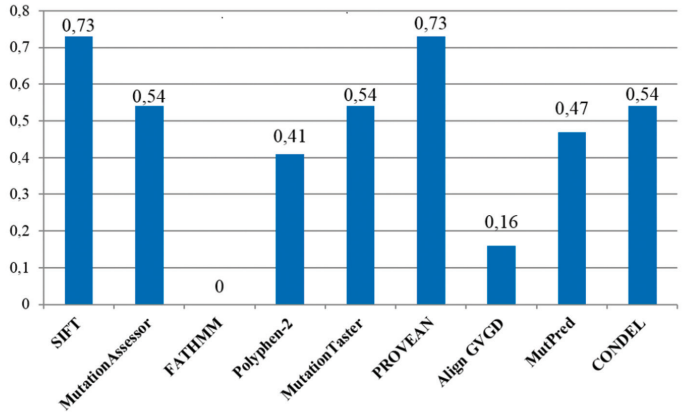

Figure 2. Histogram of the correlation coefficient (R).
Note: R - is the relationship between the known clinical value of missense variants of the GJB2 gene with in silico evaluation given by 9 computer predictive programs.

Table 3

Analytical informative parameters of computer in silico predictive programs

In silico predictive programs	Accuracy	Sensitivity	Specificity	PPV	NPV
SIFT	85.8%	66.7%	100%	100%	80%
MutationAssessor	71.5%	100%	50%	60%	100%
FATHMM	42.9%	100%	0%	42.9%	0%
Polyphen-2	71.5%	66.7%	66.7%	66.7%	66.7%
MutationTaster	71.5%	100%	50%	60%	50%
PROVEAN	85.8%	66.7%	100%	100%	80%
Align GVGD	57.2%	66.7%	50%	50%	66.7%
MutPred	71.5%	33.4%	100%	100%	57.2%
CONDEL	71.5%	100%	50%	60%	100%

Note: PPV - prognosticity of positive results (predictive of pathogenetic variants); NPV - prognosticity of negative results (predictive of benign variants).

Ensembl and HGMD databases, using four predictive computer *in silico* programs: SIFT, PANTHER, PolyPhen-2 and FATHMM [31]. Results of the study demonstrated the applicability of bioinformatics algorithms in predictions of the hearing impairment causing mutations effect. However, in this study, a comparative analysis of informative parameters (accuracy, sensitivity, specificity) of the programs was not carried out. The literature data, which presents the results of a comparative analysis of the parameters of computer *in silico* programs, show that of the existing *in silico* programs, not all are equally suitable for predicting the pathogenicity of missense mutations in specific genes responsible for a specific hereditary disease. For example, in testing 1118 variants of four *BRCA1*, BRCA2, *MLH1* and *MLH2* genes associated with cancer, the most accurate was Align GVGD [4]. As a result, of the bioinformatics analysis of the variants of the *RYR1* and *CACNA1S* genes, associated with malignant hyperthermia, four out of 8 *in silico* programs: MutPred, SNPs & GO, PhD-SNP and CADD were able to accurately classify the variants of the *RYR1* gene, but ambiguously evaluated variants of another *CACNA1S* gene [20]. In another study, which included 14 191 deleterious Mendelian disease causing mutations, and 22 001 neutral mutations, which were annotated as not known to be associated with any phenotypes, all based on Uniprot annotation found, that FATHMM and KGGSeq had the highest accuracy [7]. MAPP was the most accurate tool and MAPP + PolyPhen-2.1 provided the best-combined model of 74 missense substitutions of *MMR* genes (*MLH1*, *MSH2*, *MSH6* and *PMS2*) that confer colon cancer susceptibility in Lynch syndrome [5]. Using a dataset consisting of 122 credibly pathogenic and benign variants in genes associated with the RASopathy disorders and limb-girdle muscular dystrophy (LGMD), 17 *in silico* predictive programs were analyzed, and result was that MutPred was the most accurate, whith a weighted accuracy of 82.6% in the full dataset. [30].

Thus, out of 9 analyzed programs, two programs - SIFT and PROVEAN (R = 0,73) - gave the most accurate *in silico* predictive estimates of the clinical significance of missense variants of the *GJB2* gene. The obtained results can help in the of bioinformatics analysis, in the case of detection of previously not described missense variants of the *GJB2* gene associated with hearing impairment.

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ASSOCIATION OF FOUR SINGLE NUCLEOTIDE POLYMORPHISMS WITH ARTERIAL HYPERTENSION AND MYOCARDIAL INFARCTION IN THE RS (YA): ETHNIC AND GENDER FEATURES

ABSTRACT

The research of four single nucleotide polymorphisms (SNPs) association with arterial hypertension (AH) and myocardial infarction (MI) in population of the Republic of Sakha (Yakutia) depending on ethnicity and gender is carried out.

Keywords: single nucleotide polymorphisms, arterial hypertension, myocardial infarction, ethnicity gender and features, Republic of Sakha (Yakutia).

In Yakutia the cardiovascular diseases (CVD) in the structure of causes, both morbidity (19.1%) and mortality (47.4%), occupy a leading position [1]. Arterial hypertension (AH) is one of the main risk factors for the development of CVD and their complications, such as myocardial infarction (MI) and stroke. Currently in Russia, about 40% of the population (more than 42 million people) suffers from AH [2].

In recent years, a trend has been formed such as genetic cardiology, which integrates the concepts and technologies of molecular genetics for understanding the etiology and pathogenesis of CVD clinical polymorphism. The genetic approach allows creating a base for con-

ducting early diagnostics, selecting adequate treatment and prevention of CVD, which in the end will affect the quality of life of patients with cardiovascular pathology. One of the modern strategies for the search for genetic risk factors for the development of CVD is the full genome analysis of polymorphism of genes responsible for hereditary predisposition to them [5].

The aim of this study was to investigate the association of single nucleotide polymorphisms (SNRs) rs619203 gene ROS1 (6q22), rs4804611 gene ZNF627 (19p13.2), rs2549513 (16q23.1) and rs1376251 gene TAS2R50 (12p13.2) with arterial hypertension and myocardial infarction in population of the Republic of Sakha (Yakutia), taking into account ethnicity and gender.

MATERIALS AND METHODS

The research included 456 coronary heart disease (CHD) patients with the verified coronary atherosclerosis according to selective coronaroangiography (from them 396 men and 60 women) and 483 persons without clinical signs of CHD (from them 212 men and 271 women). Age of all surveyed participants made 45-64 years. Patients were recruited from National Centre of Medicine in Yakutsk (the main groups). Comparison groups were created by results of complex medical examination during departures to districts of the Republic of Sakha (Yakutia). Research period: 2007-2010. For the comparative analysis all examined persons were divided into 4 clinical groups: 1 – native patients with the verified coronary atherosclerosis (n=217), from them: men – 189, mean age 54,34 ± 0,44 yr and women – 28, mean age 53,39 ± 1,28 yr; 2 – non-native patients with the verified coronary atherosclerosis (n=239), from them: men – 207, mean age 54,76 ± 0,43 yr and women – 32, mean age 55,81 ± 1,01 yr; 3 – native persons without clinical signs of CHD (n=253), from them: men – 108, mean age 51,28 ± 0,57 yr and women – 145, mean age 51,19 ± 0,43 yr; 4 – non-native persons without clinical signs of CHD (n=230), from them: men – 104, mean age 51,09 ± 0,52 yr and women – 126, mean age 51,37 ± 0,47 yr. Yakuts are considered to be representatives of native nationality, non-native nationality – the Russians, Ukrainians and Belarussians living in Yakutia constantly.

Patients with coronary atherosclerosis were excluded if they had coronary arteries anomalies, intact coronary arteries, coronary artery bypass graft, existence of unstable angina, acute myocardial infarction in the anamnesis till 6 months. Criteria of exception for all groups: active inflammation, acquired and congenital heart diseases, cardiomyopathy, cancer and diagnosed of CHD for control groups, age are younger than 45 yr and more than 65 yr for all groups.

Genomic DNA was obtained from venous blood (10 ml) by phenol - chloroform extraction [4]. Genotyping was carried out by real-time PCR according to the protocol producer firms (probes TaqMan, Applied Biosystems, USA) on the ABI 7900HT (Applied Biosystems) according to the protocol producer firms. The following SNPs were included in this study: rs619203 gene ROS1 (6q22), rs4804611 gene ZNF627 (19p13.2), rs2549513 (16q23.1) and rs1376251 gene TAS2R50 (12p13.2). SNPs were selected by results of the Genome-Wide Association Studies which confirmed association of these SNPs with myocardial infarction. Genetic researches are conducted by staff of the Institute of Internal and Preventive Medicine. All researches are executed with the informed consent of examinees according to ethical standards of the Helsinki declaration (2000).

All data analyses were carried out with the statistical analysis software package SPSS 13.0 (SPSS Inc.). Any deviation of the genotype frequencies from the Hardy-Weinberg proportions was assessed by the χ2-test. The association between the SNPs and risk factors was estimated by criterion χ2-Pearson. Also used two-tailed the Fisher criterion for frequencies of genotypes and alleles. The association between SNPs and CHD risk was estimated by computing odds ratio (OR) and 95% confidence intervals (CI) from the multivariate logistic regression analyses. The probability level accepted for significance was $p < 0.05$.

RESULTS AND DISCUSSION

Genotypes frequencies in the natives and non-natives of Yakutia are presented in tables 1-2.

Rs619203 of ROS1 *gene (MIM 165020) (6q22)*. In non-native population the rs619203 was associated with AH (p=0.033) and MI (p=0.009). In women carrying the GG genotype was higher diagnosed the AH than carrying the genotype CC (p=0.002). In men carrying the CG genotype had higher frequency of MI than in men carrying the CC genotype (p=0.009). By results of the three-stage study (USA, 2005) the association of this SNP with IM [10] was revealed. However in the later works association of rs619203 with CHD and MI wasn't received [6, 7, and 11]. In the Russian study conducted in Novosibirsk the rs619203 was associated with anthropometric data and lipid levels [3].

Rs4804611 of *ZNF627 gene (*19r13.2*)*. The association of rs4804611 with AH in native women of the Republic of Sakha (Yakutia) was received. In native women carrying the AA genotype was higher frequency of AH in comparison with control group (77.6 vs 54.9% respectively, p=0.033). Association the rs4804611 with MI we not observed. Also no association of this SNP with MI in researches executed in the USA [6] and Germany [7] were observed. At the same time, in the earlier three-stage study (USA, 2005) the rs4804611 association with MI [10] was shown. This association was also confirmed in Japan [9]. In the Russian study association of rs4804611 with MI and

Table 1

Genotype frequencies of SNPs in patients with Arterial Hypertension (AH) and control group depending on ethnicity

SNPs	Genotype	Native				p	Non-native				p
		AH(+)		AH(-)			AH(+)		AH(-)		
		n	%	n	%		n	%	n	%	
rs619203 *ROS1*	CC	7	3,1	5	3,1		45	16	52	25,6	
	CG	56	24,5	44	27		120	42,7	75	36,9	0,033
	GG	166	72,5	114	69,9		116	41,3	76	37,4	
rs4804611 *ZNF627*	AA	170	72,6	116	70,3		158	56	119	58,3	
	AG	57	24,4	41	24,8		99	35,1	62	30,4	
	GG	7	3	8	4,8		25	8,9	23	11,3	
rs2549513 xp. 16	AA	205	88	154	93,9		210	75,3	161	78,2	
	AC	28	12	10	6,1		65	23,3	42	20,4	
	CC	0	0	0	0		4	1,4	3	1,5	
rs1376251 *TAS2R50*	CC	42	18,1	18	10,9		119	46,9	65	45,1	
	CT	94	40,5	88	53,3	0,023	101	39,8	56	38,9	
	TT	96	41,4	59	35,8		34	13,4	23	16	

Table 2

Genotype frequencies of SNPs in patients with Myocardial Infarction (MI) and control group depending on ethnicity

SNPs	Genotype	Native				p	Non-native				p
		MI(+)		MI(-)			MI(+)		MI(-)		
		n	%	n	%		n	%	n	%	
rs619203 *ROS1*	CC	5	4,3	7	2,5		9	7,4	87	24	
	CG	31	26,7	69	25		63	52,1	132	36,5	0,000
	GG	80	69	200	72,5		49	40,5	143	39,5	
rs4804611 *ZNF627*	AA	86	72,3	200	71,4		74	61,7	203	55,6	
	AG	31	26,1	67	24		38	31,7	122	33,4	
	GG	2	1,7	13	4,6		8	6,7	40	11	
rs2549513 xp. 16	AA	102	86,4	257	92,1		85	73,3	285	77,4	
	AC	16	13,6	22	7,9		25	21,6	82	22,3	0,001
	CC	0	0	0	0		6	5,2	1	0,3	
rs1376251 *TAS2R50*	CC	18	15,4	42	15		55	45,5	129	46,6	
	CT	39	33,3	143	51,1	0,002	46	38	111	40	
	TT	60	51,3	95	33,9		20	16,5	37	13,4	

levels of endogenic indicators was not revealed [3].

Rs2549513 *(*16q23.1*).* The association of rs2549513 with AH (p=0.028) in the natives and MI (p=0.001) in the non-natives we received. In native women carrying the AA genotype was high frequency of AH than women carrying the AC genotype (89.5 vs 10.5% respectively, p=0.028). Among non-native population in both gender groups and men carriers the AA genotype association with MI we received (all: 73.3 vs 21.6 vs 5.2%, p=0.001; men: 75.7 vs 20.4 vs 3.9%, p=0.041 respectively). In the Russian study (Novosibirsk) association of rs2549513 with MI was not revealed [3]. According to the Framingham study the rs2549513 was associated with CHD (MI and fatal CHD) [8].

Rs1376251 of *TAS2R50 gene (MIM 609627) (*12p13.2*).* Rs1376251 was associated with AH (p=0.023) and MI (p=0.002) among aboriginals of Yakutia. In both gender group and men carriers the CC genotype was higher frequency of AH in comparison with control group (all: 18.1 vs 10.9%, p=0.023; men: 18.8 vs 9.6%, p=0.004 respectively). At the same time MI was associated with TT genotype in comparison with control group (all: 51.3 vs 33.9%, p=0.002; men: 51.4 vs 32.4%, p=0.005 respectively). In the studies conducted in USA [6] and Germany [7] were no association between rs1376251 and MI. Though in the earlier three-stage study in USA association of this SNP with IM was shown [10]. In the Russian study in men with MI this SNP was associated with HDL level. In women with MI the rs1376251 was associated with index of waist circumference / hips circumference and TG level [3].

CONCLUSION

For the first time among population of the Republic of Sakha (Yakutia) the study of polymorphisms association rs619203 gene *ROS1* (6q22), rs4804611 gene *ZNF627* (19p13.2), rs2549513 (16q23.1) and rs1376251 gene *TAS2R50* (12p13.2) with arterial hypertension and myocardial infarction we conducted. According to our study was received the association of studied pathologies with different genetic markers in men and women of native and non-native population from Yakutia. The following associations with SNPs we received: with arterial hypertension – rs619203 of *ROS1* gene, rs4804611 of *ZNF627* gene, rs2549513 (16q23.1) and rs1376251 of *TAS2R50* gene; with myocardial infarction – rs619203 of *ROS1* gene, rs2549513 (16q23.1) and rs1376251 of *TAS2R50* gene. These genetic markers can be used for assessment of cardiovascular diseases development risk on the Russian (Yakutian) population.

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FREQUENCY OF M.1555A>G MUTATION IN *MT-RNR1* GENE OF MITOCHONDRIAL DNA AMONG DEAF INDIVIDUALS IN YAKUTIA

ABSTRACT

It has been established that the mutation m.1555A>G in the *MT-RNR1* gene in the homoplasmic state is associated with non-syndromic sensorineural hearing loss caused by the use of aminoglycoside antibiotics in many families of different ethnic origin. Earlier, the m.1555A>G mutation was detected on a small sample of patients (n = 65) in Yakutia with a frequency of 1.54%. In this study, we performed a search of the m.1555A>G mutation among additional sample of 108 hearing impaired individuals from Yakutia (Eastern Siberia, Russia). As a result, we found no mutation in this sample. When combining both samples (n=65 and n=108), the m.1555A>G mutation frequency in Yakutia is – 0.57% (1/173), and among the Yakut patients frequency of this mutation is 0.92% (1/108). The frequency of the m.1555A>G mutation among deaf patients in Yakutia is 0.57%, and is relatively low when compared with the global data.

Keywords: hearing loss, mitochondrial genome, m.1555A>G, *MT-RNR1*, Yakutia.

INTRODUCTION

It has been established that the mutation m.1555A>G in the *MT-RNR1* gene in the homoplasmic state is associated with non-syndromic sensorineural hearing loss caused by the use of aminoglycoside antibiotics in many families of different ethnic origin [3-5, 7, 8, 16]. The action of aminoglycosides is based on binding with the bacterial 16S rRNA of the small subunit of the ribosome, which results in the protein synthesis blocking. When adenine is replaced with guanine in *MT-RNR1* gene at 1555 bp position C-G pairing takes place in the human 12S rRNA site, which leads to a similarity to the A site of bacterial 16S rRNA, which is the target for aminoglycoside drugs [6] (fig. 1). Currently, most of the aminoglycoside drugs are used only for the treatment of severe infections, such as endocarditis, sepsis and tuberculosis [4]. However, in some developing countries, they are still being used as broad-spectrum drugs [9].

Earlier in Yakutia (Eastern Siberia, Russia) the m.1555A>G mutation was detected with a frequency of 1.54% in a small sample of patients (n = 65), 2.08% (n = 48) among Yakut patients, and the frequency of this mutation was 0.83% (n = 120) in the control sample of Yakuts without hearing impairment, [1]. The obtained values of the m.1555A>G mutation frequency indicated the urgency of conducting preventive diagnostics for the presence of this mutation before application of aminoglycoside antibiotics among the indigenous population of Yakutia. It is necessary to screen this mutation on larger sample of patients with hearing impairment to clarify the frequency of m.1555A>G in Yakutia.

Aim of study: To update data on frequency of the m.1555A>G mutation of the mitochondrial *MT-RNR1* gene in a sample of deaf patients in Yakutia in comparison with the world data.

MATERIALS AND METHODS

The sample of 108 hearing impaired individuals (66 female and 42 male) aged between 25 and 63 (mean age 44.7 ± 7.1 years) was selected. Ethnic composition of the sample: Yakuts - 60 patients; Russians - 20; individuals of other and mixed ethnicities - 28. Hearing impairment in the participants in the study was confirmed by an audiological study involving threshold tone audiometry using an audiometer "MAICO ST 20" (Germany) for air conduction at frequencies 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 kHz and bone conduction at frequencies 0.25, 0.5, 1.0, 4.0 kHz, step 5.0 DB. The degree of hearing loss was estimated at hearing thresholds of bet-

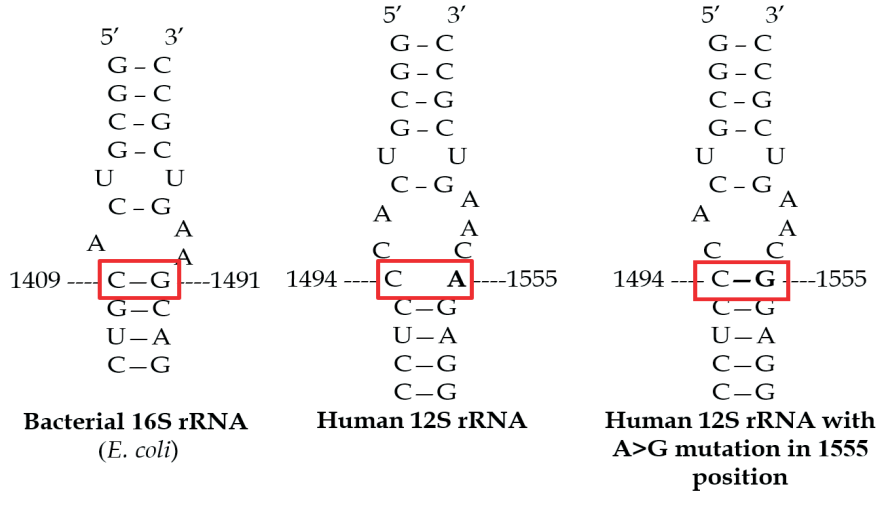

Figure 1. Molecular-genetic principle of aminoglycoside drugs action in case of m.1555A>G mutation. When adenine is replaced with guanine at the1555 bp position in the MT-RNR1 gene C-G pairing takes place in the human 12S rRNA site, which leads to a similarity to the A site of bacterial 16S rRNA, which is the target for aminoglycoside drugs [6].

ter hearing ear in the speech frequency range 0.5, 1.0, 2.0, 4.0 kHz according to the international classification.

The DNA was extracted from blood samples by a standard phenol-chloroform method with with proteinase K. The *MT-RNR1* gene of the mitochondrial genome was amplified by PCR using forward (GCTCAGCCTATATACCGCCATCTTCAGCAA; position 1247-1276) and reverse of oligonucleotide primers (TTTCCAGTACACTTACCATGTTACGACTGG; 1556-1585) flanking the 1555 bp position. The reverse primer is a mismatch primer replacing A with C at the 1557 bp position, thus in case of the m.1555A>G mutation, an artificial *HaeIII* restriction site (5 '... GG ↓ CC ... 3') is formed (fig. 2.C, the nucleotide replaced by mismatch primer is signed with black square). The results of the PCR-RFLP analysis were visualized by electrophoretic separation of the restriction products in a 3% agarose gel stained with ethidium bromide, followed by visualization in a UV-light.

All procedures in this work were conducted with the written informed consent of the participants. The local biomedical ethics committee at the FGBNU «YSC CMP» (Yakutsk, protocol No. 41, November 12, 2015) has approved this research.

RESULTS AND DISCUSSION

Among 108 hearing impaired s, the m.1555A>G mutation of the *MT-RNR1* gene was not detected (0/108). When combining results of previous [1] and the present study in Yakutia, the m.1555A>G mutation frequency is – 0.57% (1/173), and among the Yakut patients frequency of this mutation is 0.92% (1/108).

To compare the obtained data with the literature, we analyzed the global prevalence of the m.1555A>G mutation. For this purpose, data published during the period from 1999 to 2016 were used (total of 42 sources) [the list of sources is available on request]. The analyzed studies included a variety of sample scales (from 33 to 2417). Analysis showed that frequency of the m.1555A>G mutation among patients with hearing impairment in the world varies in wide range (in Australia – 0.27%, America – 0.72%, Africa – 0.97%, Europe – 1.62%, Asia – 4.42% [data available on request]. The world maximum of the occurrence among patients was recorded in Spain - 20% [10], also high incidence of m.1555A>G was registered in Morocco - 3.6% [14], China - 5.1% [2], Indonesia (5.3%) [15] and Japan - 5.4% [12]. Among Russian patients, the m.1555A>G mutation was previously registered only in the sample from St. Petersburg with a frequency of 0.8%, and was not found in the Altai Republic and in the populations of the Volga-Ural region [1]. Thus, the refined frequency of the mutation m.1555A>G in Yakutia (0.57%) corresponded to Saint Petersburg and is relatively low.

We performed additional genealogy and molecular-genetic analysis of previously described family [1], members of which were positive for m.1555A>G mutation. This family presented a variable penetrance of hearing loss. Grandmother of the proband (II:5) is positive for m.155A>G mutation but has normal hearing, possibly due to absence of

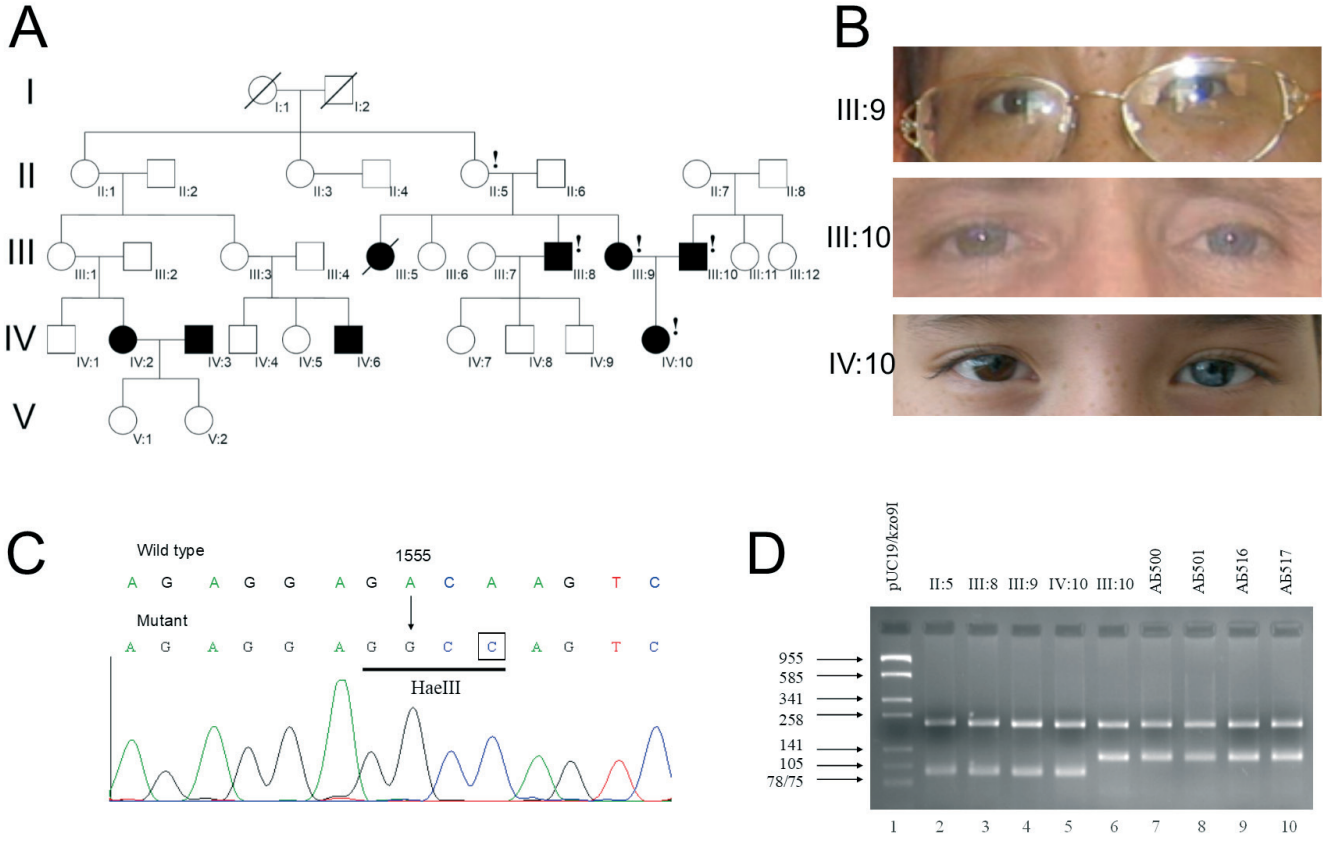

Figure 2. A. Fragment of the family tree of the proband (IV: 10), patients with hearing impairment are identified by black color, the «!» sign indicates the members who were tested for m.1555A>G mutation. B. The picture shows the iris heterochromia in the proband (IV:10) and in the father of the proband (III:10), the mother (III:9) does not have iris heterochromia. C. MT-RNR1 gene nucleotide sequence in proband (IV:10): Wild type - normal sequence, Mutant – sequence with m.1555A>G mutation. The arrow shows the nucleotide position of the mutation, the nucleotide in square is replaced in the structure of the reverse mismatch primer; the HaeIII restriction site is underlined. D. The electrophoregram of the m.1555A>G mutation PCR-RFLP analysis in a 3% agarose gel. In normal state 218 bp and 121 bp bands are observed, in case of mutation bands in 218, 91 and 30 bp are formed. (fragment 30 bp are not visualized); Left to right: lane 1 – molecular weight marker pUC19 / kzo9I; lanes 2 to 5 – samples containing m.1555A>G in the homoplasmic state; lanes 6 to 10 – samples with a normal sequence;

aminoglycosides usage history (fig. 2.A). Moreover, two cousins of the mother of the proband (III:1 and III:3) also have normal hearing and but both had deaf children (fig. 2.A). Perhaps, different penetrance is due to use of the aminoglycoside antibiotics. The m.1555A>G mutation in the homoplasmic state was previously confirmed in the proband (IV:10) and her mother (III:9) [1]. But later it was noticed that in addition to hearing loss, the proband also has heterochromia of the iris, which is also observed in the father of the proband (III:10), but the mother of the proband (III:9) has a normal color of the iris (fig. 2.B). Earlier it was reported about the possible association of m.1555A>G with the Waardenburg syndrome [11, 13], signs of which are deafness, telecant, fused eyebrows, and partial albinism [17]. The study reported a patient with a lighter skin and hair pigmentation than other relatives [13]. In this study we additionally tested three other members of the family: the probands father (III:10), the uncle of the proband (III:8) and the grandmother of the proband (II:5) (fig. 2.D). Among them, m.1555A>G was not detected only in the father of the proband (III:10), whose hearing loss reasons remain unknown and may be due to the Waardenburg syndrome. Thus, in the studied family, the iris heterochromia in the proband (IV:10) is not associated with the m.1555A>G mutation, since the proband inherited the heterochromia from the father (III:10), who did not have this mutation.

CONCLUSIONS

The frequency of aminoglycoside-induced hearing loss caused by the m.1555A>G mutation of the *MT-RNR1* gene among deaf patients in Yakutia is 0.57%, and is relatively low when compared with the global data.

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CONNECTION OF THE EEG REACTIONS IN THE CONDITION OF RECOGNITION OF EMOTIONAL LEXICA IN THE YAKUTS WITH ALLELIC POLYMORPHISM OF THE SEROTONIN TRANSPORTER

ABSTRACT

Allelic polymorphism of serotonin transporter 5-HTTLPR is connected with higher risk of vulnerability to the mental pathologies which are associated with disorders in regulation of emotional behavior. However, the influence of this polymorphism on predisposition to disorders significantly differs between different ethnic groups. The aim of our study is exploration of the 5-HTTLPR effects on the change of theta-band spectral power in EEG in the condition of recognition of written emotional sentences among Yakuts in comparison with Russians. 78 healthy Yakutian students and 82 healthy Russian students participated in the study. In EEG experiment, the participants were to find grammatical errors in the sentences written in Russian. These sentences contained implicit conditions: some of the sentences described unanimated objects, anxiety of participants, anxiety of other people, participants' aggression, or aggression of other people. The probes of blood or buccal epithelium were taken from each participant for genetic analysis. It was revealed that the frequency of occurrence of S-allele of the 5-HTT gene was significantly higher among Yakuts (73%) than that among Russians (39%). According to EEG, the emotional sentences induced higher amplitude of theta-band synchronization in comparison with the neutral sentences among all the participants. The participants with the genotypes LS and SS showed essentially smaller differences in amplitude of EEG reaction after onset of sentences from different categories in comparison with the LL genotype carriers. Our study supports the hypothesis that S- allele of 5-HTTLPR is associated with lower sensitivity to emotion-related stimuli. It could be connected with the risk of affective pathologies among people with such an allele.

Keywords: serotonin transporter (5-HTT), recognition of emotions, language comprehension, EEG, inter-ethnical comparison.

INTRODUCTION

Affective pathology is the group of mental disorders such as the depressive disorder, anxiety disorder, autism, alcohol or drug addiction, which accompanied by the dysfunctions of regulation of human emotional behavior. High risk of affective pathology in the modern society makes the elaboration of methods important for early diagnostic and prevention of such diseases in their initial stages. One of the indicators reflecting the increased risk of vulnerability to affective pathology among non-clinical subjects is the inability to distinguish emotional states of other people and difficulty in relevant expression of their own emotional states [1].

The electroencephalogram (EEG) recorded in the conditions of recognition of external stimuli reflects an individual personal ability to recognition of emotions [2; 9]. EEG can also serve as the technique of assessment of the vulnerability to affective pathology [3]. Stimuli-induced synchronization in theta (4-8 Hz) frequency band is one of the most important EEG indexes associated with emotional regulation. Theta-synchronization (or task-induced increase in spectral power) reflects the involvement of limbic structures to the process of stimulus recognition, assessment, and decision-making [2; 9]. The personal differences in the amplitudes and cortical topology of theta rhythm serve as one of the main markers of affective pathology.

The different kinds of emotion-related photographs (including facial photographs) are usually used as the external stimuli for induction of different emotional states [2; 9; 18]. Another approach consist of demonstration the samples of emotionally expressive speech to the participants [17]. The application of oral or written speech allows to simultaneously manipulate many modalities of stimulus and changing the cognitive and emotive effects to the participants. In particular, speech stimulation allows to direct the emotional loading of stimulus, or to the participants themselves, or to other people. It gives us the opportunity to use personal orientation of stimulus as a special experimental modality. Therefore, the method of speech stimulation can significantly improve the traditional approach of the facial stimulation for search for the neurophysiologic correlates of emotions perception.

One of the directions of modern science is the search for genetic markers connected with specific features of healthy people or with the risks of vulnerability to various neurologic or mental pathologies. Most often, the polymorphic variants of alleles of one of the brain neurotransmitter systems are used as such markers [6]. The serotonin (5-HT) system is strongly associated with the regulation of all aspects of emotional behavior. This is the reason of high concentration of researchers who are studying the role of allelic polymorphisms of this system in the formation of personal features of emotion perception [13]. The serotonin transporter (5-HTT) is the protein which mediates the re-uptake of serotonin from synaptic cleft [11]. Several genetically defined modifications of this protein were identified among humans and animals. One of allelic polymorphisms of the serotonin transporter is the 5-HTTLPR on genetic site SLC6A4, localized in the 17th chromosome. Among humans, this gene exists in two variants: "long" (L- allele) and short (S– allele). Respectively, each person can have one of three genotypes – LL, LS or SS. In addition, the L- allele can be either active (La) or passive (Lg). The association of 5-HTTLPR polymorphism with an anxiety disorder, depression, and bipolar disorder, and also with sensitivity to antidepressants and suicide was shown in many different studies [7; 8; 5]. However, the connection of serotonin transporter with the individual features of emotional behavior among healthy people still remains unclear. In literature, there are opposite points of view about the role of 5-HTT polymorphisms in regulations of behavior [15; 16].

According to one of hypothesizes for explanation of these differences, the behavioral effects of genotype can be modified by socio-cultural factors in essential degree, in particular, by the ethno-social specificity of people [12]. Earlier, we have carried out the comparison of the effect of 5-HTTLPR among the groups of Tuvinian and Russian participants. It has been shown that the S- allele is associated

with high anxiety level among Russians, but not among Tuvinians [14]. In this study, we check the effect of genetic polymorphism of the serotonin transporter in EEG reactions in theta frequency band among healthy young Yakuts and Russians in the conditions of recognition of the emotional and personal-oriented sentences written in Russian.

The aim of study consists of the comparison of the effects of 5-HTTPLR allelic polymorphisms on the individual abilities to recognition of emotional and personal-oriented lexis among the groups of healthy Yakuts and Russians.

THE PARTICIPANTS AND METHODS

78 young healthy Yakutian students of the Medical Faculty of the North-Eastern Federal University (age 20,1±2,3 years, 30 men and 48 women) and 82 students of the Novosibirsk State University (age 22,1±3,4 years, 31 men and 51 women) participated in the study. All participants gave the informed written consent priors to the experiment and filled out the questionnaire concerning their mental or neurological diseases or application of narcotic drugs and any other psychoactive substances. People with such diseases or using psychotropic drugs were excluded from the study. Besides, the level of general and emotional intelligence were estimated for all the participants. The groups were matched as closely as possible according to their IQ scores. The experimental protocol was approved by the ethical committee of the Institute of Physiology and Basic Medicine in accordance with the Helsinki declaration of biomedical ethics.

The samples of blood or buccal epithelium for analysis of the 5-HTTLPR genetic polymorphism have been taken from all the participants. The genetic analysis has been executed in the Institute of Cytology and Genetics of SB RAS according to the method described in the paper of Lesch et al. [10].

During the EEG experiment, 200 sentences, written in Russian, were randomly presented to each of the participants. Half of the sentences contained syntax errors, another half was grammatically correct. All the sentences were partitioned into 5 categories: "neutral sentences about unanimated objects", "participants' own anxiety" (sentences are taken from the Spielberger 's STAI), "others' anxiety" (in sentences from the category "own anxiety", 1st person pronouns are replaced with 3rd person pronouns), "participants' own aggression" (sentences are taken from the Buss-Perry aggression questionnaire), "others' aggression" (in sentences from the category "own aggression", 1st person pronouns are replaced with 3rd person pronouns). Participants were not informed about separation of sentences into several emotional-related categories before the experiments. Sentences appeared on the computer screen randomly during time period from 3 to 5 seconds. The task for the participant was to define existence of syntax errors in the presented sentences.

EEG with the events markers was recorded from all participants during the execution of linguistic task. EEG was recorded with the help of the Brain Products amplifier, Germany, with bandpass range from 0,1 to 100 Hz, with a frequency of sampling of 1000 Hz. For the Russian group, the EEG record was carried out through 128 channels located according to the International 10-5% schema with the referent on Cz and grounding electrode on AFz. For the Yakutian group, the EEG record was made through 64 channels located according to the 10-10% schema.

EEG-signals were processed by means of the EEGlab_toolbox [https://sccn.ucsd.edu/eeglab/index.php]. Under the data pre-processing, EEG was filtered in the range from 1 to 40 Hz. Eye and motor artifacts were rejected by means of the analysis of independent components (ICA, [4]). Event-related spectral perturbations (ERSPs, [4]) were applied as a measure of the brain activity connected to the task's execution and for the revealing of genetic effects.

ERSPs have been calculated for each participant on each EEG channel. Based on our previous results [9; 17;], the frequency range in theta band (4-8 Hz) during the time interval from 0 to 300 ms after the sentence's onset was used for identification of genetic effects on brain activity, because this index reflects the degree of the emotional pressure which was experienced by the participants during stimulus recognition. The repeated-measures ANOVA with the factors "sentence's category" (five emotionally differed categories), "group of participants" (Yakuts or Russians), "saggitaly" (the EEG channels oriented between frontal and posterior regions), "laterality" (the EEG channels oriented between left and right regions), and "genotype" (three genotypes of LL, LS or SS) with the correction on multiple comparisons was applied for assessment of the statistical significance of the effects.

RESULTS

The significant differences in the frequencies of population distribution of the polymorphic alleles of the serotonin transporter gene was revealed among the groups of Russians and Yakuts. The frequency of La-allele was 51% among Russians and 21% among Yakuts, whereas the frequency of S-allele was 39% among Russians and 73% among Yakuts. The frequency of Lg-allele was 6% for both groups. The significance of intergroup differences was estimated by the χ^2 criteria.

The amplitude of the theta-response after appearance of non-emotional sentences about unanimated objects was significantly lower in comparison with the responses to other categories of sentences among all the participants, regardless of their nationality and genotype. This effect was highly significant: $F = 8,48$; $p < 0,0001$, and this indicates the connection between theta responses and emotional content of speech. For the theta-synchronization, the main effect of "group" factor was significant: $F = 5,01$; $p = 0,027$. The Yakutian ethnic group showed higher amplitude of theta-synchronization (1,7±0,2) in comparison with the Russian group (1,2±0,1). This could be interpreted as bigger tendency of Yakuts to emotional response on onset of the anxious and aggressive sentences. Also, the significant interaction of factors "group" to "sentence category", $F = 2,19$; $p = 0,013$, was revealed. Among the Yakutian group, the amplitude of responses to sentences about participants' own aggression and others' aggression did not differ (respectively, 1,1±0,1 and 1,0±0,1), whereas among the Russian group, the response to sentences about others' aggression (0,3±0,1) was significantly lower than the response to sentences about participants' own aggression (0,5±0,1).

The main effect of genotype was non-significant for both groups of participants ($p = 0,6$). However, among both of groups the significant interaction of factors "sentence's category" to "genotype", $F = 2,32$; $p = 0,022$, was revealed (see Figure 1). The carriers of the LL genotype showed a differentiated pattern of the EEG response to the different categories of sentences. Among such people, the EEG responses to all five categories of sentences were different in theta-synchronization amplitude. On the contrary, among the SS genotype carriers, the EEG responses to different types of sentences were almost the same in the amplitude of theta response, and the LS heterozygotes showed differences in responses to neutral (objects) and emotional (all remaining categories) sentences only, but did not show any differences in the responses to the various categories of emotional sentences. The interaction of factors "genotype" and "ethnic group" was not revealed.

DISCUSSION AND CONCLUSION

In general, our results confirm the hypothesis that S- allele of the 5-HTTLPR is connected with weak ability to recognize other people's emotions [8; 16]. In this case, it worsens the understanding of emotional load of written speech. Yakuts and Russians have shown the identical influences of genotypes on the EEG reaction, but S-allele is found among Yakuts significantly more often than the same among Russians. Besides, it was revealed that there are the number of features of Yakuts' brain activity which distinguishes them from Russians but cannot be explained distinctly by the 5-HTTLPR influence. It is also possible to assume that the lower sensitivity to the speech emotional load in different social conditions can have a different influence on behavior, which explains the different

connection of a genotype with the risk of pathological development among the Caucasian and Mongoloid populations.

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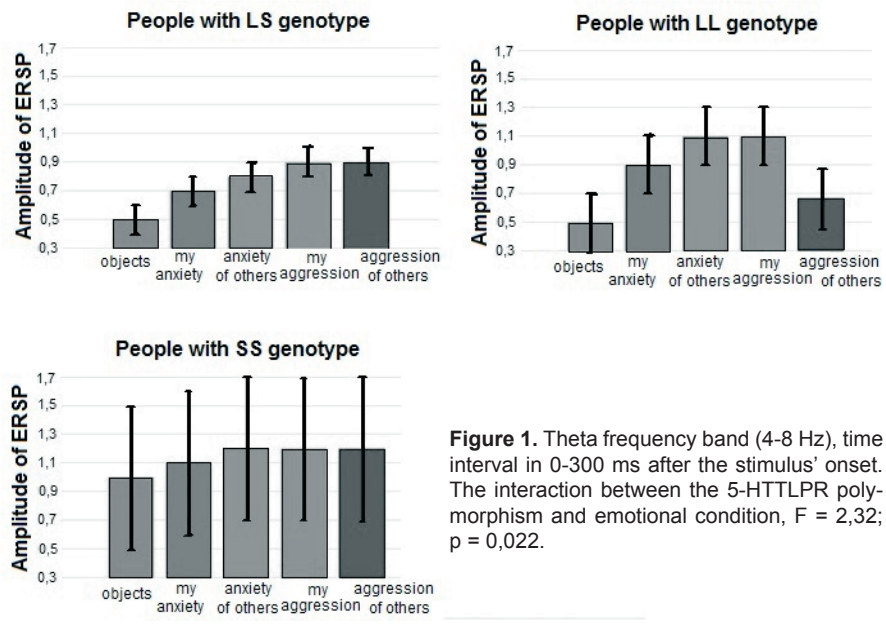

Figure 1. Theta frequency band (4-8 Hz), time interval in 0-300 ms after the stimulus' onset. The interaction between the 5-HTTLPR polymorphism and emotional condition, F = 2,32; p = 0,022.

