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Molecular-Genetic Research of Early Epileptic Encephalopathy and Cystic Fibrosis Disease in Population of Azerbaijan

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Abstract

The purpose of our research was to conduct an analysis of an Azerbaijani patient diagnosed with early epileptic encephalogatia for the first time using modern molecular genetic methods, to study the genetics of the disease. In order to research of molecular genetic study of early epileptic encephalopathy, we screened the SPTAM1 gene among children with similar diagnoses. For the first time in a patient diagnosed with epileptic encephalopathy, this associated identity of SPTAN1 has been identified, with no indication that the mutation has been localized. Among the new alcoholic diseases in the Republic of Azerbaijan are the centers for the treatment of early epileptic encephalopathy and health disease SPTAM1 gene screening. Five mutations for CFTR gene for the first time was identified in Azerbaijan population. They are as follows: Phe508del, 965,(T>C), 1000 (G>T), 1210-1211 (T>G) and 328 (G>C). Gene frequencies were equal to: Phe508del (68,75%), in two 965,T>C (12,5%) and in each of – 1000 G>T (6,25%), 1210-1211,T>G (6,25%) and 328,G>C (6,25%). We were first to describe mutation 965, T>C (Leu322Pro). Among children under study, cystic fibrosis diagnosed kids' amount consisted 5,37%.

Keywords: inherited diseases, mutation, gene, protein, nucleotide, amino acid

Introduction

Epileptic encephalopathies (EE) are a group of progressing diseases of different etiology which is expressed as neurocognitive deficit and epileptiform activity on the electroencephalogram. EE make 15% of all epilepsy forms in childhood age and up to 40% of all epileptic onsets in their first 3 years of life (Zhu *et al.*, 2017). 10 syndromic forms of EE are outlined (Saitsu *et al.*, 2010; Stabach & Morrow, 1997). Genetic factors play special role in pathologies development in around 70-80% patients and not less than 40% of all idiopathic epilepsies have got monogenic

nature (Writzl *et al.,* 2012). 35 genes responsible for EE occurrence are identified and the search is still continued. Severe genetic heterogeneity of early EEs is showed, 16 of which are inherited autosome-dominant, 13 –autosome –recessive, 4 – X-linked recessive and 2 – X-linked dominant (Hamdan *et al.,* 2012; Nonoda *et al.,* 2013).

Goal of our researches is modern molecular genetic diagnostics study of one patient with epileptic encephalopathy diagnosis from Azerbaijani family.

As to WHO information, there more than 6000 inherited diseases that have been already studied. The major part of identified genetic diseases consist of monogene natured ones. It is understood from their names that one gene is mutated and causes monogene natured inherited disease. There are around 5000 monogene inherited diseases (Trujillano *et al.,* 2017; Riordan *et al.,* 1989).

Cystic fibrosis is the widest spread monogene natured inherited disease. Cystic fibrosis is autosome recessive inritance type disease (Bailey, 2013).

Frequency of the disease (homozyous form) is 1:2500-5000 in newborns. Heterozygous carriers are born as 1:25-30 (McKusick, 2002).

In 80's of the last century, gene (CFTR) structure of cystic fibrosis disease was identified and studied by means of molecular-genetic methods. Gene CFTR locates on the long arm of the seventh chromosome (7q31). The size of the gene is 190 kb and covers 27 exons. CFTR gene takes part in synthesis of transmembrane protein sized 170 kDa (Bailey, 2013).

For now more than 700 mutations of CFTR gene have been identified, and most of them are rarely encounted. Inheritance type for cystic fibrosis is autosome-recessive (Vissers *et al.,* 2016).

Pathogenic variants in the CFTR gene are causative for cystic fibrosis, an autosomal recessive disorder. Cystic fibrosis is a multisystem disease affecting epithelia of the respiratory tract, exocrine pancreas, intestine, hepatobiliary system, and exocrine sweat glands. Morbidities include progressive obstructive lung disease with bronchiectasis, frequent hospitalizations for pulmonary disease, pancreatic insufficiency and malnutrition, recurrent sinusitis and bronchitis, and male infertility. Pulmonary disease is the major cause of morbidity and mortality in cystic fibrosis. Meconium ileus occurs at birth in 15%-20% of newborns with cystic fibrosis. More than 95% of males with cystic fibrosis are infertile (OMIM®: 219700; GeneReviews - PMID: 20301428) (Bailey, 2013).

