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MOLECULAR GENETIC CHARACTERISTICS OF MEFV GENE IN FAMILIAL MEDITERRANEAN FEVER IN AZERBAIJAN

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Purpose of the work was to study mutations of MEFV gene of the Familial Mediterranean Fever disease in the population of the Republic of Azerbaijan. For this purpose, a complex of modern molecular-genetic methods based on polymerase-chain reaction has been used.

The 7 mutations of the MEFV gene have been identified: R761H M694I, M694V, V726A, R202Q, M680I and E148Q. Two mutations - E148Q and R202Q were located in exon 2, five mutations - R761H, M694I, M694V, V726A, and M680I were found in exon 10. 9 polymorphisms have been identified in the exons 2,3 and 5 of the MEFV gene.

To prevent Familial Mediterranean Fever hereditary disease in the population of the Republic of Azerbaijan, it is planned to carry out the medical-genetic counseling for families with genetic risk with the following prenatal diagnosis of the fetus in the next pregnancy.

Keywords: *population, mutation, gene, exon, chromosome, disease, nucleotide*

Introduction

Familial Mediterranean Fever Gene (MEVF) is located in chromosome 16, being precise in 16.13.3. locus. The MEFV gene belongs to the RoRet family of genes and contains 10 exons, which is 10,000 nucleotide sequences long. The synthesized pyridine protein consists of 761 amino acids, though the length of the transcript is 3.7 thousand nucleotide sequences,. The word pyrin is a Greek word for "flame", or marenostrin expressed in mieloid cells means "our sea" in Latin. MEFV gene is located between the genes responsible for the kidney polycystosis and Rubinstein Teybi syndrome. It has autosome-recessive type of heredity. Autosome-dominant hereditary species were also recorded [1,2].

Out of 177 MEFV gene mutations found, 154 are missense mutations. The most common mutation is M694V, which occurs in 30-67% cases. The disease has a severe clinic and results in amyloidosis [3].

The mutation V726A stays in the second place and occurs in 5-35% of patients. M694V and V726A constitute 75% of all mutations found. The molecular-genetic analysis of these mutant-carrying haplotypes revealed that they belonged to the same ancestor haplotype. In the process of evolution, the ancestor haplotype has been diverted [4,5,6].

Most mutations occur at the exon 10. Approximately 70% of patients living in the Mediterranean Sea have one of five mutations (M694V, V726A, M694I, M6801, E148Q) [7,8].

The Family Disease Fever was first studied by Raymond in 1948 and was termed "periodic disease" based on periodic recurrence of the disease. Disease usually gives up to 30 years of age. It is a rare frequency hereditary disease [9].

The disease occurs mostly on the Mediterranean coasts and in the Asia Minor communities: the majority in Armenians, Turks, Seafard and Ashkahazi Jews, Arabs, and less in Greeks, Spaniards and Italians. The heterogeneity of the disease among the people living on the Mediterranean Sea is 20%, and the rate of births of homozigous children equals 1: 1000-1: 2500. It is sporadicaly met in other ethnic groups [10,11,12].

The following mutations of the MEFV gene have been identified in Turkey: E148Q, R202Q, P369S, F479L, M680GA, M680GC, M694V, M694I, K695R, V726A, A744S and R761H [8,12].

The diagnostics of the disease: what ethnic group they and their ancestors belong to - is of great diagnostic significance [5,8].

For the first time in the population of the Republic of Azerbaijan, we for the first time in Azerbaijan put up the goal: to study the molecular-genetic characteristics of the MEFV gene for the Familial Mediterranean Fever disease in ethnic Azerbaijanis.

Material and methods

The genome DNA was separated from the 200 μl venous blood. For this purpose, venous blood samples were taken from 18 patients. Patients were between 2.5-8 years old (female-5, male-13). The concentration and intactness of the separated genome DNA was tested in 0.7% agarose gel. The genome DNA was PCR-ed separately for protein-encoding exons of the MEFV gene. Positive PCR samples, that have been got by electrophoresis in the agarose gel, were purified by enzymatic method. Positive Cycle Sequencing PCR samples, got by agarose gel electrophoresis, are purified by BIGDye XT dye remover. The purified gene samples were read by the Automatic DNA sequencing AB13130xI Analysis System. The obtained nucleotide sequences were read out with Seqscape V.2.7. programme, compared to normal MEFV nucleotide sequence by Blast Ce NCBI, and then polymorphisms and relative mutations were identified. [8,10].

Studies and discussion

The molecular-genetic study of the MEFV gene has identified 7 mutations: R761H, M694I, M694V, V726A, R202Q, M680I and E148Q. The seven mutations discovered were previously identified in the communities living in the Mediterranean region, mainly in the Turkish population [4,5,8,10,11,12]. Three of 18 examined patients were heterozygotes, eight homozygotes, and seven double heterozygotes (compounds).

Table 1 lists the polymorphisms found in the MEFV gene - nucleotide substitutions, frequencies, and the located in exons.

As it shown in table 1, nine polymorphisms were found in three exons of the MEFV gene. Five polymorphisms were observed in exon 2, one polymorphism in exon 3 and three polymorphisms in exon 5.

The polymorphisms of the MEFV gene 414 A/G, 442 G/C, 495 C/A, 1422 G/A and 1428 A/G of the patients with their parents in consanguineous marriages were heterozygous.

High polymorphism rates were for 306 T/C (22.22%) and 942 C/T (16.67%) mutations. The gene frequencies for the polymorphisms of 942 C/T, for 605 G/C and 1530 T/C they were the lowest - 5.56%. The gene frequency of the remaining four polymorphisms was equal 11.11%.

