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Gene Mapping of Usher Syndrome Type IIa: Localization of the Gene to a 2.1-cM Segment on Chromosome 1q41

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Summary

Usher syndrome type II is associated with hearing loss and retinitis pigmentosa but not with any vestibular problems. It is known to be genetically heterogeneous, and one locus (termed *USH2A*) has been linked to chromosome 1q41. In an effort to refine the localization of USH2A, the genetic map of the region between and adjacent to the marker loci previously recognized as flanking USH2A (D1S70 and PPOL) is updated. Analysis of marker data on 68 Usher II families places the USH2A gene into a 2.1-cM region between the markers D1S237 and D1S229. The gene for transforming growth factor β2 (TGFB2) and the gene for the homeodomain box (HLX1) are both eliminated as candidates for USH2A, by virtue of their localization outside these flanking markers. The earlier finding of genetic heterogeneity was confirmed in six new families, and the proportion of unlinked Usher II families is estimated at 12.5%. The placement of the USH2A gene into this region will aid in the physical mapping and isolation of the gene itself.

Introduction

Usher syndrome is a hereditary disorder that causes congenital hearing loss, progressive retinitis pigmentosa, and vestibular dysfunction. The disorder is clinically heterogeneous with at least two distinct phenotypes (Möller et al. 1989). Usher type I is distinguishable from Usher type II on the basis of severity of hearing loss and extent of vestibular involvement: type I patients are profoundly deaf, whereas type II patients are "hard of hearing"; type I individuals have absent vestibular responses, whereas type II persons are normal in this regard.

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The frequency of Usher syndrome has been estimated at 3.0/100,000 in Scandinavia (Hallgren 1959; Nuutila 1970) and at 4.4/100,000 in the United States (Boughman et al. 1983). The prevalence of Usher syndrome among deaf individuals has been reported to be 0.6%–28% (Vernon 1969). Conversely, the frequency of deafness in the retinitis pigmentosa population is estimated to be 8.0%–33.3%.

One gene for Usher type II has been localized to chromosome 1q, and Usher type I families failed to show the same linkage (Kaplan et al. 1990; Kimberling et al. 1990; Lewis et al. 1990), thus demonstrating that the observed clinical heterogeneity of Usher syndrome is due to the effects of mutations in two or more separate and distinct loci. Subsequently, the genetics of Usher syndrome was found to be more complex. First came the discovery, with a set of Usher type I families in France, of a linkage to chromosome 14 (Kaplan et al. 1992), and then came the subsequent discovery of two more Usher type I linkages, one to 11p and another to 11q, in other families (Kimberling et al. 1992; Smith et al. 1992). It appears that two of the Usher type I linkages detect loci coding for less common types of Usher I, with mutations predominantly present in the people from the Poitous-Charentes region of France and the French Acadian population of Louisiana in the United States; these loci have been designated *USH1A* (14q) and *USH1C* (11p), respectively. The most common Usher type I mutation is found on a locus present on chromosome 11q (USH1B). Next came the discovery of an Usher type II family that failed to show linkage with markers on 1q41 (Pieke Dahl et al. 1993). This observation has been extended here to include several other families and has important implications because it complicates the search for the USH2A gene. The Usher gene located on 1q41 has been termed *USH2A*, and the one unlinked to that region has been designated *USH2B*. Thus, there are at least five different genetic loci involved in Usher syndrome—two coding for the milder Usher type II (one of which is linked, and the other of which is unlinked, to 1q41) and three coding for the more severe Usher type I. Although no overlap between Usher type I and Usher type II phenotypes has been observed in our large series of families, the linkage-based subtypes (Usher Ia vs. Ib vs. Ic and

Usher IIa vs. IIb) cannot be clinically differentiated from one another. This is a report of further advances toward the identification of the Usher II type gene (USH2A) on chromosome 1q41.

Families and Methods

Families

Two hundred twenty Usher type II families, including 365 affected individuals, have been ascertained. Of these, 68 families were informative. All families have been collected from the United States, Sweden, England, Ireland, Italy, Columbia, Spain, and the Netherlands. The ethnic distribution of the families is almost totally limited to persons of European extraction. Only one family included in the study is of mixed European and African background. Two families were from Colombia, and an additional two in the United States have Hispanic surnames. The final sample used in the analysis included 55 multiplex sibships and 13 simplex sibships. Within these families, 381 individuals were sampled, a group that included 137 affected individuals.