70% of all identified mutations of CFTR gene present one and the same mutation. In exon 11 in place of protein biosynthesis the mutation between nucleotides 1521-1523 causes deletion of Phenialanine in position 508. Since the synthesized protein is not structurally normal, it cannot come out from endomplazmatic reticulum and, changes are observed in its tolerance and activity (Riordan *et al.,* 1989).

All types of mutations of CFTR gene are identified as point mutations, manor and major deletions, transversions, inversions et cetera. Around half of all identified mutations are missense mutations (Nonoda *et al.,* 2013; Riordan *et al.,* 1989).

The following mutations consist 76% of CFTR gene mutations: del121kb, delf508, del1501507, 1677delTA, 2143delT, 2184insA, 394delTT, 3821delT, G542X, W1282X, N1303K, L138ins, R334W and 3849+10kb C→T (Rose & Uhl, 2010; Stabach & Morrow, 1997).

Altogether, protein synthesized by CFTR gene regulates chloride channels' activities which are located in apical membranes of epithelial cells. Disease damages function of lungs and pancreas. Diagnostic sign is increase of clorides and sodium values in kid's sweat. Only 2% of patients have got normal values of chlorides in sweat with tipical features of the disease. In these cases, molecular-genetic methods are used to identify mutation in CFTR gene (McKusick, 2002).

CFTR gene mutations causing cystic fibrosis inherited disease have not been identified for Azerbaijan Republic population.

Thus, we put up a goal to study CFTR gene mutations for population of Azerbaijan Republic, using molecular-genetic method complex.

Materıal and Methods

A child born in 2019 was diagnosed with early epileptic encephalogatia at the Azerbaijan Medical University's Teaching Therapeutic Clinic. During the compilation of the genealogy, it was determined that the patient's parents, as well as his parents, were in the marriage of close relatives. Thus, the fathers of the patient's parents are brothers. Genetic analysis of the patient and his parents was performed. For analysis, venous blood was soaked in three different DBS cards (Dry blood spot), and after drying, it was sent to the German CENTEGENE laboratory in a special envelope for genetic analysis. Direct sequencing of the SPTAN1 gene was performed by the Sanger method. Using the method, it was possible to test an existing mutation within the SPTAN1 gene. The Whole Exome

Sequencing method (CentoXome®) was used. The method was developed in CENTOGENE laboratory (Rostock, Germany).

Missens mutation of the SPTAN1 gene in a 9-month-old child diagnosed with early epileptic encephalopathy using a combination of modern molecular genetic methods: quanine nucleotide replacement with adenine nucleotide has been identified in gene 2908 (SPTAN1 2908G> A). As a result of the mutation, the amino acid glutamine was replaced by the amino acid lysine at position 970 of the protein (Glu970Lys). According to the recommendations of Centogene Laboratory and ACMG (American College of Medical Genetics), the mutation was classified as grade 3 according to the degree of importance.

Examination has been provided for 149 children-patients who applied to Scientific Research Pediatry Institute under Ministry of Health, Republic Children's Hospital and polyclinic departments in the different areas. For every patient 1 ml venouz blood has been sampled into a tube with EDTA anticoagulant solution. Later on it was absorbed to special DBS (dried blood spots) cards and dried up for an hour at room temperature, only then has been sent to the laboratory for further analysis.

Study has been carried out at "Laboratory science" Chair atthe Azerbaijan State Doctors' Advanced Training Institute after A.Aliyev and CENTOGENE laboratory in Rostock, Germany.

A part of analysis were tested on ROTOR-GENE apparatus. To do that, a panel of 6 mutations of CFTR gene: delF508, W1282X, N1303K, delT2143, 3849+10kb C→T və del2,3-21kb) was used.

The different part of analyses havs been carried out by means of fluorimetric methods, liquid chromatography and mass-spectrometry.

Results

Missens mutation of the SPTAN1 gene in a 9-month-old child diagnosed with early epileptic encephalopathy using a combination of modern molecular genetic methods: quanine nucleotide replacement with adenine nucleotide has been identified in gene 2908 (SPTAN1 2908G> A). As a result of the mutation, the amino acid glutamine was replaced by the amino acid lysine at position 970 of the protein (Glu970Lys). According to the recommendations of Centogene Laboratory and ACMG (American College of Medical Genetics), the mutation was classified as grade 3 according to the degree of importance.

The following table presents results of 8 cystic fibrosis patients identified during 149 children patients were tested for CFTR gene mutations.

Patient F.A. is double heterozygote according to two different mutations (compound form). The first nucleotide substitution has taken place in the exon 4 of CFTR gene in position 328. Guanine is changed with Cytosine. As a result of this mutation, protein synthesized changes in position 110 Asparagine aminoacid with Histedine aminoacid (328 G>C, 110 Asp>His).

