Two Finnish USH1B patients with three novel mutations in myosin VIIA

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Purpose: Usher syndrome (USH) is an autosomal recessive disorder resulting in retinal degeneration and sensorineural deafness caused by mutations in at least 10 gene loci. USH is divided into three main clinical types: USH1 (33-44%), USH2 (56-67%), and USH3. Worldwide, USH1 and USH2 account for most of the Usher syndrome cases with rare occurrence of USH3. In Finland, however, USH3 is the most common type (40%), explained by genetic and geographical isolation accompanied with a founder mutation, while USH1 is estimated to comprise 34% and USH2 12% of all USH cases.

Methods: We examined two unrelated Finnish USH1 patients by sequencing.

Results: We found three new myosin VIIA (MYO7A) mutations: p.K923AfsX8, p.Q1896X, and p.E1349K. The p.K923AfsX8 mutation was present in both patients as well as in one of 200 Finnish control chromosomes.

Conclusions: This is the first molecular genetic study of USH1 in Finland. We have found three new pathological mutations causing either premature termination of translation or replacement of an evolutionary conserved MYO7A amino acid.

Usher syndrome (USH) is a group of autosomal recessive disorders characterized by retinal degeneration, sensorineural deafness and variable vestibular dysfunction. The overall prevalence of USH ranges from 1/16 000 to 1/50 000 based on studies in Scandinavia, Colombia, the United Kingdom, and United States [1]. USH is categorized into three clinical subtypes. In the most severe form, USH1 (OMIM 276900), patients suffer from vestibular dysfunction, delayed motor development, congenital sensorineural deafness, and retinal degeneration starting in early childhood. Of all USH patients worldwide, 56-67% have USH2, and 33-44% have USH1, while only a minority of patients have USH3 [2,3]. In Finland, USH1 represents 34%, USH2 12%, and USH3 40% of USH (subtype was not determined for 14% of patients) [4]. The unique distribution of USH in Finland can be explained by population history, which includes a small number of founder settlers, isolation and genetic bottlenecks such as famines and diseases [5-7], contributing to the enrichment of two USH3A mutations, p.M120K, and p.Y176X, in the Finnish population and making USH3 the most common Finnish USH subtype [8].

Mutations in five different genes cause USH1 (Hereditary Hearing Loss Homepage; accessed May 2006). USH1B is caused by mutations in MYO7A (OMIM 276903) and it comprises 75% of USH1 cases [2,9,10]. MYO7A has 49 exons and encodes a protein of 2215 amino acids. It is an unconventional myosin with a conserved head domain, enabling movement along actin filaments, and a long tail region containing various functional domains [3]. Among other functions MYO7A has found to be involved in intracellular lysosome transport [11], opsins transport through connecting cilium in photoreceptor cells [12] and melanosome transport in retinal pigment epithelium [13].

Our screening of MYO7A from Finnish USH1 patients is the first attempt to determine the molecular background of USH1 in Finland.

METHODS

Two unrelated patients with congenital severe hearing loss were referred to the Helsinki University Eye Hospital, to diagnose or rule out possible USH. Patient 1 is 1/1 child of hearing parents. She had congenital severe hearing loss and a history of late walking. She learned to walk by 10 months with support and by two years without support. She received a cochlear implant at the age of two and communicated with speech by four years. Connexin 26 was ruled out as the causative gene by sequencing. Ophthalmological examination at age four revealed extinguished electroretinogram (ERG), and slightly granular fundus pigmentation. Her visual acuity was 20/32 in both eyes and she had no visual complaints. Diagnosis of USH1 was made based on audiological and ophthalmological findings. At age six, the patient received a cochlear implant in her second ear, to obtain a sense of direction of sound, which will be useful with the eventually deteriorating vision. DNA samples were obtained from the patient and her parents as well as from three living grandparents.

Patient 2 is 1/1 child of hearing parents. She was diagnosed with severe bilateral sensorineural hearing loss in early childhood. The family chose not to have cochlear implantation, and the patient learned to communicate by sign language. At the age of eight, she had complaints of poor night vision,
and also suffered from mild vestibular symptoms. She learned to walk by 13 months with support and by two years, two months without support. Ophthalmological examination revealed best corrected visual acuities of 20/30 in both eyes (-4.25 cyl +2.0 ax 95/-4.75 cyl +2.25 ax 90), peripheral motting of pigment in the fundi, normal Goldmann visual fields, and extinguished ERG. The diagnosis of USH1 was made, and DNA samples were obtained from the patient and her family (parents and three grandparents).