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GENETICS OF VILYUI ENCEPHALOMYELITIS

ABSTRACT

Viliuisk encephalomyelitis (VE) is a neurodegenerative disease with unknown etiology in development of which genetic factors have significant role. Study of HLA markers and other genes of immunity and analysis of exom sequencing in VE patients revealed features of the Yakut genome, predisposing to immunity dysfunction and functional insufficiency of proteolitic and phosphatase activity. In extreme environmental conditions these features may lead to dystrophic process development in the brain and distinguish type of encephalopathy as basis of VE pathogenesis.

Keywords: Viliuisk encephalomyelitis, genes of immunity, human exom, DNA sequencing.

INTRODUCTION

Viliuisk encephalomyelitis (VE) is the regional pathology of Republic Sakha (Yakutia). Study of etiology and pathogenesis of this disease since difficult nature remains subject of fundamental medicine.

At present time VE consider as primary chronic form of disease, in which a patient experience physical weakness of nervous system due to the gradual degradation of neural circuits and the death of neurons. Disease may have form of encephalopathy with stable, steady flow and minimal degrees of motor and cognitive impairments. However, in some of these patients undergone extreme physical stress (undercooling, trauma, childbirth), external factors can provoke acute inflammation of the brain (encephalitis) with a local immune response and development of a clinical picture of an acute or subacute form of the VE with possible fatal outcome [4]. Patients who survive acute inflammation develop a chronic process typical for all patients with VE. The nature of the primary neurodegenerative process in VE remains unresolved. Currently, the working hypothesis is a violation of the control function of intracellular autophagy of the neuron, inducing atrophy of tissue and spongios of the brain. The reason of which may be a violation of the molecular mechanisms that control this function. The study of the genetic component in the etiology and pathogenesis of the disease is an important aspect of the disclosure of the molecular nature of VE pathology. In recent years, modern methods of molecular genetics are being used to solve this problem.

1. Population-genetic studies of VE

The disease is known to be endemic (the focus of the disease is limited by the Vilyui river region) and afflicts only Sakha people. The disease is sporadic, group cases of the disease are not observed, but generic connections are traced. The involvement of heredity in the development of the disease was confirmed by population-genetic studies on the material of a mass neurological survey of the population of the districts of the Vilyui basin for 1969-1977 in the works of Goldfarb LG, et.al. [6]. Segregation analysis (the ratio of patients among siblings) showed the absence of a monogenic type of autosomal recessive inheritance, but does not exclude the inheritance of more than one gene, the mutual action of which predisposes to the disease. To analyze the contribution of the genetic component and environmental factors to the pathology of the VE, the Falconer-Edwards mathematical model was used to calculate the coefficient of heritability (H). The coefficient of heritability of the phenotype of the disease for relatives of the 1st degree of kinship was 22.14% in the Vilyui district and 28.94% in the other districts of the Vilyui basin [6]. This means that the disease of VE among the Yakut population is due to the genetic component by 22-29% and due to the action of external environmental factors by 71-78%. Environmental factors make a significant contribution to the development of encephalitis in patients.

2. Investigation of immunity genes

Genetic studies of VE have been initiated with immunity genes. In the work of V. V. Fefelova (1996) analysis of HLA class 1 markers using serological reactions showed a slight statistically significant increase of the HLA-b15 variant in patients with VE [7].

Genotyping of alleles of HLA 2 class (-dp, -dq, -dr) on variants of DQA1 gene was carried out in patients with VE, which revealed an ethnic difference in frequency of allele 0301 ($p < 0,05$) between patients of Yakut and European origin. At the same time, the frequency of DQA1'0301 in patients with VE was higher than in healthy Yakut patients [9]. This gives grounds for talking about the participation of immunity genes in the development of the pathogenesis of VE disease.

At a next stage of research to search for new immunity candidate genes predisposing to the VE, 7 inflammatory genes were analyzed. These are the genes of the chemokine receptors *CCR2 / CCR5,* interferon gamma, interleukins 4, 6, 10 and the stromal factor (CDF) and cytokine Rantes [8]. 17 single nucleotide substitutions (SNPs) were analyzed. As

the results of the typing of SNPs showed, none of the 17 SNPs of the 7 inflammatory genes was associated with the required disease with the required reliability [8]. Differences were revealed in the analysis of additional SNPs of the interferon gamma gene. It was shown that, the four of the eight SNPs of the interferon gamma gene were polymorphic for representatives of the Yakut population, i.e. they are specific for the Yakut ethnos. In the case-control study, a significant association of two of these alleles was shown (SNPs: rs2069718, p = 0.003, relative risk is 5.44 (OR> 1) rs2069727, p = 0.003, relative risk 7.78 (OR> 1). The strong positive association found in the group of patients aged 60-69 years [1]. IFNg mutations - (SNP; rs2069718, rs2069727, rs2430561) are located in non-coding region of a gene intron, basically 5'UTR region of the gene, which performs an important function of gene expression control. These results also note the peculiarity of the genetics of the ethnos immunity.

Clinical polymorphism of VE disease appears to be associated with a difference in the level of IFNg production. As shown in the IFNg SNP (+874) T / A gene variants (rs2430561), the low production of the cytokine IFNg binds to the variant of the gene A allele. The genotypes of TA and AA genes can be considered as factors that reduce the severity of the disease course, promoting the development of the chronic form and increase of life expectancy of the patients. The TT genotype of the patients with encephalopathy can be considered as a predisposition factor to inflammatory processes and therefore the patients carriers of this genotype are more prone to acute disease [2].

These data indicate that the pathology of VE is associated with a special genotype of the immunity of the Yakut genome and gives grounds to talk about the immunogenetic nature of the disease of the VE.

Exome sequencing of VE patients' DNA

For detail investigation of patients' genome whole exome sequencing was performed by two groups of researchers [3, 5].

In first study exome sequencing data of 12 representatives of the Yakut ethnic group was analysed. Analyzed persons lived in rural areas of Vilyui district of Yakutia, were not close relatives. Seven persons (3 men and 4 women) have VE diagnosis and five (1 woman and 4 men) were healthy. The average age of individuals included in the analysis, was 47 years old (ranged from 27 to 66 years). Exome sequencing was held in Beijing Institute of Genomics, (BGI, Beijing Genomics Institute) using the Illumina HiSeq2000 system [5].

Resulting analyses of genetic material of VE patients exome 2507 allele variants were identified, which led to non-synonymous amino acid substitutions or a shift of the reading frame or the appearance of stop codons were identified. 437 from these variants were new and may be specific for Yakut genome. For dominant model 11 variants were selected. In test of pathogenic foretelling using several computer programs it was reveal that 4 variants may be pathogenic for VE by more than two algorithms: *TMPRSS11E, SCUBE2, PPP1R36* and *PSMB6.*

SCUBE is the gene of signal peptide *CUB.*

TMPR SS11E is the gene of transmembrane serin protease.

PPP1R36 is the gene of regulator protein subunit (36) serin\treonin phosphoprotein phosphotase PP1. Protein subunit interacts with phosphotase PP1. Biological function is negative regulation of phosphotase activity.

PSMB6 is representative of B type proteasome family, subunit B6. It has endopeptidase activity of treonin type. Multi-subunit proteolitic complex is an enzyme of non lysosome degradation of proteins in the cytoplasm and nucleus of cells. Product of this gene take part in many cellular processes (metabolism, apoptosis, immunity, disease).

PPP1R36 and *PSMB6* variants are new and not present in dbSNP (the SNP NCBI database), it is typical for representatives of the Yakut population. Therefore *PPP1R36* (NM_172365:c.G1096A:p.G366R) and *PSMB6* (NM_002798:c.A541C:p.M181L) genes were selected for analyses of possible associations with VE. But significant association with VE was not revealed in group of VE patients comparing with healthy control group.

By second group of researchers sequencing was carried out at the National Institutes of Health Intramural Sequencing Center, Bethesda, Maryland, USA (4 patients) and Cornell University Sequencing Center, New York, USA (5 patients) using Illumina platform [3]. Within the framework of this project, DNA exome sequencing was performed on two patients with subacute VE and 7 patients with a documented diagnosis of chronic VE.

Analysis of the data using first a recessive model revealed no homozygous variants thus excluding the recessive inheritance of VE in studied patients. Analysis of 9 patients based on the dominant model also failed to identify rare heterozygous variants. Using largely softened parameters, two promising variants were identified in *AURKC* and *HLA-DRB1* genes, although they were also present in two patients with Spastic paraplegia living in Yakutia, and the population frequency of these variants was about 25 % (ncbi.nlm.nih.gov). This characterizes the variants as common polymorphisms, probably not related to VE. Another genetic variant in the regulatory region of *NMI* was found in 8 of 9 VEM patients studied, but again the population frequency is 5 to 24 % in different populations. Seven of 9 VE patients show variants in the coding regions of two other interesting genes-*ADAMTS14* having population frequency of zero to 0.5 %, and *SOX30* with a frequency of 2.1 % that is even higher in Asian populations, 9-12 %. The heterozygous variant of the *ADAMTS14* gene is most promising in the pathogenesis of the VE, particularly the participation of its product (enzyme metalloloproteinase) in microvascular pathology.

Analysis of the data obtained on the basis of the recessive model did not reveal rare homozygous variants, which excludes the possibility of recessive inheritance of RE in the patients studied. The next attempt to analyze the results of the exomial sequencing was done using the dominant model. But this time it was not possible to identify rare (less than 5% in the population) heterozygous variants, which would be present in each of the nine patients studied. Using the softened parameters, two promising variants in the *AURKC* and *HLA-DRB1* genes, which were present in all nine patients with RE, were identified. However, the population frequency of variants in the *AURKC* and *HLA-DRB1* genes was extremely high - about 25% (ncbi.nlm.nih.gov), which characterizes them as often occurring polymorphisms, probably not related to RE. Another variant in the regulatory part of the *NMI* gene was found in eight patients with VE from the nine investigated, but the population frequency in this case is from 5 to 24% in different populations. In seven of the nine patients studied, variants were found in the coding parts of two other interesting genes *ADAMTs14* with a population frequency from zero to 0.5% and *SOX3O* with a population fre-

quency of 2.1%, which in Asian populations is up to 9-12%. The heterozygous variant of the *ADAMTS14* gene is most promising in the pathogenesis of the VE, particularly the participation of its product (enzyme metalloloproteinase) in microvascular pathology.

CONCLUSION

Thus, the results of genetic studies of genes of immunity and analysis of exom sequencing in VE patients reveal specific features of Yakut genome, predisposing to immunity dysfunction and functional insufficiency of proteolitic and phosphatase activity. Revealed variants have not statistically significant association with VE possibly due insufficient amount of studied patients and clinical heterogeneity of disease. Nevertheless these factors may take part in induction of intracellular autophagy of neurons and atrophy of brain tissues. In condition of extreme environmental factors these peculiarities may led to the development of dystrophic processes in brain with the formation of a special type of encephalopathy as basis of VE pathogenesis.

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ANALYSIS OF REPEATED CASES OF PRENATAL DNA TESTING OF SPINOCEREBELLAR ATAXIA I IN YAKUTIA

ABSTRACT

The article presents an analysis of the repeated cases of the treatment in families burdened with spinocerebellar ataxia type 1 for the period over 10 years as a widespread rapidly progressive neurodegenerative hereditary disease, representing a specific medical and social problem in the Republic of Sakha (Yakutia). The analysis of the episodes of the repeated treatment for the prenatal DNA testing will allow to determine the competent tactics of medical care for burdened families who are at risk.

Keywords: hereditary diseases, spinocerebellar ataxia type 1, prenatal diagnostics, prenatal DNA testing.

INTRODUCTION

Spinocerebellar ataxia type 1 (SCA1) is a frequent hereditary neurodegenerative disease in Yakutia that has a dynamic mutation characterized by the prevalence of cerebellar signs, brisk tendon reflexes, presence of florid pyramidal signs, frequently with optic and oculomotor nerves damage and speech disorder [1, 2].

Infection rate of SCA1 in Yakutia doubled while reaching 46 cases for 100000 people during the last 21 years. Age of the disease onset closely correlates with the amount of CAG triplet repetitions in mutant gene [9, 11]. Besides that, therewere shown most of the patients with the repeated low-level numbers (39-55) survived to their reproductive years.

Inheritance of dynamic mutations in population is characterized by various degree of a penetrance, an anticipation phenomenon and a clinical polymorphism [1, 2, 5]. Primary medico-genetic consultation, presymptomatic and prenatal testing of the families with SCA1 are accompanied by numbers of the bioethical problems [4].

Thus, in the absence of radical methods of treatment, SCA1is an intractable problem of the regional Genetics Service as the most widespread neurodegenerative disease in Yakutia. This situation raises an issue about the opportunities and prospectsfor primary prevention of SCA1 in the Republic of Sakha [3, 4]. The repeated cases of treatments for thep renatal consultation are an indicator of effectiveness of methods of primary prevention. The analysis of the repeated cases of treatments will open problems and the hidden opportunities of the effective prenatal consultation for families with SCA1and will help to plan the further direction of researches.

MATERIALS AND METHODS

According to the register of hereditary and congenital diseases of the Medico-genetic center of the Perinatal center of National Medical Center№ 1, 1197 patients stayed on the dispensary registry in group of hereditary diseases with the autosomal dominant inheritance from whom 252 people with a spinocerebellar ataxia type 1 [6].

The research joined over-18 years-old women from the burdened families. Clinical genealogical analysis, prenatal medico-genetic consultation, talking, method of the voluntary informed consent, medical ultrasound of a fetus, invasive prenatal diagnostics by abdominal approachwith ultrasound control and diagnostics DNA methods (DNA purification, PCR, electrophoresis, detection of mutant alleles) were used in the research.

Prenatal diagnostics of diseases with dynamic mutations was carried out in the department of Prenatal Diagnostics of MGC of the NMC PC №1 in 2002 for the first time.

RESULTS AND DISCUSSION

During the last 10 years of the researches of the burdened families with SCA1, 80 treatments to the prenatal medico-genetic consultation were recorded. For thatperiod,there were the repeated casesof treatments of eleven pregnant womenaged from 23 to 32. From the Ust-Aldansky District there were four families, from the Megino-Kangalassky District - two families, from the Amginsky, Abyysky, Namsky, Churapchinsky and Khangalassky Districts – one family from each district.

Seven pregnant women were the presymptomatic carriers of a SCA1 gene, five of which inherited a disease from the father, two women inherited from mother, four pregnant women treated for repeated consultation married to the carriers of a mutant gene.

From seven female carriers of a mutant gene, six were representatives of earlier examined families during work of the international expeditions of 1992-1995 [7,8].

In our opinion, in structure of repeated treatments the important value has the time period which passed from the moment of presymptomatic DNA testing and the first treatment for the prenatal consultation.

According to our observations, from the moment of presymptomatic DNA testing to the first prenatal medico-genetic consultation it is a period of 1 to 3 years averagely. For example, according to a retrospective analysis of the treatments, it turned out that two women from the burdened families treated during the preconceptional period (before pregnancy).

The analysis of the family treatments where the hereditary carriers were husbands showed that two of four men underwent the presymptomatic DNA testing just before the prenatal consultation. However, these families were also earlier surveyed and informed about characters and features of SCA1. Family P., where the carrier is a father, and so two sons are the carriers of a pathological gene, indicates their willingness about healthy posterity birth during the first consultation. Following the results of prenatal diagnostics in these family two children were born without a pathological gene.

Thus, the total number of treatments for eleven women are 33 cases of pregnancy. Gestational age grouping of primary treatments looks in a certain way: in term up to 10 weeks of pregnancy – 6 cases, from 10 to 12 weeks of pregnancy – 11 cases, from 12 weeks and above - 16 cases among which gestational

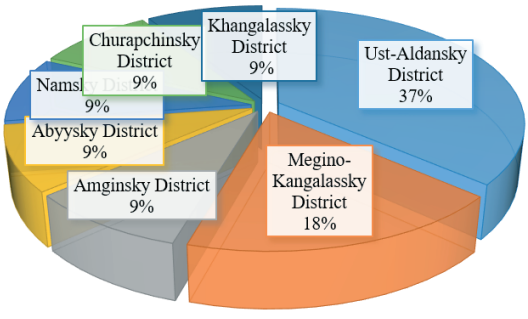

Fig. 1. Districts repeated casesgrouping scheme

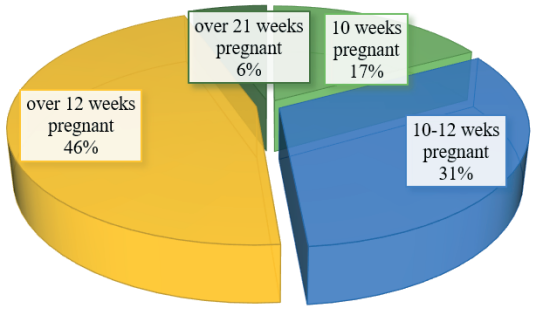

Fig. 3. Gestational age while primary treatment grouping scheme

age over 21 weeks is only 2 cases. Small amount of treatment cases of late pregnancy suggests about preliminary readiness and the conscious choice of treatment period for the prenatal help in caseof the disease.

From 33 cases, in eight episodes not-developing pregnancy was observed (up to 9 weeks of pregnancy at the time of the treatment).

Only 25 from 33 treatments to the prenatal consultation are carried outthe prenatal DNA testing. According to the gestational ages of the patients, a following distribution was observed: up to 12 weeks of pregnancy inclusively – 19 cases, over 12 weeks of pregnancy - 6 cases, among which there was 1 case of carrying out the invasive diagnostics in the gestational age of 25.

Following the results of prenatal testing with the negative DNA test result, 11 pregnant women are referred to pregnancy prolongation. At the same time in two cases with the positive DNA test result the family made the decision of pregnancy continuation. In 12 episodes with the positive DNA test result, the family made a decision to interrupt pregnancy and only three of them was in gestational age from 20 to 25.

Four families have four treatment cases for prenatal DNA testing, three families of whom were described and registered in results of the first international expeditions on studying of the Viliuisk encephalomyelitis and SCA1 in 1992-1995 when probands of our research were at the age of 10-14 years [7,8].

It is possible to assume that the international field researches of SCA1 changed views of a disease from parents of probands that subsequently affected the active and purposeful reproductive behavior of their descendants as since the early childhood there was an opinion about the possibility of anopen discussion and an active intervention in process of inheritance of SCA1. In one of such families having two heterosexual children, a daughter-in-law sought for the medico-genetic help and took the prenatal test four times; subsequently a sister of her husband (sister-in-law), also the carrier of a mutant gene, decided to do the prenatal DNA testing.

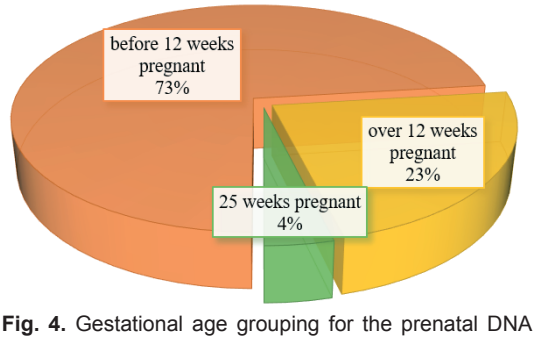

Fig. 4. Gestational age grouping for the prenatal DNA testing

CONCLUSIONS

Preliminary awareness of a family generally and, in particular, young representatives of the burdened families, promotes the early treatment for the preconception land prenatal consultation.

The conducting of the presymptomatic DNA testing to 1-3 years before pregnancy for the women from the burdened families promotes the active treatment to the prenatal consultation.

The repeated cases of the burdened families treatments to the prenatal medico-genetic consultation is an indicator of trust to prenatal diagnostics as the way of achievement of the healthy posterity birth and also efficiency of the prenatal genetic consultation in general.

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S.A. Fedorova

ORIGIN OF THE YAKUTS: MOLECULAR-GENETIC RECONSTRUCTIONS IN COMPARISON WITH THE HYPOTHESES OF HISTORIANS

ABSTRACT
This review presents the results of studies of the genetic history of the Yakut (Sakha) people conducted by researchers of the Yakut Scientific Centre of Complex Medical Problems and M. K. Ammosov North-Eastern Federal University (Yakutsk, Russia) in collaboration with the researchers of the Estonian Biocentre (Tartu, Estonia) and the Institute of Biochemistry and Genetics (Ufa Scientific Centre of RAS, Ufa, Russia), over the period 2002-2016. The obtained results are compared and contrasted with the reconstructions proposed by other groups of geneticists and with historical and archaeological hypotheses on the ethnogenesis of the Yakut (Sakha) people.
Keywords: Yakut (Sakha) people, genes, ethnogenesis, mitochondrial DNA, Y-chromosome.

INTRODUCTION

It is generally believed that the formation of the Yakut (Sakha) ethnic group in the territory of Yakutia occurred as a result of the gradual mixing of Turkic-speaking cattle-breeding tribes migrated from the south to the middle Lena River with local tribes over a long period of time. During the Soviet period, archaeological surveys were conducted in the Baikal region and Yakutia to study the earliest stages of the history of the Yakut (Sakha) people. Most modern scholars see the relation of the Yakuts to the Kurumchi culture of the Baikal region (6th to 10th centuries) [7,16,23,29]. Traditionally, the tribes of the Baikal Kurykans are considered to be the immediate ancestors of the Yakuts; apparently, they represented a union of three Turkic tribes, since they are referred to as *Uch-Kurykan* – 'three kurykans' in ancient sources [23]. Kurykans as a separate ethnic group appeared as a result of the assimilation of the aboriginal population and some Mongol-speaking groups by Turkic-speaking Teles tribes who moved to the Baikal region in the 5th-6th centuries [7].

Historians and archaeologists differ significantly in their opinions on the time when the ancestors of the Yakut people migrated to the north. Outstanding Yakut ethnographer G.V. Ksenofontov believed that the Yakuts are an ethnic group of mixed origin, which included three waves of Turkic-speaking immigrants. In his opinion, the first Yakuts began settling in the Vilyui River basin at the end of the 1st century AD; the second wave of the Yakuts migrated to the middle Lena and Vilyui from the Baikal region in the 6th-7th centuries AD; and finally, the last stage of the Yakut settlement occurred in the 11th-12th centuries, triggered by the strengthening of Mongol tribes and the complete displacement of the Yakuts' ancestors from the original place of living [20]. A.P. Okladnikov assumed two "decisive stages" in the settlement of the Yakuts' ancestors in the north. The first, in his opinion, began in the early Iron Age and ended in the 10th-11th centuries; the second stage dates back to the 15th-16th centuries [23]. According to archaeologist I.V. Konstantinov, the migration of the Yakuts' ancestors from the Baikal region occurred in the 15th century, as a compact group, which represented a fully developed ethnic community [19]. More recent researchers believe that the mass migration of the Yakuts' ancestors to the north occurred at the beginning of the 2nd millennium AD and is characterized by the appearance of the Small Houses Culture in Yakutia in the 13th century, which was later replaced by the Kulun-Atakh cattle-breeding culture. [16] On the other hand, archaeological findings (specific arrowheads, details of the bow, armor plates, cult pendant amulets, bull bones) and the appearance of runic inscriptions on the Lena petroglyphs testify

to the penetration into Yakutia of, first, Hunn-Xianbi, (in the 3d - first half of the 4th centuries AD), and later, from the 5th-6th centuries, Turkic-Mongolian groups [3].

The ratio of different-origin elements in the Yakut (Sakha) gene pool, their composition and the process of the traditional culture formation still require further research. Regarding the ethnic background of the local tribes who contributed to the formation of the Yakut ethnic group, historians have two points of view. Most researchers believe that these were the Tungusic tribes [16,20,23], although the small number (only about 4%) of Tungus words in the Yakut language testifies to the contrary. According to the anthroponymic data, out of 1,083 Yakut pagan names, there are 47% of Turkic, 37% of Mongolian, 6% of Turkic-Mongolian, and 10% of Evenki origin [24]. Thus, the linguistic data indicate a weak interaction between the Yakut and Tungusic languages.

By the opinion of Prof.A. N. Alekseev, the active settlement of the Tungus in North Asia, including Yakutia, began only at the end of the 1st and the beginning of the 2nd millennium AD, and the important contribution to the formation of the Yakut (Sakha) gene pool was made by the ancient non-Tungusic population of Yakutia, most likely by proto-Yukaghirs, proto-Samodeic or other ancient tribes whose ethnonyms were not preserved [3]. According to a legend, when Tungus and Yakut people came to the territory of Yakutia, they met local tribes - Sortols, Dirikineis, Khara Sagyls. The legends mention various ethnic groups who allegedly inhabited the territory of Yakutia, including Tumats and even the Kyrgyz [8], which indicates a sufficient diversity of the local and migrant populations of the time. A. N. Alekseev proposes that the local tribes inhabiting the middle Lena River since the ancient times and 'turkized' after the migration of small groups of southern cattle-breeders formed a significant layer of proto-Yakuts: "It was not newcomers who reproduced; the number of people who spoke their language grew ... the entire Eurasia experienced such turkization; that is why the Turkic-speaking ethnic groups are now so numerous nowadays ... "[3].

Since the 1990s, to answer the questions about the origins of individual ethnic groups, the molecular genetics methods have been widely used in addition to the generally accepted ethnographic, archaeological, and linguistic approaches. There appeared a new scientific branch - Genetic Archaeology, which allows modelling of past events basing on the study of human genome in modern populations. The intensive studies of the genetic structure of the modern Yakut (Sakha) population and the genetic relationships of the Yakuts with other peoples of Siberia were undertaken in the 2000s by three groups of researchers - scientists of the Research Institute of Medical Genetics, Siberian Branch of the Russian Academy of Medical Sciences (Tomsk, Russia) [26,28]; researchers of the Max Planck Institute for Evolutionary Anthropology (Leipzig, Germany) and the Institute of Health, Academy of Sciences of the Sakha Republic (Yakutia) (Yakutsk, Russia) [6,9,18] and scientists of the Yakutsk Scientific Centre of Complex Medical Problems, M. K. Ammosov NEFU (Yakutsk), in cooperation with colleagues from the Institute of Biochemistry and Genetics of the Ufa Scientific Center, Russian Academy of Sciences (Ufa, Russia) and the Estonian Biocenter (Tartu, Estonia) [1,2,4,5,10-13,15,17,27] These studies were carried out mainly using two complementary genetic systems - Y-chromosome and mtDNA, which allow describing the features of the male and female gene pool and autosomal markers panels. In recent years, the development of new molecular genetic technologies has enabled the whole genome analysis.

This review presents the results of our studies of the genetic history of the Yakut (Sakha) people in comparison and contrast with the reconstructions proposed by other groups of geneticists and with the hypothesis about the ethnogenesis of the Yakut (Sakha) people, proposed earlier by historians and archaeologists.

The origins of the Yakut (Sakha) people in the light of genetic archaeology data

The analysis of the lineages of Central, Vilyui and Northern Yakuts from 16 regions of the Sakha Republic (Yakutia) (n=215) showed that the vast majority of male Sakha (over 80%) are descendants of one male founder with a Y-chromosome lineage belonging to haplogroup N3 [5,11-13,15]. Our data confirmed the results of studies by other groups of geneticists on the analysis of the Yakut lineages of Ust-Aldan region (n=109) [26,28] and eight central, Vilyui and northern regions of the SR (Y) (n=178) [18]. It was also found out that the extremely low diversity of male lineages in the Yakuts is compensated by the high diversity of the female gene pool lineages [5,10-13,15], which was later confirmed by studies of other authors [6,9,18].

On the Y-chromosome phylogenetic tree, the N3-lineages of the Yakuts are combined into one specific branch [1] In previous works, the structure of this branch was considered as "star-like", that is having one ancestral haplotype [18,26,28]. According to Kharkov et al., 2008, the generation time of specific N3-lineages in the Yakut population is 4.45 ± 1.96 thousand years; according to B. Pakendorf, 2006 - 880 ± 440 years. Unlike the other authors, we consider the Yakut branch in the structure of the N3-network of neighboring ethnic groups of Southern and Western Siberia, Chukotka and Kamchatka, as one containing two closely related dominant haplotypes (Fig.1) and, accordingly, undergone **to two successive expansions** [5] According to our estimates, the separation of N3-branch, specific of the Yakuts, of Y-chromosome tree and the first expansion began ~ in the 4th-5th centuries A [5]. After the separation of the Yakut branch ~ 1,600-1,700 years ago, a secondary expansion followed ~ 900 years ago. The first date corresponds to the time when the Yakut language started to separate from the ancient Turkic languages, according to G. G. Levin [21]; the second one coincides with the estimated time of migration of the last, most extensive wave of the Turkic-speaking ancestors of the Yakuts into the Middle Lena basin. The time of the secondary expansion of N3-lineages, according to our estimate, corresponds to the values proposed by B. Pakendorf. We assume that the Turkic-speaking ancestors of the Yakuts could have migrated to the north long before the rise of the Genghis Khan Empire. An indirect confirmation of this supposition can be the fact that the Yakuts do not have the so-called 'C3-lineage of Genghisides', widespread in the territories that were under the rule of the great Khan [5]. Apparently, in the ancestral tribe in the 4th-5th centuries the men of one kin were dominated, while the ancient Yakut population whose expansion began ~ 900 years ago should have carried the both dominant haplotypes.

What region did the Yakut (Sakha) N3-lineage appear in? The maximum frequency of the "Yakut" N3-lineage is characteristic only for Yakutia: from 70 to 90% in various ethnogeographical groups

of the Yakuts, Dolgan (50%), Evenks (47%), and Evens (29%) [5,12,17]. Phylogenetic studies established that the "Yakut" lineage is not a derivative of the N3-lineages of the neighboring peoples - Mongols, Buryats, Chukchi, Eskimos, Koryaks, Nanais, Japanese and Han Chinese [2,17]; thus, the origin of this lineage, apparently, is not connected with the territories adjacent to Baikal from the east and south (Buryatia, Mongolia, the Amur River basin, China), either with the territory of ancient Beringia (Chukotka and Kamchatka). The N3-lineages of the Yakuts are also different from the N3-lineages of the peoples of the Volga-Ural region and Scandinavia. The appearance of the more ancient dominant "Yakut" haplotype (haplotype 1 in Fig.1) in Southern Siberia, in regions west of Baikal seems to be more likely, since its close phylogenetic derivatives are present in Tuvinians, Tofalars, and Soyots. The second dominant haplotype (haplotype 2 in Fig.1) and its derivatives are found only in the populations of Yakutia, respectively, its multiplication and secondary expansion occurred in the territory of Yakutia.

Researchers of the Institute of Medical Genetics (Tomsk) previously hypothesized on the autochthonous origin of the N3-Yakut lineages. Prof. V.A. Stepanov suggested that the male Yakut gene pool was formed on the basis of the local Evenk component, and the Turkic language was acquired as a result of the cultural dominance of the Turkic-speaking elite, which left no significant trace in the Y-chromosome pool [26]. Later, the authors expressed the opinion that these lineages were acquired by the Yakuts indirectly through the Evenks from even more ancient aboriginal tribes that had formerly lived in the territory of modern Yakutia and were assimilated by the Tungus [28]. The authors explain an unusually high frequency of N3-lineages in the male Yakut gene pool (over 80% of the population as a whole), in contrast to the Evenks (25-33%), by a significant increase in the number of Yakuts in recent centuries, which led to a random sharp increase in the frequencies of individual lineages with the predominant founder haplotype in the population. Unlike this, we consider the emergence of a "Yakut" N3-lineage in Eastern Sayan region, rather than in the territory of modern Yakutia, since close STR-haplotypes are present among the ethnic groups living to the west of Baikal. However, in our view, the final answer to the question "where

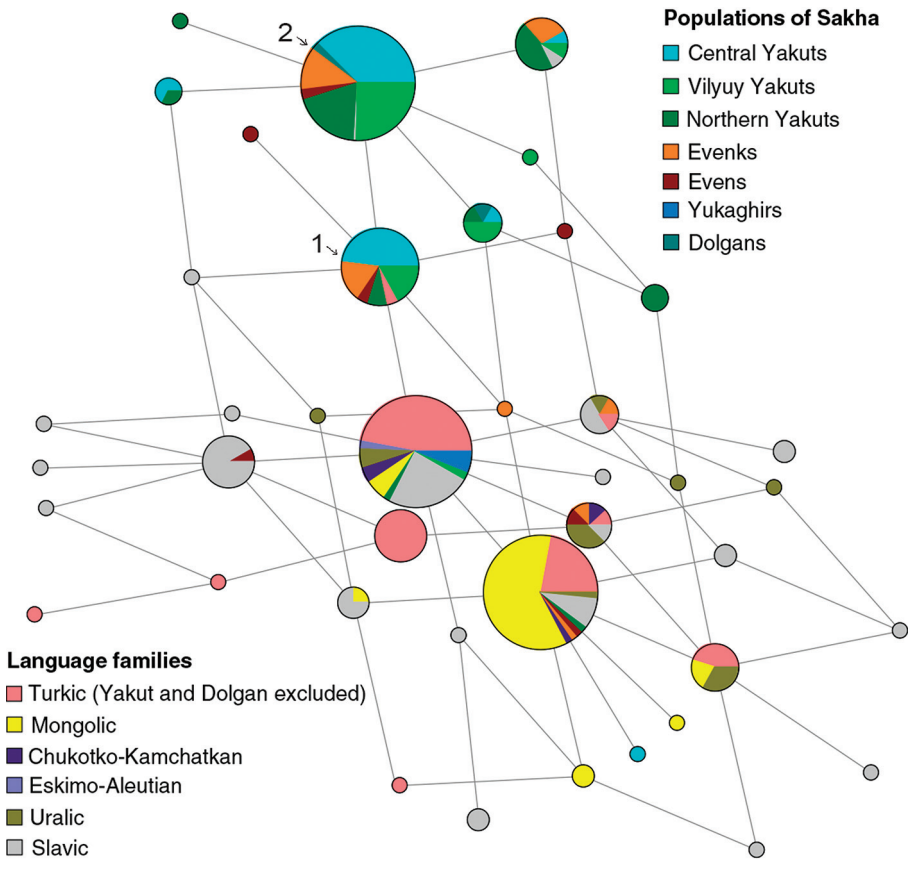

Fig.1. Phylogenetic network of N3-haplotypes of Y-chromosome of the ethnic groups in Yakutia and adjacent regions. [5] The circles indicate microsatellite haplotypes, the area of the circle and the sectors inside the circle is proportional to the haplotype frequency. The dominant "Yakut" haplotypes 1 and 2 are marked by the arrows.

and when did the N3-lineage of the Yakuts originate?" could only be given in the future by DNA analysis of the ancient populations of Yakutia and Southern Siberia.

What ethnic groups are the closest to the Yakut (Sakha people?

The study of the genetic relationships of the Yakuts with neighboring peoples showed a close genetic similarity of the Yakuts with the Evenks of Yakutia [5,12,13,15]. We propose that the close genetic relationship of the Yakuts to the Evenks of Yakutia is primarily due to the origin of these peoples from the common South Siberian genetic pool (and if the origin of the Yakuts is connected with the regions west of Lake Baikal, then the Tungus - most likely with the territories east of Lake Baikal), as well as the mixing of territorially close ethnic groups to Yakutia over a period of at least 900 years. Some ethnographic studies noted that Yakut men often and willingly married Evenk women, while Yakut women rarely married Evenks [25]. Most likely, these marriage traditions explain the high content of the common lineages in the female gene pool (50-65%) in different ethnogeographic groups of the Yakuts and Evenks [12] and the relatively low content of the N3-lineages in the male gene pool of the Evenks.

It is curious that we found the "Genghis Khan" haplotype in the Evenks of Yakutia; it occurs with high frequency among the ethnic groups that were under the power of Genghisides, which indicates a relatively recent male flow of genes from Mongols to Evenks [5]. The obtained genetic evidence supports the hypothesis of the relatively recent (in the beginning of the 2nd millennium AD) appearance of the Tungus in the territory of Yakutia., proposed by Prof.A. N. Alekseev [3]. The large-scale expansion of the Tungus and Turks through Siberia, which apparently occurred within the last 2,000 years, assimilated and/or displaced the ancient population of Yakutia to even more northern territories. Therefore, it is not surprising that in comparison with the Evenks of Yakutia, the peoples of the Arctic (Yukaghirs, Evens, Dolgans, and Nganasans) differ significantly from the Yakuts in the composition of the gene pool components and the content of specific mtDNA and Y-chromosome [5,12].

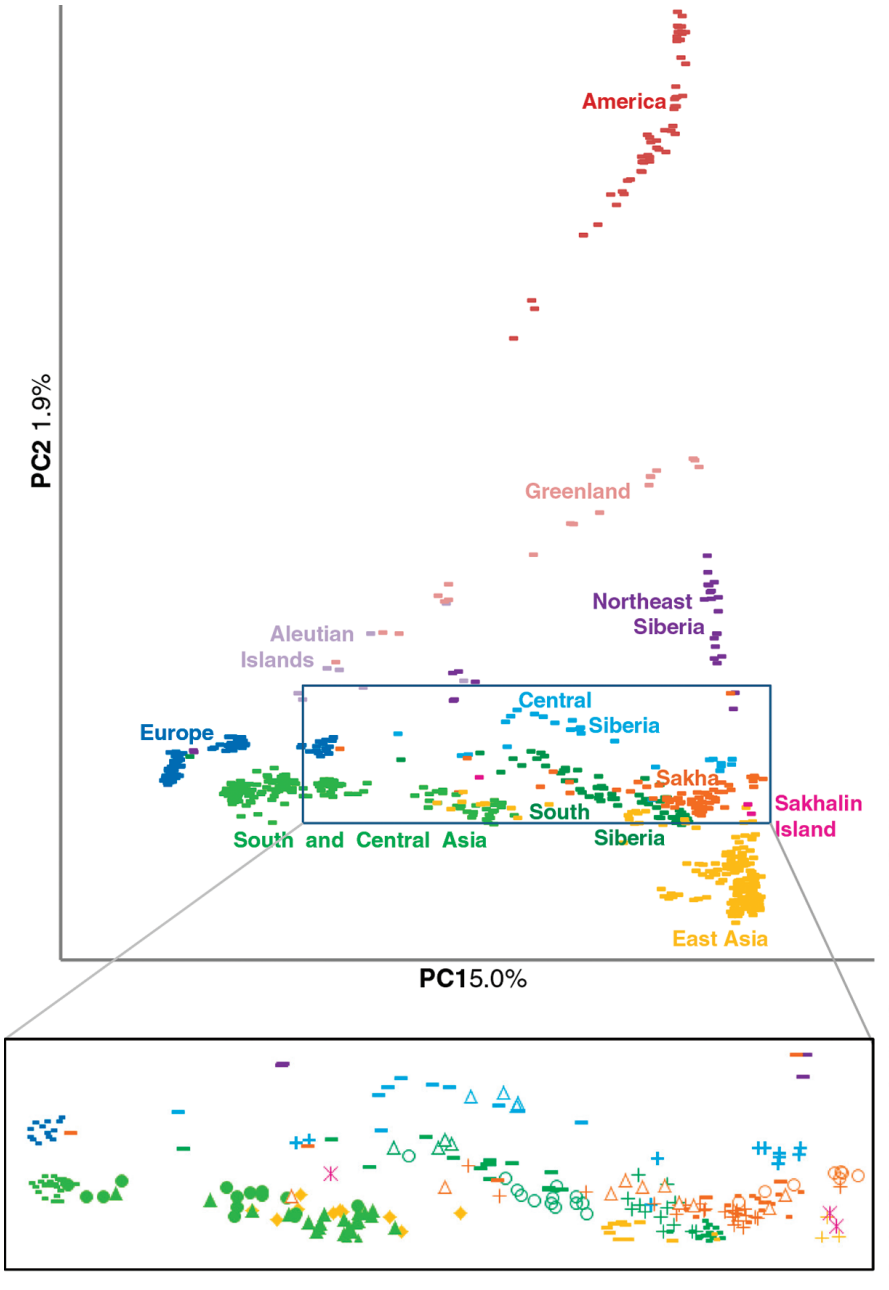

Fig.2. PC-map of the locations of populations in Yakutia among other populations in Eurasia and America, compiled with the use of 600,000 autosome SNP. [5]

Further, by the degree of genetic proximity and their genetic characteristics, the peoples of Southern Siberia - the Buryats, Tuvinians, Altaians, Mongols, Khakases - are closer to the Yakuts. Unlike the Yakuts, the Turkic-speaking peoples of Southern Siberia have a greater proportion of the European and South Asian components in their gene pool [5,27]. Even greater genetic distances separates the Yakuts from the peoples of western Siberia (Kets and Selkups), Southeast Asia (Han Chinese, Koreans, Japanese), Central Asia (Uzbeks, Uighurs), Chukotka and Kamchatka (Chukchi, Eskimos, Koryaks, Itelmen) (Fig. 2).

How large is the European component in the Yakut (Sakha) gene pool? How does the hypothesis of the Aryan origin of the Yakut (Sakha) people look in the light of the genetic archaeology data?

In the 1990s, the hypothesis about the "Aryan" origin of the Yakut (Sakha) people was brought about by the geneticist V. V. Fefelova on the basis of the analysis of two markers of the HLA system (HLA-A and HLA-B on the human 6th chromosome) was popular [14]. According to the results of immunogenetic studies, the Yakuts demonstrated a high frequency of the "Indo-European" gene HLA-A1 and haplotype HLA-A1, B17 was established, which was considered by the author of the hypothesis as the presence of a powerful ancient Caucasoid component in the Yakut gene pool, the content of which reaches 30%.

At the beginning of the 21st century, when first studying the female gene pool of the Vilyui Yakuts gene pool, indeed, the data were obtained on the high content of mtDNA lineages of European origin (17%), but the authors explained this fact by intensive processes of assimilation by the Russian-speaking population [22].

According to our data, the analysis of the mtDNA of 423 people from the Central, Vilyui and Northern ethnogeographical groups showed that the high content of the mother lineages of European origin is characteristic primarily of the Vilyui Yakuts (16%), for Central and Northern it accounts for 7%; thus, on average for the three ethnogeographic groups of Yakuts it makes 10%. The Caucasoid component of the Yakut gene pool is also low on paternal lineages - from 4% in the Vilyui Yakuts to 11% in Central Yakuts, with the average at 7% of the total pool [11,12]. It was found that the content of these lineages in the Yakut (Sakha) gene pool is explained not only by mixing with the Russian-speaking population who have been migrating to Eastern Siberia since the 17th century, but also by the presence of an ancient European component in the ancestral Yakut population, characteristic of all the Turkic-speaking peoples of Southern Siberia [5,12]. It is known that the formation of ancient Türkic groups equally involved both Caucasoid and Mongoloid populations. The origin of the ancient West-Eurasian lineages in the gene pool of the Yakut ethnos is apparently connected with the pre-ethnic stage of the formation of the Yakuts as

a separate ethnic group - the Scythian-Siberian, subsequent Hunn-Sarmatian and ancient Turkic times. Some of these lineages originated in the Middle East, migrated along the Eurasian Steppe Corridor through Central Asia and Southern Siberia, and appeared in the territory of Yakutia with the Turkic-speaking ancestors of the Yakuts (Sakha) [5].

Thus, the genetic archeology data confirm the presence of the ancient Caucasoid component in the Yakut (Sakha) gene pool, which was proposed by Prof. V. V. Fefelova basing of the results of HLA system studies; however, we estimate the content of this component as being much lower at less than 10%, rather that 30%.

