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CLINICAL – AUDIOLOGICAL AND CLINICAL - GENEALOGICAL ANALYSIS OF CASES OF HEARING LOSS IN THE REPUBIC OF BURYATIA

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In this paper we presented for the first time the results of the clinical-audiological and clinical-genealogical research of hearing impairments in the Republic of Buryatia. The sample included Buryats (47.9%), Russians (46.1%) and representatives of other ethnicity (6%), amounting 165 people. As the result of the clinical-audiological analysis, 70.3% (n=116) of individuals had bilateral deafness of the sensorineural type, and 29.7% (n=49) had bilateral hearing loss of varying severity. The segregation analysis was carried out in 17 Buryat and 18 Russian families made it possible to assume the hereditary nature of cases of hearing loss, segregating according to the autosomal recessive mode of inheritance only in Russian families (segregation frequency SF = 0.25, at t = 0.64). The frequency rate of segregation (SF = 35, at t = 0.38) of the pathological trait in Buryat families turned out to be higher than theoretically expected for the autosomal recessive type of inheritance (SF_0 = 0.25), which indicates the presence of other types of inheritance and other forms of hearing impairments caused by non-hereditary reasons. The results of this study and the expeditionary material will be the basis for further study of the molecular genetic etiology of deafness/hearing loss in Buryatia.

Keywords: clinical-audiological analysis, clinical-genealogical analysis, hearing impairment, hereditary burden, Republic of Buryatia

Introduction. For the majority of hereditary diseases associated with organs of hearing, a large number of genes have been identified with a significant variety of mutations contributing to their development [6, 9-11; 13, 14, 18, 22, 25-29, 31], also regional and ethnic differences in the spectrum and frequencies of identified mutations have been manifested [3, 7, 15, 19-23, 30, 36]. Hereditary hearing impairments (HI) are genetically heterogeneous and manifest with different penetrance, which requires a special approach to the development of molecular diagnostics methods for genetically different forms of deafness [12, 24]. Recently, a significant number of works have been published on the successful identification (using various strategies of WES - exome sequencing) of genetic factors leading to hearing loss (HL) and the list of genes associated with hearing loss is constantly expanding (Hereditary Hearing Loss Homepage: http://hereditaryhearingloss.org). To search for the molecular genetic causes of rare forms of deafness in humans at the first stage of research, a thorough clinical and genealogical analysis of families of deaf people with large pedigrees is required.

It is known that the accumulation of a rare genetic disease due to the founder effect can occur in small isolated human populations. Most of the genes associated with one or another rare genetic disease, including those associated with hearing impairment, were first identified in families with large branched pedigrees with numerous affected individuals from isolated populations with a high endogamy index (Ashkenazi Jews, Finns, Sami, as well as inbred families from the Middle East and South Asia) [4, 8, 11]. In such populations, there is high probability of detecting new genes for human mendelian diseases. In Russia, the study of the fundamental foundations of rare (monogenic) human diseases can be carried out using the example of endogamous populations of the peoples of the Caucasus, the Volga-Ural region, Siberia and indigenous peoples of the North.

As a result, the studies of congenital forms of deafness (as one of the most frequent mendelian human diseases) in poorly studied regions of the world, such as the territory of Siberia, are especially relevant. Earlier, according to the contribution of *GJB2* gene mutations (Cx26) among samples of patients with hearing impairments from Siberian regions, the following were described in detail: the Altai Republic [16], the Sakha Republic (Yakutia) [5, 33] and the Tyva Republic [2, 34, 37]. The study of hereditary deafness in the Republic of Buryatia is a logical continuation of research among the populations of Siberia, which makes it possible to close many “blank spots” concerning the issues of genetic epidemiology of hereditary forms of deafness.

The aim of this study is to conduct audiological and clinical-genealogical analysis of the families with hearing impairment in the Republic of Buryatia, which will serve as the basis for further study of the molecular genetic etiology of HI in peoples of Eastern Siberia.

Materials and methods.

The patients

During the expedition work of the Yakutsk Scientific Center for Complex Medical Problems (Yakutsk) in the Republic of Buryatia 165 (n=160 unrelated) deaf

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people were examined in the city of Ulan-Ude. Among them, males accounted for 41.2% (n=68), females - 58.8% (n=97). The average age 48.7±14.9 years. Ethnic composition of the sample: Buryats - 79 people (47.9%), Russians - 76 people (46.1%) and representatives of other ethnicity - 10 people (6%) (Metis (Buryats/Russian) - 2, Mongol - 3, Nanai- 1, German - 1, Uzbek -1, Chuvash -1, Evenk - 1). The characteristics of the sample of the subjects are presented in Table 1.