The second change in CFTR gene happened in the exon 8. In the position 1000of the exon Cytosine nucleotide was substituted with Thymine nucleotide, and in the result protein biosynthesized got change of Arginine amnoacid with Triptophan aminoacid in position 334 (1000 C>T, 334 Arg>Trp). In father of the patient F.A. 328 G>C (110 Asp>His) CFTR gene mutation was found in heterozyous state, in his mother - 1000 C>T (334 Arg>Trp) mutation also in heterozygous state.

Five patients - D.S., A.T., A.N., R.F. and B.A. had got the same deletion in exon 11 of CFTR gene genin between 1221-1223 nucleotides in hetero- and homozygogous form. As a result of the mutation, biosynthesis of protein shows deletion of Phenylalanine aminoacid (Phe508del).

B.D. patienthas got homozygous form of CFTR gene mutation as a result of change of Thymine nucleotide with Cytosine nucleotide in position 965 (965, T>C/965, T C). During protein biosynthesis, Leucine aminoacid was substituted wit Proline aminoacid in position 322. Prior to us mutation 965, T>C has not been described in Azerbaijan.

Some of mutations Phe508del (patients A.T.,A.N., R.E., B.A.) identifications have been done with ROTOR-GENE apparatus. Other part of mutations has been identified through directly sequencing.

Only one of 5 identified mutations (Phe508del) is the mostly spread type. The rest four mutations: 328(G>A), 965(T>C), 1210-1211(T>G) and 1000(G>T) are not the often encountered and treated as rare mutations of CFTR gene.

Identified mutations have been found in intron 9 and exons 4, 8, 9 and 11.

Eight patients have been examined, and out of 16 CFTR genes: 11 identified Phe508del mutated gene (68,75%), two manifested 965,T>C mutated gene (12,5%), the rest each one gene revealed – 1210-1211,T>G (6,25%), 1000, G>T (6,25%) and 328,G>C (6,25%) mutations.

Among tested patients frequency of index patients with cystic fibrosis was 5,37%. If showing in fractions, then CFTR gene frequency was 0,0537.

Table 1. Results of molecular genetic studies of CFTR gene

Patient	Gene mutation	Protein mutation	Genotype	Method
D.Kh.	1521-1523 exon 11 1210-1211T>G intron 9	Phe508del	Compound	Direct sequencing
D.S.	1521-1523del exon 11	Phe508del	Homozygote	Direct sequencing
F.A.	328 G>A exon 4 1000 G>T exon 8	Asp>110His Arg>334Trp	Compound	Direct sequencing
B.D.	965 T>C	Leu322Pro	Homozygote	Direct sequencing
A.T.	1521-1523del exon 11	Phe508del	Homozygote	Genetic panel
A.N.	1521-1523del exon 11	Phe508del	Homozygote	Genetic panel
R.E.	1521-1523del exon 11	Phe508del	Homozygote	Genetic panel
B.A.	1521-1523del exon 11	Phe508del	Homozygote	Genetic panel

Thus, as a result of screening done in Baku city of Azerbaijan Republic children hospitals, eight children-patients with cystic fibrosis diagnosis were identified for 5 mutations of CFTR gene. One mutation of these, i.e. 965 (T>C) has not been found and described prior to us.

To prophylaxy cystic fibrosis in the Republic, it is recommended to carry out genetic screening of newborns, to consult genetically risky families and to provide prenatal diagnosis of fetus during the following pregnancies.

Conclusıon

1. Missens mutation of the SPTAN1 gene in a 9-month-old child diagnosed with early epileptic encephalopathy using a combination of modern molecular genetic methods: quanine nucleotide replacement with adenine nucleotide has been identified in gene 2908 (SPTAN1 2908G> A).
2. As a result of the mutation, the amino acid glutamine was replaced by the amino acid lysine at position 970 of the protein (Glu970Lys).
3. For the first time five mutations of CFTR gene: Phe508del, 965,(T>C), 1000 (G>T), 1210-1211 (T>G) and 328 (G>C) have been identified.

4. Gene frequencies for mutations are equal: Phe508del (68,75%), in two 965,T>C (12,5%) and for each one – 1000 G>T (6,25%), 1210-1211,T>G (6,25%) and 328,G>C (6,25%).Mutasiyaları gen tezlikləri bırabərdi:
5. Mutation 965, T>C (Leu322Pro) has not been described prior to us.
6. Among all examined children, frequency of cystic fibrosis was 5,37%.

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