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GENETIC RISK FACTORS OF THROMBOFILIA IN A YOUNG ISCHEMIC STROKE PATIENT (CLINICAL CASE REPORT)

ABSTRACT

Stroke is a multifactorial disease. The stroke pathogenesis is caused by a combination of several risk factors. A determination of the genetic predisposition to stroke is especially important for young patients. Understanding the stroke development mechanisms is extremely useful for adequate treatment and secondary prevention of the disease. We present the case report of ischemic stroke in the young patient with basilar artery thrombosis and severe neurological symptoms. Diagnostics included the clinical, neurological, neuroimaging, ultrasound, immunological, molecular-genetic examination. Genotyping of polymorphisms of hemostasis system's genes *FV, FII, FVII, FXIII, FGB, ITGA2, ITGB3, PAI-I* was conducted. It was found the carrying of the three polymorphisms associated with the thrombosis and ischemic stroke risk: genotype GA polymorphism - 455 G> A gene *FGB*, genotype CT polymorphism 807 C> T *ITGA2* gene, 4G / 4G genotype of polymorphisms 5G> 4G gene *PAI-I*. Thrombophilia due to the carrying of these genetic variants in combination with an antiphospholipid syndrome, altered immune status, were predisposition factors of vertebro-basilar's system ischemic stroke in the young patient.

Keywords: ischemic stroke, vertebro-basilar system, thrombosis, *FV* gene, *FII* gene, *FVII* gene, *FXIII* gene, *FGB* gene, *ITGA2* gene, *ITGB3* gene, *PAI-I* gene

INTRODUCTION

Most strokes are multifactorial diseases, and the combination of several risk factors is important in its pathogenesis, including genetic ones. Despite the significant advances in the diagnostic technologies had been achieved recently, the significant share of strokes is currently regarded as cryptogenic, i.e. a disease of unknown etiology. Determining the possible cause of stroke, especially in young patients, involves conducting a wide range of diagnostic procedures, including genetic examination. Despite the limited using of these molecular genetic studies in clinical practice, the testing for a carriage of known variants of risk gene polymorphisms can be useful for understanding the pathogenic mechanisms of the disease and selecting adequate treatment and effective secondary stroke prevention. In this article, we present the clinical case of ischemic stroke due to basilar artery thrombosis in the young patient, with an interpretation of the alleged genetic risk factors.

Clinical case report. Patient R., female, 21 years old, admitted to Regional Vascular Center (Yakutsk) with diagnosis: Ischemic stroke in the vertebro-basilar system due to basilar artery thrombosis, for the emergency indications, in serious condition. The disease developed acute during physical exercise (training in the gym). Suddenly developed a headache, dizziness, nausea, general weakness. The arterial pressure was 108/60 mm Hg, pulse was 112 per minute. In anamnesis: patient was observed with endemic goiter by endocrinologist, using iodine preparations constantly, there are no other chronic diseases, arterial pressure was with a tendency to hypotension (90/60 mm Hg). The intake of oral contraceptives is denied, there were no pregnancies. Relatives of the first line are healthy; the patient's cousin suffered a subarachnoid hemorrhage due to an arterial aneurysm rupture and was received surgical treatment.

At admission to the hospital, the patient's condition was severe. No pathological abnormalities were detected in the somatic status. Skin and visible mucous membranes were clean with normal color. In neurological status: consciousness's depression to soporus; craniocerebral innervation disorders with the bulbar symptoms (anarthria, dysphagia, aphonia); oculomotor disorders (divergent strabismus); motor disorders (deep tetraparesis with plegia in the lower extremities, bilateral pathological pyramidal signs), moderate meningeal syndrome.

The brain magnetic resonance imaging (MRI) scan determines the acute ischemia zones in the pons 2.3 x 2.6 cm in size, and in the cerebellum left hemisphere 2.1 x 2 cm in size. Screening MR-angiography of Willis Circle determines the total basilar artery occlusion. The blood flow in the posterior cerebral arteries (PCA) on both sides was supported through the posterior connective arteries (PCoA). MRI study conclusion: Total occlusion of the basilar artery. Areas of acute ischemia in the pons and in the left cerebellum hemisphere (Figures 1 and 2).

Laboratory blood value showed: potassium level – 3.2 mmol / l, sodium – 146 mmol / l (normal), transient hyperglycemia to 7.6 mmol / l, in complete blood count – leukocytosis 15.9 x 10^9 / l, platelets 289 x 10^9 / l (normal), increased level of blood sedimentation rate in dynamics from 15 to 57 mm / h. The study of blood culture for sterility – there is no growth. It was performed the blood coagulating testing in dynamics. Fibrinogen level was 2.9-4.2 g / l (norm 2-4 g / l); international normalized ratio (INR) in dynamics: 1.12-1.2; activated partial thromboplastin time (APTT) increased with anticoagulant therapy (from 23.3 to 63.2); prothrombin index (PTI): 48.4 – 73.5; thrombin time – 15.7 seconds; protein C (activated Coagulation factor XIV) – 79% (norm 70-130%); protein S (cofactor protein C) – 58% (normal 60-140%); D-dimer – 0.27 ng / ml. The value of lupus anticoagulant was 0.87 (normal 0.8-1.2 conventional units).

Immunological examination data were as follows: no lupus Le cells. In addition, it was detected the disorders in level of blood circulating immune complexes: increased level of anti-streptolysin O (750 IU / ml) and C-reactive protein – 48 mg / l. Enzyme-linked immunosorbent assay (ELISA) were as follows: immunoglobulins G (IgG) of cytomegalovirus, simplex herpes virus – detected; ELISA on ANA (antinuclear antibody, ANA) – not detected; ELISA for antiphospholipid syndrome (APS) – IgG increased.

Data of cardiovascular system examination detected: electrocardiogram - sinus rhythm (with a transient tachycardia to 118 per minute), the heart electric axis is vertical, nonspecific changes in the myocardium in the lower wall. On the 24-

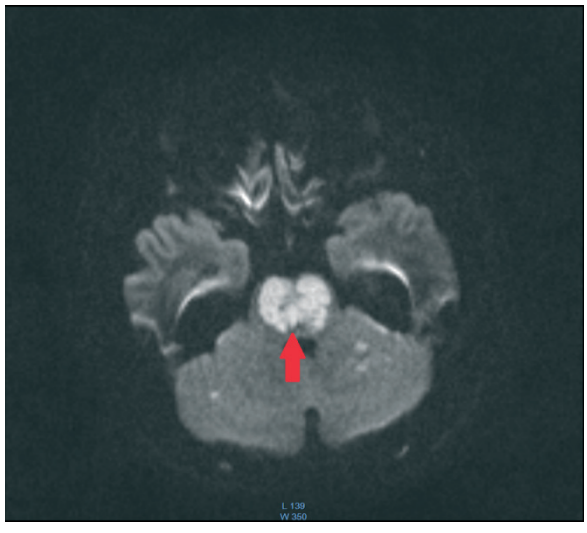

Figure 1. Patient P, 21 years old. Magnetic resonance imaging of the brain, diffuse-weighted image, B-factor 1000. Zone of acute ischemia (arrow) in the pons.

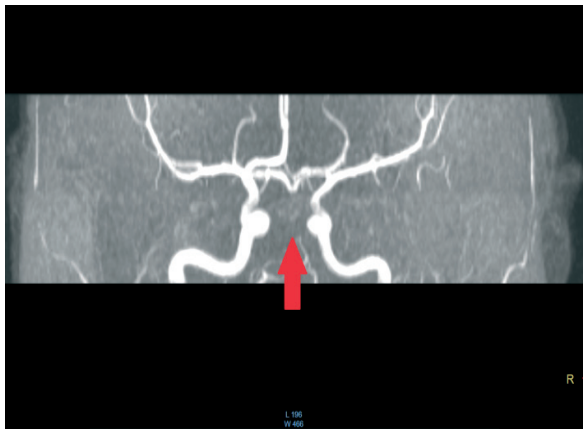

Figure 2. Patient P, 21 years old. Magnetic resonance imaging of the brain. Time-of-flight angiography. There is no MR signal from the basilar artery (arrow)

hour blood pressure monitoring, it was not registered hypertension. Ultrasound examination of the heart detected that the global systolic function of the left ventricle (LV) is normal, the ejection fraction is 66%, LV diastolic function has 1 type, 1st degree mitral regurgitation, the heart cavity is not dilated, the disturbed myocardium zones of the left ventricle are not revealed. Ultrasound examination of brachiocephalic and cerebral arteries revealed a blood flow decrease in the vertebral arteries from both sides. Vertebral artery had small diameter on both sides. Echo graphic signs of functional vasospasm along the right middle cerebral artery. The occlusion of the right posterior cerebral artery it was suspected.

A molecular genetic study was conducted at the National Center of Medicine-Republican Hospital No. 1 (Yakutsk). It was performed the genotyping of SNPs of genes of hemostasis system including polymorphism 20210 G> A of gene *FII* (rs1799963), polymorphism 1691 G> A of gene *FV* (rs6025), polymorphism 10976 G> A of gene *FVII*; polymorphism G> A of gene *FXIII* (rs5985), polymorphism - 455 G> A of gene *FGB* (rs1800790), polymorphism 807 C> T of gene *ITGA2* (rs1126643), polymorphism 1565 T> C of gene *ITGB3* (rs5918), polymorphism 675 5G> 4G of gene *PAI-I* (rs34857375). The results of the genetic research: genotype GG of polymorphism 20210 G> A *FII*, genotype GG of polymorphism 1691 G> A *FV*, genotype GA of polymorphism 10976 G> A *FVII*, genotype GG of polymorphism G> A *FXIII*, genotype GA of polymorphism G> A *FGB-455*, genotype CT of polymorphism 807 C> T *ITGA2*, genotype 1565 T> C of polymorphism TT *ITGB3*, genotype 4G4G of polymorphism 675 5G> 4G *PAI-I*. Patient was counseled by hematologist, the diagnosis: Congenital thrombophilia, mutations in genes *FVII*, *FGB*, *ITGA2*, *PAI-I*. Secondary antiphospholipid syndrome, hormone-induced.

The brain MR scan on the 34th day of the disease determined residual the post ischemic gliosis changes in the pons measuring 2 x 2.5 cm. Gliosis changes have small cysts measuring 0.2 cm. Gliosis post ischemic changes in the left hemispheres of the cerebellum measuring 1 x 0.8 cm. There is a restoration of blood flow along the basilar artery. Conclusion: Post ischemic gliosis in the pons and in the left hemisphere of the cerebellum. Restoration of blood flow in the basilar artery.

The patient received the intensive basic stroke therapy, anticoagulant, neuroprotective therapy, nootropics, symptomatic treatment, early neurorehabilitation. Later the patient received a long course of rehabilitation treatment with a good positive dynamics, with regress of focal symptoms to mild tetra paresis, dysarthria. The use of anticoagulant therapy continued for several months.

Discussion. We presented the case report of ischemic stroke (IS) in a young patient. An acute impairment of cerebral circulation developed due to the occlusion (thrombosis) of a large artery of the vertebra-basilar system, which caused ischemia in the brainstem and cerebellum. It was develop the severe cerebral and focal manifestations.

The diagnostic algorithm of the disease included a differential diagnosis between cerebral vascular dissection; cardioembolysm due to the congenital or acquired heart defects, rhythm disturbances, volume formations in the heart cavity, endocarditis; thrombosis due to violations of blood coagulation properties; antiphospholipid syndrome of various genesis; systemic diseases; vasculitis; sepsis; congenital anomalies of cerebral vessels.

According to the survey data, the cardiac, septic cause of the disease, the anomalies of the development of cerebral vessels were excluded. The examination results indicated the blood coagulating impairments, immunologic status disorders. These changes could arise as a nonspecific reaction to a serious acute disease, or be a provoking factor of a stroke. To diagnostics the coagulation system hereditary defects, it was performed the genotyping of eight polymorphisms of genes associated with thrombosis risk. It was genotyped the polymorphism 20210 G> A of gene *FII*, polymorphism 1691 G> A of gene *FV*, polymorphism 10976 G> A of gene *FVII*, polymorphism G> A of gene *FXIII*, polymorphism -455 G> A of gene *FGB*, polymorphism 807 C> T of gene *ITGA2*, polymorphism 1565 T> C of gene *ITGB3*, polymorphism 675 5G> 4G of gene *PAI-I*.

Diagnosis of the pathogenesis type of stroke is extremely important for adequate treatment and secondary prevention of stroke. Nevertheless, according to the literature, 20%-40% of ischemic strokes are cryptogenic, that is, with unidentified (unspecified) etiology [2]. Now most strokes are recognized as multifactorial diseases, several risk factors may be important in pathogenesis, including genetic ones. According to some authors, stroke is a polygenic disease [9]. Carrying out several polymorphisms of the predisposition of certain genes can lead to a higher risk of stroke than the carriage of one such polymorphism [12]. The stroke predisposing candidate genes include genes *FV, FII, FGA / FGB / FGG, FVII, FXIIIA1, vWF, FXII, SERPINE1, ITGB3 / PLA1 / PLA2 / ITGA2B, ITGA2, GP1BA, ACE , AGT, NOS3, APOE, LPL, PON1, PDE4D, ALOX5AP, MTHFR, MTR* and

CBS [9].

The most frequent thromboses predisposing gene variants are the some polymorphisms of genes of factor V (Leyden mutation), prothrombin (factor II), MTHFR [3, 4, 10, 11, 15]. Hereditary thrombophilia with concomitant risk factors, such as surgical operation, trauma, prolonged immobilization, a pregnancy and puerperium, oral contraceptives using, antiphospholipid antibodies and hyperhomocysteinemia may increase the risk of cerebral venous thrombosis. Similarly, the joint inheritance of two or more known mutations also significantly increases the risk [4]. Pathological conditions of the vessel walls (for example, due to atherosclerosis or inflammation) can lead to activation of coagulation through the internal mechanism and increase the likelihood of thrombotic incidents [1, 19].

Factor V is required as a cofactor for activating factor Xa, which in turn activates prothrombin [1]. The "Leiden" mutation is considered as one of the most significant factors of the thrombosis genetic risk [1, 4], the genotypes AA and GA of this polymorphism increase the venous thrombosis risk and ischemic stroke risk, including arterial thrombosis at a young age [20].

Prothrombin (factor II) is the key protein of the coagulation cascade from which thrombin is formed, it converts fibrin into fibrinogen [1]. Polymorphism G20210A of the gene *FII* is associated with an increased prothrombin level in plasma and is a very significant genetic risk factor for venous thrombosis [1]. Variants GA, AA of polymorphism 20210 G> A of gene *FII* are associated with IS risk, venous thrombosis, thromboembolism [20]. "Leiden" mutation, the G20210A prothrombin gene mutation and a deficiency of protein S and C, cause a reduction in the control of thrombin generation. Deficiency of antithrombin causes a decreased neutralization of thrombin. Both these mechanisms are responsible for venous thrombosis [4].

Factor VII (vitamin-K-dependent clotting factor VII) binds to factor III and further activates the "external" coagulation mechanism with significant damage to blood vessels. Increased activity of factor VII is associated with a risk of thrombosis [1]. The study [14] showed that two *FVII* polymorphisms, -670C and IVS7 seven or higher, are independent risk factors for ischemic stroke in young adult patients. Genotypes AA 10976 G> A of *FVII* gene and GA 10976 G> A of *FVII* gene are associated with a decrease in the factor VII level in the blood and with a decrease in the risk of myocardial infarction [20].

Factor XIII (fibrin-stabilizing factor) FXIII A1 forms cross-links of fibrin molecules, thus stabilizing thrombus. The biologically active form consists of globules of two types: alpha and beta. Polymorphism of V34L in the alpha globule was associated with a reduced risk of venous thrombosis, myocardial infarction and stroke [1, 5]. Variants of GT, TT of polymorphism G> A of the gene *FXIII A1* (rs5985) are associated with a decrease in the venous thrombosis risk [20].

Elevated levels of beta-fibrinogen (FGB) in plasma are associated with a risk of cardiovascular disease. Polymorphism -455 G> A gene *FGB* associated with increased fibrinogen levels [1, 7]. Carriage of the genetic variants GA, AA of this polymorphism significantly increases the risk of stroke [20]. The study [12] examined the association between the IS risk and C677T polymorphism of methylenetetrahydrofolate gene (*MTHFR*), polymorphisms 455G and T148C of beta-fibrinogen gene (*β-FGA*), polymorphisms ε2-4 of apolipoprotein E gene (*APOE*), polymorphism I/D of angiotensin converting enzyme gene (*ACE*), polymorphism G894T of endothelial nitric oxide synthase gene (*eNOS*). The results of this study showed that two polymorphisms (*ACE* I/D and *β-FG* T148C) are significant synergistic contributors to the disease risk. For example, the combination of *ACE* DD and *β-FG* 148CC, *ACE* DD and *β-FG* 148CT, *ACE* ID and *β-FG* 148CC resulted in high risk of IS [12].

The plasminogen activator inhibitor-1 gene (*PAI-1*) is associated with the stroke pathogenesis [18]. Plasminogen activator inhibitor-1 (PAI-1) is an inhibitor of fibrinolysis, as well as a marker of inflammation. Polymorphism 4G / 5G gene *PAI-1* is associated with PAI-1 levels in plasma in different ethnic populations [18] and thromboembolism [1]. Carriage of genetic variants of 5G / 4G and 4G / 4G of this polymorphism increases the level of PAI-I in the blood, reduces fibrinolytic activity and increases the risk of cardiovascular diseases [20]. The aim of the study [18] was to determine the possible potential relationship between 4G / 5G polymorphism of gene *PAI-1* and the PAI-1 level, IS risk in young Asian Indians. The PAI-1 level was significantly higher in patients than in the control group (p = 0.03). The variant involving 4G allele showed both genotypic (p = 0.0013, $\chi 2$ = 10.303; odds ratio [OR] = 3.75) as well as allelic association (p = 0.0004, $\chi 2$ = 12.273; OR = 1.99) with IS. It was also found that the homozygous variant 4G / 4G is associated with higher levels of PAI-1 (p = 0.005) [18]. However, the study [16] did not establish the association between insertion/deletion (4G/5G) polymorphism of plasminogen activator inhibitor *PAI-1* gene and IS in German child population. The distribution of the 4G/5G genotypes was no different in childhood stroke patients and controls [16]. The same results were established in study [17] which suggests that the 4 G/5 G polymorphism of the *PAI-I* gene is not a risk factor of ischemic stroke in Polish children.

Integrin alpha-2 platelets (glycoprotein IIa) ITGA2 is the main platelet receptor of collagen. *ITGA2* polymorphisms are associated with coronary heart disease and myocardial infarction [1, 6]. In several studies, the relationship between the -GA807T polymorphism of the gene *ITGA2* (rs1126643) and IS were studied, but the results were inconsistent [8]. It was performed a meta-analysis of studies on the association of polymorphism -C807T *ITGA2* (rs1126643) with IS, a total 15 studies with stroke cases (n=2242) and control groups (n=2408). The authors suggest that the polymorphism C807T of gene *ITGA2* may be a susceptible predictor of the IS risk [8]. Variants CT, TT of polymorphism 807 C> T *ITGA2* are associated with IS risk and thromboembolism [20]. It was established the cardiovascular disease risk and thromboembolism are associated with polymorphism 1565 T> C *ITGB3* (genotypes CT and CC) [20].

Study [13] investigated the association between polymorphisms of the two integrin genes (C807T of *ITGA2* gene and T176C of *ITGB3* gene) and IS risk, plasma lipid and lipoprotein levels. As expected, total cholesterol, triglycerides and low-density lipoprotein were significantly higher in the patient's group compared with the control group (p<0.05). The genotype and alleles frequencies of -C807T *ITGA2* were significantly different between patients and the control group (p <0.05), but no differences were found between groups in the frequencies of genotypes and alleles of T176C *ITGA3*. Allele T *ITGA-2* had a relative IS risk higher in 1.266 times (OR = 1.226, CI 95%: 1.053-1.428) than allele C. In addition, the total cholesterol level was higher in T-allele carriers compared with non-carriers (p <0.05). Thus, the T allele of polymorphism C807T *ITGA2* is associated with ischemic stroke [13].

As follows from the above, the most studied genetic factors of thrombophilia can contribute to the development of both venous and arterial strokes. In the devel-

opment of the disease, in most cases, a number of factors are important. Hereditary predisposition to defects of thrombus formation, especially in young patients, can become a key link in the pathogenesis of a stroke.

Thus, the patient R., 21 years old, with ischemic stroke in the background of basilar artery thrombosis was genotyped on the most significant polymorphisms of genes that affect the hemostatic system. The carriage of three variants of polymorphisms associated with the risk of thrombosis and ischemic stroke (GA genotype of the polymorphism -455G> A *FGB*, the CT genotype of the polymorphism 807 C> T *ITGA2*, the 4G / 4G genotype of the polymorphism 5G> 4G *PAI-I*). In the presented clinical case, the participation of the products of these genes in the disease pathogenesis manifested the hyperactivation of thrombus formation on the probably altered endothelial site of the large artery of the vertebrobasilar system. The contribution to the disease development included the genetically determined thrombophilia in combination with the altered immune status, antiphospholipid syndrome. In this clinical case, the observation of the patient for the long period with the monitoring of the blood coagulation, immune status, blood lipid spectrum, general clinical trials continued anticoagulant therapy for secondary stroke prevention is expedient.

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THE HYPOTHESIS OF THE DEVELOPMENT OF DIABETIC RETINOPATHY IN THE YAKUTS AT TYPE 2 DIABETES MELLITUS

ABSTRACT

The authors reported the results of the examination of patients with diabetic retinopathy (DR) at type 2 diabetes mellitus (DM), ethnic Yakuts, permanently residing in the areas of the Leno-Amga interfluve to the third generation, registered in the state register of the SD of the RS (Ya).

It is assumed that the development of DR at type 2 diabetes in ethnic Yakuts is due to the fact that the genetic (internal) factors involved in the metabolism of lipids and carbohydrates, the inflammatory process and oxidative stress, under the influence of environmental risk factors and hormonal expression in a certain age period, mainly in meno- and andropause, cause metabolic disorders and oxidative stress with an energy deficit in cells with active cleavage of ATP. Further chronic course of the inflammatory process and generalized oxidative stress lead to atherogenic damage of the vascular wall, causing pathological changes inherent to DR.

Keywords: diabetic retinopathy, type 2 diabetes mellitus, polymorphic variants of genes, Yakuts, hypothesis.

On 20, December 2006 the 61st UN General Assembly adopted the UN resolution, in which for the first time in the history of the United Nations the worldwide threat of non-infectious chronic disease - diabetes mellitus (DM) and its associated complications, entailing both a high risk for health of all nations, and huge economic losseswas announced.

Priority of national health systems in the world is to reduce blindness from diabetes by one third.

According to the World Health Organization data, the incidence of diabetes doubles every 10-15 years. Annually, more than 800,000 cases of DR are registered for the first time, including an increasing number of patients with type 2 diabetes [] (Kang P., 2012). Every year throughout the world up to 40 000 patients lose their vision] (Shadrichev FE, 2012). Real growth rates of morbidity greatly exceed these data, allowing you to identify diabetes as the largest non-infectious pandemic [] (US Census Burea, 2000).

The pathogenesis of DR is based on genetic, metabolic, hemodynamic, biochemical and immunological factors [(Neroev VV Sarygina OI Levkina OA, 2009; Moshetova LK, 2012; Frank NR, 1991, Geyer O., 1997; Feghhi M. et al., 2011; Bianchi L. et al., 2012]. To date, the pathogenesis of DR and type 2 diabetes are not fully investigated due to their long and asymptomatic treatment. Simultaneously DR problem is being discussed as a natural result of the development of diabetic changes in the retina [] Nesterov AP, 1997). The problems of the etiology, clinical manifestations, hereditary and genetic characteristics, prevention and prediction of diabetic retinopathy in patients with type 2 diabetes, including in some ethnic groups have not been fully studied [] (Balasubbu S. et al., 2010; Chun MY et al., 2010). Currently, medical genetics in ophthalmology is becoming increasingly important as the fundamental science that explains the etiology, pathogenesis, clinical polymorphism of multifactorial diseases, enabling the development of prevention and treatment of hereditary diseases. Genetically determined eye diseases play a significant role in shaping the entire spectrum of hereditary diseases in different groups of population. However, their share is 30 - 46% of cases, while in Saudi Arabia it reaches up to 84%.

In the Republic of Sakha (Yakutia) from 1960 to 2003, a five-fold increase in the incidence of type 2 diabetes was noted, especially among the indigenous population. The largest share (57%) of patients with type 2 are the Yakutsof the working age of 41 - 60 years, those aged over 60 constitute 33%. Results of population studies indicate the relative genetic uniformityofthe Yakuts (Sakha) in «Tat» C - allele of the Y-chromosome, the prescription of 6100 ± 940 years [Fedorova SA].

Purpose of this research is to study the clinical and genetic aspects of diabetic retinopathy in type 2 diabetes in the Yakuts to work out a hypotheses of the disease development.

MATERIAL AND METHODS

A group of scientists under the expedition conditions surveyed the natives –the Yakuts, residing in 5 districts of Lena Amginsky interfluveof Central Yakutia (Megino-Kangalasskii, Churapchinsky, Tattinsky, Amginsky, Ust - Aldan), where the predominant ethnic group of the Yakuts accounts over 97% of total population of the territory. According to the 2010 Census, the population of the Republic of Sakha (Yakutia) amounted to 958 thousand people.,of which the share of the rural population was- 35.9%, and in the studied areas 25.3%, i.e. 70.5% of the total rural population. The largest by population is MeginoKangalasskii-ulus (district) - 8%.

We used own data of retinopathy screening of theYakutsof Leno-Amginskyinterfluve held during expedition trips in the study area, the results of ophthalmic,reographic, morphometric, clinical and genealogical and genetic methods, laser retina photocoagulation with subsequent development of hypotheses of DR development in the Yakuts.

Sampling of patients of studied groups was formed by 1500 Yakuts, residing in rural areas of Lena Amginskyinterfluveof theRepublic of Sakha (Yakutia).

Main study group comprised 129 patients with DR in type 2 diabetes, ethnic Yakuts, residing in areas of Leno-Amginsky interfluve to the third generation and registered in the state register of DM RS (Y). Search of hereditary load to the DR in type 2 diabetes was performed in 432 patients, who had relatives with DR. In 78 patients with DR in type 2 diabetes genetic studies were conducted. Laser coagulation was held in 57 patients,rheographic research of ICA system and intraocular vessels was conducted in 40 patients.

The control group was formed of 162 healthy individuals older than 40 years, ethnic Yakuts living in identical environments, who are not in relationship with patients or other members of the study group to the third generation, without clinical and laboratory signs of diabetes and a family history of retinopathy and SD, with the presence of biallelic marker «Tat» C - allele Y-chromosome.

In both groups were women older than 50 years, the Yakuts to third generation. In the study group an increased BMI,

hyperglycemia and hypertension (p ≤ 0,05) werenoted. All patients were examined by an ophthalmologist with vizometriya, tonometry, ophthalmoscopy, furthermore theywere examined by an endocrinologist and neurogeneticist.

Isolation of DNA of patients was held in FGNU «Institute of Health of the Republic of Sakha (Yakutia)» (Yakutsk, Russia). Genotyping of polymorphic variants of genes was held in conjunction with the Department of Neurogenetics National Institute of Neurological Disorders (NINDS / NIH) (Betezda, USA). Haplotypingof polymorphic gene variants and statistical processing of genetic data were held jointly with the Institute of Biochemistry and Genetics, Ufa Scientific Center, Russian Academy of Sciences (Ufa, Russia). We used polymorphic variants of groups of genes involved in the metabolism of lipids and carbohydrates LIPC (Pr-514 variants, Ser193Asn), LPL (variants Int8, Int6, ser447tyr), ADPIQ (variants 276, Y111H, 45, -11377), PPARg (variants 18, 38, 477), LEP (variant 2549 C / A); oxidative stress (protein uncoupling mitochondrial oxidative phosphorylation) - UCP 2 gene (-862, ala55val, C'UTR 3) and inflammatory actions of TNF-alfa (-308, -857, -1031, -863), IL6 (-572, - 172), RSTN (-639, -420, 156, 298). Representativeness of the received results was provided by using standard and combined research methods.

RESULTS AND DISCUSSION

As a result of the study, the majority of patients with DR had the duration of type 2 diabetes up to 5 years (Fig. 1). DR in men was noted 2 times more often in the primary diagnosis of the prior disease. At the duration of type 2 diabetes over 5 years the proportion of DR reduces.

Clinical features peculiarities of DR in type 2 diabetes in group of ethnic Yakuts were noted in the distribution ofDR detection time depending on the duration of type 2 diabetes and sex of the patients (Fig. 1).

When analyzing the distribution of DR patients' age in type 2 diabetes according to sex it was revealed that most DR susceptible persons of both sexes were at the age of 50 -59 years. In this case, both gender groups, age group of 30 - 49 years there has been a uniform distribution of DR (31.4% and 31.3%, respectively). Increase of the proportion of DR in men was determined at the age of 40 - 49 years. With increasing of age of the patients there is a decrease in the proportion of DR in men and in the age group of 70 years and over 2 times dominated women. These results confirm the literature data on the rapid decompensation of type 2 diabetes in men.

In both gender groups preproliferative stage of DRprevails.Males proliferative stage exceeds twice the stage in women. These findings point to the role and specificity of internal factors in the defeat of fundus structures in men.

DR patients with type 2 diabetes revealed hemodynamic disturbances in the form of a significant reduction in blood flow and increase of ICA structure and intraocular vascular tone as the progression of DR, in contrast to patients with type 1 diabetes. The data obtained can be used in clinical practice as a criterion for determining the evaluation of the dynamics of blood flow deficit and vascular tone. DR is clinically characterized by abnormal choroidal or retinal vasculature with lesions of retinal neurons. It is known that photoreceptor degeneration is always accompanied by a weakening of retinal arterioles [48].

Diabetic retinopathy was observed in 43% of cases, mainly from their parents and siblings. Hereditary burden was in the sons of proband men - 39% , daughters of proband women -48%,daughters of probandmen - 27%

As a result of studies for the first time an association of polymorphic variants of genes involved in lipid and carbohydrate metabolism (lipoprotein lipase (LPL), adiponectin (ADIPOQ), periksisom-proliferator-activator receptor - gamma (PPARG), inflammatory processes: interleukin-6 (IL - 6) and Factor TNF-alpha (TNF - α) and oxidative stress (uncoupling protein 2 gene / UCP-2) wasrevealed. Simultaneously differences of identified polymorphic variants of genes depending on the sex of the patients (Table 1) were revealed. The role of polymorphic variants of leptin genes (LEP), resistin (RETN) and hepatic lipase (LIPC) in the development of DR in type 2 diabetes in the Yakuts has not been established.

First in Yakut ethnic group markers of increased risk of DR in type 2 diabetes: haplotypes GTGC (-11377C / G, 45T / G, 276G / T, 331T / C) gene Adipoq, haplotype TC (-1031T / C,-863C / A) gene TNFα, haplotype AC (rs285 (A / G), rs328 (S447X, C / G)) gene LPL, polymorphic markers-866G / A, Ala55Val (C / T) and 3 ¢ UTR I / D gene UCP-2 were revealed.

It is possible that the pathogenesis of DR in type 2 diabetes are fundamental links between involutional changes in the retina, environmental factors (environment, lifestyle, social and psychological problems, quality and nutrition, dietary habits), duration, severity and extent of the main decompensation disease accompanied by metabolic syndrome provided a genetic predisposition (family polymorphism with type 2 diabetes, and haplotypes of polymorphic variants of candidate genes associated with DR), which was established by us in ethnic Yakuts. Probably as a result of a single, complex, multi-stage pathological process in the retina of a patient with diabetic retinopathy involutionary para-inflammation transfers into acute inflammation with active chronic exposure to glucose toxicity, lipotoxicity and inflammatory cytokines contributing to the development of systemic inflammation. In this acute inflammatory process causes known functional and organic damage to the basic structures of the fundus (retinal tissue, blood vessels, optic nerve).

The period of development of diabetic retinopathy, reflecting generalized microangiopathy of the patient with type 2 diabetes indicates about the severity of diabetic vascular lesions. The average duration of diabetic retinal damage in

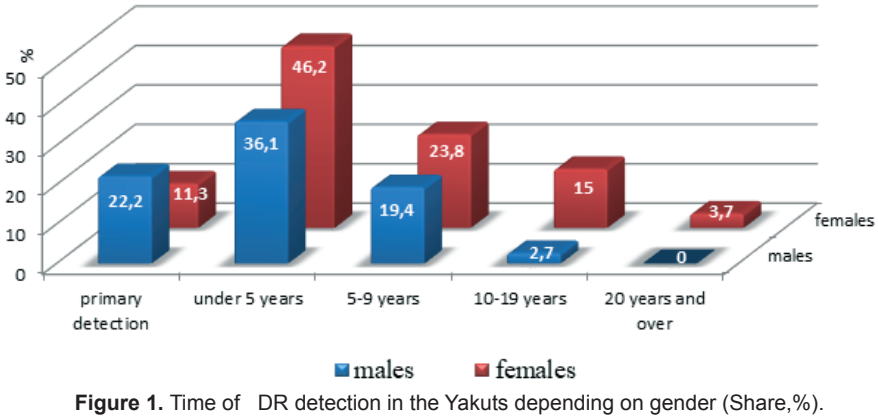

Figure 1. Time of DR detection in the Yakuts depending on gender (Share,%).

Distribution of polymorphic variants of genes depending on the duration of their expression and gender in patients with DR in type 2 diabetes

Polymorphic variants of genes / participation	Gender			Empirical term of gene expression (s
	Both sexes	Males	Females	
Gene, interleukin - 6 (IL - 6) / inflammation		aallele G rs1800796 (-572G / C) IL - 6	no	1,5
		genotype G / C allele and C Rs2234683 (-172G / C) IL - 6		
Lipoprotein lipase (LPL) / lipid metabolism	alleleArs 285 (G/A) LPL	Allele Ars 285 (G/A) LPL	alleleArs 285 (G/A) LPL	1,7 – 2,1
		Genotype CGrs 328 (C/G) LPL	no	
Tumor necrosis factor - α (TNF-α) / Inflammatory process	alleleTrs 1799964 (- 1013 T/C) TNF - α	alleleTand genotypeTTrs 1799964 (- 1013 T/C) TNF - α	alleleTrs 1799964 (- 1013 T/C) TNF – α, genotypeTTis missing	3,4
Geneofuncouplingprotein-2 (UCP-2) / oxidative stress		genotypeGA rs659366 (-866 G/A) UCP-2	Is missing	3,4
Periksisom gene-proliferator-activator receptor - gamma (PPARG) / lipid and carbohydrate metabolism		genotypeCT and allele Trs3856806 (1431C/T, Ser 477 Ter) PPARG	genotypeCCandalleleCrs1801282 (34C/G) PPARG	4,3
Adiponectin gene (ADIPOQ) / / lipid and carbohydrate metabolism		Is missing	genotypeCC rs17366743 (Y111H, 331T/C) ADIPOQ	5,6 – 6,3

the Yakuts with type 2 diabetes was 3.6 years. At the same time in one third of patients primary DR is detected in establishing primary diagnosis. At the same time long-term preservation of non-proliferative stage of diabetic retinopathy in patients with type 2 diabetes for more than 17-20 years, especially in women should be paid attention to.

Males Yakuts, unlike female Yakuts, with DR having type 2 diabetes, a fundamental factor in the development of the disease is the genetic abnormalities, associated with a greater proportion of hereditary defects in genes LPL, PPARG, IL - 6, TNF - α, UCP-2.

It is possible that such a time transfer ofDR development is confirmed in some patients with the presence of protective factors of internal body and vice versa, while in others - a genetic predisposition to the disease. It should be noted that these patients live in identical environments. However, in homogeneity of identified gender manifestations, clinical course and the presence of a genetic polymorphism in patients with DR in Yakut ethnic group have been revealed.

The obtained results of the study to identify the association of polymorphic variants of the genes of cytokines and adipokines with depression showed that in patients with type 2 diabetes gender differencesare determined .This points to the crucial role of hormonal expression and the background state of the organism of patients on the development of DR, especially during menopause and and ropause.

Uncoupling protein 2 (UCP2) is an enzyme that prevents the development of insulin cells of the pancreas and the inner mitochondrial membrane transporter, which dissipates the proton gradient, releasing the stored energy in the form of heat. Therefore, it is important in protecting against obesity. Probably provided pronounced oxidative stress also its function is impaired, which is manifested in the clinical features of the metabolic syndrome in patients with DR.

The results of our study are consistent with the opinion of the researchers on the existence of a «thrifty» genes and U-allele developed during evolution and ensuring energy supply with moderate consumption of food and hunger [].

CONCLUSION

Thus, the development of diabetic retinopathy in type 2 diabetes mellitus in ethnic Yakuts presumably due to the fact that genetic (internal) factors involved in lipid and carbohydrate metabolism, inflammation and oxidative stress, under the influence of risk factors of the environment and hormone expression in certain age period, mainly in the meno- and and ropause cause metabolic and oxidative stress with an energy deficit in the cells of the active cleavage of ATP. Further chronic inflammatory process and generalized oxidative stress lead to atherogenic damage of the vascular wall, causing pathological changes characteristic of DR. The proposed mechanism of genetically-induced diabetic retinopathy in type 2 diabetes mellitus in ethnic Yakuts is shown in the form of a scheme (Fig. 2).

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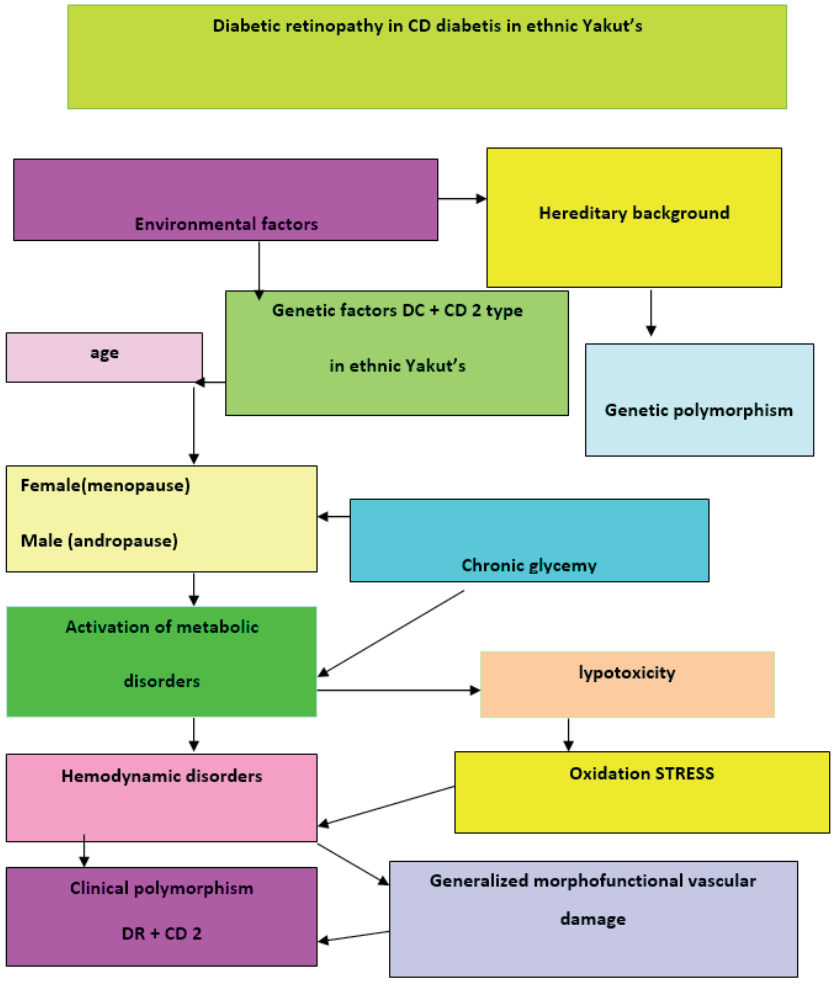

Figure 2. The mechanism of DR in type 2 diabetes in ethnic Yakut's.

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THE ISSUE MATERIALS. ORIGINAL RESEARCH

O.V. Begiev, Ya.V. Vinokurova, V.G. Begiev, A.S. Golderova

FEATURES OF DEVIANT BEHAVIOR IN CHILDREN WITH HEARING IMPAIRMENT IN THE RS (YA)

ABSTRACT

In article the tendency to deviant behavior, social and psychological adaptation and adaptability of children with a hearing disorder is considered. Reliable distinctions on expressiveness of some indicators in the comparative analysis of scales of deviant behavior of children with a hearing disorder are received and children without similar violations (control group). In the Sakha (Yakutia) Republic according to the Ministry of Labor and Social Welfare disability on hearing makes 2,5 for 1000 the population. Results of a research have confirmed interrelation of deviant behavior with adaptability of children with a hearing disorder.

Keywords: deviant behavior, adaptability, social and psychological adaptation, children with a hearing disorder.

According to World Health Organization about 10% of the population of the planet has this or that hearing disorder. On one thousand newborns 3-4 children are born with problems of hearing, 50% of cases of a hearing disorder are connected with a genetic factor. According to the Ministry of Labor and Social Welfare, 2 443 people, being hard of hearing persons live in the Sakha (Yakutia) Republic (2,5 for 1000 of the population).

Children with hearing impairment are much more difficult to adapt to the society than healthy children, or rather, the society is extremely difficult to accept children with the limited opportunities of health (LOH). Because of this, children with hearing impairment may develop deviant behavior [13].

«Deviant behavior — one of types of deviant behavior connected with violation of the relevant age, social norms and rules of conduct characteristic of the microsocial relations and small gender and age groups. It is possible to call this type of behavior anti-disciplinary» [1].

Deviant behavior can arise not only from external factors (coercion and humiliation surrounding the deviant or lack of attention and love from them), but also from the internal (predisposition to deviation, behavior or curiosity) [3,9]. The person can become a deviant not only because the society in which the individual currently resides does not accept it, but also because of the personality characteristics of the person.

Psychological features of children with hearing impairments attracted the attention of teachers and psychiatrists since the middle of the XIX century. The development of Russian surdopedagogics of that time is connected with the pedagogical activity and research of such famous scientists as V.I. Fleury, A.F. Ostrogradsky, N.M. Lagovsky, F.A. Rau. Thus, V.I. Fleury described the features of the development of deaf movements, indicating inaccurate coordination, uncertainty of movements.

N.M. Lagovsky wrote about presence at deaf children of the remains of hearing which can be made active and developed. He possesses one of the first attempts of creation of classification of the people having a hearing disorder. In compensation of the broken mental development at deafness N.M. Lagovsky noted a big role of visual perception [2, 16].

The deviant behavior at children and teenagers with LOH often is reaction to adverse conditions (social or microsocial) in which they appear, then, as socially acceptable ways of behavior weren't created yet. Disadaptation and deviant behavior are closely connected and influence at each other [11]. When carrying out correctional work it is necessary to consider not only a mental, but also social condition of the child [15], especially the child with LOH. However the help to the children subject for any reasons for deviations, is generally limited to tasks of correctional schools in which there are such children.

Rise in crime among minors shows small efficiency of the existing system of work on prophylaxis of deviant behavior and the fact that it is referred generally on social aspect and doesn't interact with personal disturbances in any way. In modern society along with body height of positive deviations amplify as well negative deviations: alcoholism, crime, use of psychoactive substances and immoral behavior [4]. According to sociologists, today the demoralization characteristic of 85% of the population of the country in a varying degree. Apathy to agents of achievement of the goal, corruption, cynicism, extremism become typical reactions to an anomy.

