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MULTIPLE EXOSTOSIS HONDRODYSPLASIA: MOLECULAR-GENETIC CAUSES

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ABSTRACT

Multiple hereditary exostosis (OMIM 133700, OMIM 133701) is a genetically heterogeneous disease manifested by generalized forms of skeletal lesion with numerous progressive deformities of bones and joints. The type of inheritance is autosomal dominant with a high incidence of familial cases. The authors made a review of domestic and foreign literature on the molecular and genetic causes of multiple hereditary exostosis. The main molecular genetic causes of multiple hereditary exostosis are mutations in the genes of exostozin 1 (*EXT1*) (OMIM 608177) and exostozin 2 (*EXT2*) (OMIM 608210).

The study of *EXT* genes, their mutations and the causes of malignant transformation is both a fundamental problem that contributes to understanding the patterns of pathogenesis, and a socially significant task of diagnosing, preventing and predicting this disease

Keywords: multiple hereditary exostosis, multiple osteochondroma, exostozin, mutation, *EXT1*, *EXT2*.

Multiple hereditary exostosis (MHE) or multiple osteochondroma (MO) (OMIM 133700, OMIM 133701) is a genetically heterogeneous disease manifested by generalized forms of skeletal lesion with numerous progressive deformations of bones and joints [14, 32].

MHE belongs to the number of common hereditary diseases. According to a number of researchers, the disease occurs mainly among Caucasoid populations. The incidence of the disease in different Caucasoid populations ranges from 1.3 to 2 per 100.000 population or 1 to 7.000 orthopedic patients. High levels of MHE prevalence were found in the populations of the indigenous residents of Guam and Poingassi Indians (Table 1). The incidence of the disease among the Asian population has not yet been identified [42].

Multiple osteochondroma is an autosomal dominant inherited disease with a very large proportion of family cases [11]. Multiple osteochondromas are initially diagnosed before 4 years of age and are represented by generalized forms of skeletal lesion with numerous progressive deformations of bones and joints, shortening and secondary changes in bone- muscular system. By the age of 18-20, the growth of MHE ceases [1, 9, 22, 28]. Osteochondromas (tumors of bone tissue with cartilage) are today the most common among all primary benign bone tumors (50%). In 15% of cases, these tumors arise in the context of a hereditary syndrome called multiple osteochondrosis (MO), an autosomal dominant skeletal disorder characterized by the formation of multiple bone tumors with cartilage in children's metaphyses. MO is caused by various mutations in *EXT1* or *EXT2*, as a result of which large genomic deletions (single or multiexonical) are responsible for up to 8% of MO cases [15, 45].

Beighton et al. (1993) analyzed the skeletons of adults in the Museum of Pathological Anatomy in Vienna. The museum was founded in 1796 by Emperor Franz II, and now it is in Narrenturm, which was previously the object of detention of persons with insanity. The museum contains 44.000 museum exhibits. Beighton et al. (1993) described the skeleton of a man with multiple exostoses, who died in 1842 from a rupture of an aortic aneurysm (probably syphilitic origin) [20].

Krooth et al. (1961) in his article reports that the first descriptions of the disease were made by Stocks and Barrington (1925), who summarized the clinical description of 1189 cases from literature from around the world and conducted a study of 21 patient from 6 large families with diaphyseal aclasis (multiple exostoses). Patients were from the Chamorro tribe, Micronesians, who live in the Mariana Islands. The frequency of diaphyseal aclasis in the Chamorros, Guam was estimated at 1 in 1000. Among 21 cases from Guam, tumors were found on examination in all men, in women only in half of them [18, 27, 46].

T.P. Vinogradova believes that the endochondral ossification disorder is the basis of this disease. According to M.V. Volkov (1974), this disease accounts for 27% of all primary tumors and tumor-like skeletal dysplasia in children, bone-cartilaginous exostoses among benign bone tumors occur in 40% of cases. The disease is well studied in clinical, radiologic, morphological and genetic aspects. The fact of its hereditary transmission in 75% of all observations was proved [4, 5].

