A.E.Yakovleva, A.L. Danilova, D.A. Petukhova,
A.L.Sukhomyasova., N.R.Maksimova

SEARCH FOR MUTATIONS IN THE *EXT1* AND *EXT2* GENES AMONG PATIENTS WITH HEREDITARY MULTIPLE EXOSTOSES IN THE REPUBLIC OF SAKHA (YAKUTIA)

DOI 10.25789/YMJ.2020.72.08

The article presents the results of the fisrt molecular genetic study in the *EXT1 and EXT2* genes among patients with hereditary multiple exostoses (HME) ant their relatives. A rare nonsense mutation c.751C>T (p.Gln251*) in exon 5 of *EXT2* gene in a heterozygous state was detected. That was the cause of HME among patients of the Yakut ethnic group. At present we are conducting the investigation to search mutations in *EXT1 and EXT2* genes in other families with HME.

Key-words: *EXT1, EXT2,* Hereditary Multiple Exostoses, multiple osteochondromas

Introduction. Hereditary Multiple Exostoses (HME) or multiple osteochondromas (MO) (OMIM #133700, #133701) is a genetically heterogeneous disease with an autosomal dominant mode of inheritance, which accounts for 16.7 to 44% of all benign tumors, tumor-like and dysplastic skeletal lesions [9]. Also, in some cases (from 0.5 to 5%), it is possible to transform individual exostoses into a secondary chondroma or chondrosarcoma [3]. This disease is manifested by the development of 2 or more bone outgrowths on long tubular bones. The number of exostoses can vary significantly with the course of the disease and can range from 15 to 18. In most cases, bone changes are asymptomatic and develop from cartilage, increase in size in the first decade of life, and stop growing during puberty, when the growth plates close [4].

Disease incidence in the world in various populations ranges from 1.3 to 2 per 100 thousand population or 1 per 7000 orthopedic patients, up to 80% are family cases [3]. It was found that in 90% of cases, HME is associated with mutations in the *EXT1* (OMIM # 608177, 8q24.11) and *EXT2* (OMIM # 608210, 11p11.2) genes. Depending on the ethnic group, the frequencies of the pathogenic variants *EXT1* and *EXT2* differ [10].

According to the "Republican Genetic Register of Hereditary and Congenital Pathology", 85 patients with HME from 41 families were registered in the Republic of Sakha (Yakutia). 70 patients of them are from 33 Yakut families, 5 patients are from 1 Evenk, 8 patients are from 5 Russian families and one case is from Tatar and Ukrainian family. The disease was registered in 16 uluses and in Yakutsk from 36 administrative-territorial units of the Republic of Sakha (Yakutia). The prevalence of this disease in the Republic of Sakha (Yakutia) was 8.85 per 100 thousand population. Until now, molecular genetic studies on the search for mutations among patients with HME have not been carried out in Yakutia. In this regard, the aim of this work is to search for mutations in the *EXT1* and *EXT2* genes among patients with HME and their relatives in the Republic of Sakha (Yakutia) using modern molecular genetic methods.

Materials and methods. *The patients.* The material for the molecular genetic study included 55 DNA samples of individuals with a clinically established HME diagnosis and 11 DNA samples of their relatives without clinical manifestations from 31 unrelated families. Of the 55 patients, 28 were males and 27 were females; 45 (81.82%) - Yakuts by ethnic origin, 6 (10.91) - Russians, 2 (3.63) - Evenks, and 1 (1.82%) - Ukrainian and Tatar. Informed consent was obtained from all individuals for this study. The work was approved by the local Committee on biomedical ethics of the Medical Institute of the Ammosov Northeastern Federal University (Yakutsk, Protocol No. 8 of November 11, 2016). All individuals were registered in the Medical and Genetic center of the Republican Hospital №1 RS(Ya) of the National Centre of Medicine.

Molecular genetic analysis. DNA was isolated from whole blood by the standard method of phenol-chloroform extraction [5].

