Genetic heterogeneity of Usher syndrome type II

S Pieke Dahl, W J Kimberling, M B Gorin, M D Weston, J M R Furman, A Pikus, C Möller

Abstract
Usher syndrome is an autosomal recessive disorder characterised by retinitis pigmentosa and congenital sensorineural hearing loss. A gene for Usher syndrome type II (USH2) has been localised to chromosome 1q32-q41. DNA from a family with four of seven sibs affected with clinical characteristics of Usher syndrome type II was genotyped using markers spanning the 1q32-1q41 region. These included D1S570 and D1S81, which are believed to flank USH2. Genotypic results and subsequent linkage analysis indicated non-linkage of this family to these markers. The A test analysis for heterogeneity with this family and 32 other Usher type II families was statistically significant at $p < 0.05$. Further clinical evaluation of this family was done in light of the linkage results to determine if any phenotypic characteristics would allow for clinical identification of the unlinked type. No clear phenotypic differences were observed; however, this unlinked family may represent a previously unreported subtype of Usher type II characterised by a milder form of retinitis pigmentosa and mild vestibular abnormalities. Heterogeneity of Usher syndrome type II complicates efforts to isolate and clone Usher syndrome genes using linkage analysis and limits the use of DNA markers in early detection of Usher type II.

(Underline)}

Clinical methods

SUBJECTS

Families in the Boys Town National Research Hospital (BTNRH) Usher syndrome project have been ascertained and clinically evaluated by methods that have been previously described. The families in the project are predominantly of northern European extraction. During the course of this study, one family (735) was encountered through self-referral. Detailed records for family 735 were obtained primarily from previous studies done at NIH in 1988. These records were consistent with a diagnosis of Usher syndrome type II. Following genetic analysis at BTNRH, certain family members were re-examined at the Eye and Ear Institute of Pittsburgh in 1991 to verify the clinical diagnosis of Usher syndrome type II.

OPHTHALMOLOGY

Visual psychophysical testing of family members included visual acuity, contrast sensitivity, colour vision, Goldmann perimetry, and light and dark adaptation studies. Electrophysiological evaluations included an electro-oculogram (EOG), and rod-cone responses measured by uniform field, Ganzfeld electrotinography (ERG). The clinical evaluation included intraocular pressures, slit lamp biomicroscopy at the anterior segment and fun
dus, and indirect ophthalmoscopy. Fundus changes were documented using standard fundus photography.

AUDIOLGY
Audiological evaluations were accomplished in different centres over many years but routinely included traditional measures of pure tone and speech discrimination thresholds, tympanometry, acoustic reflex testing, and, for one patient, auditory brainstem response (ABR) measurement. All audiological testing was accomplished in standard sound isolated testing suites using commercially available calibrated instrumentation.

VESTIBULAR STUDIES
The vestibular test battery included: (1) an oculomotor screening battery to assess saccades, pursuit, and nystagmus; (2) static postural testing; (3) Halpike's manoeuvres; (4) alternate binaural bithermal caloric irrigations with 30°C and 44°C water; (5) computerised dynamic posturography (Equitest); and (6) rotational testing using the sinusoidal harmonic acceleration test.

LABORATORY METHODS
SAMPLE COLLECTION
Blood samples from family 735 were originally collected in 1990. Non-linkage results prompted a repeat sampling which was completed and regenotyped in 1991 to verify that no sample mix up had occurred. Genomic DNA of all families was extracted from lymphocytes in 3 to 5 ml of venous blood or from lymphoblastoid cell lines using an Applied Biosystem 340A nucleic acid extraction machine.

GENOTYPING
RFLP and STRP genotyping was done according to standard protocols. DNA markers were chosen based on locus order and genetic distances listed in the CEPH consortium linkage map of chromosome 1. Recent evidence from the analysis of dinucleotide repeat polymorphisms on 1q indicates that the USH2 gene lies between D1S70 and D1S81 near PPOL (Weston and Kimberling, unpublished data).

ANALYSIS
Linkage analyses were performed with genotyped data from 32 type II Usher syndrome families (besides family 735), comprising 252 subjects (81 affected). US was assumed to be inherited as a fully penetrant autosomal recessive disorder and was treated as the affected status locus in all analyses. Pairwise and multipoint linkage tests and tests of locus order were performed using the LINKAGE 5.1 program package. Linkage data were then analysed for heterogeneity using the A test computed with the HOMOG computer program. The location scores at five equidistant points between D1S70 and D1S81 were used in the A test analysis and α, the proportion of unlinked families, was evaluated between 0 and 1.0 at 0.05 intervals.