Emergence of deviant behavior at teenagers is usually referred to manifestations of crisis of awkward age. "The teenage deviation which is shown in the most various forms gains lines of the mass phenomenon which differs in steady tendencies of growth, wide circulation on teenage and sometimes even children's part of youth. For 2014 in Russia every 20th crime has been committed by minors or with their participation. In only one year 54 369 minors who have committed crimes" [10] have been revealed.

Thus, at present, despite the increase in methods and methods of work on prevention with minors, crisis situations in all spheres of life lead to an increase in deviation among adolescents. Referring to official statistics and scientific research of scientists, we can talk about the apparent increase in deviant behavior among adolescents [4, 10]. The greatest difficulties in adapting to the requirements of modern complex economic, political and spiritual life are experienced by adolescents with various developmental disorders [6, 7, 9].

The purpose of the study is to reveal the propensity to deviant behavior in children with hearing disorder.

METHODS AND MATERIALS OF A RESEARCH

The research has been conducted on the basis of the special (correctional) comprehensive school - a boarding school of the Yakutsk. 7 boys (33.3%), 14 girls (66.7%) have been examined 21 school students of 8-12 classes, from them. Control group 25 school students of comprehensive school № 24 of Yakutsk, have made of them 14 boys (56%), 11 girls (44%). The following techniques of psychological diagnostics have been used: determination of tendency to deviant behavior (Oryol A.N.) [8]; technique of diagnostics of social and psychological adaptation (Rogers-Daymonda) [12];

multilevel personal questionnaire “Adaptability” (A.D. Maklakova, S.V. Chermyanina) [5]. Statistical data processing was carried out on the personal computer with use of the computer SPSS programs. Equality of selective averages was checked by parametrical t-criterion of Student for independent selections, the correlation analysis of indicators of blood has been carried out by bilateral criterion of Spirmen.

RESULTS AND DISCUSSION

Determination of tendency to deviant behavior by a technique Oryol of A.N. (1998) at pupils of correctional school with a hearing disorder (n=21) has revealed the following features on scales:

“the tendency to overcoming norms and rules” is revealed at 16 (76,2%) teenagers predisposition to denial of the standard norms and values, examples of behavior, i.e. tendency to oppose own norms and values group, a tendency “to break tranquility”, and at 5 (23,8%) - tendency to follow stereotypes and the standard standards of behavior.

“the tendency to addictive behavior” at all 21 (100%) respondents is absent that demonstrates good social control of behavioural reactions;

“the tendency to the self-damaging and self-destroying behavior”, i.e. low value of own life, tendency to risk, the expressed need for thrills, sadomasochist tendencies is revealed at 5 (23,8%) the examined persons; at 16 (76,2%) - are absent readiness for realization of the self-destroying behavior;

“tendency to aggression and violence”, i.e. existence of aggressive tendencies it is revealed at 7 examinees (33,3%); at 14 (66,7%) - it isn’t revealed, they note unacceptability of violence as cures of problems and not typicality of aggression as way of an exit from the frustrating situation;

“strong-willed control of emotional reactions”, i.e. unwillingness or inability to control behavioural manifestations of emotional reactions at all 21 (100%) examinees was weak;

(deviations from the standard norms) at all 21 (100%) examinees the low level of social control had “tendency to delinquent behavior”.

A comparison was made of the scores of the propensity scales for deviating behavior according to the Oryol A.N. method in the two study groups [14]. The results of the comparative analysis showed that the statistically significant differences in the high significance level ($p < 0.001$) were found on the “propensity to overcome norms and rules” scale. On average, the propensity to overcome norms and rules in a group of children with hearing impairment is higher than in children without similar pathologies. This indicates that children with hearing defect experience insufficient communication experience and as a result there are difficulties in understanding the norms and rules of behavior in various situations. “Tendency to addictive behavior” in group of children with a hearing disorder were higher, than in control group. The most significant distinctions are revealed on tendency scales “to the self-damaging behavior” ($p < 0.001$), “to aggression and violence” ($p < 0.001$) that demonstrates insufficient formation of emotional control at children with acoustical defect. Children with a hearing disorder have indicators of “strong-willed control of emotional reactions” ($p < 0.001$) and “tendencies to delikventny behavior” ($p < 0.001$) also were significantly high in comparison with control group (fig 1).

Diagnosis by the method of K. Rogers and R. Diamond, evaluating the socio-psychological adaptation and the personality traits associated with it, indicates that in all the surveyed adolescents the scores of all scales (adaptation, acceptance of others, internality, self-perception, emotional comfort and desire for dominance) vary Within the limits of the norm. Analysis of data on the multi-level personal questionnaire “Adaptivity” (AD Maklakova, SV Chermyanin) found that in children with hearing impairment:

“psychological fastness” at 12 (57,1%) appeared below an average, i.e. the low level of a behavioural regulation and a certain predilection to psychological failures, lack of adequacy of a self-rating and a real perception of reality. At 9 (42,9%) examinees psychological fastness is higher than an average that indicates the high level of psychological fastness and a behavioural regulation, a high adequate self-rating and a real perception of reality.

“communicative abilities” and “moral normativity” at 21 (100%) the examinee indicators are in norm limits.

“personal adaptive potential” at 5 examinees (23,8%) were in group of high and normal adaptability, i.e. easy adaptation to new conditions, collective, adequate orientation in a situation, fast elaboration of strategy of the behavior, not conflictness and high emotional stability is characteristic of them. At the others 16 (76,2%) examinees the satisfactory adaptability, i.e. a possibility of development of various accentuation when changing habitual conditions and activity is revealed. These persons, as a rule, have low emotional stability, asocial failures, manifestation of aggression and conflictness are possible. The faces of this group demand individual approach, constant observation, correctional actions.

For the purpose of assessment of interrelation of deviant behavior (Oryol A.N.) and adaptabilities (Maklakova A.D., Chermyanina S.V.) at children with a hearing disorder has carried out the correlation analysis which has revealed 5 significant interrelations:

between scales “tendency to overcoming norms and rules” and “moral normativity” ($p<0,05$), confirming insufficient formation of understanding of norms and rules which in turn leads to problems of respect for norms and rules of public behavior;

between scales “tendency to addictive behavior” and “moral normativity” ($p<0,05$), pointing that children with a hearing disorder because of inadequacy have estimates of the place in collective and not ability of maintenance of the standard standards of behavior there is a danger of «leaving» from reality by means of change of a mental state, for example, by means of psychotropic drugs;

between scales “strong-willed control of emotional reactions” and “psychological stability($p<0,05$), confirming low control of behavioural and emotional regulation;

between scales “strong-willed control of emotional reactions” and “communicative abilities” of high significance value ($p<0,05$), i.e. because of not formation of emotional control there are difficulties in creation of contacts with people around;

between scales “tendency to delinquent behavior” and “the personal adaptive potential” ($p<0,05$).

Thus, development of the personality and consciousness of children with a hearing disorder takes place in difficult conditions. Because of limited opportunities of spontaneous assimilation of social experience arise various degree delay of process of processing of information, difficulty of adaptation and communication with surrounding people that in turn can lead to emergence of deviant behavior.

Conclusions

1. Children with a hearing disorder have predilection to deviant behavior with disturbance of strong-willed control and

emotional reactions.

2. At assessment of social and psychological adaptation of children with a hearing disorder of aberrations it wasn't taped, however such state is possible within educational institution.

3. Adaptability assessment at children with a hearing disorder showed depression of an indicator from norm, i.e. predilection to psychological failures, signs of different accentuations and implication of aggression and of conflict is observed.

4. Implications of predilection to deviant behavior at children with a hearing disorder are expressed stronger, than at children without similar pathology.

5. The deviant behavior at children with a hearing disorder is interconnected with adaptability level, therefore, it is possible to assume that than adaptability level at children with a hearing disorder is lower, predilection to deviant behavior is expressed to those.

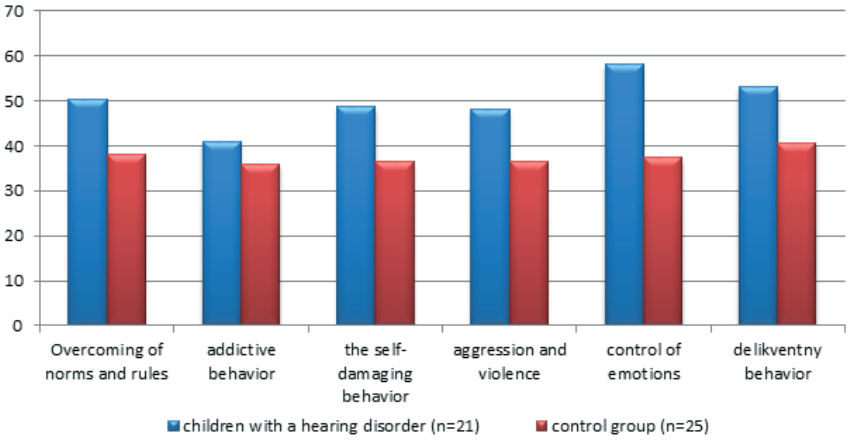

Figure 1. The comparative characteristic on indicators of scales of tendency to deviant behavior at children with a hearing disorder and control group

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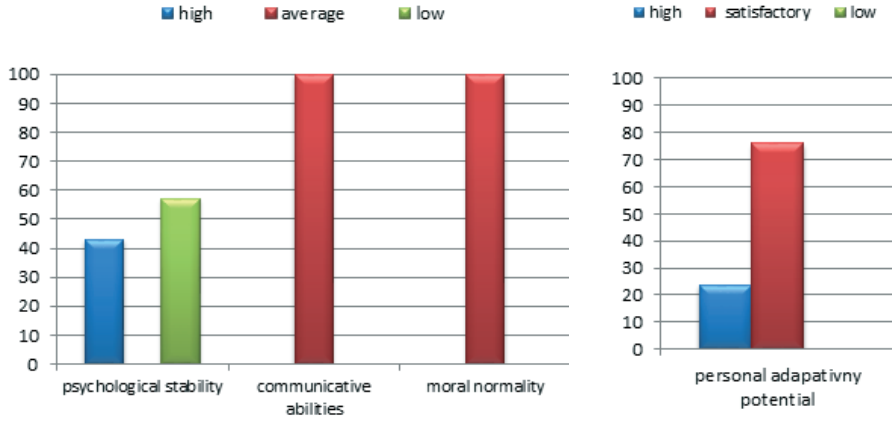

Figure 2. Results of the multilevel personal questionnaire «Adaptability» (Maklakova A.D., Chermyanina S.V.) at children with a hearing disorder (%).

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STRESS-PROTECTIVE ACTIVITY OF THE EXTRACT *FORNICIUM UNIFLORUM L.*

ABSTRACT

The results of studies on the adaptogenic properties of Fornicium uniflorum L. extracts has been given in the present article. The aim of the study was to evaluate stress-protective activity of extracts from Fornicium uniflorum L. at immobilization stress in white rats. The dry extract from Fornicium uniflorum rhizomes has stress protective properties in 18-hour immobilization stress simulated in white rats. It has been revealed that the given plant remedy prevents from the development of the "Selye's triad" signs: hypertrophy of renal glands, involution of immune competent organs (thymus and spleen), as well it prevents heavy destructions in the stomach mucosa.

Keywords: adaptogens, phytoecdysteroids, Fornicium uniflorum L., immobilization stress, antioxidant activity.

INTRODUCTION

At the present stage of the social development, there is a growing incidence rate of so called "civilization diseases"; there are recorded new, previously unknown nosological entities and syndromes caused by the depression of adaptive mechanisms, especially in people living in unfavorable environmental conditions. In the Extreme North conditions, a human body is mostly subjected to the influence of the whole complex of unfavorable factors which exhaust the adaptive resources, affect the health and bring about diseases. In this connection, the increase of the non-specific resistance of the body by pharmacological remedies – adaptogens is regarded as a priority. For this purpose, the development of the remedies of plant origin seems to be the direction with good prospects, as they are free from the main disadvantages of chemotherapeutic drugs such as drug habituation, toxicity, the development of side reactions during chronic administration of the drug.

The group of the plant adaptogenes is rather numerous, many of which were anciently used in the folk medicine as tonics and health improving means. A mechanism of their effect remained unknown for a long time. In the modern concept, the biologically active substances responsible for the pharmacological effect of the plant adaptogenes are conditionally divided into three groups: phenolic compounds, tetracyclic triterpenoids (triterpenoid glycosides) and oxylipins. These compounds are kin to endogenic mediators and hormones of the central stress-realizing systems of the body, namely, sympathicoadrenal and hypothalamic-pituitary-adrenal ones.

However, these mechanisms are not by far solitary ones; there are many other biologically active substances contributing to adaptogenic properties of a plant [15]. Phytoecdysteroids fall into the group of such substances; they are polyhydroxylated sterins, structurally identic or kin to true molting hormones. They are practically contained in all plants but the difference in their concentration reaches to 8-9 orders [8]. Physiologically, the phytoecdysteroids influence the body of a human and warm-blooded animal in a variety of ways: they regulate metabolism being ligands for endocellular and membrane receptors; they are capable to change homeostasis of the body; have an effect on the growth, differentiation and scheduled cell death; they have psychostimulant, adaptogenic,

stimulating, immunomodulating and other effects [1, 5, 6, 10, 13]. Unfortunately, in spite of the high biological activity of ecdysteroids, at present, there is only one officinal ecdysteroid-containing plant – *Leuzea carthamoides*, various medicinal forms of which are used in the clinical practice. In this connection a search of new natural sources of phytoecdysteroids and the study of their composition and properties is a promising direction of the modern pharmacological science.

We have developed a method for preparing the dry extract from the rhizomes of *Fornicium uniflorum* L. – another ecdysteroid-containing plant; besides it contains flavonoids, amino acids, vitamins and polysaccharides [4].

The aim of the present work was to evaluate the stress-protective activity of the *Fornicium uniflorum* dry extract in immobilization stress induced in rats.

MATERIALS AND METHODS

Experiments were carried out on the male and female Wistar rats weighing 180-200 g observing the rules of the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasburg, 1986) and the order of the RF Health Ministry N 267 concerning the GLP rules (19.06.2003). The animals were divided into 4 groups: intact group, control group, the first and second experimental groups of animals. The rats of the first experimental group received intragastrically the aqueous solution of the *Fornicium uniflorum* extract at a dose of 10 ml/kg for 7 days, preventively before exposure to stress (once a day, 30 min. before feeding). The rats of the second experimental group received a preparation of comparison – dealcoholized extract from *Leuzea carthamoides* at a dose of 5.0 ml/kg. The animals of the control group received the distilled water in the same volume and according to the analogous scheme. On the 7th day of the experiment the animals of the control and experimental groups were exposed to immobilization stress. The immobilization stress was simulated by fixing the animals in supine position for 18 hours [11]. After the experiment, the animals were decapitated under ether narcosis; the blood was taken for biochemical analyses, the organs (adrenal glands, thymus, spleen, stomach) were removed; then the evidence of alterations in the organs after stress was estimated; the intensity of destructive injuries in the stomach mucosa was evaluated by calculating the Pauls' index [2]. The intensity of free radical oxidation processes was evaluated taking into account the accumulation of malonic dialdehyde (MDA) in the blood serum [7]. The state of the antioxidant system was judged by the activity of catalase [3] and superoxide dismutase (SOD) and the content of the reduced glutathione [12]. The statistical processing was carried out by the standard method with the use of the Student's t-criterion.

RESULTS AND DISCUSSION

The studies have revealed that the 18-hour immobilization stress in rats is followed by the development of the classic "Selye's triad": the hypertrophy of adrenal glands, involution of immune competent organs and injury of the stomach wall mucosa (Tables 1 and 2). In the rats receiving the *F. uniflorum* extract, there was noted a reduction in the severity of the "Selye's triad" signs: the mass of adrenal glands was 30% lower, the mass of thymus and spleen was 48 and 41% higher respectively, as compared to the same indices in the rats of the control group. The immobilization stress in the rats of the control group caused the injuries in the stomach wall mucosa in the form of droplet hemorrhages, erosions and stripe-like ulcers. In the rats receiving the *F. uniflorum* extract, stress-induced injuries in the stomach wall mucosa were less expressed. No erosions of the stomach wall mucosa in the rats of the first experimental group were noted as distinct from the animals of the second experimental group. Deep injuries in the stomach wall mucosa in the form of stripe-like ulcers neither were noted in the both experimental groups. Thus, the administration of the tested remedy to the rats rendered the marked gastro-protective effect in exposure to stress.

The preventive introduction of the *F. uniflorum* extract had the marked antioxidant effect: the MDA concentration was 27% lower; the catalase and SOD activities were higher by 32% and 70% respectively, and the reduced glutathione was 23% higher as compared with the same indices in the animals of the control group (Table 3). The indices of the antioxidant activity in the rats receiving the tested remedy were comparable with those in the rats receiving *L. carthamoides*.

The findings of the studies demonstrate that the *F. uniflorum* extract has the marked stress-protective property and prevents the development of the stress-induced reaction, namely, the hypertrophy of adrenal glands, involution of immune-competent organs and destructions in the stomach wall mucosa in rats exposed to immobilization stress. It is known that the activation of free radical oxidation plays an important role in the development of stress-induced injuries in the organs; it results in the impairment of the structural integrity in the lipid layer of cell membranes and decreased activity of membrane-bound enzymes. The many-hours-long immobilization is followed by extensive production of free radicals and intensification of the LPO processes, as evidenced by the increase of the MDA content in the blood serum and decrease in the activity of the antioxidant protection enzymes in the rats of the control group. The stress-protective effect of the tested remedy is associated with its inhibiting influence on the FRO and LPO processes. Besides the substances of the phenolic nature, the phytoecdysteroids, presumably, also contribute to the realization of the antioxidant activity of the *F. uniflorum* extract, as they have direct antiradical properties and are capable to increase the level of the endogenic antioxidant protective system in animals [14].

CONCLUSION

The *F. uniflorum* extract has the marked stress-protective property in the 18-h immobilization stress induced in rats. The tested remedy protects from the development of the classic "Selye's triad": the hypertrophy of adrenal glands, involution of immune competent organs and prevents the development of destructive processes in the stomach wall mucosa. The stress-protective effect of the *F. uniflorum* extract is due to the marked antioxidant activity of phenolic compounds and phytoecdysteroids contained in the plant. The antioxidant effects of the given substances are due to the direct antiradical effect, as well to their indirect influence in the free radical processes and their capability to increase the level of the endogenic antioxidant protective system. The effect of ecdysteroids is not limited to antioxidant properties; they also have hypolipidemic, anti-sclerotic and anti-diabetic properties; they promote the functions of the liver, kidneys and modulate the activity of the immune system. The stress-protective effect of *F. uniforum* is due to its capacity to regulate mechanisms of adaptation in the body at stress. The findings of the research testify the applicability of the *F. uniflorum* extract as an adaptogenic remedy in stress, physical and psycho-emo-

tional strain, in extreme environmental conditions and as a remedy for prevention of various diseases.

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Table 1

The influence of the *F. uniflorum* extract on the evidence of the "Selye's triad" signs in rats exposed to 18-hour immobilization stress

Animal groups	Relative mass of organs (mg/100g)		
	thymus	spleen	adrenal glands
Intact (n=6)	47,0± 2,88	491,6±37,84	16,8±1,77
Control (n=8) (stress+ H2O)	34,6± 3,89	282,6 ±20,56	25,0± 2,24
Experimental 1(n=8) (stress+ *F. uniflorum*)	51,5± 5,79*	400,8± 29,42*	17,5± 1,72*
Experimental 2 (n=8)	40,2± 3,00	404,0±26,36*	17,6± 0,75*
(stress+ *L.carthamoides*)	40,2± 3,00	404,0±26,36*	17,6± 0,75*

Note: * – hereinafter the difference is significant in comparison with the control when $p \leq 0.05$

Table 2

The influence of the *F. uniflorum* extract on the evidence of the injuries in the stomach mucosa of rats exposed to 18-hour immobilization stress

Indices	Animal groups			
	Intact (n=8)	Control (stress+H2O) (n=8)	Experimental 1 (stress +F. *uniflorum* extract) (n=8)	Experimental 2 (stress+*L. carthamoides* extract) (n=8)
Hemorrhages, %	0	100	40	50
Mean hemorrhages per a rat	0	4,8	2,5	2,7
PI for hemorrhages	0	0,38	0,12	0,13
Erosions, %	0	60	0	30
Mean erosions per a rat	0	2,6	0	0,6
PI for erosions	0	0,13	0	0,02
Stripe-like ulcers, %	0	50	0	0
Mean ulcers per a rat	0	1,0	0	0
PI for ulcers	0	0,04	0	0

Note: PI – Pauls' index.

Table 3

The influence of the *F. uniflorum* extract on the intensity of the FRO processes and the state of the antioxidant system in rats exposed to 18-hour immobilization stress

Показатель	Groups			
	Intact (n=8)	Control (stress+H2O) (n=8)	Experimental 1 (stress +*F. uniflorum* extract) (n=8)	Experimental 2 (stress+*L. carthamoides* extract) (n=8)
MDA, µmol/l	4,8±0,21	10,3±0,55	7,5±0,28*	7,8±0,62*
Reduced glutathione, mmol/ml	0,73±0,041	0,56±0,024	0,69±0,07*	0,75±0,050*
Catalase, mkat/l	25,5±1,13	15,4±0,97	20,4±2,03*	25,8±1,54*
SOD, activity unit	11,2±0,78	4,3±0,31	7,3±0,65*	6,8±0,27*

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METHODS OF DIAGNOSIS AND TREATMENT

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EVALUATING THE EFFICIENCY OF NEURAL NETWORK PROGNOSIS OF HEALTH QUANTITATIVE INDICATORS IN PATIENTS WITH DISEASES OF THE HEPATOPANCREATODUODENAL ZONE

ABSTRACT

Objective was in the public health study to select and assess practical application possibilities of optimal biosatistical methods for monitoring the functioning of artificial neural networks trained for predicting the quantitative health indicators in patients with hepatopancreatoduodenal zone diseases.

Methods. The study was conducted on materials of 385 patients with hepatopancreatoduodenal diseases who underwent in-patient treatment in hospitals in the city of Kursk. There was used the internally developed information system "System of Intellectual Analysis and Diagnosis of Diseases" (Certificate of State Registration of the Program for Computers n. 2017613090). The application provides functionality for creation, configuration, training and practical application of the artificial neural network, multilayer perceptron. Hyperbolic tangent was used as an activation function.

Results. There is presented the experience of selection and practical application of mathematical methods for controlling the operation of the artificial neural network in assessing the quantitative health indicators in patients with peptic ulcer, cholecystitis and pancreatitis. There is shown the expediency of the primary interpretation of the output layer neuron OUT value where $OUT \in \mathbb{R} \wedge OUT \in (-1; 1)$ to the scale and units of measurement of the evaluated health indicator with subsequent statistical processing of the array of obtained values. The optimal mathematical methods include: the calculation of the means (and their errors) for the arrays of empirical and resulting from the neural network operation values, with subsequent comparison of the arrays using the χ^2 criterion and determining the significance level α. The next step is to estimate the forecast mean error (ME), the forecast mean square error (MSE), the forecast mean absolute error (MAE), the maximum forecast error for the 99th and 95th normal distribution percentiles, the mean percent error (MPE) and the average absolute percentage error (MPAE). An example of a tabular representation of the analysis data is given.

Conclusion. The most convenient and informative mathematical methods for assessing the operation quality of the artificial neural network predicting the quantitative health indicators in patients with hepatopancreatoduodenal zone diseases are various types of forecast errors (mean error, mean absolute error, mean percentage absolute error, etc.). It is expedient to calculate the maximum absolute error of the forecast (for p = 0.05 and p = 0.01), which increases the visibility of the results, as well as the χ^2 criterion, that allows the estimation of hypothesis' significance that there are no differences between the arrays of calculated and empirical quantitative indicators.

INTRODUCTION

The organization of public health in modern society is based on the systematic approach to decision-making [12, 7, 11]. Adapted mathematical programme complexes [4, 5, 6], as well as management information systems based on the principles of artificial neural networks (ANN), gain importance in questions of predicting health indices in patients with peptic ulcer, cholecystitis and pancreatitis [13, 1, 10]. Such tools are considered as the most promising and convenient due to their ability to process complex data [2, 8], demonstrating signs of cobweb causality [3]. However, intelligent systems require mechanisms to control their functioning.

The purpose of our study was in the public health study to select optimal biosatistical methods for monitoring the functioning of artificial neural networks designed for predicting the quantitative health indicators in patients with hepatopancreatoduodenal zone diseases and assess their practical application possibilities.

MATERIALS AND METHODS

The study involved 385 patients undergoing in-patient treatement in hospitals in the city of Kursk for the hepatopancreatoduodenal zone diseases – peptic ulcer, cholecystitis and pancreatitis. The mathematical apparatus was implemented in the internally developed software – "System of Intellectual Analysis and Diagnosis of Diseases" (Certificate of State Registration of the Program for Computers n. 2017613090). The system posesses functionality to create, configure, and train ANNs of multilayer perceptron type. Hyperbolic tangent was selected as an activation function. The dimension of the input vector of the network was 16. There was used the information on the presence of bad habits, sex, the year of birth, the presence of disability, hereditary factor, the history of occupational hazards, etc. The network included 10–14 neurons in each of the 3 hidden layers. The age of probable hospitalization was used as the main output. Overtraining of the network was monitored by cross-checking method, for which the training set was randomly broken into two parts. The information on 355 patients was used for training, and control was carried out on the material of 30 clients.

RESULTS AND DISCUSSION

The activation function – the hyperbolic tangent takes the values $OUT \in \wedge \mathbb{R} \wedge OUT \in$ (−1; 1) that in this form cannot be interpreted by the user for evaluation of quantitative patient's health indexes. The obtained value (y) can be adapted using the statistics of the training set according to the formula (1):

$$y_{dN} = y \times \max(z_{max} - Mz, Mz - z_{min}) + Mz,$$

where Mz, z_{max} and z_{min} – respectively, the mean, maximum and minimum values of this output in the training set.

The adapted value of y_{dN} is quite suitable for further practical processing and has the same units of measure as the output of the training set, for example, years. Nevertheless, this value needs statistical verification. In our study, we applied the following calculated indexes:

1. The standard error mz of the mean

empirical Mz, calculated from the objective data of the output z of the training set.

2. The average calculated My_{dN} and its standard error my_{dN} are determined on the base of the values of the y_{dN} obtained as a result of the ANN operation. Calculations of the means and their standard errors are conducted traditionally.

3. The χ^2 criterion was chosen to confirm or refute the hypothesis that there is no difference between the empirical and forecasted indexes in the sample population. Calculation of the criterion is carried out according to the formula (2):

$$\chi^2 = \sum \frac{(z - y_{dN})^2}{y_{dN}}$$

Where z – empirical values of the index, y_{dN} – the predicted value of the index.

The significance of the hypothesis (α) is estimated by the comparison of the criterion χ^2 with the critical points known from the tables for the degree of freedom k = n − 1.

4. The forecast mean error is calculated as the arithmetic mean of the difference between the empirical and predicted value of the studied quantitative index (with changes from [9]) (3):

$$ME = \frac{\sum d}{n}$$

where $d = z - y_{dN}$.

The sign of this indicator allows assessing whether the forecast is too high or too low, and its absolute value indicates the size of the error.

5. The forecast mean square error MSE allows to evaluate the operation of the ANN as a whole and is calculated as (4):

$$MSE = \frac{\sum d^2}{n}$$

6. The forecast mean absolute error MAE is calculated similarly to ME using formula (5):

$$MAE = \frac{\sum |d|}{n}$$

For MAE, it is possible to calculate the standard error mMAE according to the general rules.

7. The MAE and mMAE values obtained in the previous calculation stage are useful for calculating the visually useful value – the maximum absolute forecast errors $|d_{max99}|$ (6) and $|d_{max95}|$ (7):

$$|d_{\max 99}| = MAE + zp_{99} \times mMAE$$

$$|d_{\max 95}| = MAE + zp_{95} \times mMAE$$

where zp_{99} и zp_{95} – 99th and 95th percentiles of the standard normal distribution, respectively.

$|d_{max99}|$ and $|d_{max95}|$ are numbers that do not exceed the forecast absolute error |d| with a probability of 99 or 95%.

8. The mean percentage error MPE, like ME, allows estimating the prediction offset (8).

$$MPE = \frac{\sum \frac{d}{z}}{n} \times 100$$

For properly trained ANN |MPE| should not exceed 5%.

9. The mean percentage absolute error MPAE is similar to MAE, but allows estimating the error in the scale of the index (9).

$$MPAE = \frac{\sum \frac{|d|}{z}}{n} \times 100$$

If MPAE ≤ 10%, the prediction accuracy is considered high, if 10% < MPAE ≤ 20% – good, if 20% < MPAE ≤ 50% – satisfactory and if MPAE > 50% – unsatisfactory. MPE and MPAE are normalized and therefore can be used to compare ANN outputs that have unequal units of measurement and scale, as well as to compare different ANNs.

It is possible to use both the entire training set and a subset with smaller number of units for statistical evaluation of the ANN operation quality. If the latter case the units for calculation are selected randomly.

The results of the calculation can be presented in the form of a table similar to Table. 1. Such presentation of the analysis' results allows the researcher lacking special mathematical knowledge to get acquainted with the evaluation of the quality of the ANN training in a visual form, and also to monitor the operation of the trained network in clinical conditions.

The results of the ANN training for forecasting the age of probable hospitalization in patients with hepatopancreatoduodenal zone diseases, n = 385

Index	Age of probable hospitalization
Mean empirical, Mz, years	52,86
Standard error, mz, years	0,83
Mean calculated, MydN, years	53,31
Standard error, mydN, years	0,83
χ2 criterion	47,13
Significance level, α	≤0,001
Forecast mean error , ME, years	−0,45
Forecast mean square error, MSE, years 2	6,02
Forecast mean absolute error, MAE, years	1,87
Standard error of MAE, mMAE, years	0,08
The error of the forecast will not exceed (p95 percentile), years	2,00
The error of the forecast will not exceed (p99 percentile), years	2,06
Mean percentage error, MPE, %	−1,01
Mean percentage absolute error, MPAE, %	4,03

CONCLUSION

The most convenient and informative mathematical methods for assessing the operation quality of the ANN predicting the quantitative health indicators in patients with hepatopancreatoduodenal zone diseases are various types of forecast errors (mean error, mean absolute error, mean percentage absolute error, etc.). It is expedient to calculate the maximum absolute error of the forecast (for p = 0.05 and p = 0.01), which increases the visibility of the results, as well as the χ^2 criterion, that allows the estimation of hypothesis' significance that there are no differences between the arrays of calculated and empirical quantitative indicators.

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V.G. Banzaraksheev, Ya.G. Razuvaeva

PATHOMORPHOLOGICAL AND PATHOPHYSIOLOGICAL EVALUATION OF LIVER CHANGES IN WHITE RATS WITH EXPERIMENTAL DYSLIPIDEMIA AND ITS CORRECTION

ABSTRACT

In the experiment, a morphofunctional assessment of liver changes in rats with dyslipidemia and its phytotherapeutic correction was performed. It was found that the appointment of an atherogenic diet is accompanied by an increase in the total blood cholesterol, triglycerides, low density lipoproteins cholesterol and a decrease in the level of high density lipoproteins cholesterol. Against this background, the liver develops pathomorphological changes in the form of fatty hepatosis, necrobiosis and circulatory disorders. In addition, an increase in malonic dialdehyde concentration and inhibition of catalase activity are observed in rats liver homogenates. At the same time, the course introduction of phytoremedy normalizes the lipid metabolism, raises the activity of catalase in liver homogenates and reduces the content of malonic dialdehyde, and also reduces the severity of pathomorphological changes in the liver. Apparently, the polyvalent effect of phytoremedy is due to the content in its components of a complex of biologically active substances that exert lipid-lowering, antioxidant and hepatoprotective effects.

Keywords: dyslipidemia, hepar, lipid peroxidation, phythoremedy.

INTRODUCTION

In the 21st century the steady growth of atherosclerosis-associated cardiovascular pathology continues to be the main problem of Healthcare all over the world including Russia [2]. Besides, in many regions dyslipidemia is a common and significantly modified risk factor of atherosclerosis [6]. It is well known that the pathophysiological role of dyslipidemia, regardless of its etiology and pathogenesis, lies in its injuring

influence on the vascular endothelium via activation of oxidative mechanisms of atherogenesis [8]. Another important target of dyslipidemia is the liver – a key organ of the lipid homeostasis where the proatherogenic effect of dyslipidemia results in the early impairments of its morpho-functional state [9]. Undoubtedly, a complex rational pharmacotherapy directed at the anti- dyslipidemic, antioxidant and hepato-protective correction is one of the ways to interrupt such a "vicious circle" of disturbances. In this context, the use of multicomponent remedies of plant origin is of interest; they have polyvalent effect due to their rich content of biologically active substances and render a complex effect on the body [7].

The aim of the study is to estimate patho-morphological and pathophysiological alterations in the liver of the rats with experimental dyslipidemia and its correction by plant remedies.

MATERIALS AND METHODS

The experiments were carried out on male and female Wistar rats weighing 180-190 g. The animals were kept in the standard vivarium conditions observing a similar care, nutrition, and light and temperature regimen according to the GLP rules (Order N. 708H dated 23.08.2010) and the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasburg, 1986). The test report was approved by the ethics committee of the Institute of General and Experimental Biology SB RAS (Report N. 6 dated 12.10.2016).

The animals were distributed into 3 groups: intact, control and experimental ones. Dyslipidemia was simulated in rats by atherogenic diet for 12 weeks [10]. Pharmacotherapy of dyslipidemia was carried out by introduction of the plant multicomponent remedy to the animals of the experimental group. The remedy was developed on the base of formulae described in the *rGyud bzhi* – the main source of Tibetan medicine [12]; it contains the rhizomes of *Zingiber officinalis* L., fruits of *Rosa cinnamomea* L., beet-roots (*Beta vulgaris* L.), fruits of *Malus baccata* (L) Borkh., fruits of *Crataegus sanguinea* Pall., rhizomes of *Glycyrrhiza glabra* L., bark of *Cinnamomum cassia* Blume and other components. The plant remedy was intragastrically administered to rats in the form of decoction at a dose of 1 ml/100 g daily during the whole period of the experiment. The animals of the control group received the same volume of the purified water according to the analogous scheme.

The state of the lipid metabolism was estimated by determination of the total cholesterol content (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) with the use of fermentative method [3]. To estimate the intensity of lipid peroxidation (LPO) processes in the homogenates of the liver the concentration of the malonic dialdehyde (MDA) was determined [11]. The state of the antioxidant protection (AOP) was estimated by the catalase activity in the liver homogenate [5].

To carry out patho-morphological studies the paraffin liver sections were stained by hematoxylin-eosin [4]. To reveal neutral fats in the liver parenchyma, the quick-frozen sections, prepared on the cryostat, were stained by cerasine red and the degree of the fat dystrophy of the liver was determined by semiquantitative method using the five-grade scale for the lipid content estimation [10].

The statistical data processing was made with the use of the Excel programs package; the significance of the differences was determined with the use of the Student's t-criterion.

RESULTS AND DISCUSSION

The disturbances of the absorption and transport of nutritional lipids form the basis of exogene-induced postprandial dyslipidemia [3]. In this case the mechanism of its development comes to the increased formation of triglyceride-rich chylomicrons in the intestines and the following accumulation of remnants formed in the blood after their lipolysis. It is known, that the pathophysiology of circulating remnants involves their penetration into the arterial wall, macrophagal phagocytosis and their transformation into "foam cells" – a morphological basis of atherosclerosis [6, 8]. The experimental studies showed that the 12-week administration of the atherogenic diet including high caloric fatty nutrition resulted in significant disturbances of the lipid metabolism (Table 1). The lipidogram of the control animals has shown the increase in the TC content by 38%, TG – by 2.2 times, LDL-C – by 5 times and the 22% decrease in the level of anti-atherogenic HDL-C. At the same time, in the experimental group of animals, the course treatment with the plant remedy was followed by the decrease of TC, TG and LDL-C contents by 23%, 42% and 36% respectively and the 18% increase of HDL-C as compared to the control.

It is known that any disturbances in the lipid metabolism are followed by the impairment in the functional state of the liver – a key organ of the lipid homeostasis; moreover, the occurrence of pathological processes in it is likely not only to cause dyslipidemia, but to give a boost to the development of the lipid dysmetabolism [3, 9].

The findings of the pathomorphological studies of the animals' liver have shown that the atherogenic diet results in the development of the fatty hepatosis in rats; the average level of the liver fat in the control was 4.6±0.22 (Fig. 1). In all animals of the control group there were noted significant morphological signs of the diffuse liver fat in the form of lipid drops of different sizes; they invade the whole cytoplasm of hepatocytes. Particularly, in 5 animals the steatosis spread all over the whole liver acinus and in 38% of rats it invaded 2/3 of the hepatic tubule, mostly in the periportal area. Besides, the hepatocytes with necrobiotic and necrotic alterations and local spot necrosis were revealed all over the whole liver parenchyma in animals of the control group. Against the background of the focal-diffuse liver steatosis and necrotic alterations in hepatocytes, the tubular-radial structure of the acini appeared erased in some animals.

Along with the above patho-morphological alterations, there were noticed the signs of circulation disturbances in the liver. Particularly, in individual central veins there was revealed the blood filling and hemorrhagic suffusion of the parenchyma, mild dilation of perisinusoid space in most cases and erythrocyte stasis in capillaries. Besides, some portal tracts were slightly dilated; in their stroma there was noted moderate focal lympho-macrophagal infiltration with individual polymorphonuclear cells (Fig. 2). Also, there was noticed the margination and leukopedesis in the vessels; in the stroma of the acinus there was revealed the mild cellular, mostly, lymphohysteocytic infiltration along the sinusoidal capillaries.

The given findings of the experimental studies conform to the information in the literature; the atherogenic diet reportedly results in the marked deformation of the segmental structure of the liver, focal and diffuse inflammatory infiltration, apoptosis of hepatocytes along the portal tracts and micro- and macro-dripping liver steatosis [9].

Table 1

Influence of the plant remedy on the indices of lipid metabolism in induced dyslipidemia in rats

Indices	Intact (n=8)	Control (n=8)	Experimental (n=8)
TC, mmol/l	2,76±0,40	3,81±0,42	2,94±0,04*
TG, mmol/l	3,97±0,35	8,87±1,07	5,15±0,12*
HDL-C, mmol/l	1,82±0,05	1,42±0,21	1,68±0,15
LDL-C, mmol/l	0,15±0,01	0,81±0,02	0,52±0,02

Note: * – hereinafter the difference is significant in comparison with the control when p≤0.05.

However, the course administration of the complex remedy to animals significantly restrained the development of the liver steatosis. For example, in 2 animals from the experimental group, there was noted no fatty hepatocyte infiltration; the cytoplasm of cells had the granular form. A zonal fatty hepatocyte dystrophy was noticed in 6 animals, but in most cases the infiltration had a dust like and micro-drop character, therefore, it was revealed only by histochemical method and on specimens with high resolution of the microscope (Fig. 2). It is worthy to note, that hepatocytes containing lipids, were located in the zone of the portal tract and in individual cases they spread till the centrolobular zone. The level of the liver steatosis in the experimental group of rats averaged 2.6±0.30 (p≤0.001) being 43% less than the same index in the control. On the background of the tested plant remedy use, only individual liver cells underwent necrotic alterations and the tubular structure of hepatocytes remained intact.

It is known that the chronic dyslipidemia as an endogenic prooxidant inhibits antioxidant potential of the body and activates the oxidative stress [8]. In the previous studies we have established that, along with the changes in the ratio of atherogenic and antiatherogenic lipoprotein fractions, the induced dyslipidemia is characterized by hypoantioxidantemia and hyperlipoperoxidemia and, particularly, by the increase in the level of modified forms of LDL [1].

The findings of the studies demonstrated the signs of the AOP depression and acceleration of the peroxidation processes in the animals of the control group. Against the background of the induced dyslipidemia, the MDA content increased by 39%, the catalase activity decreased by 25% as compared to the data in the intact group (Table 2).

However, the administration of the plant remedy to the animals of the experimental group was characterized by less marked alterations in the indices of peroxidation and parameters of the antioxidant potential. Particularly, the MDA concentration in the liver homogenates of the control animals was 38% lower and the catalase activity was 16% higher as compared to the same indices in the control.

Conclusion

Thus, the experimental studies have shown that the atherogenic diet administered to rats significantly disturbs the lipid metabolism; as a result, it suppresses the antioxidant system of the body and leads to the excessive accumulation of peroxidation products. The given pathophysiological processes in laboratory animals are followed by patho-morphological alterations in the form of fatty hepatosis, necrobiosis and circulation failure in the liver. However, the course preventive phytotherapy favorably influences the lipid imbalance, diminishes peroxidation processes and decreases the manifestation of injuries in the liver architectonics. Obviously, such polyvalent effect of the plant remedy is due to the content of the spectrum of biologically active compounds having the complex hypolipidemic, antioxidant and hepatoprotective effects [7]. It is assumed that the tested plant remedy will break new ground for a rational and effective etiopathogenetic therapy of lipid metabolism disturbances.

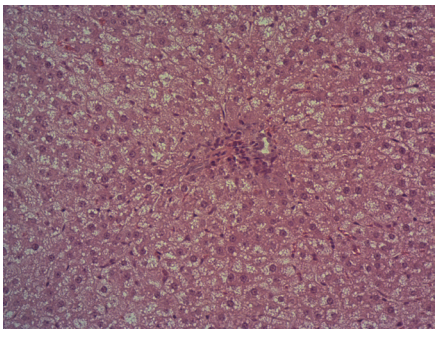

Fig.1. The liver of the control group animal. Hematoxylin-eosin staining. Optical zoom 10x20.

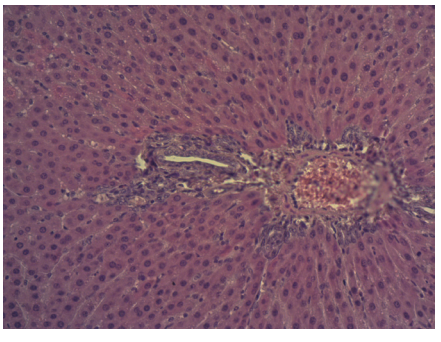

Fig.2. The liver of the animal treated with the complex remedy. Hematoxylin-eosin staining. Optical zoom 10x20.

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Table 2

Influence of the plant remedy on LPO and AOP indices in the liver of the rats with induced dyslipidemia

Indices	Intact (n=8)	Control (n=8)	Experimental (n=8)
MDA, nM/g	0,051±0,50	0,071±1,20	0,044±0,85*
Catalase, mkat/g	3,88±0,28	2,94±0,16	3,43±0,15

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ORGANIZATION OF HEALTH, MEDICAL SCIENCE AND EDUCATION

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ASSESSMENT OF POPULATION, AGE AND SEX STRUCTURE CHANGE IN TERRITORIAL SUBJECTS OF THE RUSSIAN FEDERATION IN THE TERRITORY OF THE FEFD

ABSTRACT

Successful functioning of territorial programs of the preferential provision of medicines (PM) is possible at the correct assessment of population, changes of age and sex structure as bases of formation of requirement and objective justification of the application medical institutions taking into account medico-demographic indicators and structure of incidence of the population in territorial subjects of the Russian Federation in the territory of the FEFD.