Audiological examination. The survey was used to find out complaints about the state of hearing, the presence of discharge from the ear, tinnitus, dizziness. For each patient, the following information was obtained: life history, medical history (including previous illnesses), information about allergic reaction, injuries and / or operations, use of ototoxic drugs, contact with industrial noise. The otologic examination was carried out by the unified algorithms on the KaWe Combilight otoscope. The complete audiological examination was performed using a tympanometer and an audiometer "AA222" ("Interacoustics", Denmark). Audibility thresholds were measured by 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 in 5.0 dB increments. The degree of hearing loss was assessed by hearing thresholds of better-hearing ear in the voice frequency range (VFR) in accordance with the international classification under which I degree is equal to 26-40 dB in VFR, II degree - 41-55 dB, III degree - 56-70 dB, IV degree - 71-90 dB, deafness > 90 dB.

Clinical-genealogical analysis. For each participant of the study there was an individual card that included the following information: the participant's last name, first name, and patronymic, as well as their parents; age; ethnicity up to the third generations; place of birth and residence; and profession. The individual map included information about the main ENT diagnosis, the probable cause of hearing loss, age at the time of onset of hearing loss, the presence or absence of hereditary burden and concomitant diseases. After collecting the necessary information (family history), a pedigree was compiled for each proband for clinical and genealogical analysis. The information about otorhinolaryngologic diagnosis, potential cause of HI onset of HI, presence or absence of relatives with HI, including concomitant diseases in the individual patient's card. The pedigrees compiled on the basis of all obtained data were subjected to subsequent clinical-genealogical analysis.

To confirm the hereditary nature of deafness/hearing loss and clarify the type of inheritance, a segregation analysis was performed. To take into account possible distortions related to the features of material collection (insufficient awareness of the proband about close/distant relatives and their health), the expected segregation frequency (SF) was calculated using the proband method [17, 32, 39]. The following formulae were used for calculations:

to calculate the probability of registering a trait in families:

$\pi = \sum n/\sum r$ (1)

where: π - probability of registration; n - number of all probands in all siblings; r - number of those affected in all sibstances;

to calculate the expected segregation frequency of a trait in families:

$SF = \sum r - n/\sum s - n$ (2)

where: SF - expected segregation frequency, r - number of affected individuals in all siblings, n -number of all probands in all siblings, s - total number of siblings in siblings;

calculate the standard deviation:

$\sigma = \sqrt{SF (1 - SF)/\sum s - n}$ (3)

where: σ - standard deviation, SF - segregation frequency, s - total number of siblings in siblings; n - number of all probands in all siblings;

test the hypothesis about the type of inheritance:

$t = SF0 - SF/\sigma$ (4)

where: t - student's t-test, SF_0 - theoretically expected segregation frequency, SF - segregation frequency, σ - standard deviation.

Ethical approval. All examinations provided in this study have been conducted with the informed written consent of all participants or their parents. This study was approved by the local Biomedical Ethics Committee of Federal State Budgetary Scientific Institution "Yakut Science Centre of Complex Medical Problems", Yakutsk, Russia (Protocol #7, August 27, 2019).

Results clinical-audiological analysis. Based on the results of clinical and audiological analysis, the sample was divided into two groups - deafness and hearing loss of varying severity, the criterion for distinguishing which is a different degree of HL. Of the 165 examined individuals, deafness was diagnosed in 116 (70.3%) people, the remaining 49 (29.7%) individuals - HL.The results of clinical-audiological analysis are schematically shown in Figure 1.

All 116 deaf individuals had bilateral permanent sensorineural HL. Of all patients 62 individuals believe that they have HL from birth/infancy (congenital). Prelingual (period before speech formation, up to 2-3 years) form of HL is noted by 30 people, postlingual (after speech formation) form - 24 individuals, of which 17 began to HL in preschool age (from 3 to 7 years old), the rest 7 - at school age. The main reasons for HI are subjectively considered: heredity - 26 people, past infectious diseases - 10 people, past organ/tissue inflammation - 25 people, possibly injuries received in childhood - 11 people, possibly toxic effects of antibiotics - 1 person, found it difficult to answer - 1 person, do not associate with anything - 40 people. Objectively, during otological examination, the majority of the examined among the otologic problems are noted: otitis media - 26 people, tiniitis - 32 people, dizziness - 25 people. Otologic problems are presented in detail in Figure 1.