To establish the molecular genetic cause of HME, mass parallel sequencing (MPS) was carried out using the Trusight One Sequencing panel (Illumina, USA), which includes 4800 genes with known clinical significance. The MPS method allows to obtain complete information about the nucleotide sequence of the desired DNA region and includes three main stages: preparation of DNA libraries, sample preparation, and sequencing. Sequencing of the sample was carried out on a high-performance sequencer MiSeq, (Illumina, USA), all stages of sample preparation were carried out according to the manufacturer's instructions of Illumina [1, 11].

To confirm the results, direct Sanger sequencing was carried out on an ABI 3500 genetic analyzer (Life Technologies, USA), using specially designed primers. A complex of basic molecular genetic research methods was used: polymerase chain reaction (PCR), agarose gel electrophoresis, direct Sanger sequencing. For PCR, flanking primers 5'-GACTGGTAAGGAAACACTTAC-3 '(forward), 5'-CATGTCCAGTAAAGAG-

YAKOVLEVA Alexandra Eremeevna - Research assistant of the Research Laboratory 'Molecular Medicine and Human Genetics', M.K. Ammosov North-Eastern Federal University, alexerem2013@yandex.ru, **MAKSIMOVA Nadezhda Romanovna** - Doctor of Medical Sciences, Head of the Research Laboratory 'Molecular Medicine and Human Genetics', M.K. Ammosov North-Eastern Federal University, **SUKHOMYASOVA Aitalina Lukichna** - Candidate of Medical Sciences, Deputy Head of the Research Laboratory 'Molecular Medicine and Human Genetics' Ammosov North-Eastern Federal University, Head of the Medical - Genetic center of the Republican Hospital # 1 - National Centre of Medicine, **DANILOVA Anastasia Lukichna** - Candidate of Biological Sciences, Senior Researcher of the Research Laboratory 'Molecular Medicine and Human Genetics', M.K. Ammosov North-Eastern Federal University, **PETUKHOVA Diana Aleksandrovna** – Project Engineer of the Research Laboratory 'Molecular Medicine and Human Genetics', M.K. Ammosov North-Eastern Federal University.

CAATG-3' (reverse) were used. Primers of the *EXT2* gene were selected using the NCBI / Primer-BLAST program and synthesized at Evrogen Closed Joint Stock Company (Moscow) [8]. The genome assembly number is Genome Reference Consortium Human GRCh38 (GCA_000001405.15). The Bioinformatics program MutationTaster was used to predict the pathogenicity of the found variants (http://www.mutationtaster.org/).

Bioinformatic analysis. The initial analysis of the data obtained as a result of the MPS was carried out directly in the MiSeq system itself. The data were aligned to the reference sequence GRCh37 (hg19). The obtained variants were filtered using the Sophia DDM v4 program (Sophia Genetics, Switzerland). The *EXT2 g*ene transcript: NM_001178083 was selected to annotate the identified variants. The clinical interpretation was carried out in accordance with the Russian guidelines for the interpretation of human DNA sequence data obtained by MPs methods [2]. The following databases were used to verify the results: ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), OMIM (https://www.omim.org), Exome Variant Aggregation Consortium (http://exac.broadinstitute.org/), dbSNP build 153 (https://www.ncbi.nlm.nih.gov/snp/), dbVar (https://www.ncbi.nlm.nih.gov/dbvar/), Exome Variant Server (https://evs.gs.washington.edu/EVS/), Leiden Open Variation Database system (https://databases.lovd.nl/shared/genes/*EXT2*)