According to A.V. Rusakov (1959), "cartilaginous exostoses - this is not just a tumor-like tissue, but integral parts of the viciously developing bone organs". He, like all other authors, considered exostoses as the dystopia of the skeletal-derived mesenchyme, which determines the growth of bones in length. A.V. Rusakov et al. consider that exostoses retain the same reactivity as normal germ cell cartilage. This is confirmed by the fact that during periods of increased child's growth there is also an increased growth of exostoses [5].

The disease occurs in two forms: multiple exostosis chondrodysplasia and solitary bone - cartilaginous exostosis.

In the case of solitary lesions, tumors that are immobile in relation to the bone, of various sizes and forms, are revealed; skin over them is usually not changed.

Most authors consider single and multiple exostoses as two forms of a single process in essence. Along with this affirmation in literature and clinical practice, solitary neoplasm is often regarded as a benign tumor - chondroma (achondroma) or osteochondroma. The fact of inheritance of single exostoses is not established [4]. Bone - cartilaginous exostosis of large size can exert pressure on vessels or nerve trunks, causing pain.

MHE is usually detected in childhood and adolescence, most often in the second decade of life. There was a significant predominance of males.

Clinical symptoms of MHE depend on the form of the disease, localization, size of exostoses, their shape and relationship with surrounding organs and tissues. According to S.T. Zatsepin (2001) clinically exostoses can manifest themselves very differently, since they can cause many secondary symptoms. Doctors are well aware that exostoses have different shapes: a relatively wide base and a thin, sharp end; a narrow base, ending with a rounded or spherical tip, mostly cartilaginous; some exostoses almost simultaneously with growth ossify; others have a large cartilaginous non-salient "cap" [5].

At the multiple form of exostosis chondrodysplasia, symptoms such as short stature, slanting, valgus deformity of knee joints are often at the forefront. The location of exostoses in the region of the spine with their growth towards the

vertebral canal can cause compression of the spinal cord [11].

The main signs of exostoses are: localization in the metaphyseal or in the metadiaphyseal zone; osteochondral exostosis can have a wide or narrow leg, which is a continuation of the cortical layer of the bone itself and the medullary cavity; the bulk of exostosis represents a bone structure, the outer surface of it can be flat or with subulate outgrowths. The localization of exostoses in frequency corresponds to the growth zones with the greatest potency - this is the lower zone of growth of the femur, upper brachial, tibia, etc. The growth of exostosis continues usually during the period of bone growth, but sometimes the increase in its size is also noted after the closure of growth zones [12].

The most frequent localization of MHE is the metaphysis of long tubular bones. Lesions of the distal metaphysis of the femur, proximal metaphysis of the humerus and tibia occur in 48% of all bone-cartilaginous exostoses [5, 36, 43].

One of the serious complications of the course of bone-cartilaginous exostoses is their malignancy [4, 11]. According to S.T. Zatsepin, L.P. Kuzmina (1971), the transformation of exostoses into chondrosarcoma was noted in 12.5% of patients, according to Adler (1983), about 20%. V.V. Balberkin (1994) notes that among 29 cases of malignant osteochondral cartilage exostoses chondrosarcoma is diagnosed in 25 cases and in 4 - osteogenic sarcoma. More often malignancy of exostoses occurs in patients with a multiple form of exostosis chondrodysplasia (72%). The prevalent localization of malignancy of exostoses is the pelvic bone, less often the scapula, ribs and spine [5, 30].

According to A.M. Gerasimov and A.A. Razzakov (1985), an analysis of the components of proteoglycan aggregates of cartilage extract of exostoses with increased growth activity (with the signs of uniform and uneven bone formation) made it possible to reveal an increase in the content of hyaluronic acid by 5-10 times; the ratio of chondroitin-4-sulfate and chondroitin-6-sulfate was 85:15 at a norm 50:50. The ratio of proteins from proteoglycan cartilage aggregates of exostoses with increased growth activity was close to the ratio characteristic for cartilage of newborns and some other growing tumors, while in the extracts of cartilage of the ilium wing (Lat.: *ala*) of the same patients it corresponded to the age norm [5]. The proteins *EXT1* and *EXT2* form a hetero-oligomer complex, which functions in the biosynthesis of proteoglycan of heparan sulfate [34]. Pacifici (2017) believes that most HME cases are associated with function loss mutations in *EXT1* or *EXT2* that encode the glycosyltransferase responsible for the synthesis of heparan sulfate (HS), resulting in HS deficiency [38].