In another group with HL (n = 49), 37 individuals have the bilateral persistent HL of the sensorineural type, 11 individuals of the mixed (conductive and sensorineural) type, and one person of the conductive type. Of these, 14 individuals have a congenital form of HI, 33 people - juvenile (15 people - prelingual, 18 people - postlingual) and 2 people noticed HL in adulthood. Subjectively, of the main causes of HI are noted: heredity - 13 people, transferred infectious diseases - 6 people, transferred inflammation of

Table1

Characteristics of the samples

Sample investigated		Total individuals n (%)	Total unrelated n (%)	Sex		Middle age:
				Male	Female	
Total		165 (100%)	160 (100%)	68 (41.2%)	97 (58.8%)	48.7±14.9
Ethnicity	Buryats	79 (47.9%)	74 (46.2%)	31 (39.2%)	48 (60.8%)	44.2±13.9
	Russians	76 (46.1%)	76 (47.6%)	32 (42.1%)	44 (57.9%)	54.7±14.0
	Other*	10 (6%)	10 (6.2%)	5 (50%)	5 (50%)	39.5±12.1

Note: * - individuals of mixed and other ethnicities

organs/tissues - 15 people. Among the otologic problems in most cases tiniitis (n=11) and dizziness (n=9) were also noted. Otologic problems and the causes of HI in this group of subjects are presented in detail in Figure 1.

Thus, in accordance with the clinical and audiological analysis of the samples (n=165), the majority of individuals (92.7%) showed a persistent bilateral impairment of sound perception according to the sensorineural type (n=116 - deafness, n=37 - hearing loss). In total, 52% (n=87) of the subjects had juvenile HL, 46% (n=76) of individuals noted congenital form, two people 1,2% (n=2) noticed HI only in adulthood (at 30 and 37 years, respectively).

Clinical-genealogical analysis. Empirical data on the pedigrees of 160 probands allowed us to assume the probability of transmission of the disease by autosomal recessive type, since 97% (n=155) of probands had hearing parents who did not complain of hearing impairment. The rest of the probands (3%, n = 5) with deaf parents (both parents are affected - in 3 probands, one parent - in 2 probands), the family had hearing siblings and/or close relatives.

To establish or refute the hypothesis of AR type of inheritance, segregation analysis was carried out. To obtain correct results, this analysis was carried out separately in 17 Buryat (Table 2) and 18 Russian families (Table 3), in which repeated cases of HL (burden) are observed. In the analysis, only siblings in each nuclear family were taken into account, excluding half-siblings and indirectly (according to relatives) registered affected. During the analysis, nuclear families with one child were excluded from the material, as well as isolated cases in three generations (unburdened), since in these families (both hearing parents) it is not possible to check the segregation of the trait.

Thus, the analysis includes burdened nuclear families with two or more children with hearing parents. As a rule, the “proband” Weinberg method is most often used to calculate the segregation frequency (SF) [1, 38]. The essence of the method consists in calculating the ratio of the total number of affected siblings (without probands) to the total number of all siblings (without probands).

When establishing the hereditary nature of the pathological trait (deafness/HL) in Buryat families, the probability of registration (π) of the trait (probability completeness) by the Fisher method was:

(1) π = 17/44 = 0.38.

