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Evaluation of mutations MYO7A gene in a family with Usher syndrome

The Usher syndromes (USH) are a group of clinically and genetically heterogeneous autosomal recessive disorders characterized by bilateral sensorineural congenital hearing loss, retinitis pigmentosa (RP), and in some cases, vestibular areflexia. It is the most common cause of deafness accompanied by blindness, accounting for 6% of the congenital deaf population and 18% of all patients with RP. The prevalence of USH ranges from 3.5 to 6.2 cases per 100,000 (2).

Usher syndrome I is the most severe form, characterized by profound congenital deafness, RP, cataract formation and vestibular areflexia (3). People with Usher syndrome II display congenital moderate-to severe hearing loss, RP and normal vestibular responses. USH II is the most common form of Usher syndrome accounting for over half of reported cases (2). USH III has a progressive hearing loss, variable vestibular problems and RP (2)

The majority of the Usher type I cases are caused by mutations in the MYO7A gene (Usher IB) while mutations in the USH2A gene (Usher II A) are the cause of most cases of type II. Usher syndrome type III, caused by mutations in the USH3 gene is frequently seen only in Finland (3). Liu et al. suggested that identification of MYO7A as a gene that underlines both syndromic and non-syndromic deafness recommends comprehensive screening of non-syndromic deafness families for MYO7A mutations.

The aim of our study was to present a family, in which in one generation (five siblings – 3 women and 2 men) in four of them were found symptoms of Usher syndrome (one brother was unaffected). A mother and four children of two affected patients were normal. The unaffected brother and father were not available for study. (A father of this family was dead). Below we present the pedigree of the family (Fig. 1).

All affected patients had been deaf-mute from birth and vision disorders had been gradually developing in them. Ophthalmologic examination showed the presence of cataract and retinitis pigmentosa in both eyes (Fig. 2, 3).

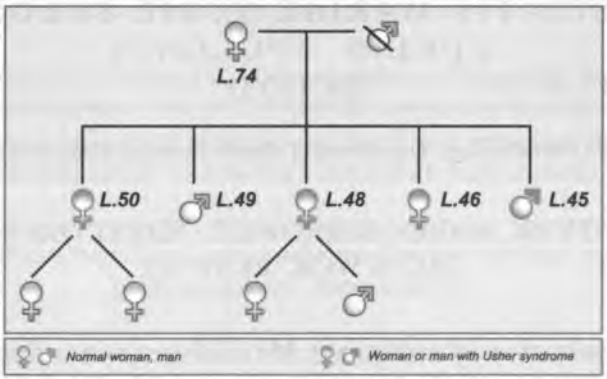

Fig. 1. Schema of Usher syndrome inheritance in the described family

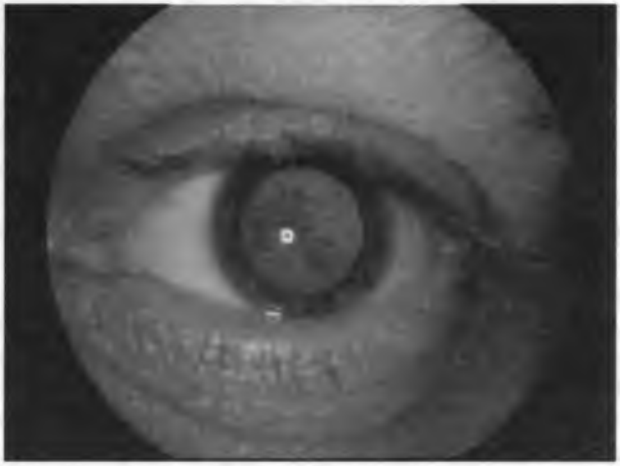

Fig. 2. Initial cataract (subcapsular posterior)

Fig. 3. Peripherally visible pigmentary changes of the retina

It was impossible to determine their visual acuity. In all the affected patients cataract extraction was performed combined with lens implantation. After operation patients felt considerable subjective improvement of vision. We also have performed genetic analysis of this family. According to a paper of Liu et al. we chose exons 4, 7, 28 of MYO7A gene as a possible point of mutation in our patients.

MATERIAL AND METHODS

The whole blood (2 ml) was taken from affected sibs, their mother. The isolation of DNA was performed using Hirt's metod. The obtained specimen of DNA were diluted to concentration of 0.5 μg/μl – suitable for PCR reaction. Exons 4, 7, 28 of MYO7A were amplified from genomic DNA by the polymerase chain reaction (PCR) using intronic primers: exon 4, forward (F) 5'-CCG GCC CCT TCC CCT GAA GT-3' and rewerse (R) 5"-CTC ACG TAG ATG AGG TGG TCC CGG-3'(227-bp product); exon 7, F 5"-ACC AGA GTT CCG AGGGTG-3' and R 5"-AGG GGC CTG GGT CTA TTC-3 (260-bp product); exon 28, F 5"-ACT GGC TGC TAG GAGGA-3" and R 5"-ATT GCT CTC CCC ACA GTG-3' (280-bp product).

The amplification conditions were: 95°C for 10 min, then 30 cycles of 94°C for 20 sec., 60° for 15 sec., 72°C for 20 sec., with a final extension for 10 min at 72°C. For mutation analysis, the PCR products were run through an 12.5% non-denaturing polyacrylamide gel at 15°C and single-strand conformational polymorphism (SSCP) were detected using silver staining. Analysis of exons 4, 7, 28 for affected and unaffected family members did not demonstrate differing patterns relative to normal controls (Fig. 4, 5, 6).