DNA samples from 100 anonymous blood donors representing different regions of Finland were provided by the Finnish Red Cross Blood Transfusion Service and studied as controls.

Genomic DNA was extracted either from whole blood using the Puregene™ Genomic DNA Purification Kit (Gentra systems, Minneapolis, MN) or from saliva using Oragene™ kits (DNA Genotek Inc., Ottawa, Ontario, Canada). The intronic primers used for direct sequencing of the 49 exons of the MYO7A gene were as published [10,14] or designed for this study (Table 1).

Amplification conditions were 95 °C for 7 min or 10 min (exon 31), then 30 cycles of 95 °C for 45 s, 60 °C (exon 23 and exon 31), or 56 °C (exon 41) for 45 s, 72 °C for 30 s or 45 s (exon 31) and final extension time of 10 min at 72 °C. Amplification enzymes were AmpliTaq Gold™ (Applied Biosystems, Foster City, CA), FastStart Taq DNA polymerase (Roche, Mannheim, Germany) and Dyazyme II DNA polymerase (Finnzymes, Espoo, Finland). PCR products were sequenced either directly or after gel extraction with the QIAquick® Gel Extraction Kit (Qiagen, Hilden, Germany). Sequencing was performed with an ABI2720 Automated DNA sequencer using the ABI PRISM BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The patients’ sequences were compared to known MYO7A sequence (GenBank accession NM_000260). Protein sequences were compared with ClustalW (European Bioinformatics Institute).

Control samples were analyzed by PCR amplification of MYO7A exons 23 and 41 and subsequent restriction enzyme

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<th>Exon</th>
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<tr>
<td>23</td>
<td>5’-CAGAGGTGGGGAAGTCAGAG-3’</td>
<td>5’-AAAACTTGGAGAGGGCGTTG-3’</td>
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<tr>
<td>31</td>
<td>5’-CTGAGGCTAGGAAAGCATTG-3’</td>
<td>5’-ATGCTAACAGAGGACTG-3’</td>
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<tr>
<td>41</td>
<td>5’-GTTTGTCTGCCTCAAATG-3’</td>
<td>5’-GGCAGTACAGACTCTAAGG-3’</td>
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digestion. Amplified exon 23 fragments were digested with Sac I (New England BioLabs Inc., Beverly, MA) at +37 °C for 4 h and exon 41 fragments were digested with BstF5I (SibEnzyme, Novosibirsk, Russia) at +65 °C for 4 h. Both mutations eliminated the respective restriction sites. Digested PCR products were run through 1.5% agarose gels for 2 h at 150 V.

**RESULTS**

Patient 1 was found to be heterozygous for two novel mutations: a deletion c.2766_2779del14, resulting in a frameshift and a new termination codon 22 nt upstream (p.K923AsX8), and a single nucleotide change c.5686 C>T, causing a nonsense mutation p.Q1896X. The patient’s father and paternal grandfather were found to carry the p.Q1896X mutation. The mother carries p.K923AsX8 and the maternal grandmother has normal alleles, while the maternal grandfather is deceased (Figure 1). Patient 2 is heterozygous for the c.2766_2779del14 deletion and for nucleotide change c.4045 G>A, resulting in an amino acid change p.E1349K in a conserved FERM domain of MYO7A. We compared protein orthologues from five different species (Mus musculus (NP_032689), Rattus norvegicus (NP_703203), Danio rerio (NP_694515), Drosophila melanogaster (NP_523571), Caenorhabditis elegans (NP_508420)) and the glutamic acid at position 1349 is conserved in all (Figure 2). Patient 2’s mother and maternal grandfather are heterozygous for the deletion, and her father and paternal grandmother are heterozygous for the p.E1349K mutation (Figure 1).

Screening of the control samples revealed the p.K923AsX8 mutation in one of 200 control chromosomes. None of the 200 control chromosomes carried the p.Q1896X or the p.E1349K mutations.

The origins of the mutations were traced to the birthplaces of the documented or most likely carrier grandparents. The grandparents carrying the deletion p.K923AsX8 and the mutation p.E1349K were born in the same region of western Finland, whereas the p.K923AsX8 control carrier’s grandparents came from former eastern Finland. The grandparent carrying the mutation p.Q1896X was born in the region of central Finland.