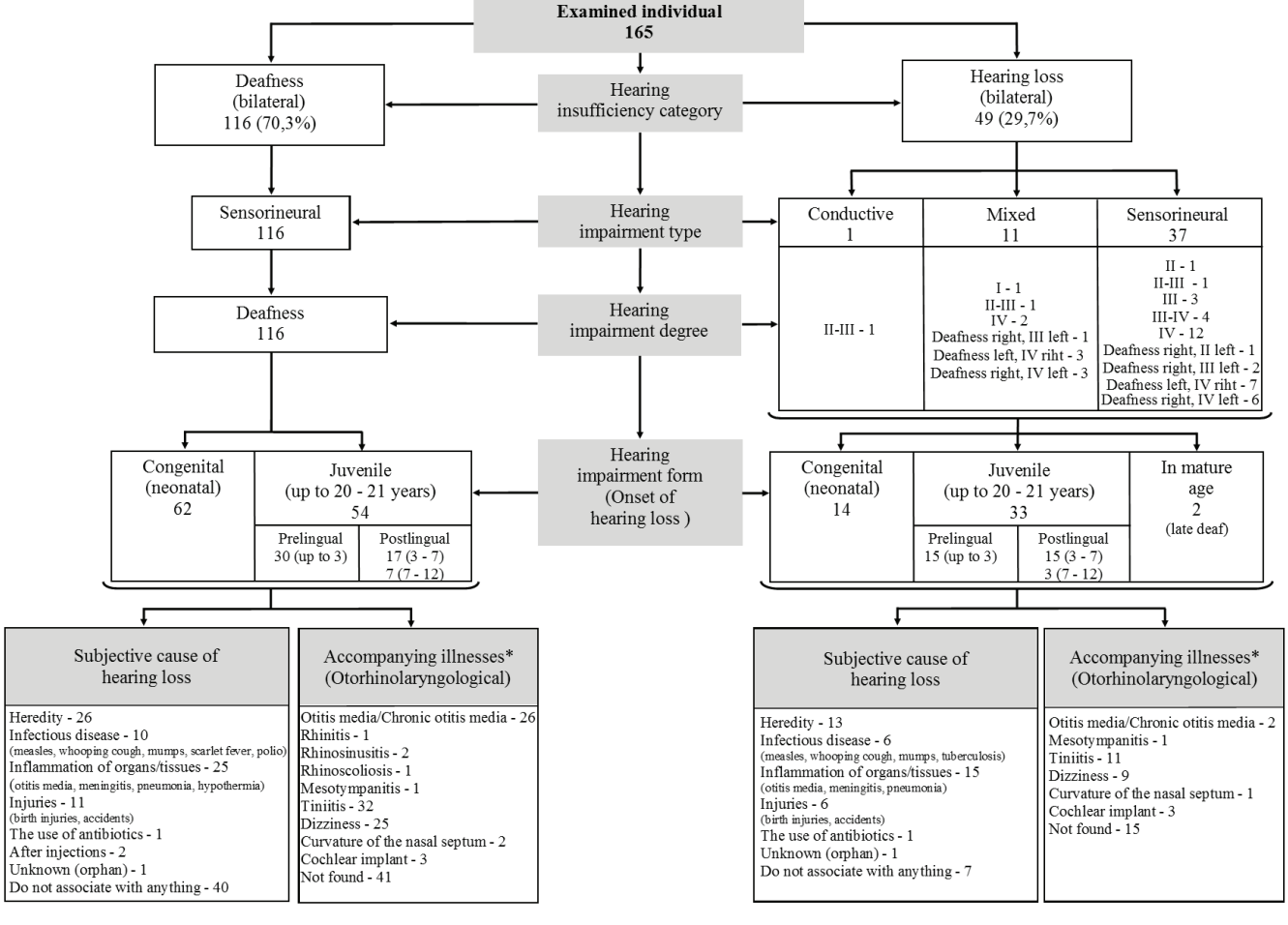
The obtained probability of registering a trait (π = 0.38) indicates its hereditary nature and corresponds to multiple incomplete registration, where $0 < \pi \leq 1$ [32, 39].

The segregation frequency (SF) or the expected proportion of the affected for all siblings was:

(2) SF = 44 - 17/94 - 17 = 27/77 = 0.35.

(3) $\sigma = \sqrt{0.35(1 - 0.35)/94 - 17} = 0.05$.

The segregation frequency (SF = 0.35) turned out to be higher than theoretically expected in autosomal recessive inheritance (SF_0 = 0.25). Further, the comparison of the obtained frequency with the theoretically expected frequency was carried out for different types of inheritance (SF_0 = 0.25 - AR, SF_0 = 0.50 - AD) using the student's t-test (4). As a result, negative values were obtained ($t = 0.25 - \sqrt{0.35/0.05}$, $t = 0.50 - \sqrt{0.35/0.05}$), which refuted these types of inheritance, where $t_{AR} > 2.58$, $t_{AD} < 2.58$.



The results of clinical and audiological analysis. * one individual may have several concomitant ENT diseases

Table2

Segregation analysis in Buryat families with signs of deafness/hearing loss

Sibship's size	Nuclear families/ probands (n)	Number of siblings with affected children				Total number children		
		2	3	4	5	Affected (r)	Healthy -	Total (s)
2	3	3				6	3	6
3	2	2				4	2	6
4	2		2			6	2	8
6	3	1	1	1		9	9	18
7	2	1	1			5	9	14
8	4	3			1	11	21	32
10	1		1			3	7	10
Total	17	10	5	1	1	44	53	94

Table3

Segregation analysis in Russian families with signs of deafness/hearing loss

Sibship's size	Nuclear families/ probands (n)	Number of siblings with affected children		Total number children		
		2	3	Affected (r)	Healthy -	Total (s)
2	10	5		10	10	20
3	2	1	1	5	2	6
4	2	2		4	2	8
5	3	2	1	5	10	15
8	1	2		4	7	8
Total	18	12	2	28	31	57

When establishing the hereditary nature of a pathological trait in Russian families, the probability of registration (π) of a trait according to Fisher's method was:

(1) π = 68/74 = 0.64.