Fig. 4. Electrophoretic separation of amplificons of exon 4, MYO7A gene;
line 171 –l.48 (age of patient), line 172 – l. 74 (age of patient),
line 173 – l.45 (age of patient), line 174 – l.50 (age of patient), line 175 – healthy control,
line 176 – l.18 (age of patient), line 177 – l.46 (age of patient)

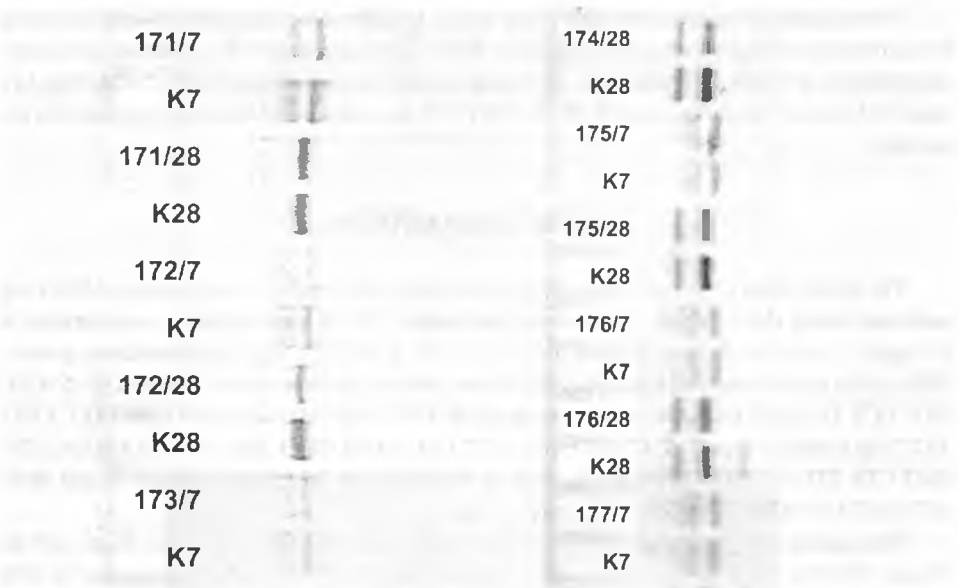

Fig. 5, 6. Electrophoretic separation of amplicons of exons 7, 28;
line 171/exon 7/l. 48 (age of patient), line K7 – negative control of exon 7, line 171/exon 28/l.48 (age of patient), line K28 – negative control of exon 28,
line 172/exon 7/l. 74 (age of patient), line K7 – negative control of exon 7, line 172/exon 28/l.74 (age of patient), line K 28 – negative control of exon 28,
line 173/exon 7/l. 45 (age of patient), line K7 – negative control of exon 7, line 173/exon 28/l.45 (age of patient), line K 28 – negative control of exon 28,
line 174/exon 7/l. 50 (age of patient), line K7 – negative control of exon 7, line 174/exon 28/l.50 (age of patient), line K 28 – negative control of exon 28,
line 175/exon 7/healthy control, line K7 – negative control of exon 7, line 175/exon 28/healthy control, line K 28 – negative control of exon 28,
line 176/exon 7/l. 18 (age of patient), line K7 – negative control of exon 7, line 176/exon 28/l.18 (age of patient), line K 28 – negative control of exon 28,
line 177/exon 7/l. 46 (age of patient), line K7 – negative control of exon 7, line 177/exon 28/l. 46 (age of patient), line K 28 – negative control of exon 28.

CONCLUSIONS

1. We did not find any mutations in the investigated 4, 7, 28 exons of MYO7A gene in all the examined patients.

2. There is a possibility that defective mutations occur in the other exons of MYO7A gene or in the other genes.

3. The cataract extraction with an intraocular lens implantation improved the visual function in the described patients.

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SUMMARY

Usher syndrome is characterized by congenital deafness and pigmentary retinopathy. It occurs at the rate of 3–6 cases per 100,000 people and is inherited in the autosomally recessive manner. The aim of our study was to present a family in which in one generation (five siblings – 3 women and 2 men) in four of them were found symptoms of Usher syndrome. All the affected patients had been deaf-mute from birth and vision disorders had been gradually developing in them. Five members of this family were screened using SSCP for MYO7A mutations. We did not find any mutations in the investigated 4, 7, 28 exons of MYO7A gene in all the examined patients. There is a possibility that defective mutations occur in the other exons of MYO7A gene or in the other genes.

Badania mutacji genu MYO7A w rodzinie z zespołem Ushera

Zespół Ushera charakteryzuje się wrodzoną głuchotą oraz retinopatią barwnikową. Występuje z częstotliwością 3–6 przypadków/ 100 000 osób. Dziedziczy się zwykle w sposób autosomalnie recesywny. Celem naszej pracy było przedstawienie rodziny, w której w jednym pokoleniu (pięcioro rodzeństwa –3 kobiety i 2 mężczyzn) u czworga rodzeństwa stwierdzono objawy zespołu Ushera. Wszyscy chorzy nie słyszeli od urodzenia (byli głuchoniemi) i stopniowo rozwijały się u nich zaburzenia widzenia. Pięcioro członków rodziny było badanych w kierunku obecności mutacji genu MYO7A. Nie stwierdziliśmy mutacji w badanych eksonach 4, 7 i 28 genu MYO7A. Prawdopodobnie mutacje występują w innych eksonach lub w innych genach.