The most common cause is mutations in the *EXT* (exostozine) genes, which account for 90% of all cases of MHE [9].

Recently 3 genes have been described: *EXT1, EXT2* and *EXT3*, which mutations lead to multiple exostosis chondrodysplasia. Stickens et al. (1996) showed that three genes were identified by an analysis of the genetic linkage in chromosomes 8q24.1, 11p11-13 and 19p [19]. Ahn et al. (1995) defined two main regions, the changes in which lead to MHE: 8q24.1 and 11p11.2. In 1995 in the region 8q24.1, the *EXT1* gene was cloned, coding sequence of which was 2238 bp. (746 amino acids) [16, 35, 37, 43].

Mutations of the *EXT2* gene occur 3 times less frequently than in the *EXT1* gene, only single descriptions are known for the *EXT3* gene [27]. *EXT1* and *EXT2* encode glycosyltransferase involved in the synthesis of heparan sulfate. The *EXT1* gene (OMq 608177) (8q24.11-q24.13) contains 11 exons, *EXT2* (OMIM 608210) (11p12-p11) - 16 exons, 438 mutations in the *EXT1* gene and 205 mutations in the *EXT2* gene (OMIM 600209) are described [9, 12, 13, 27] (Table 2).

We analyzed the literature data describing the analyzed mutations in various populations of the world (Table 3).

Wuyts et al. (1998) analyzed the genes *EXT1* and *EXT2* in 26 EXT families from 9 countries. Out of 26 families, 10 had an *EXT1* mutation and 10 had an *EXT2* mutation. 12 of these mutations have not been previously described. From a review of these and previously reported mutations, it was concluded that mutations in the gene *EXT1* or *EXT2* are responsible for most cases of multiple exostoses. Most mutations in these 2 genes cause premature termination of EXT protein, while missense mutations are rare. Therefore, the development of exostoses is mainly due to the loss of the function of the *EXT* genes, which agrees with the hypothesis that the *EXT* genes have tumor suppressor function [39].

In 23 out of 43 examined Japanese families, Seki et al. (2001) found 21 mutations, 18 of which were new. 17 (40%) of the 23 families had a mutation in *EXT1* and 6 (14%) had a mutation in *EXT2*. From 17 families with mutations of *EXT1*, in 13 were those that cause premature stopping of the function of the protein *EXT1*, and 4 showed missense mutations. In contrast to the results of Seki et al. (2001), Xu et al. (1999) found more mutations in *EXT2* than in *EXT1* in Chinese patients (33% and 14%, respectively) [41, 44]. Raskind et al. (1998) re-

Table 1

The prevalence of mhe in different populations

Population	Number of population, per 1.000 of pop.	Geographical location	Population (country)	Reference
Chamorro	32	Guam Isl. (USA)	65 (21 case, 1 :1000)	[30]
Poingassi Indians	0.583	reservation of Poingassi (Manitoba, Canada)	1298 (1:77)	[30]
All Europe	–	Europe	1,3-2	[39,40]
Russian Federation				
Karachaevtsi	194,3	Karachay-Cherkess Republic	0,9	[7]
Tatars	2012,6	Republic of Tatarstan	4,8 (1:20927)	[7]
Bashkirs	1199,7	Republic of Bashkortostan	0,06	[2]
Chuvashi	814,8	Republic of Chuvashia	0,34	
Russian	3795,6	Rostov Region	0,16	
Russian	626,1	Kostroma Region	0,04	
Russian	4522,9	Krasnodar Territory	0,28	
Russian	1199,6	Kirov region	0,11	
Russian	1296,7	Tver Region	0,39	

Table 2

Mutations in the genes *EXT1* and *EXT2*

Mutation type	Gene	
	EXT1	*EXT2*
Missens / nonsense	147	70
Splicing	47	24
Regulatory	1	6
Minor deletions	150	57
Minor insertions	51	26
Indel- mutations	9	5
Large deletions	27	17
Complex restructuring	6	0
	438	205

ported that the mutations found in *EXT1* in Caucasoids and in Japanese patients were more identified in family cases than in sporadic ones [19].