CLINICAL RESULTS
FAMILY HISTORY
The ancestry of family 735 is predominantly of Irish, Scottish, and Welsh origin. There is no known consanguinity. There is a positive family history of adult onset diabetes in both the maternal and paternal sides. The father reports a mild hearing loss resulting from environmental factors. There are no instances of RP or other hearing loss outside the sibship reported here. Usher syndrome type II was originally suggested as a probable diagnosis when pediagry analysis indicated that the RP was segregating with congenital hearing loss in four of seven sibs (fig 1) shows six of the sibs, insufficient DNA prevented the seventh from being included in the study).

OPHTHALMOLOGY
All affected sibs reported symptoms of RP from late childhood or early adolescence. In 1988, the parents and affected sibs underwent ophthalmological evaluations at the NIH National Eye Institute. I 8 had no abnormal findings. The fundus of I 9 was normal except for a few macular 'brusen', which had been noted in previous examinations. Affected sibs II-1, II-5, II-6, and II-7 displayed a pattern of retinal degeneration that appeared different from RP exhibited in typical Usher syndrome. Clinical examination showed motting of the retinal pigment epithelium with individual and coalescent greyish 500 μ spots. Despite the evidence of extensive retinal degeneration, few characteristic intraretinal bone spicules pigment deposits were seen. Blood vessels were attenuated, visual fields were constricted, EOGs were subnormal, and ERGs were distinguished or showed thresholds that were extremely depressed in a rod-cone pattern. The diagnosis of 'RP sine pigmento' with hearing loss was made.

In 1991, after DNA testing of family 735 showed non-linkage to markers in the 1q32-q41 region, the parents, affected sibs II-5, 6, and 7, and unaffected sib II-4 were re-examined at the Eye and Ear Institute of Pittsburgh. The father displayed mild bilateral diabetic retinopathy. II-4 was normal. The three affected sibs presented with increased visual acuities, worsened symptoms of night blindness and tunnel vision, further attenuation of retinal blood vessels, increased motting of the RPE, but still few intraretinal pigment deposits (fig 2). The general appearance of each fundus resembles an early stage of RP.

AUDIOLGY
All affected sibs showed symptoms of congenital hearing loss, verified in early childhood. Patient II-1, evaluated at the age of 34, exhib-
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Figure 1: Abbreviated pedigree of family 735 with chromosome 1q DNA marker types of each subject shown underneath, illustrating no consistent segregation of haplotypes with affected status.

<table>
<thead>
<tr>
<th>Chromosome 1q DNA markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
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Figure 2: Diagram illustrating the distribution of DNA marker types among family members.

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<thead>
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<tbody>
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<tr>
<td>1</td>
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<td>2</td>
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Figure 3: Audiogram showing speech and suprathreshold hearing thresholds for patient II-5.

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>Speech Threshold (dB)</th>
<th>Suprathreshold Threshold (dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>400</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>800</td>
<td>10</td>
<td>20</td>
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At age 16, patient II-7 complained of headaches, dizziness, vomiting, and tinnitus.
overs are highly improbable, these results are not compatible with linkage to markers on 1q32–1q41. Retyping of family 735 using DNA from the second samples confirmed that the original results were valid and no sample mix up had occurred. No instance of non-paternity was found in over 30 polymorphisms which have been tested for the family.

**LINKAGE ANALYSIS**

The conclusions are further supported by a significantly negative set of multipoint lod scores for family 735 with chromosome 1q markers that span the region between CRP and D1S102. Fig 4 shows the multipoint likelihood distribution for only family 735 compared with that of all 32 Usher II families. The peak lod score of the Usher II sample approaches 15 between markers D1S70 and D1S81. At mid-way between D1S70 and D1S81, family 735’s lod score is less than −2·00, consistent with the hypothesis that family 735’s Usher gene is not in the USH2 region. This contention was further reinforced by Ott’s HOMOG A test for heterogeneity, which resulted in a $\chi^2_{10} = 5·255$, $p < 0·05$, supporting heterogeneity. Of the 33 families, only 735 showed a low posterior probability of linkage at 0·02. The distribution of the posterior probabilities for the remaining families was: 0·71 (one family), 0·80 to 0·90 (six families), and > 0·90 (25 families). There is no statistically significant evidence of any other unlinked families in this sample. The $\chi^2$ was reduced to 0·00 when family 735 was removed from the sample.

