Localization of the Usher syndrome type ID gene (Ush1D) to chromosome 10

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The Usher syndromes (USH) are a group of autosomal recessive diseases characterized by progressive pigmentary retinopathy and sensorineural hearing loss. Five USH genes have been mapped and at least one additional gene is known to exist. By homozygosity mapping in a consanguineous family, a sixth USH gene has been localized. Clinical findings in the four affected children are consistent with established diagnostic criteria for Ush1. Linkage to known USH loci was excluded, and using two genomic DNA pools, one from the affected children and the other from the parents, 161 polymorphic markers evenly spaced across the autosomal human genome were screened. The location of the Ush1D gene was defined by the only region showing homozygosity by descent in the affected siblings, a 15 cM interval on chromosome 10q bounded by D10S529 and D10S573.

INTRODUCTION

Deaf–blindness in humans is most frequently caused by the Usher syndromes (USH), a group of disorders characterized by autosomal recessive inheritance and dual sensory impairments. Affected persons have sensorineural hearing loss and become visually impaired secondary to progressive pigmentary retinopathy (PPR). To date, three phenotypically distinct forms of USH have been described and mapped to five different genomic regions (Table 1) (1).

Although PPR occurs with all types of USH, phenotypic differentiation is made by assessing auditory and vestibular function. Usher syndrome type I (Ush1) is characterized by congenital, severe-to-profound hearing loss and vestibular dysfunction; Usher syndrome type II (Ush2), by congenital, moderate-to-severe hearing loss and normal vestibular function; and Usher syndrome type III (Ush3), by progressive hearing loss (1,2). Ush1 is heterogeneous, with loci on chromosomes 14 (Ush1A) (3), 11q (Ush1B) (4,5) and 11p (Ush1C) (5). Ush2A and Ush3 have been mapped to chromosomes 1q and 3q respectively (2,6). The Ush1B gene has been cloned and encodes myosin VIIA (7). These five localizations account for ~90% of clinical cases of USH. At least one additional USH gene exists. A few families with Ush1 and nearly 10% of Ush2 families do not map to any of the reported USH loci (8). In the case of Ush1, the absence of a sufficient number of unmapped families and the possibility of further heterogeneity have precluded the identification of new loci by classical linkage analysis. However, rare recessive genes also can be localized by homozygosity mapping. A first cousin marriage that produced four children with the clinical diagnosis of Ush1 has given us the opportunity to use this methodology to identify a fourth Ush1 locus, Ush1D (Fig. 1).

RESULTS

Clinical description

A first cousin union of parents of Pakistani origin produced four children with clinical findings consistent with established diagnostic criteria for Ush1 (1); there are no unaffected progeny. Each child has a history of prelingual profound auditory impairment and uses sign language for communication as hearing aids are unhelpful; pure tone audiometry has confirmed the presence of profound sensorineural hearing loss. Developmental milestones in one child are consistent with congenital vestibular dysfunction, and in all children, vestibular and oculomotor tests to assess vestibular function show no response to caloric stimulation of the vestibular end organs. Fundoscopic examination in each child has confirmed the presence of bilateral progressive pigmentary retinopathy.

Linkage mapping using pooled DNA samples

After excluding linkage to known USH loci (Table 1), two genomic DNA pools, one from the affected children and the other from the parents, were used to screen 161 short tandem repeat polymorphic markers (STRPs) evenly spaced across the autosomal human genome. PCR products were resolved on 6% denaturing polyacrylamide gels by silver staining. Seven shifts to allelic homozygosity were observed in the affected sibling pool.

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Each of these STRPs was evaluated in the pedigree by typing adjacent flanking markers and reconstructing haplotypes to establish whether homozygosity by descent (HBD) was possible. The only autosomal region showing HBD flanked GATA87G01. Based on consistent allelic homozygosity, a 15 cM region bounded by D10S529 and D10S573 was identified as the location of the Ush1D gene (Fig. 2). Lod scores calculated using MAPMAKER/HOMOZ (9) confirm this linkage.

DISCUSSION

In the family we describe, the finding of consanguinity between parents provides a high degree of assurance that the form of USH in the affected children results from the recessive inheritance of a single ancestral gene (10). By screening the entire genome, we have shown that in the affected children, the only autosomal region that is homozygous by descent is flanked by markers D10S529 and D10S573.

Recently, the Ush1B gene was cloned and shown to encode a cytoskeletal protein, myosinVIIA (7), implicating other genes that encode unconventional myosins and their interacting proteins as candidates for other forms of USH. On this basis, the 15 cM Ush1D interval contains three candidate genes, vinculin (VCL), annexin VII (ANX7, synexin) and ankyrin-3 (ANK3, ankyrin-G). VCL is a cytoskeletal protein associated with the cytoplasmic face of cell–cell and cell–extracellular matrix adherens-type junctions where it interacts with other proteins to anchor F-actin to the membrane (11); ANX7 is a calcium-dependent membrane binding protein that fuses cell membranes and acts as a voltage-dependent calcium channel (12); and ANK3 belongs to a class of peripheral membrane proteins that interconnect integral proteins with the spectrin-based membrane skeleton (13). Two other candidate genes in the same interval are transcription integral proteins with the spectrin-based membrane skeleton (13). Two other candidate genes in the same interval are transcription factor 6-like 2 (TCF6L2) and collagen type XIII (COL13A1). The former activates transcription of each mtDNA strand by heavy-strand promoters (14), and the latter is a short chain collagen (15). Previous work has implicated mtDNA mutations in non-syndromic hearing loss (16,17), and atypical collagens play an important role in forming an inner ear matrix that can withstand the stresses associated with the repetitive movements necessary for sensory transduction (18). Unfortunately, no evidence of expression of these genes could be demonstrated by PCR amplification of either fetal cochlear- or retinal-specific cDNA libraries.