The analysis of age and sex composition in territorial subjects of the Russian Federation in the territory of the FEFD reveals detailed features at a negative gain of male population, and is result of evolution of reproduction of the population. The main reason of the Russian «female overpoise», including territorial subjects of the Russian Federation in the territory of the FEFD – a consequence of the Great Patriotic War of the 20th century, participation of the Russian Federation in the local and international conflicts. Also male «supermortality» at able-bodied age and on diseases for the studied period (2010-2014) remains very high.

Keywords: subjects, economics, evaluation, Far, Eastern, Federal, District, FED, coefficient of demographic loading, indicators, reproduction, working, population, Russia, Russian Federation.

INTRODUCTION

The important direction in work on implementation of programs of supply of medicines of socially unprotected segments of the population is formation of requirement and justification of the application of medical institutions. Formation of requirement has to begin from the physician of primary link or narrow experts on acquisition of medicines and has to be made taking into account data of the personified account, a medico-demographic situation and structure of a case rate of the population in territorial subjects of the Russian Federation in the territory of the FEFD for successful functioning of regional programs of supply of medicines. Certainly, at the same time the rest of medicinal preparations in drugstores and in warehouses, the consumption of drugs for the previous period, and also history of their sales and appointment has to be considered.

MATERIALS AND METHODS OF A RESEARCH

Comparative systems analysis; information and analytical; assessment of dynamic rows; statistical data handling by means of the plate Microsoft Excel

2010 processor.

RESULTS AND DISCUSSION

From 2010 for the beginning of 2015 population in territorial subjects of the Russian Federation in the territory of the FEFD was reduced by 1,72%, (table No. 1).

At the same time population of Yakutia approaches 1 million inhabitants. The Republic of Sakha — the only region in the Far East where the population steadily doesn't decrease, but grows. According to data of regional service of statistics, for 2015 in the Republic of Sakha (Yakutia) it became 2979 inhabitants more. As of January 1, 2016 nearly 959, 9 thousand people live in the Republic.

In all other territorial subjects of the Russian Federation of the Far East region, according to regional statistics, population becomes less every year.

Migration for 1992-2002 has made 88,5% of the general reduction of number of inhabitants in the region. The peak of migratory outflow has fallen on 1992-1995 when there was a process of intensive outflow of the population of northern territories of the federal district.

Decrease in volumes of intensity of migration doesn't demonstrate in recent years stabilization of migratory processes and furthermore, economy and a social situation in regions of the FEFD [4], (table No. 2).

The main reason for reduction of migration has rather economic character, owing to a difficult social and economic situation in certain territories that shows migration percent within the region at a smaller ratio to migration percent from other regions of Russia and because of her limits in the FEFD (table No. 3).

Rate of decrease in unemployment rate (table No. 4) in territorial subjects of the Russian Federation in the territory of the FEFD during 2010-2014 is almost comparable to the all-Russian indicator (29,9% - the Russian Federation and 25,8% - the FEFD) that doesn't reduce number of potentially possible migrants who have no means and conditions for realization of the intentions and are forced to postpone moving from the region.

The analysis of distribution of the population at the place of residence shows that in the majority territorial subjects of the Russian Federation in the territory of the FEFD the urban population (table No. 5) has traditionally high rate of specific weight. The maximum level of an urbanization is shown by the Magadan region (95,4% of urban population), Khabarovsk territory (81,8%), the Sakhalin region (81,4%), Kamchatka territory (77,5%).

In territorial subjects of the Russian Federation in the territory of the FEFD type of age structure of the population – stationary that corresponds also to the all-Russian type of the population (table

The population of subjects of the Russian Federation

Table 1

in FEFD (2010-2015 гг.), thousand people

	2010 year	2011 year	2012 year	2013 year	01.01.2015 year
Far East Federal district	6320	6285	6266	6252	6211
The Republic Of Sakha (Yakutia)	959	958	956	956	956
Kamchatka territory	323	322	320	320	317,2
Primorsky Krai	1965	1953	1951	1947	1933,3
Khabarovsk territory	1349	1343	1342	1342	1338,3
Amursk region	835	829	821	817	809,9
Magadan region	159	156	155	152	148,1
Sakhalin region	501	497	495	494	488,4
Jewish Autonomous region	178	176	175	173	168,4
Chukotka Autonomous district	51	51	51	51	50,5

Table 2

FACTORS OF MIGRATION GROWTH
per 10,000 population in the constituent entities of the Russian Federation in FEFD (2010-2014 гг.)

	2010 year	2011 year	2012 year	2013 year	2014year
FEFD	-49	-28	-32	-53	-40
федеральный округ	-49	-28	-32	-53	-40
The Republic Of Sakha (Yakutia)	-71	-102	-87	-96	-70
Kamchatka territory	-41	-51	-2	-38	-98
Primorsky Krai	-35	6	-6	-37	-20
Khabarovsk territory	-31	14	-4	-22	-19
Amursk region	-60	-74	-53	-71	-16
Magadan region	-141	-118	-137	-142	-153
Sakhalin region	-63	4	-31	-44	-59
Jewish Autonomous region	-49	-95	-89	-125	-108
Chukotka Autonomous district	-174	102	-66	-70	-30

Table 3

The distribution of the number of arrivals in the direction of movement (% of total arrivals)

	в пределах региона					из других регионов России					из-за пределов России				
	2010 year	2011 year	2012 year	2013 year	2014 year year	2010 year	2011 year	2012 year	2013 year	2014 year	2010 year	2011 year	2012 year	2013 year	2014 year
FEFD	58,9	57,0	54,3	53,6	51,9	35,1	33,4	34,2	36,5	35,3	6,0	9,6	11,5	9,9	12,8
The Republic Of Sakha (Yakutia)	64,5	68,4	65,8	67,0	63,0	29,8	26,1	30,1	30,3	34,7	5,7	5,5	4,1	2,7	2,3
Kamchatka territory	30,0	26,4	20,9	21,6	24,3	45,5	43,2	41,7	48,2	46,9	24,5	30,4	37,4	30,2	28,8
Primorsky Krai	65,1	61,4	60,5	60,7	60,2	30,3	25,7	25,7	26,9	26,5	4,6	12,9	13,8	12,4	13,3
Khabarovsk territory	51,4	49,2	47,7	46,5	45,4	42,1	44,2	38,7	43,0	38,7	6,5	6,6	13,6	10,5	15,9
Amursk region	71,2	73,8	67,0	67,6	57,8	27,7	24,7	30,7	30,3	27,7	1,1	1,5	2,3	2,1	4,5
Magadan region	48,8	45,5	41,8	39,9	35,3	44,7	46,0	51,0	54,0	53,4	6,5	8,5	7,2	6,1	11,3
Sakhalin region	62,0	47,4	47,3	44,8	47,5	34,0	38,0	42,2	45,0	43,1	4,0	14,6	10,5	10,2	9,4
Jewish Autonomous region	51,1	55,2	44,2	38,7	35,5	45,3	41,8	53,4	57,8	59,8	3,6	3,0	2,4	3,5	4,7
Chukotka Autonomous district	24,6	16,0	21,1	23,9	17,7	68,0	76,3	74,9	70,1	76,8	7,4	7,7	4,0	6,0	5,5

Table 4

THE NUMBER OF UNEMPLOYED (according to the sample survey of population on problems of employment; thousand people)

	2010 year	2011 year	2012 year	2013 year	2014year
RF	5544	4922	4131	4137	3889
FEFD	295	254	228	224	49
The Republic Of Sakha (Yakutia)	44	44	40	37	37
Kamchatka territory	13	12	11	11	11
Primorsky Krai	102	85	73	76	73
Khabarovsk territory	66	50	48	42	44
Amursk region	30	27	23	26	24
Magadan region	6	5	3	3	3
Sakhalin region	26	23	22	21	18
Jewish Autonomous region	8	8	7	7	7
Chukotka Autonomous district	1	2	1	1	1

No. 6).

Feature in change of age structure of the population in territorial subjects of the Russian Federation in the territory of the FEFD throughout the studied period (2010-2014), was growth of a share of the population is more senior than working-age and growth is aged younger able-bodied, but at reduction of a share of persons of working-age. In territorial subjects of the Russian Federation in the territory of the FEFD the specific weight of the population is more senior than working-age has grown by 2015 against 2010 by 9,9%, it is more than the all-Russian indicator for 2,3% for the studied period (across the Russian Federation – 7,6%).

It is known that not only the age structure, but also education level, qualifications in many respects can determine the labor capacity of the territory.

On the basis of types of age structure of the population of the FEFD (G. Sundberg's classification), coefficients of demographic loading can be defined (table No. 7). In the analysis of an indicator – the coefficient of demographic loading which shows how many it is the share the unemployed population of 1 000 people of the working-age occupied in economy can be established increase in loading by the population more young than working-age and more active increase in coefficient of demographic loading by the population is more senior than working-age in territorial subjects of the Russian Federation in the territory of the FEFD. The comparative analysis of a gain of persons on coefficient of demographic

Table 5

The proportion of urban and rural population in the total population in subjects of the Russian Federation in FEFD, for the period 2010-2014 (%)

	Городское население					Сельское население				
	2010	2011	2012	2013	2014	2010	2011	2012	2013	2014
RF	73.8	73.9	74.0	74.2	74.0	26.2	26.1	26.0	25.8	26.0
FEFD	74.8	74.9	75.1	75.3	75.4	25.2	25.1	24.9	24.7	24.6
The Republic Of Sakha (Yakutia)	64.1	64.6	64.9	65.2	65.3	35.9	35.4	35.1	34.8	34.7
Kamchatka territory	77.4	77.5	77.1	77.4	77.5	22.6	22.5	22.9	22.6	22.5
Primorsky Krai	76.1	76.3	76.6	76.7	76.9	23.9	23.7	23.4	23.3	23.1
Khabarovsk territory	81.8	81.5	81.5	81.7	81.8	18.2	18.5	18.5	18.3	18.2
Amursk region	66.9	67.0	67.1	67.1	67.3	33.1	33.0	32.9	32.9	32.7
Magadan region	95.5	95.7	95.8	95.3	95.4	4.5	4.3	4.2	4.7	4.6
Sakhalin region	79.8	80.3	80.8	81.2	81.4	20.2	19.7	19.2	18.8	18.6
Jewish Autonomous region	67.8	68.1	67.9	67.9	68.2	32.2	31.9	32.1	32.1	31.8
Chukotka Autonomous district	64.9	66.0	66.7	67.5	68.3	35.1	34.0	33.3	32.5	31.7

Table 6

The age composition of the population in subjects of the Russian Federation in FEFD, 2010-2014 (% of total population)

	The population younger than working					The population of working age					The population over working age				
	2010	2011	2012	2013	2014	2010	2011	2012	2013	2014	2010	2011	2012	2013	2014
RF	16.2	16.5	16.8	17.2	17.6	61.5	60.9	60.1	59.3	58.4	22.3	22.6	23.1	23.5	24.0
FEFD	17.4	17.6	18.0	18.4	18.8	63.4	62.7	61.9	61.0	60.1	19.2	19.7	20.1	20.6	21.1
The Republic Of Sakha (Yakutia)	23.3	23.5	23.8	24.1	24.4	63.9	63.1	62.3	61.4	60.5	12.8	13.4	13.9	14.5	15.1
Kamchatka territory	17.2	17.4	17.4	17.7	18.0	65.4	64.7	64.3	63.6	62.7	17.4	17.9	18.3	18.7	19.3
Primorsky Krai	15.4	15.6	15.9	16.3	16.7	63.0	62.4	61.6	60.7	59.8	21.6	22.0	22.5	23.0	23.5
Khabarovsk territory	15.7	16.0	16.4	16.9	17.4	63.5	62.9	62.1	61.3	60.5	20.8	21.1	21.5	21.8	22.1
Amursk region	18.2	18.5	18.9	19.4	19.6	62.3	61.3	60.5	59.4	58.7	19.5	20.2	20.6	21.2	21.7
Magadan region	16.8	17.1	17.5	18.0	18.3	66.3	65.3	64.2	63.0	62.0	16.9	17.6	18.3	19.0	19.7
Sakhalin region	16.8	17.0	17.3	17.8	18.2	63.5	62.7	61.8	60.7	59.6	19.7	20.3	20.9	21.5	22.2
Jewish Autonomous region	18.6	18.9	19.4	19.8	20.2	62.1	61.1	60.2	59.1	58.2	19.3	20.0	20.4	21.1	21.6
Chukotka Autonomous district	22.5	22.2	22.2	22.3	22.6	67.1	67.0	66.5	65.8	64.9	10.4	10.8	11.3	11.9	12.5

Table 7

Old-age dependency ratios in the constituent entities of the Russian Federation on the territory of the far Eastern Federal district for the period 2010-2014.

	Per 1,000 people of working age have persons working age														
	TOTALLY					The population younger than working					The population over working age				
	2010	2011	2012	2013	2014	2010	2011	2012	2013	2014	2010	2011	2012	2013	2014
RF	626	643	664	687	713	264	271	280	290	301	362	372	384	397	412
FEFD	577	595	616	640	664	275	281	291	302	313	302	212	325	338	551
The Republic Of Sakha (Yakutia)	566	584	604	628	652	365	372	381	392	403	201	276	223	236	249
Kamchatka territory	529	544	556	572	595	263	268	272	278	287	266	353	284	294	308
Primorsky Krai	588	603	624	648	673	245	250	259	269	280	343	336	365	379	393
Khabarovsk territory	574	590	609	630	653	247	254	264	275	287	327	328	345	355	366
Amursk region	606	630	654	682	703	292	302	313	326	334	314	269	341	356	369
Magadan region	508	532	558	586	612	254	263	274	285	294	254	324	284	301	318
Sakhalin region	575	595	619	647	677	265	271	280	293	305	310	326	339	354	372
Jewish Autonomous region	611	636	662	691	718	299	310	322	335	347	312	161	340	356	371
Chukotka Autonomous district	488	492	505	519	541	334	331	334	338	348	154	116	171	181	193

loading among persons is younger than working-age for 13,8%, for the studied period, we will compare with the all-Russian indicator which makes 14% and allows to consider it as a positive element in formation of a manpower in the territory of the Far East region.

Increase in coefficient of demographic loading by the population is more senior than working-age, for the studied period (2010-2014), in territorial subjects of the Russian Federation in the territory of the FEFD has shown percent of a gain of 82,4%. In the Russian Federation the gain of coefficient of demographic loading has made only 13,8%. Increase in loading by the population is more senior than working-age doesn't exclude risks of a social and economic situation in the region, testifies to a population postareniye that influences an indicator of economic loading, the number of the persons having the right for the set of social services (SSS) including on preferential provision of medicines increases.

The aging process of population can create serious problems in respect of increase in demographic load of those who are busy with work. At such ratio of the busy population and dependents it will be difficult to provide worthy life to the population living in the region [1; 4].

The age and sex pyramid of the population of the modern Russian Federation (fig. 1) was created under the influence of two groups of factors: evolutionary changes as consequences of natural decline in mortality and birth rate in the course of the demographic transition and perturbation influences connected with economic and social shocks of the 20-21st centuries [3].

The deformed, torn edges and strong

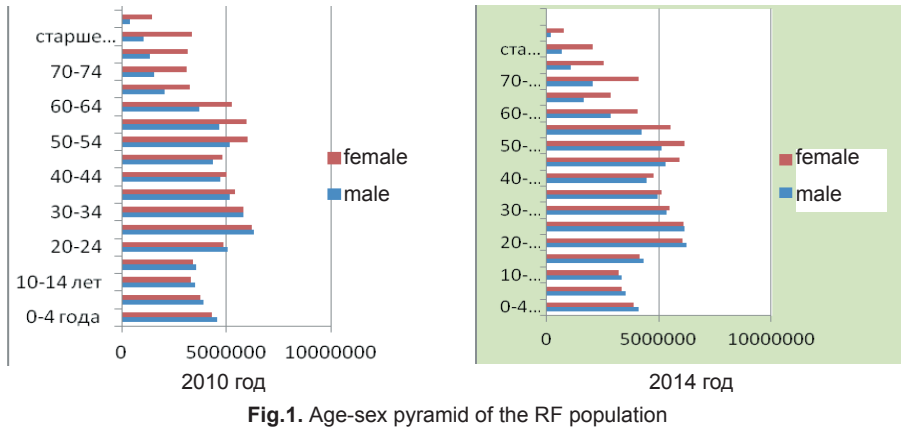

Fig.1. Age-sex pyramid of the RF population

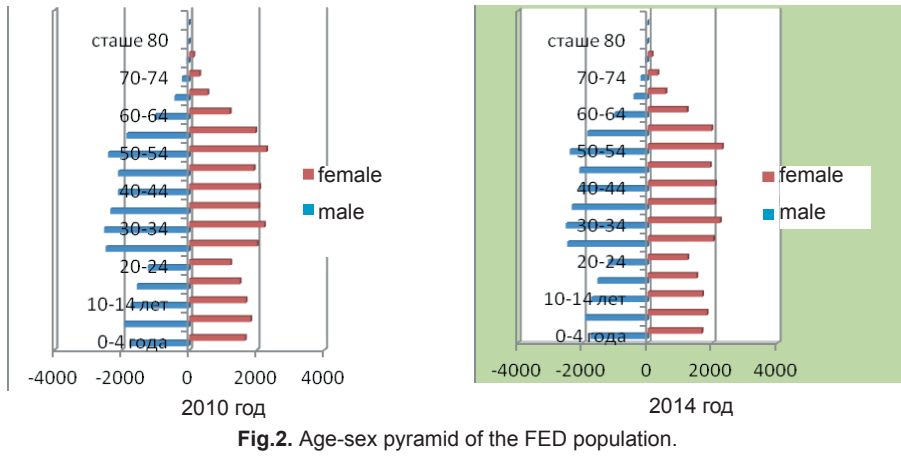

Fig.2. Age-sex pyramid of the FED population.

asymmetry of men's and female parts of a pyramid [2] were characteristic of the Russian age and sex pyramid and in territorial subjects of the Russian Federation in the territory of the FEFD during the second half of the 20th century and at the beginning of the 21st century, (fig. 1, fig. 2).

Comparison of ratios of men and women in territorial subjects of the Russian Federation in the territory of the FEFD has allowed to judge the general nature of changes of age and sex structure of the population for the studied period (2010-2014), (table No. 8).

CONCLUSIONS

The analysis of age and sex composition in territorial subjects of the Russian Federation in the territory of the FEFD reveals detailed features at a negative gain of male population, and is result of evolution of reproduction of the population. The main reason of the Russian "female overpoise", including in territorial subjects of the Russian Federation in the territory of the FEFD

Table 8

The ratio of men and women in subjects of the Russian Federation in FEFD (per 1,000 men, there women)

РФ	2010 year	2011 year	2012 year	2013 year	2014 year	Тпр, (%)
	1163	1162	1160	1159	1158	- 0,42
FEFD	1083	1082	1081	1081	1081	- 0,18
The Republic Of Sakha (Yakutia)	1058	1057	1059	1060	1060	0,19
Kamchatka territory	1018	1014	1001	996	1003	- 1,5
Primorsky Krai	1089	1085	1084	1085	1086	- 0,28
Khabarovsk territory	1101	1101	1097	1098	1096	- 0,45
Amursk region	1109	1112	1113	1115	1108	- 0,09
Magadan region	1057	1060	1060	1062	1065	0,76
Sakhalin region	1080	1077	1078	1077	1079	- 0,09
Jewish Autonomous region	1101	1103	1102	1105	1105	0,36
Chukotka Autonomous district	995	971	966	964	961	- 3,42

– a consequence of the Great Patriotic War of the 20th century, participation of the Russian Federation in the local and international conflicts. Also male "supermortality" at able-bodied age and on diseases for the studied period (2010-2014) remains very high.

The analysis of an age and sex pyramid allows to characterize not only demographic history of the state, but also to predict a demographic situation in the future including in territorial subjects of the Russian Federation in the territory of the FEFD for implementation of programs of the state support, including preferential supply of medicines and other social programs.

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HYGIENE, SANITATION, EPIDEMIOLOGY AND MEDICAL ECOLOGY

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THE ENVIRONMENT AND INCIDENCE OF MALIGNANT NEOPLASMS IN THE POPULATION OF THE DIAMOND PROVINCE OF YAKUTIA

ABSTRACT

The degree of influence of anthropogenic, techno-genetic loads on the state of the environment and the characterization of the incidence of malignant neoplasms of the population of the regions that make up the diamond province of Western Yakutia are analyzed.

Keywords: diamond province, environmental factors, neoplasms, morbidity.

To the zone of the diamond province are regarded the districts of Western Yakutia: Anabarsk, Olenek, Mirninsky, Suntarsky, Nyurbinsky, Verhnevilyuysky, Vilyuysky, Olekminsky and Lensky, whose vast territory extends from the Irkutsk region to the shores of the Arctic Ocean and occupies 22.1% (684.3 thousand km2) of the territory of the Republic of Sakha (Yakutia). In the Myrninsky, Nyurbinsky, Anabar and Oleneksky districts, the diamond mining industry is developed and oil and gas production is increasing. The Vilyuysk hydroelectric power station, the Vilyuy Reservoir are constructed, huge reserves of hydrocarbons are reconnoitered, all this has caused considerable technogenic and anthropogenic loads of the environment (E) which remain a big environmental problem of the Western Yakutia. From traditional industries in these territories, agriculture is developed, mainly the branches of meat and dairy cattle breeding, herding, farming, and also lacustrine fishing and hunting. In addition, in the Olekminsky district forestry, timber processing industry, precious stones mining are developed, and the Lensky district is a transport hub of the diamond mining, oil and gas industry, and the woodworking industry is also developed here. Climatic conditions in the regions of Western Yakutia are estimated in the range from "relatively moderate" (Olekminsky and Lensky) to "extremely extreme" (Anabasky, Olenek) [2].

The purpose of the study is to assess the degree of influence of anthropogenic, technogenic loads, environmental factors on the incidence of malignant neoplasms of the population living in the eon of the diamond province (Western Yakutia).

MATERIALS AND METHODS OF RESEARCH

The materials of Yakut republican oncological clinic (YROC) reporting were classified for the period from 1989 to 2010. Materials from the State report of the Ministry of Nature Protection of the Republic of Sakha (Yakutia) for the period from 2010 to 2014 were used [3, 4], Statistical data on the Territorial authority of Fed-

eral State Statistics Service in the Sakha Republic (Yakutia)[13 , 14], the results of their own research [1,6 - 11]. Assessment of influence of anthropogenic and technogenic loads of state of environment is carried out with use of data of E.I. Burtseva [2]. Statistical data are processed according to a common methodology using an application package.

RESULTS AND DISCUSSION

Anthropogenic load. In the regions of Western Yakutia, in connection with the rapid development of the diamond industry and the discovery of huge hydrocarbon reserves with the prospect of their industrial development, both for domestic consumption and for imports to the countries of Southeast Asia, since the second half of the 20th century, the population of the region has increased significantly. By 1990, in comparison with 1959, it was 2.4 times.

In the regions of Western Yakutia, due to the rapid development of the diamond industry and the discovery of huge hydrocarbon reserves with the prospect of their industrial development, both for domestic consumption and for imports to the countries of South-East Asia. From the second half of the XX century, the population of the region increased significantly by 1990 compared to 1959 by 2.4 times. In this, an important role was played by external migration from the countries of the near abroad and other regions of Russia. According to data of republican statistical office, the share of the visitor of the population in Mirninsky district made 93,3% of all population, in Lensky – 89,3, in Olekminsky – 51,8 and Oleneksky – 50,8%. In other areas which are a part of the diamond province: Nyurbinsky (82,2%), Suntarsky (92,9), Verkhnevilyuysky (94,8), Vilyuysky (68,9) and Anabarsky (87,2%), a majority of inhabitants makes indigenous people. The increase in the population increased the anthropogenic and man-caused environmental pressures on Western Yakutia by 178.0%. For the purpose of the most accurate determination of the effect of anthropogenic pressure on the environment, a methodology based on calculating the level of population density per 1 km2 is used (Table 1).

Relatively high coefficients of population density (people per 1 km2) in 1959 were observed in Verkhnevilyuisky (0.30), Suntarsky (0.29), Lensky (0.29), Nyurbinsky (0.29) and Viluisky (0 , 25) districts, and the lowest values are in Myrninsky (0.04) and in the Arctic districts (Anabar and Oleneksky – < 0,02). At the same time, the load on the environment was characterized as “lowered” in the Southern and Vilyuisk regions and in the Arctic as “low”.

Table 1

Dynamics of anthropogenic pressure in the regions of Western Yakutia [5,13,14]

District	Population density, the people on 1 km2			Ranging scale			Environmental load		
	1959	1990	2012	1959	1990	2012	1959	1990	2012
Lensky	0,29	0,67	0,51	2	4	4	U	R	R
Olekminsky	0,16	0,19	0,16	2	2	2	U	U	U
Mirninsky	0,04	0,57	0,44	1	4	4	L	R	R
Suntarsky	0,29	0,45	0,42	2	3	3	U	M	M
Nyurbinsky	0,38	0,56	0,47	3	3	3	M	M	M
Verkhnevilyuysky	0,30	0,51	0,51	3	4	4	U	R	R
Vilyuysky	0,25	0,59	0,45	2	4	4	U	R	R
Anabarsky	0,02	0,07	0,06	1	1	1	L	L	L
Oleneksky	0,01	0,01	0,01	1	1	1	L	L	L

Note. In Table 1, 4-5, the environmental load: L – low, U – under, M – medium, R – raised, H – high.

It should be noted that the indicators of anthropogenic load on the environment in the dynamics over the 30-year period (1959-1990) have undergone a significant change towards the growth of indicators. Thus, by the end of the analyzed period, the environmental load in the zone of the Vilyui group of areas was assessed as “elevated”, and in the southern and arctic regions as “medium” and “low” (Fig. 1).

Medico-demographic characteristics. The highest rates of birth rate of children significantly influencing indicators of a natural increase of the population were observed in Olenekskom (24,8%), Verkhnevilyuysk (21,5), Vilyuysk (22,1), Nyurbinsky (21,6), Suntar (21,0) and the Anabar (19,4) districts. The majority of the population of these areas is made by Yakuts and the small peoples of the North (SPN), their specific gravity fluctuates from 50,8 to 98,2% of total number of the inhabitants living in them. Meanwhile in Mirninskom (93,3), Lensk (89,3), the Olyokma (51,8) districts where the inverse ratio of a share of the visitor is observed (Russian, etc. nationalities) and indigenous people, birth-rate coefficients is comparative below [5,13,14] (tab. 2).

Correlation analysis revealed a strong inverse correlation between the number of newcomers in the diamond province of Yakutia and the birth rate of children in the field (r = -0.79). And between the birth rate of children and the number of indigenous peoples of the North living in the same areas, there was a direct strong connection (r = 0.88). Similar results were revealed during the correlation analysis between population growth rates and the relative density of the newcomer and the indigenous population (respectively: r = -0.79 and r = 0.70).

The results of the analysis make it possible to confirm that the maximum incidence of women with malignant neoplasms (MN) of reproductive organs in the republic is registered in industrial regions where the majority of the female population are visitors. It should be noted that the low birth rate of children causes an increase in the incidence of female (MN) of the reproductive organs. This is evidenced by the results of pair correlation, presented in Table. 3, which allow us to state that the decrease in the birth rate of children is associated with an increase in the incidence of heart failure in reproductive organs in women (r = -0.68), primarily the mammary gland (r = -0.62) [6-8].

Economic and technogenic loads of the environment. The environment of the ter-

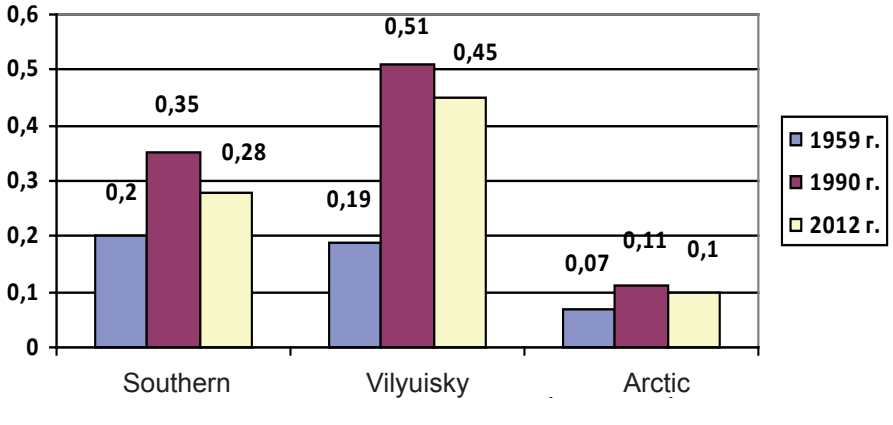

Figure 1. Anthropogenic load in the regions of Western Yakutia for 1959-2012.

Table 2

Medico-demographic indicators of the population of areas of Western Yakutia (Per 1000 population) [5,13,14]

Content	Year	Districts of Western Yakutia								
		Southern		Vilyuisky		Arctic				
		Lensky	Olekminsky	Mirninsky	Nyurbinsky	Suntarsky	Verkhnevilyuysky	Vilyuysky	Anabarsky	Oleneksky
Children were born	1990	17,2	22,2	15,4	23,7	27,3	30,2	25,8	29,0	28,1
	2000	11,6	13,1	10,5	17,6	17,1	18,6	15,7	18,7	17,8
	2005	13,4	11,6	11,1	17,2	15,2	17,4	15,4	20,7	13,7
	2011	15,3	16,5	13,3	21,6	21,0	21,5	22,1	19,4	24,8
Mortality the population	1990	6,8	9,4	3,8	7,8	8,3	8,3	7,7	9,5	9,0
	2000	11,4	12,1	6,6	10,6	8,8	8,9	10,1	11,3	8,3
	2005	12,5	13,6	6,7	10,9	10,7	10,0	10,1	11,7	12,9
	2011	10,9	13,7	6,9	9,4	10,1	9,9	9,4	10,9	13,2
Growth the population	1990	10,4	12,8	11,7	17,9	19,0	21,9	18,1	19,5	19,0
	2000	0,2	1,0	3,9	6,9	8,3	9,6	5,6	7,4	9,5
	2005	1,1	2,1	4,4	6,3	4,5	7,3	5,3	8,5	0,7
	2011	4,4	2,8	6,4	12,1	10,9	11,6	12,7	8,6	11,7
Yakuts and SPN(%)	1990	10,0	41,6	4,4	82,2	92,9	94,8	68,9	87,2	49,2
	2011	10,7	48,2	9,7	95,2	98,0	98,2	86,3	96,4	50,8
Russian and other nationalities (%)	1990	90,0	58,4	95,6	17,8	7,1	5,2	31,1	12,8	50,9
		89,3	51,8	93,3	4,8	2,0	1,8	13,7	3,6	49,2

ritories of the regions of Western Yakutia is experiencing enormous economic and man-caused stresses on the part of mining enterprises (Table 4). In particular,

Table 3

Indicators of childbirth and morbidity of female malignant neoplasms of reproductive organs in industrial areas of Western Yakutia

District		Years	Birth rate, 0/00	Years	Total women with MN, 0/0000	Including, 0/0000			
						mammary gland	body of the womb	uteral cervix	ovary
Southern	Olekminsky			1989-1998	66,1	31,4	17,7	4,6	12,4
		2000-2010	13,3	2001-2010	85,2	32,5	33,9	6,5	12,3
	Lensky	1980-1990	18,3	1989-1998	58,3	26,1	17,1	3,7	11,4
		2000-2010	14,2	2001-2010	99,7	54,2	20,9	11,8	12,8
Vilyuysk	Mirninsky	1980-1990	17,9	1989-1998	42,3	27,6	6,2	3,8	4,7
		2000-2010	12,0	2001-2010	86,3	47,0	16,8	11,7	10,8
	Suntarsky	1980-1990	24,9	1989-1998	19,2	9,2	5,4	0,8	3,8
		2000-2008	16,7	2001-2010	37,6	14,6	10,0	4,6	8,4
	Nyurbinsky	1980-1990	23,3	1989-1998	24,1	9,6	5,5	2,8	6,2
		2000-2008	17,4	2001-2010	30,3	15,9	6,8	3,8	3,8
	Verkhnevilyuysky	1980-1990	25,3	1989-1998	24,5	8,5	7,5	2,8	5,7
		2000-2008	17,4	2001-2010	23,0	7,4	10,1	1,8	3,7
	Vilyuysky	1980-1990	23,6	1989-1998	33,6	17,8	9,6	2,1	4,1
		2000-2010	16,3	2001-2010	43,7	24,1	12	2,3	5,3
Arctic	Anabarsky	1980-1990	26,8	1989-1998	26,0	10,4	5,2	0,0	10,4
		2000-2010	19,3	2001-2010	25,0	10,0	5,0	5,0	5,0
	Oleneksky	1980-1990	28,5	1989-1998	24,5	14,7	4,9	0,0	4,9
			18,4	2001-2010	29,4	24,5	0,0	4,9	0,0
The correlation coefficient between the birth rates for 1980-1990 and the incidence of heart failure for 2001-2010.					-0,68	-0,62	-0,18	-0,81	-0,38

the environment of Myrninsky district experiences a high load from discharges of contaminated sewage into surface water bodies and disturbed lands (extraction of rock from the bowels of the earth), medium - from transport, average annual emissions of pollutants into the atmosphere. Mirninsky district is represented as the main cross-border zone, which is directly influencing environment of the areas located in a flood plain of the lower current of the Vilyuy River. Besides, OS of the Vilyuysk district experiences high strain from the gas industry located in his territory. In other regions of the Vilyuysk group (Suntarsky, Nyurbinsky, Verkhnevilyuysky and Vilyuysky) which are more agricultural load of OS from branch is estimated ranging from "raised" to "medium" (tab. 5) now.

The environment of the Olyokminsky district experiences the raised strain from transport, agriculture, extraction of mountain weight at extraction of construction material, emissions of pollutants in the atmosphere, dumpings of the polluted sewage. The main loading on the environment of Lensky district is connected with increase in volumes of the mountain weight taken from an earth subsoil at oil production, gas, dumpings of pollutants into the atmosphere, dumpings of sewage, motor transport, by expansion of the areas of the broken lands.

In the Arctic districts of the Western Yakutia agriculture load of the environment insignificant. So, the environment of the Anabarsky district tests from reindeer breeding "under", and Oleneksky – "low" loading (tabl.6).

So, in 2001-2010 in comparison with 1989-1998 the number of the diseased per 100 thousand population has increased in Lensky by 96,5 people (at the average annual rate of a gain – 9,2%), in Mirninsky district – on 85,0 (6,2) and Anabarsky – on 15,4 (1,3%) persons. In other regions of the Western Yakutia the situation close to stabilization has been stated, at fluctuations of average annual indicators from + 0,8% in Verkhnevilyuysky to - 0,05% in Oleneksky areas.

Despite it, on the level of annual indicators of incidence during 2001-2010 the first 5 places have reserved (in decreasing order of indicators) Lensky (267,0), Olyokminsky (215,0), Mirninsky (188,0), Vilyuysky (176,0), Nyurbinsky (171,0) areas, i.e. where the enterprises that extract diamonds, oil and gas are working with the greatest intensity.

In Mirninsky, Lensky and Anabarsky districts growth of the general indicators of oncological incidence of the population has happened at the expense of high average annual rates of a gain of indicators

Table 4

Assessment of economic and technogenic loads of the environment of territories of the Western Yakutia

Index	Districts of Western Yakutia								
	Southern		Viluysky					Arctic	
	Lensky	Olekminsky	Mirninsky	Suntarsky	Nyurbinsky	Verkhnevilyuysky	Vilyuysky	Anabarsky	Oleneksky
Loading agricultural on the environment	R	R	H	R	R	M	M	U	L
Transport loads of the environment	U	R	M	U	U	L	U	L	L
Extracted from the bowels of the earth of the rock mass until 2002 - million m3	506,6	0,5	1931,6	0,25	14,5	*/	12030,5	13,9	*/
level of load of the environment	R	L	H	L	L	*/	H	L	*/
Annual emissions of pollutants in the atmosphere for 1995-2005 - thousand tons	6,67	2,83	7,06	1,41	4,85	2,05	1,15	0,81	0,3
level of load of the environment	M	U	M	L	M	U	L	U	L
Dumpings of the polluted sewage:-one million m3	5,20	2,45	30,3	*/	1,60	5,80	*/	2,10	*/
level of load of the environment	R	M	H	*/	U	R	*/	M	*/

*/ - data not available

Table 5

The load of agriculture on the environment of the regions of Western Yakutia [13,14]

District	Years	Cattle		Horses		Deer	
		Beast	Environmental load	Beast	Environmental load	Beast	Environmental load
Lensky	1995	6355	M	861	L	-	-
	2008	2154	L	1190	L	-	-
	±голов	-4201	-	+329	-	-	-
Olekminsky	1995	12773	R	5232	R	2470	M
	2008	11098	R	5461	R	3338	R
	±голов	-3675	-	+229	-	+868	-
Mirninsky	1995	2585	L	720	L	100	L
	2008	1986	L	511	L	16	L
	±голов	-599	-	-209	-	-86	-
Suntarsky	1995	27561	H	10308	H	-	-
	2008	27562	R	10809	H	-	-
	± голов	-9689	-	+501	-	-	-
Nyurbinsky	1995	30369	H	10389	H	-	-
	2008	19411	H	10017	H	-	-
	± голов	-10958	-	-372	-	-	-
Verkhnevilyuysky	1995	22420	H	7177	R	-	-
	2008	14060	R	7344	R	-	-
	± голов	-8360	-	+167	-	-	-
Vilyuysky	1995	22420	H	8880	H	-	-
	2008	14051	R	6427	R	-	-
	±голов	-8369	-	-2453	-	-	-
Anabarsky	1995	-	-	-	-	21262	H
	2008	-	-	-	-	15485	U
	± голов	-	-	-	-	-5777	-
Oleneksky	1995	-	-	-	-	11961	R
	2008	-	-	-	-	2207	L
	± голов	-	-	-	-	-9754	-

Note. ± the heads – in 2008 in comparison with 1990.

of malignant new growths of bodies of a digestive tract (5,5; 3,1 and 5,6% respectively). In particular, in Mirninsky district annual indicators of colon cancer cases from 1989-1998 to 2001-2010 have grown by 1,6 time, rectal cancer – in 2,0, a liver cancer– in 1,4 and pancreas cancer– by 2,6 times. The similar situation is observed in Lensky (2,3; 2,0; 1,4 and 2,6 times, respectively) and Nyurbinsky (colon cancer – 3,0; pancreas cancer – 2,1 times) districts . In the increase in the total indicators of cancer morbidity in Western Yakutia, a significant contribution was made by the high average annual rates of increase in the incidence of malignant respiratory diseases: in Mirninsky - 5.5%, Lensky - 3.1 and Anabarsky - 5.6%.

Of particular concern is the relatively rapid growth in the majority of regions of the diamond province of the overall morbidity rates of MN in men - sex, and in women - in reproductive organs. Thus, in the male population, the highest mean annual rate of increase in the incidence of malignant genital organs is different: Vilyuisky (13.1%), Verkhnevilyuisky (12.8), Myrninsky (9.2) and Lensky (6.1%), and in female - Mirninsky (9.2%), Suntarsky (6.9), Oleneksky (6.3) and Lensky (6.1%) districts.

Average annual rates of a gain at MN of urinary bodies are high (Lensky – 10,8, Mirninsky – 8,6, Nyurbinsky – 7,5, Olyokminsky – 4,8), nervous system and a brain (Mirninsky – 9,0%, Vilyuysky – 9,7, Verkhnevilyuysky – 5,7%), a thyroid gland (Nyurbinsky – 20,7%, Mirninsky – 13,5, Suntar – 9,6%) and lymphatic and haematogenic tissue (Anabarsky – 19,5%, Vilyuysky – 7,3, Suntarsky – 5,9%).

In conclusion, we note that the results of the correlation analysis indicate that the factor “the volume of rock mass extracted from the bowels of the earth” had a strong direct relationship with the general indicators of oncological morbidity in the population of Western Yakutia (r = 0.62) and the straight line with hemoblastoses (r = 0.19).

Indicators the characterizing “annual emissions of pollutants in the atmosphere” had direct link with the general indicators of cancer of digestive organs (r = 0,43) and breath (r = 0,39). Thus, when carrying out anticarcinogenic fight in regions of the Western Yakutia, a zone of intensive industrial development important development of the actions directed first of all to decrease in impact on a human body of anthropogenic, technogenic factors of the environment is represented.

Morbidity of the population of Western Yakutia by malignant neoplasms and its average annual growth rate over periods from 1989 to 1998 and from 2001 to 2010.