**Conclusion**

We conclude that family 735 represents a variant of Usher syndrome type II and propose that the locus be designated USH2b when it is identified. The locus on 1q would be USH2A to be compatible with the previous suggestion of USH1A, USH1B, and USH1C for the Usher type I loci. The variant of Usher type II may be distinguished from the common Usher type II by a form of RP with fewer pigment deposits and mild vestibular defects, but these observations must be verified with additional Usher IIb families.

**Discussion**

Localisation and isolation of a gene relies on an accurate clinical diagnosis. Clinical characteristics of family 735 did not differ significantly enough to prevent the inclusion of that family into the Usher type II sample for linkage analysis. However, after non-linkage was shown, further examination and re-evaluation of family 735 was warranted to prevent inadvertent inclusion of additional Usher IIb families into the common Usher IIa sample. The affected subjects in family 735 display a variant of RP, namely RP sine pigmento. Heckenlively outlined the early stages of RP as being characterised by generalised diffuse depigmentation of the RPE, relatively normal retinal vessels, but with an abnormal but not

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**Figure 2** Fundus photographs of three affected sibs from family 735 show relatively few pigment deposits, characteristic of early RP.

**Molecular results**

**HAPLOTYPES**

Fig 1 shows the chromosome 1 haplotypes of family 735 in the most likely phases assuming fewest crossovers between markers, after all possible 735 parental phases were considered. Affected status does not cosegregate with the DNA markers D1S70 and D1S81 which flank USH2. For this phase, three double crossovers are necessary if one USH2 gene is placed between D1S70 and D1S81. Assuming the locus order is correct and that double cross-
extinguished ERG, night blindness, and some-
what constricted visual fields. Little or no
pigment deposition is seen in almost all RP
patients initially, and this particular stage is
more persistent in some cases. This descrip-
tion is very similar to retinal findings of family
735 (fig 2). Family 735's pattern of hearing loss
does not significantly deviate from that of
typical Usher II, and similar variation within
Usher II families has been observed. The
origin of this variation can be found in a
variety of environmental or genetic factors
unrelated to Usher syndrome, or by the fact
that the psychoacoustic test is inherently sub-
tect to some variation. If there is a progressive
component, it is much less pronounced than
reported for proposed subtype Usher type
III.\(^1\)\(^9\)\(^7\) Hearing loss appears to have pro-
gressed in II-5, but one might postulate that
chronic hypothyroidism is somehow involved
since she is the only sib showing a progressive
loss and hypothyroidism has been implicated
in progressive hearing loss. Vestibular abnor-
malities within Usher syndrome type II have
been previously reported.\(^1\)\(^7\) Mild vestibular
abnormalities as displayed by family 735 could
be a way to distinguish phenotypically
between the two subtypes of Usher II before
DNA analysis, if similar defects were consist-
tently observed in other families unlinked to
1q32-41 markers. Vestibular findings are too
variable in family 735 to be considered diag-
nostic without such verification.

Extensive genetic heterogeneity in Usher
syndrome has become increasingly apparent as
research progresses. With this report, muta-
tions at at least five different loci can cause
Usher syndrome: three for type I which have
been localised to chromosomes 11p, 11q, and
14q, and two for type II, one localised to 1q and another located somewhere else. It is reasonable to assume these genes are functionally related; some may code for similar functions or code for enzymes in a common pathway vital for normal vision and hearing. The identification and cloning of the Usher genes is necessary to discover the different role each plays.

There are two important implications with regard to these findings. First, the use of genetic markers for prenatal diagnosis of Usher type II will be confounded by heterogeneity. Although the unlinked type is relatively uncommon, a small error, equal to a value no greater than the fraction of unlinked families, will be associated with linkage based predictions. The problem will not be overcome until the specific genes and mutations associated with Usher syndrome are identified. Second, the process of pinpointing the precise segment containing the USH2a gene depends on observation of critical crossovers and false results from inclusion of a variant family in our sample might not be detected. If that family provides what appeared to be a critical crossover, the search for the USH2a gene could be misdirected. Some clinical parameter that allows the subdivision of the sample into Usher II and II variant without regard to marker typing results is required. We are currently expanding our sample, since accumulation of further Usher IIb families may provide a clinical distinction between different Usher II types. Although family 735 is highly informative, other linked Usher II families must be ascertained before the second locus can be found by linkage analysis. After the genes are located, other methods could be used to characterise the causative factors.

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