Comparative human–mouse mapping suggests that Jackson circler (jc), waltzer (w) or Ames waltzer (Av) may represent the mouse model of Ush1D. Each of these mutants is recessive and shows the typical circling, head-tossing and hyperactive behavior associated with deafness and vestibular dysfunction (19). Although Jackson circler has not been studied for inner ear anomalies, in the other two mouse mutants, degenerative changes of the membranous labyrinth have been described (20–22). None has any reported retinal pathology; it is worth noting, however, that shaker-1 (sh-1), the mouse model of Ush1B, also has no evidence of pigmentary retinopathy (23). Interestingly, Waltzer–shaker-1 double heterozygotes (+/− sh-1/) become deaf at 3–6 months and have changes similar to +/+ homozygotes in the organ of Corti, stria vascularis and spiral ganglion (21,22). This phenotype suggests an interaction between myosinVIIA and the waltzer gene, making the latter a strong candidate for the Ush1D gene.

MATERIALS AND METHODS

Genomic DNA preparation and STRP typing

Genomic DNA was extracted from whole blood by following a standard protocol (24), quantitated by spectrophotometric readings at OD260, and diluted to 20 ng/µl for amplification by PCR. In the two pooled DNA samples, equal quantities of DNA from each individual were used to ensure equal amplification of all specimens. STRP screening set version 6, developed from markers generated by the Cooperative Human Linkage Center and made available through Research Genetics Inc., was used in this study (25).

PCR was performed with 40 ng of pooled DNA in a 6.4 µl reaction mixture containing 1.25 µl buffer (100 mMTris–HCl pH 8.8, 500 mM KCl, 15 mM MgCl2, 0.01% w/v gelatin); 200 µM each of dATP, dCTP, dGTP and dTTP; 2.5 pmol of forward and reverse primers; and 0.25 U Taq polymerase. Forty cycles of amplification were completed at 94°C for 30 s, 55°C for 30 s and 72°C for 30 s. Reaction products were resolved on 6% denaturing polyacrylamide gels (7.7 M urea) and visualized by silver staining. Individual samples were genotyped in a similar manner using 40 ng of DNA template in each reaction.

LOD score calculations

LOD scores were calculated using MAPMAKER/HOMOZ, a computer package based on an algorithm especially designed for

Table 1. Phenotypic and genotypic data for the Usher syndromes

<table>
<thead>
<tr>
<th>Usher type</th>
<th>Phenotype</th>
<th>Chromosome</th>
<th>Gene</th>
<th>Screening markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ush1</td>
<td>Congenital severe-to-profound hearing loss; absent vestibular function; PPR</td>
<td>Ush1A–14q</td>
<td>?</td>
<td>D14S267, D14S250, D14S78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ush1B–11q</td>
<td>MyoVIIA</td>
<td>D11S911, D11S272, D11S937</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ush1C–11p</td>
<td>?</td>
<td>D11S902, D11S921, D11S888</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ush1D–10q</td>
<td>?</td>
<td>D10S529, D10S202, D10S573</td>
</tr>
<tr>
<td>Ush2</td>
<td>Congenital moderate-to-severe hearing loss; normal vestibular function; PPR</td>
<td>Ush2A–1q</td>
<td>?</td>
<td>D1S237, D1S474, D1S229</td>
</tr>
<tr>
<td>Ush3</td>
<td>Congenital moderate-to-severe progressive hearing loss; normal vestibular function; PPR</td>
<td>3p</td>
<td>?</td>
<td>D3S1308, D3S1299, D3S1279</td>
</tr>
</tbody>
</table>
Figure 1. USH1D pedigree demonstrating homozygosity by descent in the affected progeny over an interval of \( \sim 15 \) cM bounded by \( D10S529 \) and \( D10S573 \); the coefficient of inbreeding is 1/16 (☐, male; ☠, female; ■, affected male).

Figure 2. Multipoint map over the interval defined by \( D10S529 \) and \( D10S573 \) (loc1, \( D10S529 \); loc2, \( D10S188 \); loc3, \( D10S218 \); loc4, \( D10S195 \); loc5, \( D10S202 \); loc6, \( D10S69 \); loc7, \( D10S219 \); loc8, \( D10S201 \); loc9, \( D10S551 \); loc10, \( D10S573 \); the highest two-point lod score, 2.56, was obtained with \( D10S195 \) at \( \theta = 0 \).

homozygosity mapping (9). The frequency of the Ush1D gene was set at 0.001, and the disease was coded as fully penetrant and recessive. Allele frequencies are based on reported data. Because the family immigrated from Pakistan and allele frequencies were not based on this specific ethnic and geographic background, calculations were repeated imposing a lower bound of 10% on allele frequencies to guard against inflated LOD scores (9).

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REFERENCES