In a study of 82 Japanese patients with hereditary multiple exostoses Seki et al. (2001), 4 patients developed malignancy of the tumor, and their mutations (3 in the *EXT1* gene and 1 in *EXT2*) were different, indicating that the malignant transformation did not directly was associated with a specific mutation in *EXT1* or *EXT2*, but was more likely to be associated with other genetic factors. Loss of heterozygosity was found in chondrosarcoma not only in the *EXT* loci, but also in others, such as 10q (RET, 164761) and 3q [41].

Depending on nationality, about 56-78% of the mutations are found in the *EXT1* gene, and in the EXT2 gene, 21-44% mutations. Most mutations are

Table 3

Mutations in the *EXT1* and *EXT2* genes responsible for MHE in the surveyed populations of the world

Mutations in the *EXT1* gene	Mutations in the *EXT2*	Method of study	Number of subjects	Population (country)	Reference
gene	Method of study	MLPA	33 patient	Poland	Jamsheer, et.al., 2014
14	6	Direct sequencing by Sanger on a genetic DNA analyzer	43 families	Japan	Seki, et.al., 2001
45	9	1. MLPA 2. Direct sequencing by Sanger on a genetic DNA analyzer	90 patients	Southern Italy	Ciavarella, et.al., 2013
11	8	Direct sequencing by Sanger on a genetic DNA analyzer	23 patient	Germany	Heinritz, et.al., 2009
29	16	1. MLPA 2. Direct sequencing by Sanger on a genetic DNA analyzer	48 patients	China	Li, et.al., 2017
1	2	Direct sequencing by Sanger on a genetic DNA analyzer	4 probands from 4 families	China	Wu, et.al., 2013
-	1	Direct sequencing by Sanger on a genetic DNA analyzer	25 patients	China	Wang, et.al., 2012
-	1	Direct sequencing by Sanger on a genetic DNA analyzer	23 patients	China	Tian, et.al., 2014
9	4	MLPA	33 patients	Latin America	Delgado, et.al., 2014
2	-	Direct sequencing by Sanger on a genetic DNA analyzer	2 probands	Taiwan	Lin, et.al., 2014
1	-	Direct sequencing by Sanger on a genetic DNA analyzer	4 patients	China	Zhang, et.al., 2013
1	4	Direct sequencing by Sanger on a genetic DNA analyzer	8 patients	China	Xu, et.al., 2017
5	4	Direct sequencing by Sanger on a genetic DNA analyzer	46 patients from 10 families	China	Kang, et.al., 2013
5	4	1. T-NGS 2. Direct sequencing by Sanger on a genetic DNA analyzer	10 probands from 10 families	China	Guo, et.al., 2017
1	-	Direct sequencing by Sanger on the genetic DNA analyzer	9 probands	Iran	Akbaroghli, et.al., 2017
1	-	1. Hiseq2000, Illumina 2. Direct sequencing by Sanger on a genetic DNA analyzer	2 probands, 200 control (100 female, 100 male)	China, province Fuczyan'	Hong, et.al., 2017
28	9	MLPA	39 patients	Spain	Sarrion, et.al., 2013
30	15	Direct sequencing by Sanger on the genetic DNA analyzer	112 patients from 71 family	Japan	Ishimaru, et.al., 2016
-	2	Direct sequencing by Sanger on the genetic DNA analyzer	5 probands from the same family	Zhejiang Province, China	Ruan, et.al., 2018
35	17	1. MLPA 2. Direct sequencing by Sanger on a genetic DNA analyzer	153 patients from 114 families	Brazil	Santos, et.al., 2018
11	5	1. MLPA 2. Direct sequencing by Sanger on a genetic DNA analyzer	14 patients from 9 families	Prague (Czech Republic)	Medek, et.al., 2017
35	12	Direct sequencing by Sanger on a genetic DNA analyzer	92 patients from 26 families		Wuyts, et.al., 1998
			3	Morocco	
			27	Netherlands	
			7	Italy	
			2	Germany	
			1	France	
			3	Turkey	
			1	Great Britain	
			9	USA	
			39	Belgium	

point-like. It is assumed that *EXT1* and *EXT2* genes are carcinogenesis suppressor genes, since their participation in malignant transformation of cartilaginous and bone tissue has been established [6, 8, 10, 19, 21].