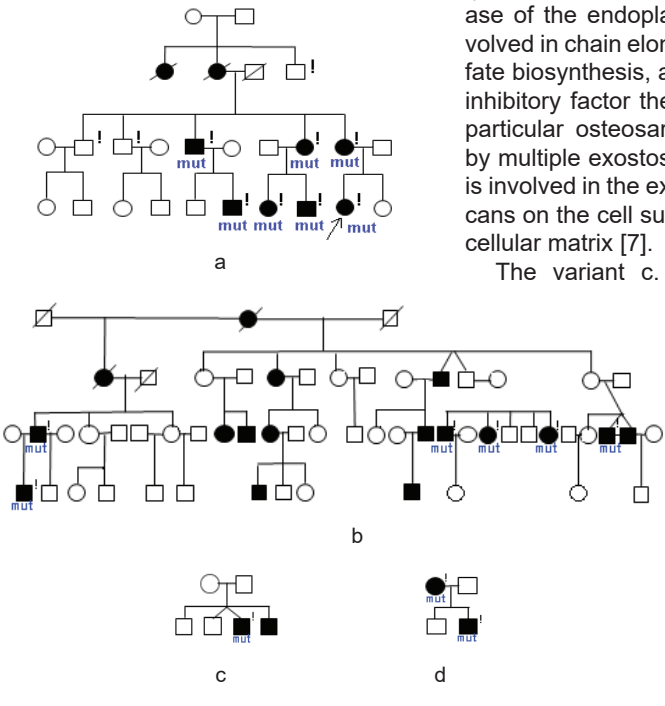
Results and discussions. For the study of mutations, the G's family with the largest number of HME was selected (Fig. 1, A). For the first time the proband addressed to the Medical and Genetic center of the RH1-NCM at the age of 10 with complaints of tumor-like formations in the shoulder area. There were exostoses of large sizes, shortening of the left hand. Then, at the age of 14, the patient had complaints of increased exostosis, curvature of the forearm bones, ulnar deviation of the hands, and restriction of movement in the arms. There were large exostoses, shortening of the left arm. From the age of 14, the patient began to complain of an increase in exostosis, curvature of the forearm bones, ulnar deviation of the hands, and movement limitation in the hands. At the time of the last examination, the proband was 20 years old.

As a result of MPS and the filtration of nucleotide sequence variants, the nonsense mutation c.751C>T (p.Gln251*) in exon 5 of the *EXT2* gene in the heterozygous state was revealed at the proband.

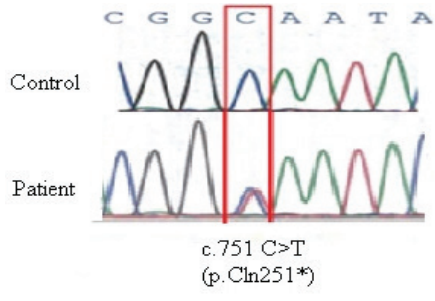
The *EXT2* gene is located on the 11th chromosome at the 11p12-p11 locus, consists of 14 exons and two exons at the alternative splicing site, encodes a type II transmembrane glycosyltransferase of the endoplasmic reticulum, is involved in chain elongation in heparan sulfate biosynthesis, and can also act as an inhibitory factor the growth of tumors, in particular osteosarcomas, accompanied by multiple exostoses. The gene product is involved in the expression of proteoglycans on the cell surface and in the extracellular matrix [7].

The variant c. 751C>T (p.Gln251*) of the *EXT2* gene was detected in the Leiden Open Variation Database system (https://databases.lovd.nl/shared/genes/EXT2). According to the LOVD database, this mutation has been detected in members of two European families. The result is presented with no detailed description of the clinical picture of patients with HME [6, 9]. Further, the direct Sanger sequencing was performed in 54 individuals with HME clinical diagnosis. As a result, 16 (29.09%) of 55 patients with HME had this nonsense mutation c.751C>T (p.Gln251*) in exon 5 of the *EXT2* gene in a heterozygous state (Fig. 2). All 16 patients with this mutation come from Yakut families. The direct Sanger sequencing was also performed on 11 relatives of patients with HME, and the mutation was not detected.

Thus, the diagnosis of HME was confirmed by contemporary methods of the molecular genetic studies in 16 patients from 4 unrelated Yakut families (Fig. 1, B-D).



Picture 1. Pedigrees of families of patients with hereditary multiple exostoses.



Picture 2. Chromatogram fragment of the exon 5 gene *EXT2*
Control – healthy; Patient – patient with hereditary multiple exostoses with nonsense mutation c.751C>T (p.Gln251*)

Conclusion. As a result of the search for mutations in the *EXT1* and *EXT2* genes among 55 patients from 31 unrelated families of different ethnic origin with a clinically diagnosed HME, a rare nonsense mutation c.751C>T (p.Gln251*) in the exon 5 of the *EXT2* was revealed among 16 (29.09%) patients from 4 unrelated Yakut families.