Table 1

No	Polymorphism	Exon	Number	Frequency (%)
1.	306 T/C (D102D)	2	8	22.22
2.	414 A/G (G138G)	2	4	11.11
3.	442 G/C (E148Q)	2	2	5.56
4.	495 C/A (A165A)	2	4	11.11
5.	605 G/C (R202Q)	2	2	5.56
6.	942 C/T (R314R)	3	6	16.67
7.	1422 G/A (G474G)	5	4	11.11
8.	1428 A/G (G476G)	5	4	11.11
9.	1530 T/C (D510D)	5	2	5.56

Table 2 lists the mutations causing the disease, gene frequencies, and exons in which the MEFV gene is identified.

Table 2

MEFV gene mutations, frequencies and exons in Azerbaijan Republic

Mutations	Mutation number	Frequency (%)	Frequency (fraction)	Exon
E148Q	3	9,1	0,0909	2
R202Q	5	15,2	0,1515	2
M680I	4	12,1	0,1212	10
R761H	9	27,3	0,2727	10
M694I	5	15,2	0,1515	10
M694V	1	3,0	0,0303	10
V726A	6	18,2	0,1818	10

The highest frequency of the MEFV gene among the 18 patients was 27.3% for the R761H mutation. Mutation V726A (18.2 %) was in the second place, and M694I (15.2%) was in the third place.

Two R202Q and E148Q mutations were found in exon 2 (28.57%) of MEFV gene, and five mutations - M860I, R761H, M694I, M694V and V726A – were reealed in the exon 10 of the gene (71.43%).

R202Q mutation was in two patients, mutation E148Q was heterozygous in one patient, and two patients had compound form (R202Q/E148Q). The homozygous form of the R761H mutation was recorded in two cases, but the same mutation in compound condition (R761H/M694I) was found in four cases with M694I mutation. The M694I mutation was separately met with two mutations - M694V and R202Q (M694I/M694V and M694I/R202Q) as compounds. M680I mutation was found to be homozygous in two patients (M680I / M680I). The mutation of the V726A was identified in three cases to be homozygous. It should be noted that in patients with the homozygous form of mutations parents had consanguineous marriges.

According to world literature, five mutations: M694V, V726A, M694I, R202Q, M680I and E148Q found up today, constitute 75% of all mutations [5,9,6,11]. Five of the seven mutations found in our studies belong to the same group, and constitute 57.6% of total mutations found.

In order to prevent Familial Mediterranean Fever disease, parents of 18 patients have been consulted by geneticist for a healthy child prognosis for the next pregnancy and 25% of the risk of affected child. As the majority of families are in reproductive age, we have got their consents to carry out fetus prenatal diagnosis during their next pregnancies.

Conclusion

Results of molecular genetic researches for the MEFV gene in patients with the diagnosis: periodic disease - are presented. Seven mutations of MEFV gene were identified, they are: R761H, M694I, M694V, V726A, R202Q, M680I and E148Q. Two mutations E148Q and R202Q are located in exon 2, and the rest R761H, M694I, M694V, V726A, M680I five mutations in exon 10.

To carry out prophylaxes of periodic disease to families with genetic risk of affected child birth, medical genetic consultation is planned to be conducted with the following prenatal molecular genetic diagnostics of fetus in the first trimester of pregnancy.

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AZƏRBAYCANDA AİLƏVİ ARALIQ DƏNİZİ HƏRARƏTİ XƏSTƏLİYİNİN MEFV GENİNİN MOLEKULYAR GENETİK XARAKTERİSTİKASI

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Molekulyar-genetik metodların kompleksindən istifadə edərək MEFV geninin 7 mutasiyası identifikasiya edilmişdir: R761H M694I, M694V, V726A, R202Q, M680I və E148Q. İki mutasiya - E148Q və R202Q iki saylı ekzonda, beş mutasiya - R761H M694I, M694V, V726A, M680I on saylı ekzonlarda identifikasiya edilmişdir. MEFV geninin 2,3 və 5 saylı ekzonlarında 9 polimorfizm müəyyən edilmişdir.

Azərbaycan Respublikasının əhalisində Ailəvi Aralıq Dənizi Hərarəti irsi xəstəliyinin profilaktikası məqsədilə genetik riskli ailələrin tibbi-genetik konsultasiyasının və növbəti hamiləlikdə dölün ana bətnində prenatal diaqnostikasının aparılması planlaşdırılır.

Açar sözlər: *populyasiya, mutasiya, gen, xromosom, xəstəlik, nukleotid*

МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКАЯ ХАРАКТЕРИСТИКА ГЕНА MEFV СЕМЕЙНОЙ СРЕДИЗЕМНОМОРСКОЙ ЛИХОРАДКИ В АЗЕРБАЙДЖАНЕ

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С использованием комплекса молекулярно-генетических методов у населения Азербайджана исследован ген MEFV Семейной Средиземноморской лихорадки. Идентифицировано семь мутаций гена MEFV: R761H, M694I, M694V, V726A, R202Q, M680I və E148Q. Две мутации - E148Q и R202Q расположены в экзоне 2, остальные пять - R761H, M694I, M694V, V726A, M680I в экзоне 10. В экзонах 2,3 и 5 идентифицировано 9 полиморфизмов.

Обсуждаются пути профилактики наследственного заболевания Семейной Средиземноморской лихорадки у населения Азербайджана с последующей пренатальной диагностикой плода в будущей беременности с разрешения супругов в семьях с высоким риском данного генетического заболевания.

Ключевые слова: *популяция, мутация, ген, хромосом , заболевания, нуклеотид*