The obtained probability of registering a trait (π = 0.64) indicates its hereditary nature and corresponds to multiple incomplete registration, where 0 <π ≤ 1.

The segregation frequency estimate, taking into account the registration probability (π = 0.64), was SF = 0.25:

(2) SF = 28 - 18/57 - 18 = 10/39 = 0.25.

The obtained values of the segregation frequency turned out to be equal for the expected AR inheritance (SF_0 = 0.25) and prove the correctness of the setting of the type of disease inheritance in the analyzed 18 Russian families.

Thus, the performed segregation analysis confirmed the hereditary character of the sign of deafness / hearing loss (according to Fisher's method 0 <π = 0.64 ≤1) segregating according to the AR type (SF = 0.25, at t = 0.64) only in Russian families. The obtained segregation frequency (SF = 0.35, at t = 0.38) of the pathological sign in 17 Buryat families turned out to be higher than the theoretically expected for the AR type of disease transmission.

Discussion. For the first time, the clinical-audiological and clinical-genealogical analysis of cases of HI in the Republic of Buryatia was carried out. As the result of this analysis of the samples (n = 165), according to the degree of HI, 70.3% (n = 116) of individuals had bilateral deafness, the remaining 29,7% (n=49) had bilateral HL, of varying severity. Among the reasons that influenced HI, 49.7% (n = 82) of the surveyed indicated exogenous factors, mainly the pathological effects of various infectious and inflammatory diseases transferred in childhood (mainly otitis media), as well as various injuries. A hereditary cause of HL was indicated by 21.8% (n = 36) of individuals. HI was not associated with anything in 28.4% (n = 47) people, in this group, in some patients, the hereditary nature of the disease may be hidden.

The segregation analysis was performed to confirm the hereditary nature of deafness/HL and to clarify the type of inheritance. Taking into account the fact that deafness/HL is extremely heterogeneous and the frequency/likelihood of the manifestation of these genes differs in ethnic populations, the segregation analysis was carried out separately for 17 Buryat and 18 Russian (n=18) families in which the inheritance of the pathological trait was noted. (repeated cases of transmission - burdened). The empirical observations of the pedigree data of families allowed us to assume the likelihood of transmission of the disease in an autosomal recessive mode of inheritance, since all probands had hearing parents, and similar signs were found in siblings, cousins, or second cousins. At the first stage of the analysis, the obtained probability of registering the trait (deafness/HL) in Buryat families was π = 0.38, in Russian families π = 0.64. These values corresponded to multiple incomplete registration (0 <π ≤ 1), which makes it possible to use this value (π) to calculate the segregation frequency. Further, the performed segregation analysis confirmed the hereditary nature of the sign of deafness/HL (according to Fisher's method 0 <π = 0.64≤1) segregating in an AR manner (SF = 0.25, at t = 0.64) in Russian families. The obtained segregation frequency (SF = 35, at t = 0.38) of the pathological trait in 17 Buryat families turned out to be higher than theoretically expected for the AR type of inheritance (SF_0 = 0.25).

Conclusion. Thus, the performed segregation analysis suggested the hereditary nature of cases of hearing impairment, segregating according to the autosomal recessive mode of inheritance only in Russian families. It should be noted that when testing one or another hypothesis of inheritance, the assessment of the segregation frequency was complicated not only by isolated cases in the family, but also by the late nature of the manifestation of the disease (in our sample, they are 52%, n=87). The difficulty lies in the fact that when compiling pedigrees in such families, siblings may not be taken into account, who do not have any symptoms or have a mild degree of HL (HI, before the progression begins). Under such circumstances, the frequency of healthy families with sick children in the sample will inevitably be overestimated. Another problem may be associated with the presence of other types of inheritance and other forms of hearing loss caused by non-hereditary reasons in some genealogies with hereditary burden.

We hope that in the future the results obtained in the course of this work will allow to develop the most optimal approach to the molecular genetic study of hereditary hearing impairments in the Republic of Buryatia, the results of which

will complement the information on the genetic etiology of deafness/hearing loss in populations of Eastern Siberia.

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