Approaches have been developed for molecular genetic laboratory diagnostics of the identified mutation, which can be used to confirm the diagnosis of HME, predict, and prevent adverse outcomes.

At present, we are conducting the investigation to search for mutations in the *EXT1* and *EXT2* genes in other families with HME.

The research is conducted under the state target program of the Ministry of Science and Higher Education of the Russian Federation: project FSRG-2020-0014 "Genomic of Arctic: epidemiology, hereditary and pathology". Molecular genetic studies were carried out in the Arctic Innovation Centre of the "Ammosov Northeastern Federal University".

References

1. Кушлинский Н.Е. Генетические исследования при опухолях костей / Н.Е. Кушлин-

ский, Ю.С. Тимофеев // Вестник ТГУ. – 2013. - Т. 18, №6. – С. 3265-3273 [Kushlinsky N.E. Genetic research for bone tumors / N.E. Kushlinsky, Yu.S. Timofeev // Tomsk State University Journal. – 2013. V. 18, №6. – P. 3265-3273. (In Russ.)]

2. Руководство по интерпретации данных последовательности ДНК человека, полученных методами массового параллельного секвенирования (MPS) / О.П. Рыжкова, О.Л. Кардымон, Е.Б. Прохорчук [и др.] // Медицинская генетика. – 2018. – №2. – С. 3-23. [Guidelines for the interpretation of massive parallel sequencing variants (MPS) / O.P. Ryzhkova, O.L. Kardymon, E.B. Prohorchuk [et al.] // Medicinskaâ genetika. – 2018. – №2. P. 3-23. (In Russ.)]

3. Чеснокова Г.Г. Изучение структурных аномалий и точковых мутаций генов EXT1 и EXT2 при множественной экзостозной хондродисплазии и спорадических злокачественных новообразованиях: автореф. дис. … канд. биол. наук: 03.00.15 / Г.Г. Чеснокова. – М., 1999. С. 23. [Chesnokova G.G. Study of structural anomalies and point mutations of the EXT1 and EXT2 genes with multiple exostosis chondrodysplasia and sporadic malignant neoplasms: author,s abstract.Kushlinsky N.E. Genetic Research for Bone Tumors / Kushlinsky N.E., Timofeev I.S.// Tomsk State University Journal. – 2013. V. 18, №6. – P. 3265-3273.]

4. Hennekam R.C. Hereditary Multiple Exostoses / R.C. Hennekam // Journal of Medical Genetics. - 1991; 28: 262-266.

5. Maniatis T. Molecular Cloning - A Laboratory Manual / T. Maniatis, J. Sambrook, E.F. Fritsch // Cold Spring Harbor Press (New York). - 1982. – P. 76-85.

6. Novel *EXT1* and *EXT2* Mutations Identified by DHPLC among Italian Patients With Multiple Osteochondromas / Pedrini E., A. De Luca, Valente E.M. and others // Human Mutation. - September, 2005; 26(3):280. - P. 1-10. doi: 10.1002/humu.9359.

7. Pacifici M. Hereditary Multiple Exostoses: New Insights into Pathogenesis, Clinical Complications, and Potential Treatments / Pacifici M. // Curr Osteoporos Rep. 2017; 15:142–52. doi:10.1007/s11914-017-0355-2.

8. Primer Designing Tool [Electronic resource]. – A link: http://www.ncbi.nlm.nih.gov/tools/primer-blast. − Title. – 05.11.2017.

9. The Genotype-Phenotype Correlation of Hereditary Multiple Exostoses / Alvarez C., Tredwell S., M. De Vera [and others] // Clin Genetics, August, 2006; 70(2):122-30. doi: 10.1111/j.1399-0004.2006.00653.x.

10. The Putative Tumour Suppressor *EXT1* Alters the Expression of Cell-Surface Heparan Sulfate / C. McCormick, Y. Leduc, D. Martindale and others // Nature Genet. -1998. - Vol.19. - P. 158-161.

11. TruSight One Sequencing Panel Series. Reference Guide. 2018. Available at: https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/trusight_one/trusight-onsequencing-panel-reference-guide-15046431-03.pdf. Accessed 25 Apr 2019.