Table 6

Morbidity of the population of Western Yakutia by malignant neoplasms and its average annual growth rate over periods from 1989 to 1998 and from 2001 to 2010. (Per 100 thousand population) [4-6]

Localization	Year	District of the western Yakutia								
		Lensky	Olekminsky	Mirninsky	Suntarsky	Nyurbinsky	Verkhnevilyuysky	Vilyuysky	Anabarsky	Oleneksky
1	2	3	4	5	6	7	8	9	10	11
Malignant neoplasms - total	1989-1998	170,0	217,0	103,0	174,0	174,0	175,6	166,0	111,3	177,3
	2001-2010	267,0	215,0	188,0	169,0	171,0	160,9	176,0	126,7	178,4
	growth rate	9,2	-0,1	6,2	-0,3	-0,1	-0,8	0,3	1,3	0,05
Including:	1989-1998	8,2	5,8	5,8	7,8	4,2	5,0	5,6	0,0	0,0
	2001-2010	3,6	3,5	2,3	2,7	5,5	2,3	4,4	1,4	15,5
- Lip, tongue, throat	growth rate	-7,9	-4,9	-8,85	-10,0	2,45	-7,45	-2,4	0,0	0,0
Digestive organs, total	1989-1998	59,2	83,4	28,7	93,5	76,3	94,6	78,4	47,9	82,6
	2001-2010	81,2	66,0	48,5	80,8	68,0	60,0	72,8	47,3	70,8
	growth rate	3,2	-2,2	3,4	-1,4	-1,1	-4,4	0,7	-0,1	-1,5
– gullet	1989-1998	8,6	15,9	2,3	30,2	26,8	36,0	21,9	20,1	32,5
	2001-2010	10,6	10,5	4,2	13,7	17,4	9,4	13,6	2,5	14,7
	growth rate	2,1	-4,0	6,2	-7,6	-4,25	-10,0	-4,5	0,05	-7,65
– stomach	1989-1998	23,7	35,8	10,7	19,3	24,7	25,7	22,9	17,7	20,0
	2001-2010	24,1	26,1	15,0	22,8	17,0	11,7	17,9	9,9	24,4
	growth rate	0,2	-3,1	3,2	1,8	-3,6	-7,5	-2,3	-5,1	2,0
– colon	1989-1998	5,8	7,8	4,8	11,2	1,9	5,8	9,0	0,0	5,0
	2001-2010	13,4	8,0	7,7	5,5	5,8	4,7	12,1	7,5	12,2
	growth rate	8,7	0,2	4,8	-6,8	11,8	-2,0	3,0	0,0	16,5
– rectum	1989-1998	6,7	3,9	4,3	5,8	5,2	3,8	3,5	0,0	5,1
	2001-2010	13,2	6,2	8,8	8,2	5,4	4,2	5,4	0,0	0,0
	growth rate	6,3	4,7	7,4	3,5	0,3	1	4,4	0,0	0,0
– liver	1989-1998	6,9	13,8	3,6	22,8	13,9	19,5	18	10,1	17,5
	2001-2010	10,3	10,5	4,9	25,9	14,3	26,7	16	22,4	17,1
	growth rate	4,1	-3	3,1	1,3	0,3	2,9	-1,2	8,3	-0,2
– pancreas	1989-1998	7,5	6,2	3,0	4,2	3,8	3,8	3,1	0,0	2,5
	2001-2010	9,6	4,7	7,9	4,7	8,1	3,3	7,8	5,0	2,4
	growth rate	2,5	-2,8	10,2	1,1	7,8	-1,4	8,7	0,0	-0,4
Respiratory organs, total	1989-1998	31,9	55,8	19,7	40,6	46,1	44,2	39,1	22,5	45,0
	2001-2010	54,8	53	26,9	35	34,4	39,3	37,7	32,3	46,5
	growth rate	5,5	-0,5	3,1	-1,6	-2,9	-1,1	-0,3	5,6	6,7
– larynx	1989-1998	2,9	5,7	2,3	0,8	2,8	3,4	1,0	2,5	2,5
	2001-2010	5,4	2,9	3,6	2,0	1,5	0,0	1,9	2,5	4,9
	growth rate	6,4	-6,5	4,6	9,6	-6,0	-8,9	6,0	0,05	1,1
– trachea, bronchi, lung	1989-1998	26,5	46,9	16,1	39	40,9	0,0	38,1	20,0	40,0
	2001-2010	49,4	50,1	23,3	33	32,9	39,3	35,8	29,8	41,6
	growth rate	6,4	0,6	4,2	-1,6	-2,1	-0,05	-0,6	4,0	0,4
Bones and articular cartilage	1989-1998	1,4	3,2	1,4	3,5	2,8	2,9	1,1	25,0	47,4
	2001-2010	3,4	1,5	1,9	0,4	2,3	0,0	3,5	0,0	2,4
	growth rate	9,3	-7,3	3,2	-10	-1,9	0,0	12,3	0,0	-10,0
Skin (including melanoma)	1989-1998	6,0	5,5	4,9	0,8	2,5	2,4	2,4	2,6	5,1
	2001-2010	9,3	4,3	8,3	3,9	9,3	3,2	2,3	2,5	4,9
	growth rate	5,0	2,4	5,4	17,2	14,1	2,9	-0,4	-0,4	-0,4
Female reproductive organs - total	1989-1998	58,3	66,1	42,3	19,2	24,1	24,5	33,6	26,0	24,5
	2001-2010	99,7	85,2	86,3	37,6	30,3	23,0	43,7	25,0	29,4
	growth rate	5,5	2,5	7,4	6,9	2,3	-0,6	2,7	-0,4	6,3
- mammary gland	1989-1998	26,1	31,4	27,6	9,2	9,6	8,5	17,8	10,4	14,7
	2001-2010	54,2	32,5	47	14,6	15,9	7,4	24,1	10,0	24,5
	growth rate	7,5	0,3	5,4	5,2	5,1	-1,3	3,1	-0,4	5,2
- cervix	1989-1998	17,1	17,7	6,2	5,4	5,5	7,5	9,6	5,2	4,9
	2001-2010	20,9	33,9	16,8	10,0	6,8	10,1	12,0	5,0	0,0
	growth rate	2,05	6,7	10,5	6,4	2,15	3,0	2,25	-0,40	0,0
- uterus	1989-1998	3,7	4,6	3,8	0,8	2,8	2,8	2,1	0,0	0,0
	2001-2010	11,8	6,5	11,7	4,6	3,8	1,8	2,3	5,0	4,9
	growth rate	12,3	3,5	10,8	19,1	3,1	-4,3	1,0	0,0	0,0
- ovary	1989-1998	11,4	12,4	4,7	3,8	6,2	5,7	4,1	10,4	4,9
	2001-2010	12,8	12,3	10,8	8,4	3,8	3,7	5,3	5,0	0,0
	growth rate	1,15	-0,1	8,7	2,0	-4,75	-4,25	2,6	-7,05	0,0
Male sexual organs - total	1989-1998	5,8	5,0	4,0	3,9	3,5	2,0	2,1	0,0	5,1
	2001-2010	10,5	5,8	8,8	3,2	4,0	6,7	7,2	5,0	4,9
	growth rate	6,1	1,5	9,2	-1,9	1,2	12,8	13,1	0,0	-0,4
- prostate	1989-1998	5,4	4,4	2,1	3,1	0,7	1,0	0,7	0,0	5,1
	2001-2010	8,9	5,8	7,2	3,2	2,4	4,8	4,8	0,0	4,9
	growth rate	5,1	3,1	13,2	0,3	13,1	17,0	21,3	0,0	-0,3
- testicle	1989-1998	0,4	0,6	1,9	0,8	2,8	1,0	1,4	0,0	0,0
	2001-2010	1,6	0,0	1,6	0,0	1,6	1,9	2,4	5,0	0,0
	growth rate	14,9	0,0	-1,7	0,0	-5,4	6,6	5,5	0,0	0,0
Urinary organs	1989-1998	6,1	7,0	5,3	5,8	4,5	4,4	8,6	0,0	5,1
	2001-2010	17	11,2	12,1	6,3	9,3	6,5	12,1	5,0	2,4
	growth rate	10,8	4,8	8,6	0,8	7,5	3,9	3,4	0,0	-7,2
- kidneys	1989-1998	3,8	5,4	4,2	4,6	3,5	3,9	5,5	5,2	5,0
	2001-2010	9,8	5,8	8,5	4,3	7,0	6,5	8,6	5,0	2,4
	growth rate	9,9	0,7	7,3	-0,6	8,0	5,2	4,5	-0,4	-7,1
- urinary bladder	1989-1998	2,3	1,6	1,1	1,2	1,0	0,5	3,1	2,5	0,0
	2001-2010	7,2	5,4	3,6	2,0	2,3	0,0	3,5	0,0	0,0
	growth rate	12,1	13	12,6	5,8	7,8	0,0	1,1	0,0	0,0
– central nervous system	1989-1998	3,0	2,9	1,6	2,3	3,1	1,9	1,0	5,1	0,0
	2001-2010	3,9	2,2	3,8	2,4	3,1	3,3	2,3	0,0	2,4
	growth rate	2,6	-2,7	9,0	0,45	0,05	5,7	9,7	0,0	0,0
– thyroid gland	1989-1998	3,0	5,5	1,7	0,8	0,7	1,4	2,1	0,0	4,9
	2001-2010	3,9	4,7	7,7	2,0	4,6	1,9	3,9	7,5	4,9
	growth rate	2,6	-1,5	13,5	9,6	20,7	3,1	8,0	0,0	0,0
– hemoblastosis	1989-1998	12,0	7,7	8,0	4,2	7,3	5,2	3,5	2,5	5,0
	2001-2010	14,2	7,3	11,7	7,5	5,8	5,6	6,6	14,9	7,3
	growth rate	1,9	-0,5	3,9	5,9	-3,2	0,7	7,3	19,5	3,8

(Per 100 thousand population) [4-6]

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EPIDEMIOLOGY OF PARKINSON'S DISEASE IN THE RS (YA)

ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative disorder in the world after Alzheimer's disease. The prevalence of the disease varies widely in different ethnic and geographical groups. The purpose of the research is to study the epidemiological picture of PD in the population of the Sakha (Yakutia) Republic. We used the next sources of information: 1) own data collected during the examination of patients; 2) retrospective analysis of medical records about patients of the neurological department; 3) data from annual reports of neurologists; 4) the results of selective population studies conducted by the Department of neurology and psychiatry of Medical Institute of NEFU. All information was entered in the database «Register of patients with Parkinson's disease in the Sakha (Yakutia) Republic». The prevalence of PD in Yakutia was 67 per 100,000 of the adult population. The disease was more common in women than in men: 79.7 versus 52.9 per 100,000. The prevalence of the disease in Yakutsk was 76.5 per 100,000 population, and in the regions it varied widely from 9.8 to 185.6 per 100,000 population. The highest frequency of PD was found in the central regions (Gorny, Khangalassky), in the regions of the Vilyui group (Vilyuisky and Verkhnevilyuisky), as well as in Tattinsky, Abyisky, Verkhnekolymsky and Lensky regions. The prevalence of PD increased in the older age groups: in the 40-49 age group this parameter was 12.9 per 100,000, and in the 70 years and older group it was 527.5 per 100,000. We did not reveal statistical differences in prevalence of PD among the Yakut (75.4 per 100,000) and Russian (73.4 per 100,000) population. The incidence of PD persists at a low level, but over the past 5 years, there has been a tendency to increase it: if in 2011 it was 1.42 per 100,000, in 2016 it would be 3.86 per 100,000 population. Thus, we conducted an epidemiological study of PD in Yakutia for the first time, identified regional features, found that the disease with the same frequency occurs in the Yakut and Russian population.

Key words: Parkinson's disease; epidemiology; prevalence; incidence.

INTRODUCTION

Parkinson's disease (PD) is one of the most common age-dependent neurodegenerative diseases [4]. If PD is extremely rare up to 40 years, among those over 60 years of age prevalence reaches 1%, and among those over 80 years old - 4% [8].

The epidemiological data of the disease varies widely in different ethnic and geographical groups [6]. For example, prevalence of PD in France is 308 [9],

Great Britain – 128 [12], and Egypt – 436 per 100,000 population [10]. In Russia, the epidemiological situation of PD is heterogeneous, and prevalence rates range from 17 to 139.9 per 100,000 population [5, 7]. This situation is probably due to different designs of the studies conducted, as well as the hypodiagnosis of the disease itself due to low awareness of the population, the difficulties in differential diagnosis with other motor disorders accompanied by parkinsonism [3, 5].

Therefore, epidemiological studies of PD is important for determining potential risk factors and improving understanding of the course of the disorder. In addition, these data are used for effective planning of medical care and rational use of health resources [2, 11].

The purpose of the research is to study the epidemiology of PD in the population of the Sakha (Yakutia) Republic.

MATERIAL AND METHODS

The study was conducted at the Department of neurology and psychiatry of the Medical Institute of M.K. Ammosov North-Eastern federal university (NEFU) and the clinical bases of this department: the neurological department of Republican Hospital Number 2 – The Center of emergency medical aid, University Clinic of NEFU in the period from 2015 to 2017. The study was approved at the meeting of the Local Committee for Biomedical Ethics of the Yakutsk Scientific Center for Complex Medical Problems (Protocol No. 43 of 9 November 2016, Decision No. 2).

We used the next sources of information: 1) own data collected during the examination of patients; 2) retrospective analysis of medical records about patients of the neurological department; 3) data from annual reports of neurologists; 4) the results of selective population studies conducted by the Department of neurology and psychiatry of Medical Institute of NEFU. All information was entered in the database "Register of patients with Parkinson's disease in the Sakha (Yakutia) Republic".

At the end of the research period, following indicators were calculated: prevalence – the total number of patients with PD in the population, calculated for 100,000 of the population; incidence – the number of new cases of PD in the population for 1 year, calculated for 100,000 of the population.

Epidemiological data were calculated for the adult population of Yakutia, for individual regions, and for males and females. The prevalence was calculated among the Russian and Yakut population.

RESULTS

The population of the Yakutia at the time of data processing (March 2017) was 959,689 people, including persons of 18 years and over - 698,735 people, persons 40 years and over - 371,637 people. During the period of the study in the Yakutia, there were 468 cases of PD. The prevalence of the disease was 67 (95% CI: 60.9-73.0) per 100,000 of the adult population, which is almost 2 times lower than the global figure.

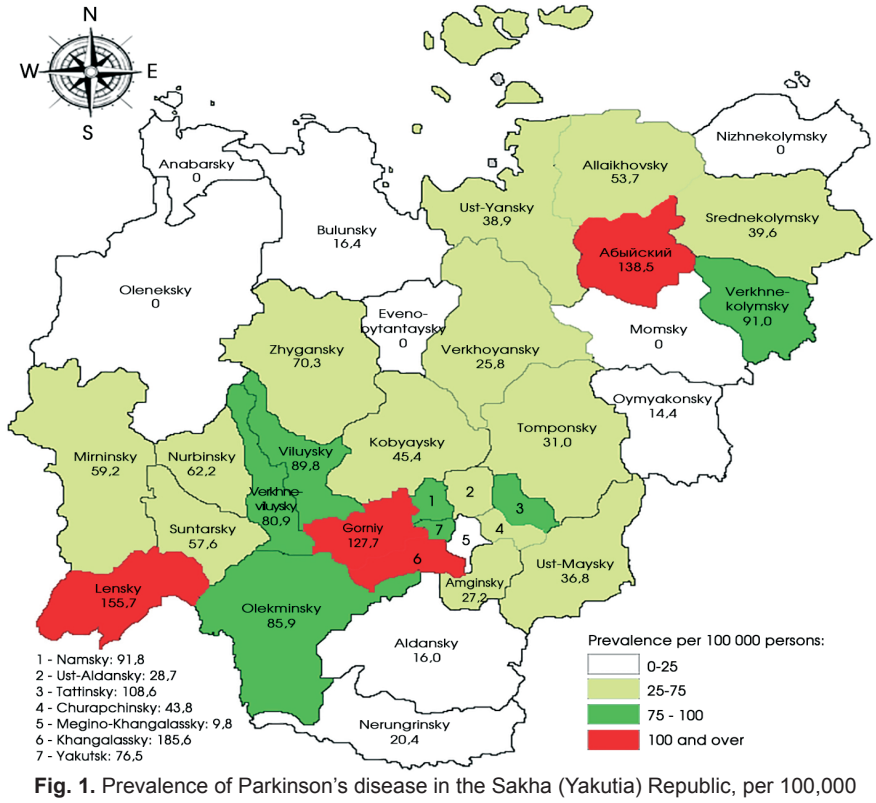

Fig. 1. Prevalence of Parkinson's disease in the Sakha (Yakutia) Republic, per 100,000 population

PD was more common in women [79.7 (95% CI: 80.2-100.9) per 100,000] than in men [52.9 (95% CI 40.9-55.1) per 100,000], which can be explained by the greater concern of women with their state of health, as well as by the shorter life expectancy of males. In general, the ratio of men and women was 1: 1.65.

PD was identified in 29 of the 34 administrative regions of the Yakutia and in Yakutsk. The prevalence of the disease in the capital of the republic, in the city of Yakutsk, was 76.5 (95% CI: 65.7-87.4) per 100,000 people, and in the regions varied from 9.8 to 185.6 per 100,000 (fig. 1).

The highest frequency of PD was found in the central regions (Gorny, Khangalassky), in the regions of the Vilyui group (Vilyuisky and Verkhnevilyuisky), as well as in Tattinsky, Abyisky, Verkhnekolymsky and Lensky regions. In this regions of the Yakutia prevalence of PD coincide with global indicators. Lower prevalence levels were recorded in the northern regions: Ust-Yansky, Bulunsky, Srednekolymsky, Verkhoyansky and others, and in the two southern regions – Aldansky and Neryungrinsky.

We have identified areas that are "free" from PD: Anabarsky, Momsky, Nizhnekolymsky, Oleneksky, Eveno-Bytantaisky. The absence of PD cases in these administrative-territorial regions can be explained by insufficient specialized care for patients with neurological diseases. However, this fact is probably also due to a low population, including elderly and senile patients and, correspondingly, a smaller expected number of patients with PD.

A study of the prevalence of PD among the age groups showed that the frequency of occurrence of the disease increases in older age groups: in the 40-49 age group the prevalence was 12.9 (95% CI: 6.6-19.3) per 100,000 population, in group of 70 years and older – 527.5 (95% CI: 454.4-600.6) per 100,000 population (Fig. 2).

The study showed that the prevalence of PD in women is higher in all age categories, except for the group of 70 years and older, where this parameter for men was 560.4 (95% CI: 427.6-693.2) per 100,000, for women – 511.9 (95% CI: 424.5-511.9) per 100,000 (Fig. 3).

According to the results of the All-Russian Population Census in 2010 in the Yakutia lived 958,528 people, of which 466,492 –Yakut ethnic group and 353,649 - Russian [1]. We have standardized the population by ethnic group for the adult population of the republic for 2017 to compare the frequency of occurrence of PD. As a result, it was revealed that the disease was spread with practically the same frequency among the Yakut ethnic group and among the Russian population. Thus, this indicator was 75.4 (95% CI: 66.2-84.6) per 100,000 for the Yakut population and 73.4 (95% CI: 62.9-83.9) per 100,000 for the Russian population.

The incidence of PD in 2011 was 1.42 per 100,000 of the population. Within 5 years there has been a steady increase in this indicator, which reflects an improvement in the diagnosis of the disease. So, in 2016 the incidence of PD was 3.86 per 100,000

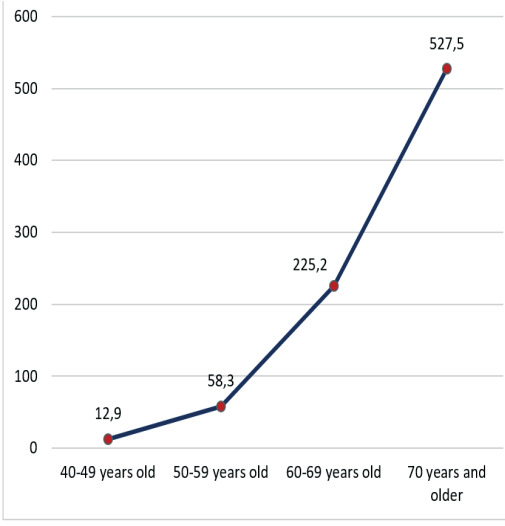

Fig. 2. revalence of PD in different age groups, per 100,000 population

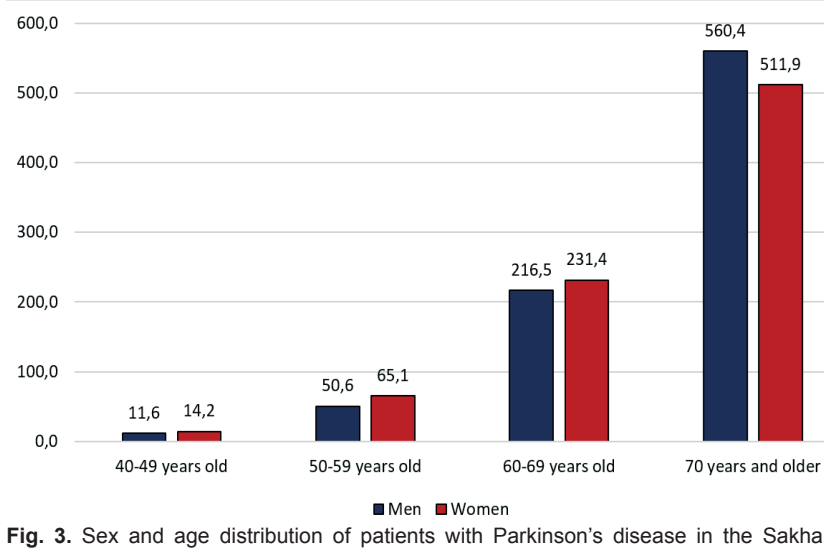

Fig. 3. Sex and age distribution of patients with Parkinson's disease in the Sakha (Yakutia) Republic

The incidence of Parkinson's disease in the Sakha (Yakutia) Republic in 2011-2016, per 100,000 of the population

Year	2011	2012	2013	2014	2015	2016
Incidence	1.42	1.25	1.39	2.29	2.58	3.86

of the population (Table 1). However, the indicator does not reach the international level, which requires further improvement of the quality of care in this category of patients.

CONCLUSION

The prevalence of PD in the Yakutia is heterogeneous. The disease is more evident in those rural areas where the work of a neurologist is well established. The prevalence of patients with female PD is due to women's concern for their health and longer life expectancy. The wide gap between epidemiological indicators in the neighboring areas indicates the role of medical support in the active detection of PD. In addition, in some parts of the region, especially in northern areas, cases of PD are not identified, which may be explained by the small number of elderly and senile patients and the low expected number of patients. The differences in frequency of PD among the Yakut and Russian population were not found. The increase in the incidence of PD in the last 5 years is associated with the appearance of a parkinsonologist in the Yakutia.

Thus, in order to improve the organization and increase the availability of specialized medical care for patients with PD, the organization of a educational base for neurologists, the introduction of new methods of diagnosis and treatment in practice, improving the social adaptation of patients in the Sakha (Yakutia) Republic, there is a need to create a center for extrapyramidal pathology.

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DYNAMICS OF THE STRUCTURE OF GASTROENTEROLOGICAL MORBIDITY IN PATIENTS OF DISPENSARY GROUPS OF 20- YEAR OBSERVATION

ABSTRACT

The dynamics of the structure of gastroenterological morbidity is analyzed on the example of 134 patients p. Vilyuchan of the Suntarsky district, who were under clinical observation for 20 years since 1980 and were subjected to repeated examinations (in 1990, 1997 and in 2001).

Keywords: Disease, digestive organs, structure of morbidity, dispensary groups, dynamics.

MATERIALS AND METHODS

Prophylactic medical examination in 1980 passed 422 people (171 men and 251 women), with a total adult population of 495 people, or 85.25% of the population. According to the size of the population p. Vilyuchan, by the time of the second inspection in 1990, out of the first examined, 189 people had left, 135 out of them in connection with the departure and 54 due to death. In 1990, with an adult population of 484 people, 77.3% were examined and 233 people were examined again. More than half of the contingent was people in Age from 18 to 40 years. Individuals aged 60 years and over were 17.6%.

In 2001, 69.19% of the 422 adults surveyed for the out-of-hospital population were examined, including 134 patients registered on dispensary since 1980. According to the data received, in 1980, 76 people were practically healthy (18% of those surveyed), in 1990 - 14 (3.7%), of the 134 people in 2001, only 6 people were practically healthy (4, 48%).

RESULTS AND DISCUSSION

General incidence of adult population p. Vilyuchan of the Suntar region is characterized by high indicators both in 1980 and 1990 and has a tendency to increase in subsequent years.

In the structure of the general incidence of digestive diseases, the leading place is occupied by the leading one. In 2nd place are diseases of the blood circulation system, in 3rd place - in 1980 and 1990 diseases of the respiratory system.

In 2001, the third place went to diseases of the genito-urinary organs (Fig. 1). At the 4-th place in the structure of the general incidence of the adult population - diseases of the musculoskeletal system and connective tissue.

In the dynamics of medical examination for 1980-1990-2001, the growth rates of the incidence of the population is observed in many classes of diseases: the circulatory system, digestive system, musculoskeletal system and connective tissue, nervous system, urogenital organs due to urolithiasis and uric acid Diathesis. Especially it should be noted the growth of helminthiases.

Those who were practically healthy during the preventive examination were 180.1 ‰ in 1980, 37.7 ‰ in 1990, 20.54 ‰ in 2001

Analysis of gastroenterological morbidity in the population p. Vilyuchan, according to the data of preventive examinations conducted in 1980-1990-2001, shows a high prevalence of diseases of the digestive organs and their non-growth in dynamics.

The most common diseases of the stomach and duodenum, although in recent years, their decrease in morbidity and specific gravity (Fig. 2).

At the same time there was a statistically significant increase in the diseases of the hepatobiliary system and pancreas and their sharp growth by 2001. It is alarming the sharp growth of calculous and calculous cholecystitis in 1990 with a 4-fold increase in the number of patients with postcholecystectomy syndrome in 2001 and giardiasis cholecystitis. The prevalence of colon diseases continues to be high in 2001, but the highest incidence rates of colon diseases, exceeding the 1980 data by 5.7 times, were noted in 1990 (Fig. 3).

In p. Vilyuchan in the course of 20 years, was followed by 134 people undergoing medical check-up, both in 1990 and in 2001, of which 103 were examined in 1997.

44 patients out of 103 gastroenterological patients, examined in 1997, in 1980 were practically healthy, 11 of which were in those years in childhood and adolescence. By age - these are people who by 2001 have become older (compared to 1980) for 20 years.

In the structure of morbidity the pathology of the digestive organs predominates with a consistent increase in indices. Invariably the 2nd place is occupied by diseases of the circulatory system. At the same time, the number of diseases of the circulatory system has increased.

Of particular note is the sharp increase in diseases of the genitourinary system (urine-stone disease and urine acid diathesis), the musculoskeletal system and connective tissue (osteochondrosis), the nervous system (posttraumatic and discirculatory encephalopathy). Among gastroenterological patients, 9 people suffered from obesity. Attention is drawn to the subsequent growth of cases of tuberculosis of respiratory organs, as well as gynecological diseases, mainly

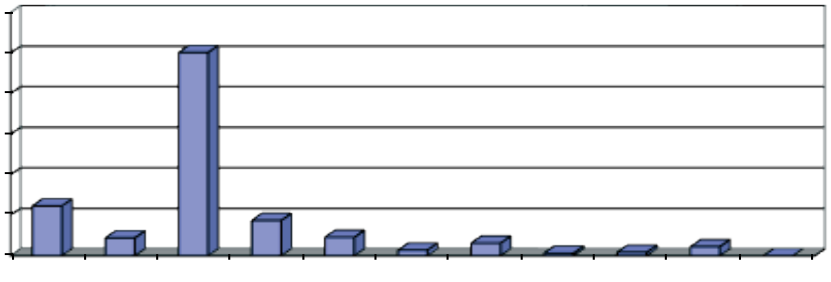

Fig. 1. Morbidity of the population p. Vilyuchan of the Suntarsky district according to the data of the medical check-up of 2001.

1 - diseases of the circulatory system; 2 - diseases of the respiratory system; 3 - diseases of the digestive system; 4- diseases of the urino-genital organs; 5 - diseases of the musculoskeletal system and connective tissue; 6 - diseases of the endocrine system; 7 - diseases of blood and blood-forming organs (anemia); 8 - diseases of the nervous system; 9 - tuberculosis of the respiratory system; 10 - gynecological diseases; 11 - other.

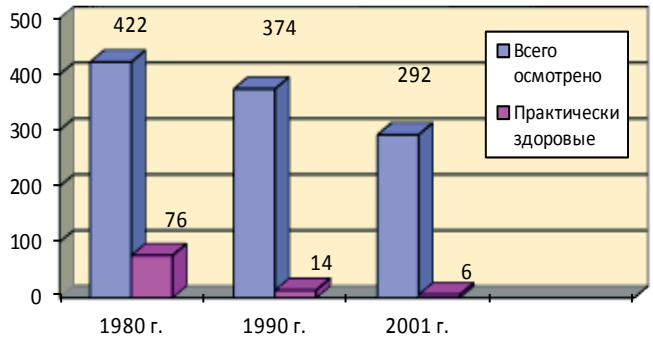

Fig.2. The contingent of practically healthy according to the data of professional examinations in dynamics.
Those who were practically healthy during the preventive examination were 180.1 ‰ in 1980, 37.7 ‰ in 1990, 20.54 ‰ in 2001

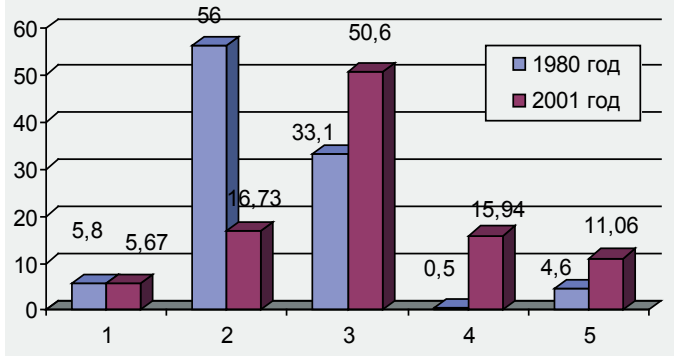

Fig. 3. Gastroenterological morbidity of the population p. Vilyuchany
1 - diseases of the esophagus; 2 - diseases of the stomach and duodenum; 3 - diseases of the hepatobiliary system; 4 - pancreas diseases (pancreatitis); 5- Bowel disease.

due to uterine myoma. In the described period, there was an increase in the incidence of diseases of food-digestive organs (Fig. 4).

The highest rates of growth in morbidity rates are characteristic for diseases of the hepatobiliary system, pancreas and intestine, mainly due to colonopathy. At the last dispensary examination in 2001, 68 patients from 103 patients of the previous examination of 1997 passed a gastroenterological examination. Among them were 28 men and 40 women. In the dynamics, the increase in the diseases of the gastrointestinal tract was revealed from 237 cases in 1997 to 293 cases in 2001, mainly in women (from 143 to 181 cases, respectively).

In the structure of gastroenterological morbidity there is a decrease in the specific gravity of diseases of the esophagus, stomach, duodenum and stomach with an increase in the diseases of the hepatobiliary system and the pancreas (Fig. 5).

At the same absolute absolute number of patients with esophageal pathology, in the compared years there was an increase in men of reflux esophagitis II and III degree of severity. A high percentage of chronic gastritis with hypoxecretion and achlorhydria in recent decades can be related to the age of patients who, by the time of the last two examinations, are older by 17-20 years.

The pattern of changes in the gastric mucosa, confirmed by morpho-endoscopic studies, was traced in 29 healthy and 40 patients with chronic gastritis, examined in dynamics at intervals of 20, 10, 7 years and 3 years. In dynamics, the growth of pathomorphological changes in the gastric mucosa as in practically healthy patients and in patients with superficial gastritis is clearly traced. Already in 1990, only one woman was found to be practically healthy, the rest of the patients, who were healthy at the first examination, had gastric changes in the gastric mucosa. The aggravation of the degregenerative de-structural changes in the gastric mucosa with a persistent decrease in the level of acid formation was noted in 28 of 40 patients with chronic gastritis.

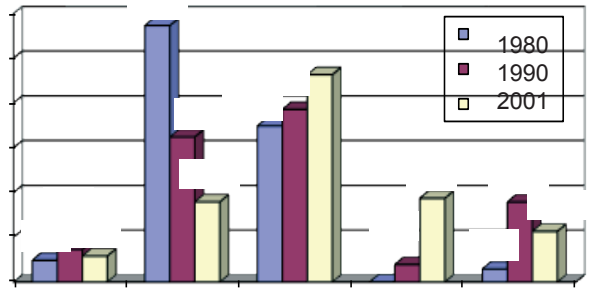

Fig. 4. Structure of gastroenterological morbidity in dynamics
1 - diseases of the esophagus; 2- diseases of the stomach and duodenum; 3- diseases of the hepatobiliary system; 4 diseases of the pancreas; 5 - bowel diseases.

The esophagogastroduodenoscopy, carried out from 1980 to 2001 in dynamics 4 times, indicates the involvement of the esophagus, duodenum, hepatobiliary system in the pathological process, as

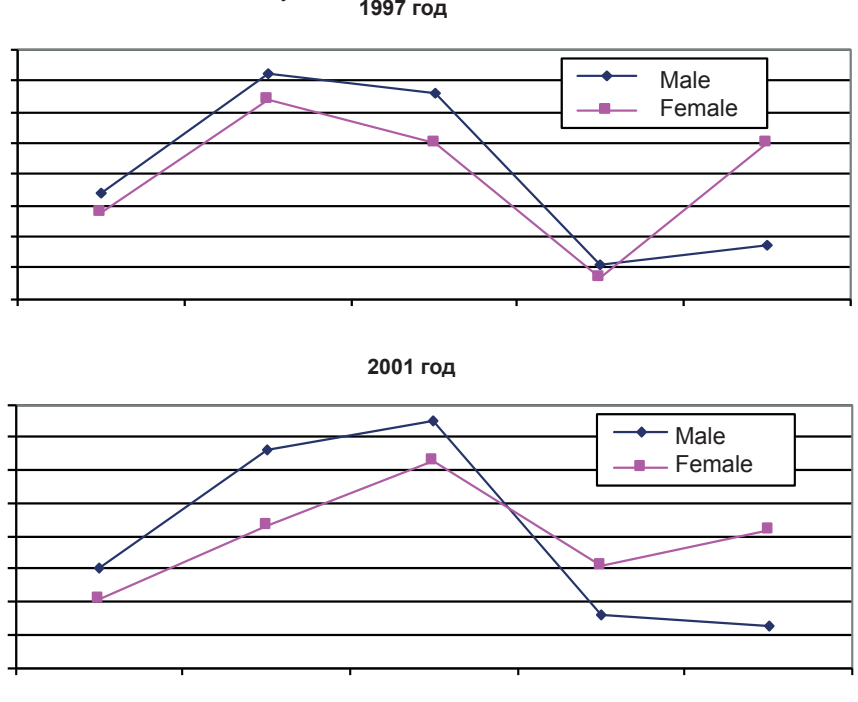

Fig. 5. Structure of gastroenterological morbidity
1 - diseases of the esophagus; 2- diseases of the stomach and duodenum; 3- diseases of the hepatobiliary system; 4 diseases of the pancreas; 5 - intestinal diseases.

well as the violation of the motor and evacuation function of the gastrointestinal tract.

Perennial endoscopic observations of practically healthy individuals and patients with various forms of chronic gastritis confirm the progression of changes in the gastric mucosa with the age of the patient and the absence of reverse development of atrophic gastritis. In patients with chronic gastritis, regardless of its clinical morphological variant, stratification of the pathology of other organs of the digestive tract aggravates the course of the underlying disease.

Among the accompanying pathology in gastroenterological patients, the leading place in 1997 was occupied by diseases of the circulatory system, exceeding the primary index by 5 times in both men and women (Table 2.4.8). Continuing to increase, the diseases of the circulatory system receded to the third place, giving way to diseases of the genitourinary organs and the musculoskeletal system. The frequency of detection of respiratory diseases in gastroenterological patients in the last decade (according to 1997 and 2001) moved to the 4-th and 6-th places. Women with age have a higher proportion of diseases of the endocrine system and anemia (Table 2.4.9).

Thus, the frequency of concomitant pathology is determined by the age and sex of the patient.

Attention is drawn to the intensive growth of osteochondrosis with the age of the patients: in men 6 times, in women 23 times. Compared with baseline data, the frequency of diseases of the musculoskeletal system exceeded in 2001 in men 10 times, in women 45 times. There is concern about the continuing increase in the incidence of urolithiasis, whose incidence in 2001 was many times higher than the primary rates. It is alarming the growth of calculous (from 0 to 20 cases) and lambliasis cholecystitis (from 0 to 15 cases).

In the structure of gastroenterological morbidity, the leading role belongs to the diseases of the hepatobiliary system, pancreas and intestines. Dynamic examination with an interval of 10 years allows to assess the degree of progression of the degregenerative and dystrophic processes of the gastric mucosa, the risk of transformation into more severe forms of chronic gastritis, the layering of complications of the esophagus and intestine.

The effect of various exogenous and endogenous factors on the development of chronic processes was traced in 102 patients with gastritis with a decreased secretion, examined in dynamics based on anamnestic data analysis for 12 factors. The average age of men was 49.04 ± 0.95 years, women - 50.92 ± 1.69 years. For 10 years (1980-1990), with a preserved average growth of 154.2 ± 0.9 cm, body weight increased from 56 ± 0.01 to 58.7 ± 0.97 kg.

Hereditary complication in oncological diseases was detected in 33 patients (32.3%) with a distinct predominance of weighting along the line of the father (19.6 ± 3.91%), the hereditary maternal burden was noted in 8.82 ± 8, 1% of patients, in the line of both parents and direct relatives - 0.98 ± 0.97 and 2.94 ± 1.67%, respectively.

Of the 102 patients, most do not smoke, including those who quit smoking on the recommendation of doctors in connection with the disease (59.8 ± 4.85%), 38.24 ± 4.81% of patients are constantly smoking at least a pack a day. Absolutely do not drink alcoholic beverages 16.67 ± 3.19% of patients, rarely consuming were 54.9 ± 4.92%. Alcohol consumption once a month was noted in 7.84 ± 2. 66% of patients who regularly drink once a week - 8.82 ± 2.81%, threw out drinking for various reasons was 9 people (8.82 ± 2.8%).

One of the risk factors for the disease of the digestive system is tooth decay, noted in all patients examined in the dynamics. An essential role in the development of the disease of the digestive system has alimentary factors. In this case, a special place is occupied by a disturbance of the diet (62.75 ± 4.7%), consisting of irregular feeding (with long intervals between meals, overeating in the evening). A satisfactory quality of nutrition was noted in 92.16 ± 2.66% of patients, good - in 6.86 ± 2.5%, unsatisfactory nutrition was established in one. In the daily diet, meat of this group is dominated by meat (79.41 ± 4.0%), milk and dairy products (82.35 ± 3.77%), bread and flour products (90.2 ± 2.94%). The presence of fish in the grocery set is noted in 33.3 ± 6.6% of patients. Fresh vegetables in nutrition are seasonal in nature and their regular intake during the survey was detected in 8.82 ± 2.85% of patients. It should be noted a certain tendency to abuse sweets and fatty foods (8.82 ± 2.81 and 17.64 ± 3.77%, respectively).

Conjugation of diseases of the digestive system with the blood group is shown in 46 patients with chronic gastritis with a decreased secretion. Among the surveyed, the largest percentage were people with A (II) blood group (41.3%). Patients with 0 (I) and B (III) had 11 blood groups (23.9%), and A (IV) blood group was detected in 5 patients (10.9%).

The average age of patients with chronic gastritis with a decreased secretion corresponded to 50 years, which, perhaps, can be attributed to factors of cancer risk in connection with the hormonal changes and metabolic changes typical for this age period.

Based on the generalization of data, the above exogenous and endogenous factors should be considered as factors contributing to the emergence of the pathological process and its chronicization.

Long-term clinical and epidemiological studies conducted in the dynamics in p. Vilyuchan of the Suntarsky district of the Republic of Sakha (Yakutia), while increasing the level of general morbidity of adults, which shows a progressive deterioration in the health of the population. The obtained data are correlated with the statistical data of the M3 PC (H). For the period 1997-2001. There was an increase in the incidence of diseases of the hepatobiliary system, mainly due to diseases of the gallbladder and bile ducts. There is a tendency to rejuvenate cholelithiasis. It is connected, as it seems to us, not only with the improvement of diagnostics, but above all the true quantitative growth of the disease. Among the reasons that lead to the growth of gastrointestinal diseases, it is necessary to identify metabolic disorders, hypodynamia, lifestyle changes associated with social and economic transformation

Hypomotor dyskinesia of the bile ducts, which predominate among the indigenous population, regardless of their age, lead to the development of secondary inflammatory processes in the gallbladder. The growth of helminthiases and diseases of the gallbladder parasitic etiology is of particular concern.

Endoscopic studies with morphological verification, conducted in dynamics in practically healthy people and in patients with chronic gastritis, confirm the progression of pathomorphological changes in the gastric mucosa with the age of the patient, which must be taken into account when developing therapeutic and prophylactic measures.

Analysis of the acid-forming function of the gastric mucosa, carried out in dynamics on the same patients, shows the presence of interrelations of the secretory function of the stomach with the change in the state of the gastric mucosa with the age of patients.

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RESULTS OF NEONATAL SCREENING ON ADRENOGENITAL SYNDROME IN CHILDREN IN THE RS (YA) FOR 10 YEARS

ABSTRACT

The results of neonatal screening in the Republic of Sakha (Yakutia) (RS (Y)) and the endocrinology department of the Pediatric Center RB-1-NTSM for ten years on congenital dysfunction of the adrenal cortex (syn. Adrenogenital syndrome) are presented in the article. Adrenogenital syndrome is a group of diseases with an autosomal recessive type of inheritance, which is based on a defect of one of the enzymes or transport proteins involved in the biosynthesis of cortisol in the adrenal cortex. Neonatal screening for adrenogenital syndrome is an effective method of early diagnosis and treatment of a disease that can prevent the development of disabling complications and death. Coverage of newborns with neonatal screening in the RS (Y) annually increases. With timely treatment of adrenogenital syndrome, the rates of physical development and puberty of the child are approaching the norm. The prevalence of adrenogenital syndrome in the RS (Y) is lower than in the Russian Federation and its regions: in the Ural Federal District, in the Siberian Federal District. The most frequent occurrence of adrenogenital syndrome is observed in Alaska residents, the lowest in China. All patients have a lossy form of the disease. Analysis of patients identified by neonatal screening did not determine significant differences in gender, place of residence. In girls, the diagnosis was made immediately after birth due to the presence of virile syndrome. A case of an incorrect determination of the sex in a girl at birth was described on the patient's medical chart and was diagnosed with hypospadias. Substitution therapy for the majority was started up to 21 days. All patients receive replacement therapy with glucocorticoids and mineralocorticoids (Cortef, Cortineff) from the time of diagnosis in an individual dosage, depending on age. Acceleration of bone age is observed only in one child, in three, a decrease in the rate of growth and a lack of body weight. The organization of neonatal screening for adrenogenital syndrome in the RS (Y) allowed achieving a high percentage of the survey of newborns, reduction of the period of examination and early initiation of substitution therapy, prevention of disability of patients.

Keywords: congenital adrenal cortex dysfunction, neonatal screening, newborn, frequency.

INTRODUCTION

Congenital adrenal cortex dysfunction (ADHD, adrenogenital syndrome (AGS), congenital adrenal hyperplasia) is a group of diseases with an autosomal recessive type of inheritance, which is based on a defect of one of the enzymes or transport proteins that participate in the biosynthesis of cortisol in the adrenal cortex [2].

For the first time the disease was described by Phillips in 1886 as pseudohermaphroditism in a girl at the age of 19 days. In 1924 O.V. Vereshchinsky for the first time in the domestic literature cited information on 12 cases of adrenal-sexual syndrome. In the years 1950-1952. F.C.Bartter, F.Albright, A.Leaf, E. Dempsye, E. Carroll, L. Wilkins deciphered the essence of this disease, the biosynthesis of hydrocortisone. VDKN is the most common pathology of the adrenal glands in children (1 case per 5000 born).

Neonatal screening contributes to the early diagnosis of ACS, especially in boys before the development of clinical symptoms, to the early onset of substitution therapy and the safe social adaptation of children. Coverage of newborns with neonatal screening in the RS (Y) annually increases. With timely treatment of adrenogenital syndrome, the rates of physical development and puberty of the child are approaching the norm.

All patients have a lossy form of the disease. Analysis of patients identified by neonatal screening did not determine significant differences in gender, place of residence. In girls, the diagnosis was made immediately after birth due to the presence of virile syndrome. A case of an incorrect determination of the sex in a girl at birth was described on the patient's medical chart and was diagnosed with hypospadias. Substitution therapy for the majority was started up to 21 days. All patients receive replacement therapy with glucocorticoids and mineralocorticoids (Cortef, Cortineff) from the time of diagnosis in an individual dosage, depending on age. Acceleration of bone age is observed only in one child, in three, a decrease in the rate of growth and a lack of body weight.