**DISCUSSION**

Our study was initiated by the clinical diagnosis of a four-year-old USH1 patient, which needed molecular verification. Sequencing of the patient’s MYO7A gene resulted in the finding of two new pathological mutations, p.K923AsX8, and p.Q1896X, causing USH1B. A subsequent patient diagnosed with USH1 was also studied and found to be a compound heterozygote for the p.K923AsX8 deletion mutation and a third novel pathological mutation, p.E1349K.

Both p.K923AsX8 and p.Q1896X cause premature termination of MYO7A translation, resulting in truncated and possible unstable MYO7A proteins. The p.K923AsX8 deletion occurs in the MYO7A coiled-coil domain at the beginning of the long MYO7A tail and p.Q1896X occurs in the C-terminal FERM domain. Both the MYO7A coiled-coil domain and the full-length tail are required for MYO7A protein dimerization and dimer structure stability [15]. The mutation p.E1349K is also located in the MYO7A tail. The mutated glutamic acid is part of the FERM domain, which mediates protein attachment to the plasma membrane [16], and is evolutionarily conserved.

Almost all known MYO7A mutations cause USH1B. However, mutations causing recessive atypical USH [17,18], recessive, non-syndromic deafness (DFNB2) [19,20] and dominant non-syndromic deafness (DFNA11) [21-23] have also been reported. The genetic background of these mutations may play an important role in phenotypic variation as some mutations cause both USH1B and atypical Usher syndrome [17,24]. However, the existence of MYO7A mutations causing DFNB2 have later been questioned as these patients might represent misdiagnosed USH1 cases [25].

The p.K923AsX8 mutation may well be the most widely spread USH1B mutation in Finland as it was shared by both patients and observed in one out of 200 control chromosomes of healthy Finnish blood donors. As the deceased maternal grandfather of patient 1 is the most likely carrier of the p.K923AsX8 deletion, both patients inherited this deletion from grandparents originating from the same region of western Finland, but the ancestors of the healthy blood donor carrying the p.K923AsX8 deletion came from former eastern Finland. Incidentally, the p.E1349K mutation was inherited from a grandparent originating from the same western region of Finland.

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**Figure 2.** MYO7A protein orthologues compared around the E1349K mutation position. The glutamic acid at position 1349 is highlighted in grey background. Homo sapiens symbolized as Hs, Mus musculus symbolized as Mm, Rattus norvegicus symbolized as Rn, Danio rerio symbolized as Dr, Drosophila melanogaster symbolized as Dm, and Caenorhabditis elegans symbolized as Ce.
Finnish patients with a combination of congenital severe sensorineural hearing impairment and progressive pigmentary retinal dystrophy were studied during the years 1965-68. At that time, the disease was called Dystrophia Retinæ Pigmentosa-Dysacusis (DRD) and included all the Usher subtypes [26,27]. The information of birthplaces of DRD patients’ parents and their geographical distribution in Finland was compared with the birthplaces of the grandparents who carried the MYO7A mutations. We found that our MYO7A mutation carrier grandparents were all born in regions where the DRD patients’ parents had been born. We suspect that some of the DRD patients were actually USH1 and carried the MYO7A mutations we now have found.

It has been estimated that 50% of cases with congenital hearing impairment are caused by connexin 26 mutations [28], and 3-6% of deaf children have USH [29]. Delayed walking is suggested to be the earliest clinical sign of Usher syndrome in deaf children [30]. Early diagnosis of USH1 is important for the families of congenitally deaf children prior to deciding whether to accept cochlear implantation. Our younger patient was already implanted in one ear at the time of the clinical and molecular diagnosis of USH1. After the diagnosis, her second ear was implanted because it was thought to be beneficial for having a sense of direction of sound when, eventually, her vision would deteriorate. The other patient did not have a cochlear implant. At the clinical and molecular diagnosis of USH1B, this patient was considered too old (14 years) to benefit from cochlear implantation. It can only be speculated whether an earlier diagnosis of USH1B would have led to her having a cochlear implantation in early childhood.

The finding of the three novel USH1B mutations in two Finnish USH1 patients suggest further prevalence for these mutations among Finnish USH1 patients. Our future studies aim to gather more samples from Finnish USH1 patients and determine the molecular background of USH1 in Finland in order to provide the patients and their families with the possibilities of precise and early molecular diagnoses, accurate genetic counselling and optimal rehabilitation.

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REFERENCES