With the development of new sequencing technologies, biochip analysis, the search for new mutations has become more extensive and rapid. Hong et. al. (2017) have shown that multiple osteochondromas (MO) are an autosomal skeletal disease with an elusive molecular mechanism. To further elucidate the genetic mechanism of the disease, a Chinese family with MO was examined and a new mutation with a change in the structure (c.335_336insA) in the gene of exotozine 1 (*EXT1*) in one patient with MO was examined by the exom sequencing. This was further confirmed by the method of direct sequencing by Sanger and comparison with 200 unrelated healthy people from the control sample [24].

Lin et al. (2014) in their studies showed that DNA sequencing revealed a mutant *EXT1* gene in both cases in which a mutation of c.447delC (p.Ser149fsX156) arose in exon1 and the nonsense mutation c.2034T> G (p.Tyr678X) in exone 10. No mutation was detected in the control group [31]. Liu et al. (2015) analyzed a large Chinese family of five generations with MHE. Exon sequencing was performed in three individuals with MHE and three healthy relatives. The study confirmed a new deletion of C in codon 442 in the exon 5 of exostozine-1 exon (*EXT1*) as the only cause. Immunohistochemical analysis revealed that the level of the EXT1 protein in patients with a new mutation in the study was lower than that in patients without an *EXT1* mutation from another family. For a deeper understanding, they analyzed the spectrum of mutations of the *EXT1* gene. The present study should contribute to a further understanding of MHE [25]. Medek et al. (2017) found in five probands various mutations of the *EXT1* gene leading to a premature stop codon (p.Gly124Argfs * 65, p.Leu191 *, p.Trp364Lysfs * 11, p.Val371Glyfs * 10, p.Leu490Profs * 31). Two mutants in the *EXT2* genome were found to have a nonsense mutation (p.Val187Profs * 115, p.Cys319fs * 46). Five mutations were new and two mutations in probands occurred de novo [23]. Sarrion et al. (2013) conducted a mutation analysis of the *EXT1* and *EXT2* genes in 39 unrelated Spanish patients, most of whom had a mild phenotype, and sought correlation of the phenotype genotype. They found a mutant allele in 37 patients, at 29 in *EXT1* and at 8 in *EXT2*. Five mutations in *EXT1* were deletions identified by MLPA. Two cases of mosaic were recorded. The authors noted a smaller number of exostoses in patients with a described new mutation compared with other mutations. Mutations in *EXT1* or *EXT2* were detected in 95% of Spanish patients. 18 of 37 mutations were new [33]. Xu et al. (2017) found a new missense mutation (c.1385G> A) in the exon 8 and a splicing mutation (c.725 + 1G> C) in the intron of the 3 *EXT2* gene that are responsible for MO [3, 44].

Our results are useful for expanding the database of known mutations in *EXT1*, *EXT2* and in understanding the genetic basis in patients with MHE, which can improve genetic counseling and prenatal diagnosis.

Conclusion

Thus, multiple exostosis chondrodysplasia is a fairly common hereditary disease with an autosomal dominant type of inheritance. The disease is characterized by the presence of multiple cartilaginous exostoses in areas of bone growth. The genes responsible for MHE are the carcinogenesis suppressor genes and are located on three different chromosomes: *EXT1* (8q24), *EXT2* (11p12) and *EXT3* (19p). The spectrum of gene mutations, leading to exostosis and malignancy, has not yet been determined. The study of *EXT* genes, their mutations and the causes of malignant transformation is both a fundamental problem that contributes to understanding the patterns of pathogenesis, and a socially significant task of diagnosing, preventing and predicting this disease.

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