MATERIALS AND METHODS

The order of the Ministry of Health of the Republic of Sakha (Yakutia) of March 20, 2006 was issued to organize screening, introduce new methods, organize diagnostic and therapeutic care. 01-8 / 4-134a «On the progress of the activities of the section of the national project» Health «on the examination of newborn children for hereditary diseases.» The Order of the Republic of Belarus No. 1-NCM dated August 31, 2006. №01-0108 / 91 «About rendering medical aid to children with cystic fibrosis, adrenogenital syndrome, galactosemia, phenylketonuria and congenital hypothyroidism, revealed by neonatal screening» [5]. The screening procedure includes blood sampling in full-term newborns on day 4 of life, in preterm patients on day 7 and determination of 17-hydroxyprogesterone (17-ONP) levels in samples using special screening kits. The level of 17-SNP in blood samples is determined by the immunofluorescence method (test kits «Delfia 17-α-OH Progesteron», Finland, and «17-α-OH-Progesterone-Immunoskrin», Russia). The following provisions are taken into account when interpreting the indicators of 17-SNP: - the level of 17-SNP for full-term children (the gestation period is more than 37 weeks, the body weight is more than 2000 gr.) Normally up to 30

nmol / l; At a level of 17-SNP 30-90 nmol / l - the result is regarded as questionable (false positive), re-determination of 17-SNP in the control spot is required; At a level of 17-SNP more than 90 nmol / l - the result is positive, the information is transmitted at the location of the child. For preterm infants (gestation period 33-36 weeks, body weight less than 2000 g.), The normative index of 17-OHP is up to 60 nmol / l. In cases where the premature baby has levels in 17-SNP within 60-100 nmol / L - the result is doubtful (false positive). In premature infants with a level of 17-SNP more than 100 nmol / L, the result is positive, information is given at the location of the child. In children with deep prematurity (gestation period of 23-32 weeks), the result should be considered positive at a level of 17-SNP above 150 nmol / l. In this case, it is necessary to send information to the hospital or to the children's polyclinic where the child is, and to re-take and screen-test the blood sample [4].

Data on neonatal screening for the period 2006-2016. Provided by the laboratory of the Medical Genetic Center (MHC) of the Perinatal Center, data on patients - the endocrinological department of the Pediatric Center of the State Bank of the Republic of Sakha (Yakutia) «Republican Hospital No. 1-National Center of Medicine». A retrospective study of stationary charts of children with a diagnosis of congenital adrenal cortex dysfunction was carried out. Data on patients are taken from the register of admission of patients with endocrinology department (form 001 / y).

RESULTS OF THE STUDY

From 2006-2016. According to the neonatal screening program in RS (Y), only 160 626 newborns were examined, the diagnosis of VDKN was established in 11 children, the coverage was 99.5%. The frequency of VDKN 1:14 is 602 newborns. In 2006, out of 5559 newborns that had undergone the study, no ACS was detected. In 2012 Of the 16832 newborns studied, the detectability of ACS was the highest and amounted to 4 people per year at a frequency of 1: 4208 newborns (Table 1). Thus, the prevalence of ACS in the Republic of Sakha (Yakutia) is 1 case at 14,602 (Figure 1), lower than in the Russian Federation 1: 7650 and its regions: in the Ural Federal District 1: 5781, in the Siberian Federal District 1: 9681. The most frequent occurrence of ACS is observed in Alaska residents 1: 280 newborns, the lowest in China 1:28 000 [3].

Analysis of patients identified by neonatal screening did not determine significant differences in gender - 5 boys (45%), 6 (55%) girls, sex ratio 1: 1.5, place of residence - urban 5 (45%), rural 6 (55%), nationality - 4 (36%) of the Yakut child, 5 (46%) Russians, and 2 (18%) other nationalities.

Table 1

Screening of newborns for mass screening In the RS (Y) for 2006 - 2016 years

Years	The number of births in the RS (Y)	Surveyed: AGS	Coverage	Revealed	Frequency
2006	13623	5559	40,8	-	-
2007	15152	14931	98,5	1	1:14931
2008	15254	10746	70,4	2	1:5373
2009	15783	15468	98	-	-
2010	15877	15662	99	-	-
2011	16173	16092	99,5	1	1:16092
2012	16922	16832	99,5	4	1:4208
2013	16611	16546	99,6	1	1:16546
2014	16964	16946	99,8	2	1:8473
2015	16469	16459	99,9	-	-
2016	15418	15385	99,8	-	-
	Всего	160 626	98.9	11	1:14 602

For 10 years 376 (0.23%) children had an elevated level of 17-ONP. Retest was performed in 214 children with elevated levels of 17-SNP, among them term infants 101 (47%), premature infants 113 (53%) (Table 2). As a result of the retest, an increased level of 17-SNP in 56 children, among them preterm infants 43 (77%), full-term children - 13 (23%). ACS was established in 11 children, respectively 45 children had a transient increase in 17-ONP. The level of increase in 17-SNP in these cases at birth varied from 65 to 1158 nmol / l. Concentrations of 17-hydroxyprogesterone may be elevated, even when there is no deficiency of this enzyme. This is due to the peculiarities of adrenal steroidogenesis, the immaturity of the axis «hypothalamus - pituitary - adrenal glands». It happens in preterm; Children with birth trauma or severe physical illness; Against intravenous infusion; In newborns with high blood bilirubin levels; At birth with low body weight at normal gestation terms. False negative results can also be determined if the mother during the pregnancy took dexamethasone for the prevention (therapy) of lung fetal diseases or so treated a newborn (with a lack of surfactant). In such cases it is recommended to check the hormone index repeatedly - after 5-7 days [4].

The condition of children with ACS at birth in 9 children was noted as satisfactory, 2 children needed resuscitation. On the Apgar scale, almost all have high scores. The physical parameters of newborns correspond to normal indices.

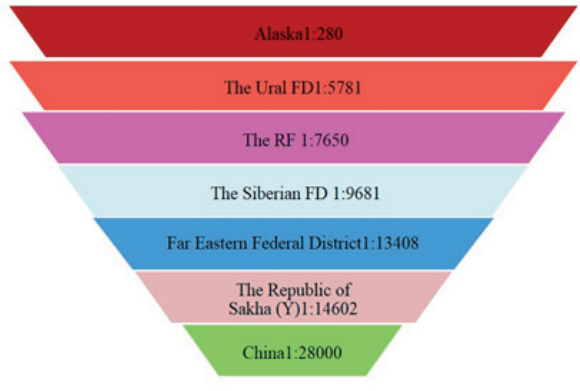


Fig. 1. The frequency of ACS in the RS (Y) in comparison with other regions

The level of 17-SNP for neonatal screening in the identified patients averaged 235.26 ± 1.09 nmol / l, (the range of oscillations from 67.17 ± 1.09 nmol / l to 413.34 ± 1.09 nmol / l). All

Table 2

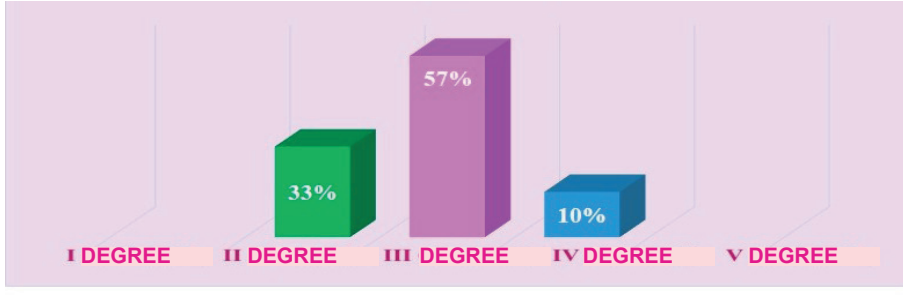
Analysis of the screening of neonatal screening

Год	Only 214 children were tested	Elevated level of 17-SNP as a result of retest in 56 children	ACS was detected in 11 children
2006	1	-	-
2007	34	1	1
2008	9	5	2
2009	5	1	-
2010	119	31	-
2011	3	1	1
2012	10	4	4
2013	5	3	1
2014	8	3	2
2015	12	5	-
2016	8	2	-

patients have a lossy form of the disease. The diagnosis of VDKN in 3 boys was put on the 21st day and the 1st month of life against the background of a saltwort crisis, in 2 boys, VDKN was detected for neonatal screening. In 4 girls, the diagnosis was made immediately after birth by the presence of a viril syndrome and the degree of virilization according to the Prader scale was II-III. In 1 girl with virilization of external genital organs of III-IV degree, sex at birth was incorrectly determined and with the diagnosis: hypospadias entered the II stage of treatment of the Perinatal Center. In 1 female, the ACS was confirmed at 5 months of age, during the examination in the psycho-neurological department No. 2, due to the irregular structure of the external genitalia (Fig. 2).

The purpose of substitution treatment for children with VDKN is not to simulate physiological secretion, but to restore the deficit of corticosteroids, the secretion of which is reduced as a result of an enzymatic defect with suppression of increased secretion of corticotropin releasing hormone and ACTH, in preventing virilization, optimizing patient growth, ensuring normal sexual Maturation and potential fertility [1].

All patients receive replacement therapy with glucocorticoids and mineralocorticoids (Cortef, Cortineff) from the moment of diagnosis in the individual dosage depending on the age (Fig. 3). Acceleration of bone age is observed only in 1 (9%) of the child, at the age of 8 years the bone age corresponds to 11-12 years. This child needs to reduce the dose of substitution therapy. In 3 (27%) children there is a decrease in the rate of growth and a lack of body weight. In this group of children, an increase in the dose of hormonal drugs is required. The remaining 5 (45%) children have a normal growth rate and bone maturation. That indicates adequate therapy.



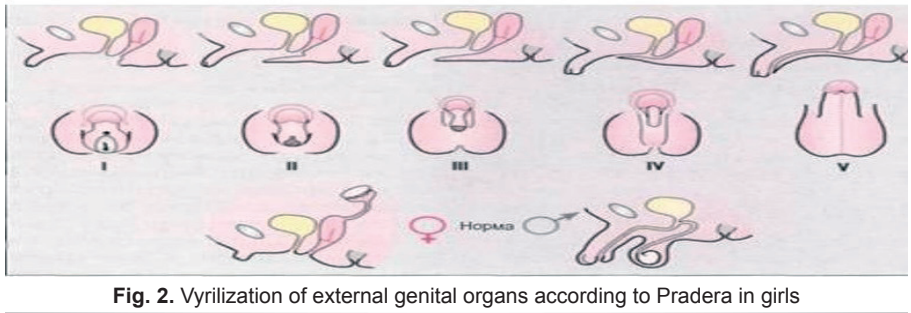


Fig. 2. Vyrilization of external genital organs according to Pradera in girls

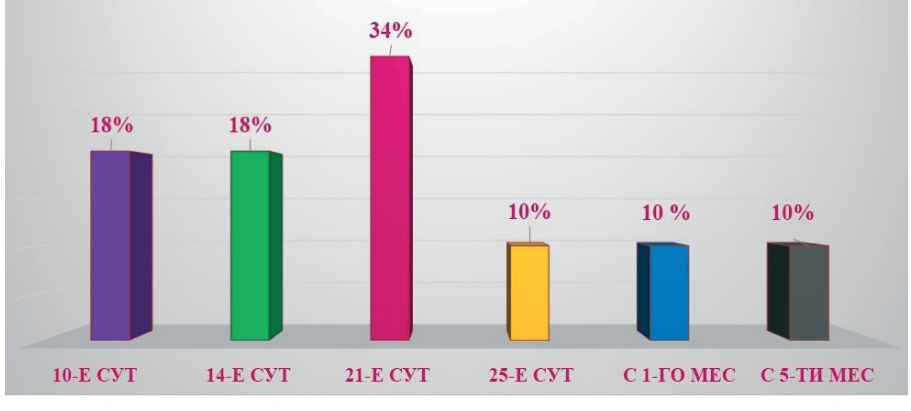


Fig. 3. Time of onset of substitution therapy

CONCLUSION:

1. Neonatal screening coverage from 40.8% in the first years of its introduction increased to 99.8%. In the period from 2006 to 2016. The frequency of ACS was 1:14 602, which shows a low incidence rate in comparison with other regions of the Russian Federation.

2. Analysis of the anamnesis, distribution by place of residence, by gender, by nationality, by the state of health at birth, by physical parameters, the Apgar score did not reveal any specific features. In all cases, there is a salt-losing form of the disease. In girls, the diagnosis of ACS is assumed at birth, due to the virilization of NGOs, in men, the diagnosis was made on the basis of clinical symptoms and neonatal screening. In most cases, the clinic begins on the 21st day of life, i.e. It is necessary for doctors neonatologists and pediatricians to send timely to the retest of newborns with increased results, to be wary of ACS, to carefully inspect the external genitalia. The average level of 17-SNP for neonatal screening was 235.26 ± 1.09 nmol / l.

3. Properly selected and timely therapy of GCS and ISS provides normal growth rates, bone maturation, sexual development and normal reproductive function.

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NUTRITION IN THE NORTH

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EPIDEMIOLOGY OF BREASTFEEDING, ITS IMPORTANCE FOR MATERNAL AND CHILD HEALTH, WELL-BEING AND SUSTAINABLE DEVELOPMENT OF SOCIETY: AN OVERVIEW OF CURRENT DATA

ABSTRACT

In this article there are presented the modern data about the breastfeeding epidemiology in the countries with the different level of income, its influence on the mother's and children's health, the possible saved children's and women's lives.

This publication was prepared by using the papers in English including the modern systematic reviews and meta-analyses on the problem of breastfeeding and published like the Breastfeeding series in Lancet (2016).

Keywords: breastfeeding, epidemiology, prevention, children's and women's health, saved lives, "Goals for the sustainable development".

Introduction

Breastfeeding has not lost the urgency and presently. It is extremely important for a survival and the further development of the child [19, 37]. The reproductive cycle not casually consists of two long components, pregnancy and chest feeding. During decades the medical practice of the most developed western countries underestimated breastfeeding. There was an opinion, that it is possible to replace it easily and without serious consequences with artificial feeding [35]. As a result there was a situation when developing countries on parameters of prevalence of breastfeeding have appeared in the best situation, rather than developed countries of the world. Modern epidemiological and biological researches confirm the fact, that the decision of mother to nurse has positive long-term effects for health and developments of children, and also for health of mothers. Probably, there are no other forms of behaviour concerning health which could have such various consequences for two essences which are involved in them - mothers and the child. Opening in the field of immunology, epigenetic and microbiom of the mother and her child, steam cells which have been made for last two decades, throw light on potential mechanisms of influence of the breastfeeding on formation of health. Other more surprising mechanisms of exclusive influence of breastt milk on health and development of the person since a birth from positions of the personified medicine are possible also.

Considering exclusive importance of breastfeeding for mother's and the child's, health and also its influence on social and economic spheres of human life, the international group of researchers has lead the analysis of existing systematic reviews and meta-analyses on English on the given problem. Results of this work have been published in Lancet in the beginning of 2016 like the Breastfeeding series and had the wide response among experts of all world, prosecute subjects of breastfeeding [6, 42].

The present publication represents translation and a statement of substantive provisions of the articles in English of this series - "Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect and " Why invest, and what it will take to improve breastfeeding practices, including questions of the breastfeeding epidemiology, its value for mother's and child's health and also a role in achievement of well-being and sustainable development of the communities.

Epidemiology of the breastfeeding in the countries with the different level of the income: rates and trends

For estimation and analysis of the breastfeeding epidemiology it is important to have precise representation about used indicators. WHO offers following indicators: the early beginning of the breastfeeding – a portion of the newborns which have been enclosed to a breast within the first hour after delivery, exclusively breastfeeding up to 6 months - a portion of children in the age of from 0 up to 5 months which are fed with exclusively breast milk; continued

breastfeeding in 1 year - a portion of children in the age of 12-15 months which receive breast milk and continued breastfeeding in 2 years – a portion of children in the age of 20-23 months which receive breast milk [43].

As only some of the rich countries (with a high level of the income) in the reports use above listed indicators additional ones were applied to an opportunity of comparison with data of other countries: a share of children ever was on chest feeding; breastfeeding in 6 months - a portion of children which were on breastfeeding since a birth and up to 6 months or is more senior also breastfeeding in 12 months - a portion of children on breastfeeding in 12 months and longer.

For the countries with a middle and low level of the income in the standardized researches following additional indicators - a portion of children on breastfeeding in the age of 4-7 months (a median 6 months) and a portion of children on breastfeeding in the age of 10-13 months (a median 12 months) were used.

The general number of the countries which data were exposed to the analysis has made 127 of 139 countries with low and middle level of the income (on their share it is necessary up to 99% from the general number of children in these countries) and 37 of 75 countries with a high level of the income. Parameters of breastfeeding were estimated for a population of children in the age of is younger than 2th years. For an estimation of dynamics of parameters of breastfeeding models of plural linear regress have been used, deviations from linear distribution [33] also were estimated.

The estimation of parameters of breastfeeding has shown that the greatest prevalence of breastfeeding among children in the age of 12 months takes place in the countries of Africa to the south from Sahara and in southern Asia, and also some countries of Latin America (a Fig. 1). In the majority of the rich countries this parameter was below 20% - from 35% in Norway and 25% in the USA up to 16% in Sweden and less than 1% in the United Kingdom.

The estimation of parameters of breastfeeding in 4 groups of the countries depending on a level of their income (low, below, middle, above a middle and high) has been lead. Data are received from national reviews of 153 countries with use of standard indicators for a population of children in the age of till 2th years. The following of 6 indicators - the early beginning of breastfeeding, a prevalence of children ever was on breastfeeding, exclusively breastfeeding in 0-5 months and in 6 months, breastfeeding in 12 months and continued breastfeeding in 20-23 months (a Fig. 2) were analyzed. The strong negative interrelation between parameters of breastfeeding in 6 months and size of the internal gross revenue on the person ($r = -0,84$; $p < 0,0001$). Doubling of the internal gross revenue has been connected with decrease on 10 % of a portion of children on breastfeeding in 12 months.

In all countries, it is not dependent on a level of the income, the majority of mothers started to nurse their children. Only in three countries - France, Spain and the USA a portion of children ever was on breastfeeding was below 80%. Nevertheless, parameters of the early beginning and exclusively breastfeeding were low for all countries.

For exception of the early beginning of breastfeeding prevalence of breastfeeding on all indicators decreased with increase of well-being of the countries. The countries with a low level of the income had high parameters of breastfeeding in all age groups but even in these countries parameters of the early beginning and exclusively breastfeeding

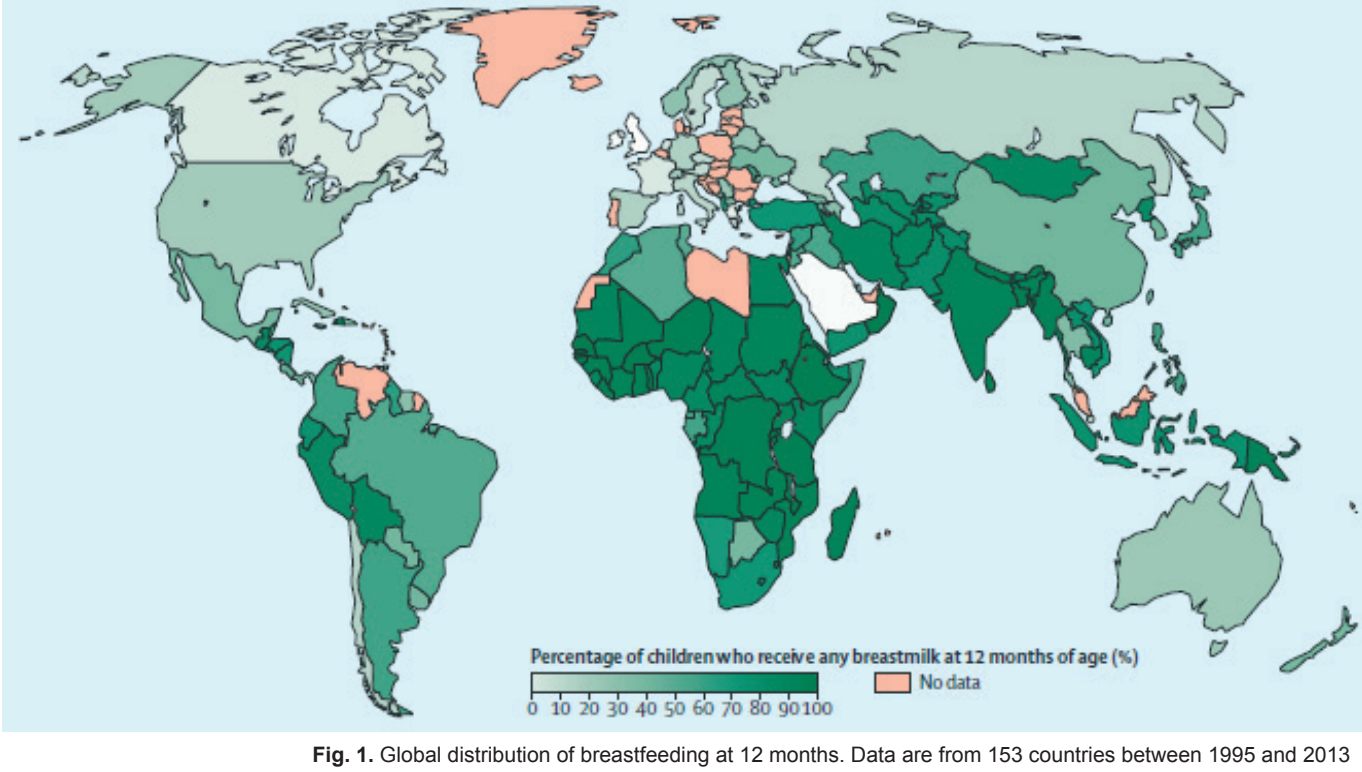
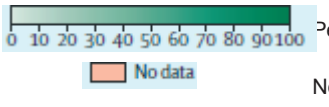


Fig. 1. Global distribution of breastfeeding at 12 months. Data are from 153 countries between 1995 and 2013



Percentage of children who receive any breastmilk at 12 months age

No data

were not satisfactory.

Surprisingly but the majority of indicators of breastfeeding have not been interconnected among themselves. Only moderate correlation communication between exclusively breastfeeding and proceeding breastfeeding in 1 year in the countries with low and an average level of the income (r=0,54) has been revealed. Parameters of exclusively breastfeeding among children in the age of 0-5 months in these countries averaged 37%.

For 20 years with 1993 for 2013 in the countries with low and middle level of the income parameters of exclusively breastfeeding have slightly grown from 24,9% up to 35,7%. In the richest families this increase has been considerably more expressed while among the poorest families it corresponded to the general trend. Continued breastfeeding in 1 year on the average has decreased in these countries - from 76,0% to 73,3% substantially due to decrease in this parameter among the poorest families.

Interest submits data on distinctions in parameters of breastfeeding between families with a different level of prosperity inside of the countries differing on a level of the income. In the countries with low and middle level of the income it has not been revealed distinctions between rich and poor families on parameters of exclusively breastfeeding. However, on parameters of a proceeding breastfeeding it is revealed, that in poor families nursed longer in comparison with rich. This tendency has been more expressed in the countries with an average level of the income [32]. Low parameters of prevalence of a proceeding breastfeeding among more provided families raise probability of that less provided mums will be developed aside substitutes of breast milk if their income will increase, a question which gains in strength in communication with decrease in levels of breastfeeding in poor populations. In the rich countries distinctions in parameters of breastfeeding depending on formation of women are revealed. Among formed mothers was more nursing, than among women with several years of formation [13, 20, 27]. For example, in the USA till 60th years the share of mothers, begun to nurse, was more, than among mothers with a low educational level but since then this social trend has replaced the orientation on opposite.

Short-term effects of breastfeeding: diseases and death rate among children of early age

Influence of breastfeeding on following

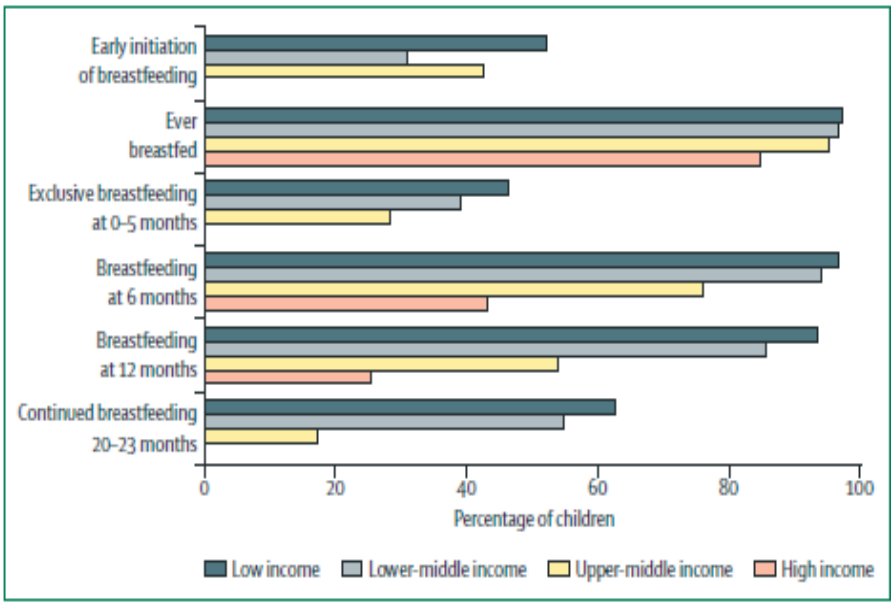


Fig. 2. Breastfeeding indicators by country income group in 2010

parameters of health of children of early age was considered: death rate, frequency of infectious diseases (a diarrhea, an infection of the low respiratory ways, an acute otitis), frequency of allergic diseases (eczema, a food allergy, an allergic rhinitis, a bronchial asthma), physical development (growth, weight, an index of weight of a body), health of a teeth (caries, an estimation of a bite).

On an example of the countries with low and middle level of the income it has been shown, that breastfeeding considerably reduces risk of death from infectious diseases. The probability of death among babies on exclusively breastfeeding in the first 6 months of a life makes only 12% in comparison with children who are not receiving breast milk [34]. In other researches it has been shown, that among children 6 months without breastfeeding higher parameters of death rate - above in 3,5 times among boys and in 4,1 times among girls, in comparison with children received breast milk even in a small amount were more younger. However, protective properties of breast milk decrease with the years [41]. These results have been confirmed by researches among children in the age of 6-23 months in which with breastfeeding connected decrease in death rate on 50%. About half of all cases of a diarrhea and third of respiratory infections could be prevented owing to breastfeeding [15]. Besides breast feeding could prevent 72% of cases of hospitalization owing to a diarrhea and 57% of hospitalization owing to respiratory infections.

The researches lead in the countries with a high level of the income, have shown, that breastfeeding, is not dependent on its duration, leads to decrease on 36% of probability of sudden death among babies. On 58% the number of cases of necrotizing enterocolitis [18], diseases with high risk of a fatal outcome in any country decreased [14]. Breastfeeding has great value for preventive maintenance of an acute otitis among children younger than 2th years, but is not found convincing data concerning children older of this age [5]. Concerning preventive maintenance of allergic diseases, such as eczema and food allergy, there are no convincing proofs of a role of breastfeeding, and there are some researches concerning its value for preventive maintenance of an allergic rhinitis among children before 5 years [23]. Also it has been shown, that breastfeeding reduces on 9% probability of development of a bronchial asthma or on 5%-6% by results of more strict the analysis of results of the cohort researches.

The researches lead in the countries with low and middle level of the income has shown that breastfeeding reduces probability of infringements of a bite on 68% [28]. The majority of researches have been limited by children of early age with milk teeth, but infringement of a bite in this age group is a risk factor of infringement of a bite at adults [29]. However breastfeeding longer 12 months and night feedings associated with increase in 2-3 times of cases of caries of milk teeth that can be connected with inadequate hygiene of an oral cavity after feeding by a breast [36].

In the researches lead in the countries

with middle level of the income it has not been revealed distinctions in growth and weight of babies to 6 months of a life [11]. But some decrease in an index of weight of a body at children which mothers participated in programs of intervention on support of breastfeeding took place.

The long-term effects of breastfeeding: obesity, noncommunicable diseases and intelligence

Health of children more advanced age and also teenagers and adult population was estimated on following parameters: levels of arterial pressure and the general cholesterol, presence of superfluous weight of a body and obesity, diabetes type 2, the estimation of intelligence (IQ factor) also was spent.

The analysis of scientific researches on studying the remote influence of breastfeeding on size of the arterial pressure, the general cholesterol of blood, risk of development of superfluous weight of a body and obesity, diabetes type 2 is resulted in systematic review and the meta-analyses presented by B.L. Horta et al. [17]. The researches taken in the analysis have been lead among children, teenagers and adult population, the majority of them - in the countries with a high level of the income. It is revealed, that longer period of chest feeding reduces risk of development of superfluous weight of a body and adiposity to 13%-20%. And, this influence extends and on the countries with low and an average level of the income. Breastfeeding reduces risk of development of a diabetes type 2 on 14 %. However, it has not been revealed interrelations between duration of breastfeeding and sizes of arterial pressure and also the maintenance of the general cholesterol in blood.

Influence breastfeeding on intellectual development of children and teenagers is proved. Breastfeeding increases factor of intellectual development (IQ) by 3,4 items in view of employment with children of the house [16]. In other researches in which intellectual development of mothers was considered influence breastfeeding on increase IQ of factor it was estimated in 2,6 items. Prospective researches according to intelligence among children in the age of 6,5 years born premature have revealed increase IQ of factor at 7 items among children received breast milk in comparison with children who were fed by formula [21, 24]. Positive influence of breastfeeding on progress of children at school has been shown in the researches lead to the Great Britain [25, 31], New Zealand [12] and Brazil [38]. Research which was spent in Brazil during 30 years has allowed reveal positive influence of breastfeeding on intelligence, progress at school and the salary of the adult person, and influence of breastfeeding on size of the received salary contacted increase IQ of factor [39].

The opinion on possible preventive effect of breastfeeding concerning diabetes type 1 expresses [18]. Besides data are obtained that breastfeeding reduces on 19 % risk of development of children's leukemia [1].

Breastfeeding and women's health

Influence of breastfeeding on health of women was spent in view of following parameters: duration of lactation amenorrhea, change of weight of a body after sorts, presence of a cancer of a breast and ovaries, diabetes type 2 and development of a bony rarefication (a mineralization of bones).

In 2015 the modern review according to influence of breastfeeding on health of mothers on its such parameters, as lactation amenorrhea, a cancer of breast and ovaries, diabetes type 2 and bony rarefication has been published [7]. Besides there are also other publications concerning a role of lactation amenorrhea in preventive maintenance of a diabetes and postnatal depression for mothers, and also in restoration of weight after delivery. The majority of researches were spent in the countries with a high level of the income, except for researches on lactation amenorrhea.

The role of breastfeeding as natural method of contraception is proved. It is counted up, that in the countries where long breastfeeding prevails, for example, in Uganda and Burkina Faso it would be possible to expect 50% of additional birthes at absence of breastfeeding [3]. Increase in duration of breastfeeding especially exclusively and mainly breastfeeding connect with longer period lactation amenorrhea [7.] Random controllable researches according to the programs of intervention directed on increase of a level of breastfeeding confirm this effect [22].

Breastfeeding is preventive maintenance of breast cancer. The analysis of 47 researches lead in 30 countries in which it was observed more than 50000 women with breast cancer and about 97000 healthy women has allowed to assume that each 12 months of feeding by a breast reduce risk of development invasive cancer of a breast on 4,3%. [8]. Among the parous women with identical number of births longer breastfeeding reduces risk of development of breast cancer on 7% [7].

The analysis of 41 researches on studying influence of breastfeeding on risk of development of ovarian cancer and a bony rarefication at women has shown that long breastfeeding reduces risk of ovarian cancer on 30% [7]. Exception of the analysis of parous women and standardization of the received data on number of births have allowed to assume, that decrease in risk of ovarian cancer can make 18%. It has been revealed interrelations between breastfeeding and mineral density of a bone.

Breastfeeding can be preventive maintenance of a diabetes type 2 among nursing women [2]. However it has not been revealed influences of breastfeeding on decrease in weight of a body of women after delivery [26]. Nevertheless, there are the researches confirming remote influence of breastfeeding on preventive maintenance of obesity. Researches on supervision over 740000 British women for a long time revealed that on the average the index of weight of a body decreases on 1 % for each 6 months of feeding by a breast [4].

The authentic interrelation between breastfeeding and depression at mothers [10] is revealed. But remains not clear breastfeeding reduces depression or absence of depression increases probability of that mother will nurse.

Estimation of the saved lives among children and mothers

For forecasting number of the saved lives among children and mothers due to achievement of an optimum level of breastfeeding the special technique "Tools according to kept lives " was on a global scale used [40]. Calculation of potentially saved lives by 2015 in comparison with 2013 has been lead [30, 34]. For optimum parameters of breastfeeding the following have been accepted: exclusively breastfeeding among children younger 1 month - 95% and among children younger 6 months - 90% and also breastfeeding among children 6-23 months - 90%. Potentially possible number the prevented death owing to all infectious diseases among children till 2th years and also 15% of death among premature children who could be connected with complications after the first week of a life among this category of babies was considered. Among women potentially possible number of the prevented death owing to

a cancer of a breast was estimated.

Use of the special tool for an estimation of the saved lives has allowed to count up, that at optimum levels of breastfeeding by 2015 it would be possible to prevent annually 823000 or 13,8% of death among children is more younger than 2th years in 75 countries with the highest parameters of infant death rate [6]. From them of 87% would have on babies up to 6 months as at this age the highest death rates and great value for their preventive maintenance are marked has exclusively breastfeeding.

It has been counted up that the global level of breastfeeding existing now warns 19464 death from a cancer of a breast annually in comparison with the conditional script if women did not nurse [6, 8]. In regions with a low level of the income but with long breastfeeding (Africa and southern Asia) is annually prevented 58% of death from a cancer of a breast in comparison from 36% from the general population included in the analysis. Also it has been calculated that it would be possible to save in addition 22216 lives annually if duration of breastfeeding would be increased up to 12 months for each child in the rich countries and till 2th years in the countries with low and middle level of the income. It is possible to assume that the effect of the saved lives will differ in the different countries. For example, in the countries of Latin America, central and the East Europe, the Countries of Independent Commonwealth and also the rich countries the effect of the saved lives will be more significant as in these countries higher prevalence of a cancer of a breast and smaller duration of feeding are marked by a breast.

For achievement of full effect from breastfeeding it should proceed till 2th years. Results of ethnographic researches show that the general duration of feeding by a breast varies from 2 till 4th years in the most traditional communities that gives a material for modern international recommendations concerning its duration [9].

Breastfeeding, well-being and sustainable development of the community

Value of breastfeeding is not limited only to its influence on health of mother and the child but also mentions the important social and economic spheres of human life [42].

Absence of breastfeeding associates with lower intelligence and economic costs which annually make approximately 302 billion dollars or 0,49 % from the world total national income.

Breastfeeding and breast milk - ecologically pure activity, allowing to receive a product within all year and consequently should be considered as the important ecological problem of a national and global level. It is counted up that for manufacture of only 1 kg of a powder milk formula it is required more than 4 000 liters of water. In the USA for packing mixes 86 000 tons of metal and 364 000 tons of a paper which, eventually, appear on a dump are annually used 550 million cans.

Breastfeeding is not mentioned by obvious image in "Purposes of sustainable development" (United Nations), however available scientific data show that perfection of sphere of breastfeeding will promote achievement of the purposes in other spheres such as public health services, food safety, education, equality, development and preservation of the environment.

Conclusion

Breastfeeding is important for all mothers and children, it is not dependent on where they live and whether are poor or rich. It protects children from such diseases as a diarrhea, respiratory infections and acute otitis. In the countries where infectious diseases are a principal cause of death breastfeeding provides significant protection but even in the developed countries it reduces death rate from necrotizing enterocolitis and a syndrome of sudden death of babies. Breastfeeding increases the human capital due to increase of intelligence. Besides breastfeeding reduces probability of superfluous weight of a body and obesity, diabetes and children's leukemia and also reduces risk of development in mothers of breast and ovarian cancer and diabetes.

Value of breastfeeding is not limited only to its influence on health of mothers and children but also mentions the important social and economic spheres of human life being the important component for achievement of "Purposes of sustainable development" (United Nations) in the field of public health services, food safety, education, equality, development and preservation of the environment.

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ACTUAL TOPIC

M.M. Shatz ,Y.B. Skachkov, A.P. Cherepanova

STREET TRAUMATISM IN YAKUTSK AS A DERIVATIVE OF STATE OF ROAD SYSTEMS

ABSTRACT

The purpose of the publication is to highlight the problem of street injuries in Yakutsk, due to the lack of reliability and safety of its sidewalks. The main causes of natural origin include flooding of surface areas of Yakutsk, due to violation of the migratory routes of many water bodies with stagnant water regime, change the vertical layout and leading to negative redistribution of surface and ground waters. Showing geotechnical problems associated with the condition of sidewalks unreliability, low quality of construction and quality of materials used, as well as the lack of control of the state. As a result, a significant part of the city sidewalks, especially in the transitional periods are a major hazard to pedestrians who receive varying degrees of injuries.

It was noted that the issues of safe, non-traumatical movement on the sidewalks in Yakutsk are relevant all year round, but they vary depending on the season. During the warmer months the main risk is related to the technical condition of the sidewalks and for moving around the pavement areas with defects or uneven surface it is enough to use attention and be careful. Often the movement in partially destroyed surfaces aggravates the process of their destruction. During the transition periods it is much more difficult, and sometimes impossible to avoid the danger. In September-October and March-April in the city are common air temperature changes with transitions through 0 ° C, with partial melting of the previously fallen snow.

As a result, when there's a strong cold wind on a large part of the sidewalk there is an ice layer, which is a special danger to pedestrians. With the first snowfall, in medical institutions there is a large number of injured people. On the icy sidewalks, in yards of houses and on the steps of the stairs in public organizations people break the bones of hands and feet, ribs, and receive more serious injuries. There are ways recommended to remedy the situation to ensure the stability and reliability of urban pavements, as well as behaviour rules reducing the risk of injury in the street.

Keywords: level of street injury, causes of unreliability of sidewalks, behavior rules.

а б в

Fig.1. Sergelyakhskoe shosse, Saisary lake: а – 2013, б – 2015, в – 2016. Photo by Skachkov Yu.

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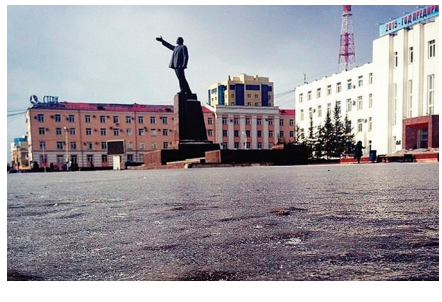

Fig.2. The ice layer on the Lenin Square. Photo by Markov A.

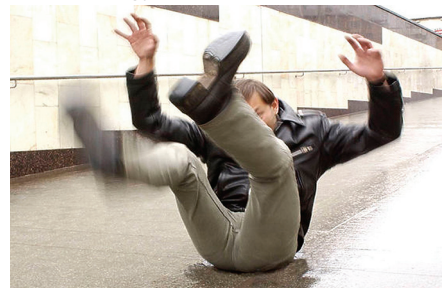

Fig.3. Fall on the icy pavement. Photo by Makeev A.

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SCIENTIFIC REVIEWS AND LECTURES

A.A. Chakhov, I.D. Ushnitsky

THE ROLE AND IMPORTANCE OF ANATOMICAL AND TOPOGRAPHICAL FEATURES OF THE MANDIBLE AT MANDIBULAR ANESTHESIA

Abstract

The work presents the characteristic of anatomy-topographic pecularities of the lower jaw. We describe in detail age changes of the branch and angular width of the lower jaw which need to be considered for carrying out adequate local anesthesia at dental interventions. The article speaks about features of anatomical structure of the lower jaw depending on gender, where indicators of the chin square contour, expressivenesses of the submental eminence and adventive eminence, a deviation of tops of corners outside and eminence edge of corners have age and gender distinctions. Besides there are data on structure change of the bone tissue of the body of the lower jaw during human life. Similar changes in anatomy and topography are undergone by the mandibular channel. So, men have a distance from tops of roots of the first and second molar to the mandibular channel more than women and have three options of passing of the channel (high, average, lower). The mandibular channel in the body of the lower jaw is S-shape bent, as much as possible adjoining to a lingual cortical plate in the field of the third and second molars, turns on 45º around the first molar, approaches a cheek cortical plate and at right angle leaves a mental opening. At the same time the channel keeps a S-shaped form also in the vertical plane. Meanwhile, patients with a full edentia have reduction of distance between the mandibular channel and alveolar shoot of the lower jaw.

It is important to note what for carrying out adequate local anesthesia of the lower jaw has a certain value position of a lingula in age aspect which is located in front of mandibular foramen. At the same time the cross section of a lingual nerve on all its extent varies in form. Collateral branches of a lingual nerve innervate retromolar space and area of the lower third molar. And the lower alveolar nerve after an exit through a mental foramen is variable and asymmetric on different sides of the lower jaw – from one powerful trunk, without branches, to a series of the small branches which are scattered in the field of foramen.

It should be noted that when carrying out conduction types of anesthesia of the lower alveolar nerve there is a probability of traumatizing chorda of a temporal muscle, medial wing-shaped muscle, wedge-shaped and mandibular ligament, wing-shaped and mandibular ligament, neurovascular bunch entering into channel of the lower jaw, a wing-shaped venous texture, a maxillary artery, etc. that can promote formation of hematoma in wing-shaped and maxillary space and movement disorder of the lower jaw.

At the same time the main reasons for development of neurologic deformations after blockade of the lower alveolar nerve are traumatizing of the nerve by needle especially at repeated anesthesia as at the same time there is no protective painful reflex, injuring by the deformed needle edge (a fishing hook), endoneural introduction of anesthetic, fast introduction of solution of anesthetic (hydropreparation) at which happens stratification of covers of a nerve and neurotoxic effect of anesthetic.

All this dictates need of carrying out the further researches directed to the improvement of blockade of the lower alveolar nerve taking into account anatomy-topographical pecularities of the lower jaw.

Keywords: lower jaw, anatomy and topography, age and sex distinctions, mandibular foramen, mandibular channel, mental foramen, lower alveolar nerve, mandibular anesthesia.

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POINT OF VIEW

A.G. Egorova, A.N. Romanova

APPLICATION OF BEE PRODUCTS IN CARDIOLOGY: EXPERIENCE, IMPLEMENTATION PROBLEMS IN MEDICAL PRACTICE

ABSTRACT

The main questions of apitherapy application in cardiology from the sources up to the present are presented in this article. Modern ideas about the efficiency of bee products application in treatment of cardiovascular diseases are presented. The indications and basic methods of apitherapy in cardiology are provided. The characteristic of the Yakut honey is given.

Keywords: apitherapy, cardiology, the Yakut honey.

Cardiovascular diseases (CVD) come a top place of both morbidity and mortality reasons in the world. The Republic of Sakha (Yakutia) is no exception. According to Federal Public Statistics Service of Yakutia, in 2012 (in comparison with 2011) a crude arterial hypertension (AH) morbidity rate of an adult population increased to 7,8%, and a crude ischemic heart disease (IHD) morbidity rate increased to 1%. Also, an IHD mortality rate increased to 1% (2012 – 178,1; 2011 – 165,1; 2010 – 176,4), including an acute myocardial infarction (AMI) mortality cases – 4,1% (2012 г. – 25,3; 2011 – 22,0; 2010 – 24,3). In contrast, a cerebrovascular diseases mortality rate decreased to 0,4% (2012 г. – 82,7; 2011 – 83,2; 2010 – 83,0) [4]. Increasing AMI and cerebral apoplexy (CA) morbidity rate and mortality rate among the indigenous population of the Sakha Republic with the pronounced tendency of “diseases juvenation” gain special attention [3; 5].

Considering a high morbidity and mortality rate of the population of Sakha Republic and Russia at large, searching of new approaches to prophylaxis and treatment of CVD is a current and socially important problem of modern medicine. An apitherapy (from lat. «apis» - bee) is a common name of a treatment method using hive products. The germination of the apitherapy started thousands years ago, and now, as a result, the organism recovery processes effectively proceed. The main products of the apitherapy are honey, flower pollen, propolis, royal jelly, bee bread, bees-wax, a bee subpestilence, bee venom and drone (male bee) homogenate. On the basis of the curative products developed by bees various agents are made (cream, ointments, infusions, tablets, etc). Apitherapy medicines are used in therapy along with a bee stinging treatment. The apitherapy is a multi-purpose specialty allowing to fight against a huge number of diseases. Today the efficiency and safety of this method doesn’t raise doubts. The hive products were traditionally applied in medical treatment of CVD. Confirmation for that are written sources of Byzantium Empire where during the reign of the Emperor Constantine VII an original encyclopedia of beekeeping was made. On the Sumerian clay tablets dated on the 3rd millennium BC there are an information about honey as a medicine. In India medicinal properties of honey and propolis were known 5-7 thousand years ago. Widely and successfully honey was applied in medical treatment of many diseases by Hippocrates (430-377 BC), Aristotle (the 4-3rd centuries BC), Claudius Galenus (131-201), Alexander Tralliysky (525-605) and many other philosophers, doctors of ancient times. Till the era of antibiotics honey was applied to repair wounds. Ibn Sīnā (Avicenna) in the therapeutic practice was applied by honey and bees-wax including for treatment of heart attacks. He considered that to aged people 45 years are more senior it is necessary to use honey, especially with the pounded walnut containing many fats for extension of youth, restoration of memory and a mind exacerbation.

In Russia beekeeping have been used for a long time. The first scientific justification of the apitherapy in medicine took place in 1864 when professor of the St. Petersburg Academy of forest management Lukomsky M. I. offered a medical treatment of rheumatic disease and gout using bee venom. Later, professor Artyomov N. M. researched the use of bee venom and gave the report that bee venom affects two major systems: nervous and vascular, and in a less degree – immune system. In 1959 the legal base in this direction was already done – the academic council of the Ministry of Health of the USSR approved the Application Instruction of using apitherapy by bee stinging [1].

In 1961 Karitskaya V. M. showed effect of Venapiolinum (bee venom) of an atherosclerosis and an idiopathic hypertensia in the Candidate’s dissertation. Kadyseva N.M. treated angina with royal jelly and had positive results. Korneva and Asafova’s researches showed influence of bee venom on myocardial contractility. In the Candidate’s dissertation Kireeva V.F. (1968) showed influence of bee venom on protein composition of blood serum and blood vessel permeability.

Fundamental research was a work of De Carly and his colleagues (1974) about the action of royal jelly on rabbits with disturbances of a coronary circulation. Authors identified a significant clinical improvement of myocardium nutritional care using ECG that confirmed clinical observations of L.E. Martsivechene (1988).

In the Candidate’s dissertation of Krylov V. N. a cardiotropic action of bee venom (Cardiopep peptide) was investigated. Orlov B.N. established that, while operating of bee venom, the volume of the regurgitating blood is increased and reflex reactions improve, also, the action of autonomous system is stimulated. In 1971 Mladenov offered to use honey in cold water for treatment of a cardioneurosis. Bezborodko S.A. with the colleagues in 1991 considered that bee venom possesses a larger role in prophylaxis of CVD, improve the ECG and rheologic qualities of a blood and indicators of lipide exchange [1].

Several research works of Okhotsky B.A. and Okhotskaya N.B. are concerned with the treatment of angina by using of bee venom and royal jelly. They considered that the hive products reduce excitability of sympathetic nervous system that conducts to angiectasia, improvement of a cardiac rhythm, rising of functional ability of myocardium, increasing inhibitory control of a cerebral cortex, and sensation of pain blocking [1].

In 1972 Gabermann E. identified that bee venom improves a venous drainage. J. Filipos and Leitch Si with the colleagues established a discernable interrelation of influence of bee venom on circulatory dynamics. Georgieva

E. and Vasilev V. in Bucharest (1976) reported about a positive effect of pollen to an atherosclerosis of coronary vessels due to depression of a Hyperlipidemia. Sinyakov A.F. (1990) believed that royal jelly plays a large role as an adaptogen. Gorbachenkov A.A. (1988) established that pollen and honey figure in aftercare of patients with MI of month-old period (40 g of pollen and 50 g of honey). Ad. Ruff (according to Kostoglodov's quote) at long consumption of honey on 700 g for patients with serious cardiovascular pathology observed the general well-being mend, rising percent of a hemoglobin and also a cardiovascular tonus. Turk I. with colleagues (1979) offered apiphit (апифит - rus.) for treatment of cardiologic patients. Lupachev V.F. treated 80 ischemic heart disease patients using Apilac for 30-60 mg a day during one month. There were noted that patients have complete pain easing, decreasing frequency of seizures, reduction of cholesterol level, and blood phospholipids increase. Makarova V.G. (1974) also established that royal jelly reduces cholesterol level, enlarges level of phospholipids in blood and tissues of a brain and liver. Similar information were obtained by other researchers (Dobrovoda I. (1976), Dzhordzhesku M.M. (1976), Okhotsky B A. and Kovriga A.T. (1984)). The last ones treated 243 patients with angina, 412 ischemic heart disease patients, and 49 patients with infarct in an aftercare period using Apilac (10 mg) with the expressed positive effect [2].

The significant contribution in developing of local apitherapy was made by professor and neuropathologist Ludyansky E.A. In 1972 in Vologda he opened an apitherapy center at the neurology department of a local hospital. Ludyansky E.A. was one of the first who showed on numerous examples that apitherapy can be applied as a medical treatment of various diseases and that it can be combined with traditional methods of other treatment. He wrote the first large manual about apitherapy (treatment by bee venom, honey, propolis, pollen and other hive products) for doctors, students of medical schools and beekeepers (1994). Ludyansky organized the All-Union conference about apitherapy treatment in Vologda in 1987. Also, he organized a school of honeybee health specialists (apitherapists) in Vologda national hospital No. 1 in which about hundred doctors had training. For the first time doctors from Russia received an academic degree on the apitherapy. In 1995 Eduard Averyanovich brilliantly defended the doctoral dissertation «The hive products in complex treatment of diseases of a nervous system».

Today many Russian and foreign clinics and research institutes intensively conduct the researches about medical. The hive products promote prophylaxis of an atherosclerosis (influence lipide exchange), improve rheological properties of blood, widen coronary vessels, render anti-ischemic, hypotensive, antibacterial and adaptogenic activity. Including the apitherapeutic products (bee venom, royal jelly, pollen, bee bread, propolis) in the list of CVD medications increases efficiency of the therapy. Medical disposal of apicomplex (bee venom + royal jelly + propolis) along with prescription of medicines to patients with an ischemic heart disease after an old myocardial infarction, esspecially of a certain age, leads to the general well-being mend, reduces the frequency of anginal attacks and blood pressure, increases effort tolerance, normalizes blood lipids. Royal jelly and bee bread are valuable metabolic agents which contain a large amount of potassium and magnesium. The hive products can help in the complex prophylaxis and medical treatment of cardiac rhythm disorder [2].

The hive products such as royal jelly, propolis, bee bread and, first of all, bee venom are quite successfully used in the Ryazan regional clinical cardiology hospital to treat patients with cardiovascular diseases. So, Soldatov E.S. carried out a clinical test to establish the efficiency of hive products in medical treatment of arterial hypertension patients in geriatric practice. Bee venom, royal jelly, propolis, bee bread, and honey prescribed to the patients at the age of 65-79 with a hypertension of II, III stages with presentations of dyscirculatory encephalopathy. There were 70 men and 72 women. Duration of the disease is from 7 to 25 years. The diagnosis was established on the basis of clinical and instrumental methods of the research. The hive products were addition to the medicamentous therapy including the use of Hydrochlorothiazide (a daily dose of 12,5-25 mg once a week) and Corinfar® retard (20-40 mg for a month). Medical purpose of bee products was caused by the lack of efficacy of medicamentous therapy in mean therapeutic doses, side effects and also hypotensive, metabolic, antisclerotic and disaggregative activity of apitherapy medications. All patients were divided into six groups with 24 persons on each. The patients of the first (control) group were treated with medicamentous therapy only. In the second group all patients were given bee stinging treatment sessions (on 6-8 times of bee stinging on a collar zone, occipital region, distal segments of extremities) during every other day for 30 days. The course dose made from 90 to 140 times of bee stinging. In the third group royal jelly was used in the form of 2% Apitonus solution in a single sublingual dose of 75-100 mg/days during the period of 30 days. In the fourth group patients were treated with bee bread (30 g b.i.d. in half hour before meals in the first half of day) for 4 weeks. To the patients of the fifth group they were prescribed 30% alcohol solution of propolis (30-40 drp t.i.d in half hour before meals). In the sixth group there were the patients were given an electrophoresis with 20% honey solution from both poles (according to Scherbakov No. 10) on a collar zone. The clinical effect was estimated on the reduction level of the AT upper indicators, and the general symptomatology in 30 days after the treatment. In all groups the patients had general well-being and sleep improvements and reduction of headaches and irritability to the the second week ending. As a result it became clear that use of bee venom with traditional therapy leads to a blood pressure reduction for 20% averagely, royal jelly – for 14, bee bread – for 10, propolis – for 7, an electrophoresis with honey – for 5% in comparison with control group [2].

Thus, the research showed that use of bee products has a positive effect on the course of hypertension, renders a significant therapeutic effect allowing to reduce a dose of hypotensive drugs and implication of dyscirculatory encephalopathy in the elderly. The hive products can be recommended as an addition to the complex therapy of hypertension in the elderly.

Medical treatment using bee venom is carried out in 170 countries of the world now. In such countries as France, Romania, Austria, Canada, Japan, China there are developed hundreds of medicines of bee products, in different pharmaceutical dosage forms. In South Korea systemic researches about apitherapy are conducted by three clinics, and in Japan there is even an association of the apitherapists specializing in treatment of various diseases only by means of bee stinging treatment. A noteworthy fact is all Japanese school students of 10th to the 12th grade are daily received 15-20 g of honey during a school breakfast and its publicly-funded. In Romania the world's largest commercial specialized clinic where with the use of apitherapy and apiphitotherapy treatment medications the numerous diseases are cured which can't be cured with the help of other medications. In the USA since 1989 there is apitherapy society which principal activities are gathering, analysis

and distribution of information about the use of this method in other countries. The American doctors for a long time apply bee stinging treatment to the patients with an atherosclerosis.

Russia, unfortunately, has no such achievements in apitherapy development yet, but has all resources for it. At the moment Russia is falling behind many foreign countries just as preparation and processing of these products, so their introduction in medical practice, despite an unconditional lead in research works and opportunities of production of biologically active bee products. It is necessary to notice that in our country apitherapy and hirudotherapy are considered as traditional methods of treatment, and in the USA – alternative. It is correct because Russia in former times made a significant amount of hive products and was famous for the red-cheeked beauties, healthy children and excellent soldiers though they ate generally honey, milk and vegetables. For any unknown reason apitherapy isn't included into the list of medical services.

The legal base of apitherapy in Russia was put by the Application Instruction of apitherapy by a bee stinging treatment approved by Ministry of Health Academic Medical Council of the USSR in 1959. Apitherapy and apireflexotherapy were included in the official list of licensed medical activities of the Ministry of Health care of the Russian Federation by its Order of 01.07.96 No. 270, however the last was already recognized invalid in 1998.

The reason for which the official medical science bewares of using apitherapy is generally that apitherapy medications can't be standardized as their natural structure can change depending on time and the place of their creation. Meanwhile, professor Skichko N. D. considers that chemical composition of bee products can slightly change depending on a location of a beehive and species of the blossoming plants [1].

Heavy metals pollution as a result of anthropogenic activity is a current environmental problem around the world, and honey bees and hive products are the pollution indicators. Tissues of bees and also honey, wax, pollen, bee bread, propolis collect all radioactive nuclides, heavy metals, pesticides and other harmful substances, and give information about pollution of the external environment. Bees, visiting plants, immediately adjoin to the environment and various pollutants and, as a result, bees are endangered to be accumulated with it. Works of the Russian researchers showed that environmental pollution by toxic elements significantly affects purity of propolis and pollen, however, heavy metals don't collect in honey and wax. But honey, wax and pollen can be the bioindicators accumulating various doses of radioactive cesium-137 and strontium-90 depending on the nature of pollution by environment radionuclides. High assay of copper and zink in a body of bees demonstrates accumulation of these elements by them.

Thus, the quality and ecological safety of bee products depend on a location of a beehive and species of the blossoming plants.

In the Republic of Sakha (Yakutia) the hereditary and qualified beekeeper Roman Ocheretyany is engaged in beekeeping within 26 years. His beehive is located in the wood in 25 km from Yakutsk. Following the results of an analytical research of honey sent to Moscow during the whole year from every quarter of Russia, the Yakut honey of Roman Ocheretyany was awarded with the gold medal «For High Quality of Honey» under the decision of Central office of the Russian National Union of Beekeepers in 2015. In 2011 our honey has taken a place in the World 's Bees Museum (in Tokyo) because of the most environmentally benign. So, arsenic, cadmium and radionuclides are actually not found in the Yakut honey. Lead is 2,7 times lower than norm by Sanitary Regulations and Norms (tab. 1-2).

Consequently, apitherapy is one of the most effective and perspective non-drug methods of treatment and prophylaxis, especially for cardiovascular diseases. However, in Russia long-term experience of use of bee products in a cardiology isn't so widely applied. We consider that apitherapy deserves closer attention and support from the powers that be, and can take the worthy place in the list of medical services.

Table 1

The test protocol of honey from Ocheretyany R.N. (Yakutia) No. 8-1-P of April 3, 2014, research laboratory of a State Scientific Institution of beekeeping of the Russian Agricultural Academy

Test Index Name	Test Method RD	Index Value		Error, % nom.
		SanPiN, mg/kg	Actual value	
Toxic element mass fraction, mg/kg:				
Arsenic, As	GOST P 52097-2003	0,5	N/D	1,0
Lead, Pb	GOST 30178-96	1,0	0,37	5,0
Cadmium, Cd	GOST 30178-96	0,5	N/D	5,0

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Table 2

The test protocol of honey from Ocheretyany R.N. (Yakutia) No. 2015/2015 of October 23, 2006, Accredited Testing Laboratory of agricultural production, Federal state institution center of agrochemical service «Khabarovsky»

Index Name	UM	Test Results	Test Method RD	ME	No. & Test Date
Cadmium mass fraction	mg/kg	0,03	GOST 26929-94 GOST 30178-96	AAS-30	AIO 006668 to 13.10.07
Lead mass fraction	mg/kg	0,2	GOST 26929-94 GOST 30178-96	AAS-30	AIO 006668 to 13.10.07
Arsenic mass fraction	mg/kg	0,04	GOST 26929-94 GOST 26930-86	КФК-3	AIO 008033 to 14.11.06
Radionuclide activity: Cs-137	Bq/kg	0	MG 2.6.1.717098	«Complex"	№03 136124-187 to 27.03.07
Concentration of volatile N-nitrosamines	mg/kg	0			

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CLINICAL CASE

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ALPORT SYNDROME IN A 16 YEARS OLD CHILD

ABSTRACT

This article focuses on a rare disease -- Alport syndrome in children. Alport syndrome has a poor prognosis as it leads to the development of chronic renal failure. In addition to the renal symptoms in children with Alport syndrome is marked hearing loss; various eye disorders; delayed physical development; congenital anomalies (deformed ears, high palate, joined or extra fingers – no more 7 signs);rarely – Lam. The article presents a clinical observation of Alport syndrome in a child 16 years of age.

Keywords: hearing loss, hereditary disease, renal insufficiency, congenital anomalies, and poor prognosis.

Hereditary nephritis (the more famous the name, Alport syndrome) is a quite rare pathology. According to official data, in Russia per 100,000 newborn babies have 17 with this malformation. In Europe 1% of all patients with chronic renal failure (CRF) is the people with hereditary nephritis. The main and only reason why children are born with Alportsyndrome, is a genetic mutation. Damaged one of the extrarenal symptoms of congenital nephritis occur later: hearing loss (first child ceases to distinguish high-pitched sounds, then normal speech); various eye disorders;delayed physical development; congenital anomalies (deformed ears, high palate, joined or extra fingers – no more 7 signs);rarely – Lam (overgrowth of smooth muscle fibers) of the esophagus, trachea, bronchi [1].

The aim: to show the features of the Alport syndrome in a child 16 years of age.

Observations: the Patient, 16 years, was admitted in the Nephrology Department of the National medical center with complaints of recurrent dizziness, weakness, fatigue, hearing loss.

From the disease anamnesis: ill since birth. Is under medical supervision of a nephrologist since 2006, the audiologist since 2008 with a diagnosis of Hearing loss 1 degree. Pre-school age observed recurrent gross hematuria, with the years, increasing proteinuria. In 2007, he was treated with prednisolone for 6 months, efficacy was not. Since the end of 2008, a decrease of hearing and was diagnosed with Bilateral sensorineural hearing loss of 1 degree. In 2009, spring was examined in the Scientific center of children's health (our center) in Moscow, where he was diagnosed with Hereditary nephropathy (Alport syndrome). The last hospitalization was in November 2016 – the patient was taken urgently, in connection with significant deviation in the urine. Constantly gets Eralfon scheme, omeprazole, folic acid. Planning comes to control examination and treatment in Nephrology BUT PDTS «RB No. 1НЦМ».

From the anamnesis of life: Child from first pregnancy occurring in the 1st half with toxicosis in the second half of the AD. Delivery at 38 weeks, labor operational.The Apgar score 8/8. Body weight at birth 2880, height 50 cm, Cried at once, a loud cry. Rash, diaper rash was not. Neonatal jaundice appeared on day 3, stayed 6 days, moderately severe. To the breast applied on the first day, sucked actively. Umbilical remnant fell on the fifth day. Discharged home on the seventh day.Natural breastfeeding to 12 months.Input solid foods from 6 months.Psychomotor development at the age up to 1 year. The disease: SARS, acute respiratory infections, chickenpox, pneumonia, and recurrent bronchitis. Preventive all age. BCG in the maternity hospital 23.08.2000.Mantoux from 14.11.08 -7 mm, 22.10.10 – negative. Medical withdrawal in 2010. The heredity is not burdened, the mother of 34 years, has a chronic disease – pyelonephritis. Allergic anamnesis burdened. Food Allergy to citrus.

Objective status at survey: assessment of the condition of the child moderate, harmonious physical development. BMI =17.1, which is the norm. Skin and visible mucous membranes pale, dry. The hair is dry. On the left hand in the upper third of the forearm in the wrist and elbow there is aarteriovenous fistula for hemodialysis. Child low power.Subcutaneous fat layer mild. Peripheral lymph nodes were not expressed. In the lungs vesicular breathing, wheezing no. The abdomen is soft, painless. The liver and spleen are not enlarged.

The patient survey.

Survey results: ultrasound of the kidneys from 26.01.2017 Expressed diffuse changes of renal parenchyma with a decrease in age sizes. Seal of the renal sinuses.

General analysis of blood from 23.01.2017 HGB – hemoglobin-116r/L.

Biochemical analysis of blood from 25.01.17 g: serum Creatinine blood 377,2 μmol/l, iron levels, blood - 7.1 μmol/l, ferritin serum was 8.2 μg/l; the phosphorus level the blood to 1.5 μmol/L.

Blood for parathyroid hormone from 25.01.17 g – 195 PG/ml

KOS blood (from 25.01.17 g): ctHb – 177 g/l mmol/L. The General analysis of urine from 25.01.17 g : protein - 1.67 g/l; leukocytes 3-5 in p/Zr; erythrocytes changed entirely in the field of view; erythrocytes unchanged entirely in the field of view.Consultation consultation from 27.01.17 g: Diagnosis of Dry rhinitis.

Helik – test 27.01.17 g: Hp(++).

Conclusion: the result is positive

Fegds from 27.01.17 g: Conclusion: Duodenogastric reflux. Catarrhal distal esophagitis.Stagnant gastroduodenopathies. Chest x-ray in direct projection from 27.01.17 g: Conclusion: In the lungs without focal and infiltrative changes. ECG from 27.01.17 g: Conclusion: sinus rhythm with heart rate of 57 beats per minute, bradycardia. The vertical position of the EOS.

Consultations of specialists: Gastroenterologist from 27.01.17 g: Diagnosis: Primary: K21.0 Gastroesophageal reflux disease with esophagitis of the lower third of the esophagus. Collateral: K29 common chronic superficial gastroduodenitis, active stage associated with H. pylori.

The survey was delivered clinical diagnosis:

Primary: Hereditary nephritis. Alport Syndrome.

Related: Bilateral sensorineural hearing loss, 1 table K21.0 Gastroesophageal reflux disease with esophagitis of the lower third of the esophagus.K29 common chronic superficial gastroduodenitis, active stage associated with H. pylori.Dry rhinitis

Complication: Chronic renal failure, terminal stage.

During the hospital stay the treatment: Mode of ward, Table 7G, end-stage renal failure with hemodialysis, and extra food. Hemodialysis for 4 hours a day. Omeprazole 20 mg-1 capsule 2 times a day.Eralfon 2000 IU, 3 times a week. Alfacalcidol 0.5 μg - 2 tablets 1 time a day. Lineks 1 capsule 3 times a day. Control of blood pressure 2 times a day.

During the hospital stay, the dynamics of the patient's condition are positive, the efficacy of the treatment is stable.

Recommendations: the Child is sent to the Department of transplantation at the RCCH Moscow, for carrying out kidney transplantation (06.02.17 g). To continue training and treatment in hospital. Strictly follow a diet. The control of the KLA, OAM, blood pressure. Correction treatment when indicated.

The plan: re-sugar curve, re-consultation of a cardiologist and a gastroenterologist.

CONCLUSIONS

1. In the absence of specific treatment, the main goal becomes slowing development of kidney disease. Children are prohibited physical activity, assigned complete and balanced nutrition.

2. The use of hormones and cytotoxic drugs does not lead to significant improvement. The main treatment remains transplantation (transplantation) kidneys.

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CASES OF PRENATAL DIAGNOSIS OF REVERSE ARTERIAL PERFUSION SYNDROME IN YAKUTIA

ABSTRACT

There are six cases of Twin Reversed Arterial Perfusion Syndrome (or Acardia) in ultrasound prenatal diagnostics in the period from 2009 – July, 2013; one of the cases is presented in the triplet pregnancy.

Keywords: pregnancy, ultrasound prenatal diagnostics, complications of monochorionic twins, Twin Reversed Arterial Perfusion (TRAP) Syndrome, Acardia.

INTRODUCTION

TRAP syndrome, or Acardia syndrome –a rare complication of monochorionic twins (frequency is 1:35000) characterized with the arterioarterial and arteriovenous anastomoses of twins cord vessels [3, 4].

A fetus with Acardia syndrome (recipient) is nonviable, and a single vascular supply source is the circulatory system of a donor twin.Term "reversed arterial perfusion" is used to characterize the bloodstream toarecipient twin: oxygenated bloodcirculates not with the umbilical vein, as it should be, but the umbilical artery, and deoxygenated blood circulates to placenta with the umbilical vein [1, 5].

With the increasing of the gestational age of the recipient twin the need of oxygenated blood is gaining too, and this factor leads to considerable increase of a heart stress of the donor twin, resulting in heart failure [2].

Accordingly, without prenatal adjustment conductionthis is largely responsible for the loss of donor fetus in 50-75% cases, especially if a tentative mass of the recipient twin is about more than 50% from a mass of the donor twin [6].

Six various cases of Acardia syndrome from November, 2009 to July, 2013, one of which was observed at triplet pregnancy, are presented.

Pregnant women were referred on a complex prenatal research from central district hospitals and health centers of Yakutsk to the genetic consultation of the National Hospital No. 1. Ultrasonography analyses were conducted on the ultrasonic device Voluson E8, Toshiba-Xario in the genetic consultation of NH No. 1. There also prenatal karyotyping was carried out.

Considering that departures of the families in the Federal Centers of Prenatal Diagnostics for laser coagulation of an umbilical artery of a fetus recipient were complicated during the described period, tactics of active non-interference was chosen.

In two described cases pregnancy came to the end with the birth of children. In one of them – neonatal loss in the 10th days of life, and in other case – the birth of the child with a cerebral palsy.

High frequency of occurrence of this pathology in Yakutia is interesting, considering the fact, that the period from 2009 to 2012 the number of birth was 64833 (2009 – 15848, 2010 – 15868, 2011 – 16195, 2012 – 16922).

CLINICAL SURVEILLANCE

Case No. 1

Pregnant woman, 35 years, the inhabitant of the suburb of Yakutsk, is referred from LLC Victory Clinic to the genetic consultation of the National medical center with the clinical conclusion of ultrasonography: Pregnancy of 13,4 weeks. Monochorionicmonoamniotic twins. The Acardia syndrome, for the decision of further actions in pregnancy care. The patient is somatically healthy. According to the gynecologic history, the patient has one births in time, the child is healthy; three induced abortions in early gestational age, without complications. This pregnancy is the fifth. The patient has no social habits. The partner is healthy.

Accordingly to the ultrasonography research the diagnosis was completely confirmed. During the prenatal karyotyping – a karyotype of fetuses 46, XY {11} - normal. The prenatal genetic consultation was carried out. The couple was ordered to conduct the pregnancy in one of the Federal Centers of Prenatal Diagnostics, considering rare type of defect and high risk of antenatal mortality, but at the gestational age of 17,5 weeks with the dynamic ultrasonography an antenatal death of fetuses was diagnosed.

Results

An autopsy of fetuses confirmed the diagnosis. The first fetus (donor): male, weight is 120 g, height is 21 cm, without anatomical defects.

The second fetus (recipient: 10 g, 8 cm. The following defects are found: absence of the calvarial bones in a facial part, orbits, nose, auricles, and acoustic meatus. In the oral cavity a small-sized tongue is detected. Fetus body is extremely edematous. Humeruses are "immured" in a skinfold of the body; forearms and hands are easily detected. The top and lower extremities are without bony skeleton, easily are bended along the axis.

Placenta, umbilical cord: one placenta, weight is 50 g, the size is 10x8, 5x1 cm, with defect of a tissue, and covers are absent. The place of an attachment of the umbilical cords isn't detected because of the serious defects of the placenta. The second fetus (recipient) has an umbilical artery with hypoplasia.

Case No. 2

Pregnant woman, 32 years, referred from Zhigansky District to the female consutive department of National Medical Center of Yakutsk with the diagnosed anomaly of the twins fetal development and a positive gynecological anamnesis. From Female consultative department of NMC the pregnant was sent to ultrasonography research in the radiology department of NMC, there were established: Pregnancy of 27/5 weeks. Monochorionicdiamniotic twins. Frank polyhydramnios of the first

fetus. Abnormal development of the twins fetus: the TRAP (acardia) syndrome. A thickened placenta. The umbilical edema of gelatin of Wharton of the first fetus.

From the anamnesis: the fifth pregnancy. The first two pregnancies completed with birth in time, children are healthy. The third pregnancy is terminated in the gestational age of 17-18 weeks for medical reasons - congenital defect of central nervous system (an acrania, spina-bifida). The forth pregnancy completed with a late spontaneous miscarriage on 20th week, without congenital defects.

With this pregnancy the patient was registered since 10 weeks. With the ultrasonic analysis in a district healthcare center at the gestational age of 11,5 weeks congenital defects was not established. The patient has the third degree of obesity. She is married at the second time, the partner (from hearsay) is healthy. Social habits: smoking to the 5 weeks of the current pregnancy. Has no professional harmfulness.

In the genetic consultation of NMC a geological monitoring was conducted. Considering the duration of gestation, the prenatal karyotyping was not conducted.

The patient was hospitalized in the pathologic pregnancy department of the municipal clinical hospital in Yakutsk, where in the 30 gestational weeks of pregnancy with a pericardial effusion and an edema of gelatin of Wharton of the first fetus (donor) the elective operative delivery is carried out.

Results

Feature of this defect is a defective fetus does not have some parts of a body and internals, including heart. Its delivery occurs at the expense of a healthy fetus. Using special formulas it is possible to know the weight of an acardiac fetus, and that is a determining factor for further pregnancy prolongation. Upon reaching a certain weight, the CVS stress of a healthy fetus considerably increases.

At the gestational age of 27,5 weeks, according to ultrasonic analysis conclusion, the recipient fetus is described as: "… the fetus with a bizarre shape is detected close to a fetal surface - it has no head, but partially has a top part of a body, thoracic organs and an abdominal cavity. It was succeeded to visualize one top extremity, fingers aren't differentiated. Lower extremities are two. In the abdominal cavity a kidney is detected. Weight - 980 g. An umbilical cord of the second fetus is short (about 20 mm). The place of an attachment of the umbilical cord of the second fetus is in a close proximity with the place of an attachment of an umbilical cord of the first fetus. In the CFM the wide network of the anastomoses is detected. The relation of mass of the acardiac fetus to mass of the anatomically healthy fetus makes 64%".

At the gestational age of 30 weeks the pregnant woman was sent to an operative delivery.

The mass of the acardiac monster (in the birth moment) was 1190 g. From the protocol of autopsy of the second fetus (recipient): female fetus, weight is 1140 g, height is 23 cm. The head is absent, the body is deformed, shortened. Procurvation of a backbone, the upper part ends blindly. The right upper extremity is edematous, a hand is with hypoplasia and has 5 vestigial fingers. The left upper extremity is absent, there is a cartilaginous apophysis d - 0,5 cm and 0,8 cm long. The lower extremities with vestigial feet, which have two fingers on the left and three fingers on the right foot.

There is one placenta, weight is 520 g, size is 24x20x2 cm. Lobularity is accurate, maternal surface is rough. The umbilical cords: the first fetus (donor) - with a diameter about 3 cm, gelatin of Wharton is extremely edematous; the second fetus (recipient) - in the diameter of 1,3 cm. Three (!) vessels are detected.

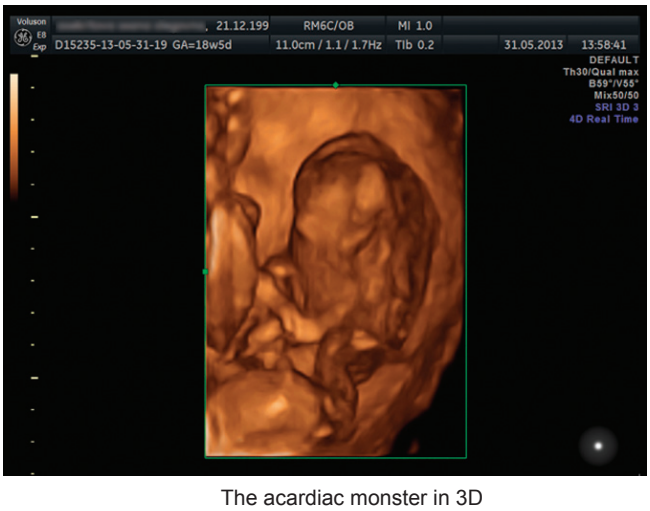
Attachment of the umbilical cords: a cord of the first fetus (donor) - paracentral, the second fetus (recipient) - velamentous.

The first fetus is female (donor), weight is 1760 g, height is 43 cm. At the birth an assessment on a scale Apgar score was 6/6 at birth. The patient was artificially ventilated in ICU. On the 10th day with stable condition the newborn girl was transferred to the neonatal pathology unit of the Maternal and Child Health Center of national hospital, NMC No1. At the present moment the child has a disability status because of cerebral palsy.

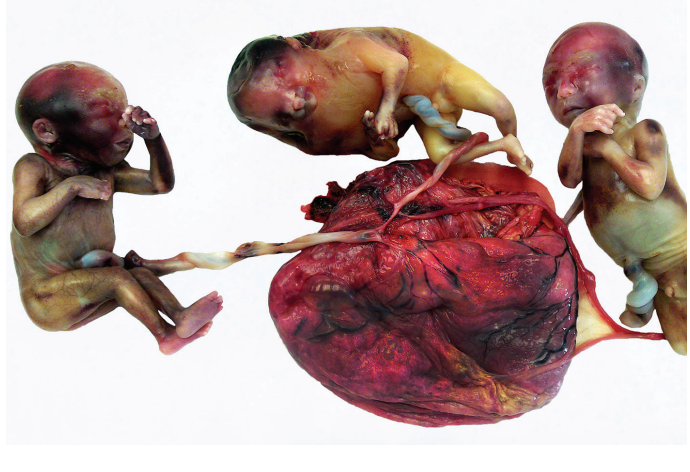
Case No. 3

The pregnant woman, 25 years, referred from Namsky District to the genetic consultation of NMC in the gestational age of 24 weeks with the clinic ultrasonic conclusion: Pregnancy 20, 5 weeks. Monochorionicmonoamnionic twins. The second fetus has an acardia syndrome. Polyhydramnios.

From the anamnesis: the first pregnancy completed with births in time and at terms, the child is healthy. Further, there were two cases of a spontaneous miscarriage in gestational age of 12 weeks. The fourth pregnancy completed with an operational delivery concerning the placental abruption in the gestational age of 29 weeks. Current pregnancy is the fifth. Registered on 20 weeks. Somatically healthy. Denies social habits. Civil marriage is the second. The partner is healthy (from hearsay).



The acardiac monster in 3D



The triplet pregnancy in the combination with the TRAP syndrome

Results

According to the ultrasonic research the gestational age was 24,2 weeks when the monochorionic twins was revealed. The second fetus has only the lower extremities, a part of an intestine and haunch bones. It has no head, the upper part of a body and the upper extremities. A subcutaneous tissue is frank edematous. Cord of the second fetus is short, and it is detected in the fixed position of a fetal surface of a placenta. The umbilical fetal cords are attached to the placenta at a close proximity to each other which gives impression that loops are bound. The first fetus is without anatomical defects, and it has a reasonable gestational age of 23,3 weeks.

The amniotic band was failed to visualize. There is one thickened placenta with signs of a premature maturity.

Conclusion of ultrasonography research: Pregnancy 24,2 weeks. Monochorionicmonoamniotic twins. Abnormal development: the TRAP syndrome of the second fetus (acardiaacephalus). The intrauterine growth retardation of the first fetus is not excluded. Moderate asymmetry of lateral ventricles of cerebrum of the first fetus. Polyhydramios. Thickening and premature maturing of a small-sized placenta.

In default of a possibility of the pregnant woman to departure to the Federal Centers of Prenatal diagnostics, with consent of the family it was procured an operative pregnancy interruption.

Results of an autopsy of fetuses confirmed the diagnosis of the ultrasonic research.

The first fetus is female, weight is 104 g, height is 37 cm, without anatomical defects.

Weight of the second fetus (donor) is 80 g, and looked like a shapeless mass with the lower extremities and a pelvis to a lumbar spine. There is no other part of the body. The sex cannot be detected – there are no external genitals. The fundament is presented. The frank edemas of extremities. From a pelvic part to the lower third of a cnemis the musculocutaneous folds are detected, which are interfering an extension of knee joints (pterygiums).

Case No. 4

A pregnant woman, 37 years, referred from the Phthisiology scientific production center to the genetic consultation of the National medical center No. 1 in the gestational age of 28,5 weeks suspected a twins congenital defects. She was hospitalized from the Eveno-Bytantaysky District with the diagnosis: Pregnancy of 28 weeks. Twins. Infiltrative tuberculosis of the right lung. Chronic hepatitis B. It is the second pregnancy, wanted. Labors in 1994, a healthy boy (given on adoption). Registered on D since 16 weeks.

The clinical ultrasonography conclusion: Pregnancy of 25,1 weeks. Monochorionicdiamniotic twins. Abdominal dropsy and antenatal death of the second fetus. Hyperechoic focal spots of the first fetus. Shorting of long bones of the first fetus. Edema of a gelatin of Wharton of the first fetus. Frank hypamnions of the first fetus. Oligoamnios of the II fetus. It is impossible to exclude a twin-to-twin transfusion syndrome.

According to the clinical conclusion the pregnant woman is hospitalized in the pathologic pregnancy department of the municipal clinical hospital in Yakutsk where the operative delivery in the gestational age of 28 weeks was carried out. The first fetus (donor) is female, weight is 1144 g, height is 38 cm, Apgar score is 6/6. She has been living for 10 days. The second fetus had a bizarre shape and weight is 2400 g (a fetus – the donor).

Results

In this case there is a wrong conclusion, though a specialist tried to describe an acardiac fetus: "… during examination of the second fetus the cardiac rate and the motion activity – aren't detected. There is a frank edema of soft tissues of a bandwidth at the head level which is about 60 mm, and at the level of an anterior abdominal wall is about 50 mm.

In edematous tissues multiple cystophorous formations (the overall is 90x72 mm) are visualized. The fetus sizes without edema of soft tissues are reasonable to 23 weeks of pregnancy.

The attachment place of an umbilical cord of the second fetus cannot be visualized. An umbilical cord of the first fetus with the signs of an edema of a gelatin of Wharton.

The autopsy of the second fetus: MCA - Acardia (Hemiacardius), corpus callosum aplasia, frontal cerebral hernia, hydrocephalus, anophthalmia, agenesia of a nose, auricles and acoustical meatuses. Hypoplasia of an upper and lower jaw bone with fragmentation. Agenesia of a trachea, extrapulmonary bronchus and lungs. Sinistral diaphragmatic pseudohernia with an eventration of loops in a thoracic cavity. Agenesia of a esophagus, liver and lien. Atresia of a fundament and rectum and genitals. Agenesia of the upper extremities, webbed toes of IV and V fingers of a left foot, agenesia of the IV finger of a right foot. Cystic lymphangioma of the soft tissues of a posterior surface of a neck and back. The umbilical cord of the second fetus has two vessels and a battledore placenta.

Case No. 5

A pregnant woman, 20 years, referred from a healthcare center of the high level to the genetic consultation of NMC with a triplet pregnancy and an ectopic first fetus, with the gestational age of 12,3 weeks. The current pregnancy is the third, wanted. The patient became pregnant in the next menstrual cycle after interruption of the second non-developing pregnancy. The husband is 21 years old. Partners are somatically healthy, have no occupational hazards and social habits. The examination was conducted on the ultrasonograph devices Toshiba Xario and Voluson E8.

Results

According to the first ultrasonic research conclusion in the gestational age of 12,4 weeks: Pregnancy of 12,4 weeks. Dichorionicdiamnionic triplets. Antenatal death, edema of the third fetus. Augmentation of the second fetus. The prenatal karyotyping of fetuses is carried out. A karyotype of fetuses 46, XY [11] and 46, XY [20] - normal. Chromosomal fetal pathology is excluded. In the gestational age of 14-15 weeks the woman is hospitalized to the gynecology department of NMC, for the decision about further prenatal care actions.

According to the ultrasonic research in the term of 15 weeks it is determined: in an uterine cavity there are three fetuses. The third fetus with parietal-coccygeal length of 74 mm (13,4 weeks) … "in energy regime" vessels are registered. Heart of the fetus isn't visualized, but the fetal movement becomes perceptible. On examination of brain structures - a cerebellum has a banana form. The bladder isn't detected. The frank edema of soft tissues, a hydrothorax are detected. Facial structures aren't visualized accurately because of the edema. The amniotic band isn't visualized accurately. During examination of the umbilical cord of the second and third fetuses it are revealed that cord vessels are in a close proximity to each other, at distance of 12-15 mm from a anterior abdominal wall. Pulse frequency of vessels of the acardiac fetus is 167 beats per minute; the donor fetus - 160 beats per minute.

At a dymanic research in the

gestational age of 22,5 weeks there are signs of a cardiovascular collapse of the second fetus (donor) – free fluid in thoracic cavity and abdominal cavity, therefore, with the consent of a family, the pregnancy was interrupted.

On autopsy the diagnosis about acardia syndrome of the third fetus was confirmed. From the protocol: a fetus weight is 360 g, height is 21 cm. The head is detected. There are no palpebral fissures. Bones of a nose aren't detected. There is a bilateral cleft of an upper lip and hard palate. In a neck, a breast to an epigastrium and a back to a loin there is a non-pitting edema of tissues. Hypoplasia of auricles, low located. Heart, an esophagus, a stomach, a liver, a pancreas are absent. Lungs without share division. In kidneys there are three spherical formations of 1,3 cm long. Adrenals in the typical place aren't detected.

There is one battledore placenta for the second and third fetuses, and velamentous placenta of the first fetus with an anastomosis of one vessel to a nearby umbilical cord with a distortion around it.

Case No. 6

Pregnant woman, 29 years, referred from LLC Victory Clinic to the genetic consultation of NMC with twins pregnancy with the acardia syndrome of the first fetus in the gestational age of 13,5 weeks. The current pregnancy is the fourth, unplanned, wanted. In the anamnesis there are one physiological labors, two abortions. The pregnancy was complicated by early toxicosis, an ARVI with temperature of about 102 degrees, an urogenital infection. The partners are somatically healthy, have no occupational hazards and social habits. The examination was conducted on ultrasonic devices Voluson Pro and Voluson E8.

Results

The conclusion of the first ultrasonography in Victory Clinic at the gestational age of 13 weeks: Pregnancy 13 weeks 5 days. Monochorionicdiamniotic twins. The Acardia syndrome: a nanocephalia, a hypodermic edema, a cervical hygroma, a hydrothorax, an omphalocele of the first fetus. A velamentous placenta with the abnormal number of vessels.

The ultrasonic research conclusion in the genetic consultation was confirmed the diagnosis. At the gestational age of 14,3 and 19 weeks it was additionally revealed: an aplasia of radial bones, oligodactyly and clinodactyly of the acardiac fetus. The prenatal karyotyping of fetuses is carried out. Chromosomal pathology is excluded.

In the gestational age of 19 weeks with the consent of the family the pregnancy was interrupted on medical authority. During the autopsy the ultrasonic diagnosis of the acardia of the second fetus was confirmed.

From the protocol: a fetus' weight is 250 g, height is 18 cm. A tissues surrounding heads, shoulders, body, the upper extremities to hands are presented by a vascular tumor (lymphangioma). Fetus head: the right parietal bone isn't formed, the brain is covered with a pachymeninx. A right orbit is covered with soft tissues, extruded. Nasal bones are absent, a rhegma of median soft tissues of an upper lip is detected. Heart and lungs aren't detected. In a thoracic cage a "slugged" yellowish tissue. Lungs, a liver, a lien are absent. There are kidneys on both sides (histologically primitive). On About the locomotion system: the right upper extremity is shortened at the expense of a shorting of forearm bones, a hand has 4 fingers and rotated inside with an acampsia of a radiocarpal joint. The left extremity is shortened at the expense of forearm bones, on a hand there are 4 distorted fingers. Lower extremities: the left foot has 3 fingers, right - 4 fingers.

CONCLUSIONS:

1. For the described period, a frequency of TRAP syndrome in Yakutia made 1: 10 805 labors. It is three times more than in other regions.
2. One of the reasons of high detection frequency of TRAP syndrome is rising of quality level of the prenatal diagnostics in the Republic of Sakha (Yakutia).
3. With the aim of frequency reduction of TRAP syndrome and reduce the endocrine pathology of women of childbearing age the extensive use of periconceptional care is highly recommended.
4. With detection of TRAP syndrome a laser coagulation of an umbilical artery of a fetus recipient is highly recommended before the progression of volemic complications.

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