Clinical Studies and Sample Collection

As each family was ascertained, a full medical and genetic history was taken. Many affected individuals in the United States were brought to Boys Town National Research Hospital for a full clinical evaluation, including audiology, vestibular studies, ophthalmology (fundus examination, electroretinography, electrooculography, fluorescein angiography), ear/nose/throat examination, and dysmorphology. The diagnosis of retinitis pigmentosa in a family requires that at least one family member have a history of night blindness, progressive reduction of peripheral vision, diminished or absent electroretinogram, and a typical fundoscopic appearance of the retina. The distinction between the type I and II phenotypes was based on audiologic and vestibular results: a type II individual was defined as one with normal vestibular responses and a mild-to-profound sloping hearing loss. Exceptions to this rule were not included in the present analysis. Also, families with patients showing atypical retinitis pigmentosa, unexplained mental retardation, or dysmorphic features were eliminated from the study. A more complete discussion of the clinical evaluation and rationale for the definition of the two clinical types of Usher syndrome have been given elsewhere (Möller et al. 1989).

Blood of informative family members was mailed to Omaha, where the DNA was extracted from pelleted nuclei by an Applied Biosystem 340A nucleic acid extraction machine. DNA from families collected in South Africa was extracted there and was sent to the United States for typing.

Molecular Analysis

PCR amplifications of target sequences were performed in a total volume of 25 μl containing 200 ng of human genomic DNA, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 0.01% gelatin, 200 mM dNTPs, 1 μM of each primer, and 1 unit of *Taq* DNA polymerase (Perkin Elmer Cetus). The $MgCl_2$ was lowered to 0.9 mM for PCR reactions containing TGFB2 primers. For polymorphism screening and genomic typing, genomic DNA was lowered to 20 ng, and dATP was 2.5 μM with the addition of 1 μCi of α^{32}P-dATP (800 Ci/mmol; NEN) to each reaction. All primers used in this study were synthesized on a Cruachem PS 250 automated oligonucleotide synthesizer using standard phosphoramidite chemistry. SSCP typing for the HLX1 locus was done according to the method of Nishimura et al. (1993).

Cytogenetics

Blood specimens were cultured for prometaphase analysis by using a modification of the method of Ryback et al. (1982).

Genetic Analysis

Linkage analysis of DNA markers was performed by using the LINKAGE program, version 5.03 (Lathrop et al. 1985). Both two-point and multipoint analyses were done. The two-point results were done only to assure consistency and as a check on the multipoint results. LOD scores across the entire span of markers (i.e., D1S245 to PPOL) were generated by doing a rolling multipoint analysis using overlapping sets of five contiguous marker loci with the test locus (five-point analysis). The LOD scores plotted and used in the HOMOG analysis were those from the middle segment of each four-marker locus set. The pattern of inheritance of Usher syndrome was assumed to be recessive with full penetrance for the affected homozygote. The frequency of the Usher type IIa gene was assumed to be 1/140. The actual frequency of Usher type II is not known, but it is generally felt to be approximately the same as that for Usher type I. DNA marker haplotypes for normal and USH2 chromosomes were established by inspecting the parental chromosomes. Heterogeneity analysis was done with multipoint data generated as outlined above and using the HOMOG (Ott 1991) program.

Results

Organization of the Markers within the D1S70–PPOL Region

A genetic map of the USH2A region was constructed using marker data from the Usher type II families presented in this report. The markers in the region are listed in table 1, which also gives other pertinent information about each marker. All the markers are short tandem repeats (STRP); except for HLX1 and D1S70, there are no other known RFLPs or VNTRs in the region. Our data for D1S70 are a composite of results from typing both its RFLP and STRP sites. The SSCP polymorphism for HLX1 was typed in selected families, and, once it was found to crossover with Usher type IIa, its typing was discontinued.

Table 1

Characteristics of the Chromosome 1q41 Dinucleotide Repeat Polymorphisms Used in Mapping Usher Type IIa

Locus	No. of Alleles	Heterozygosity	Size Range (kb)	Reference
D1S245	10	.83	235–253	Weissenbach et al. 1992
D1S70	4	.62	179–185	Weston et al. 1994*a*
D1S217	5	.66	130–142	Weissenbach et al. 1992
D1S556	12	.83	109–139	Weston et al. 1994*b*
D1S237	10	.77	172–192	Weissenbach et al. 1992
D1S229	8	.78	191–207	Weissenbach et al. 1992
D1S227	7	.68	111–125	Weisenbach et al. 1992
TGFB2	5	.52	156–168	Weston and Kimberling 1994
D1S320	7	.70	191–205	Weissenbach et al. 1992
ADRPT	6	.63	85–99	Fougerousse et al. 1992

Although the recent reports of chromosome 1q maps (Dracopoli et al. 1991; NIH/CEPH Mapping Group 1992; Weissenbach et al. 1992) provide a good framework for mapping in that general region, there is still ambiguity regarding the relative positions of markers not common to the various published maps. In order to make use of all the marker data available, a combined map of the region, using all informative markers, needed to be constructed. We first attempted to determine the placement of the set of markers D1S245, D1S237, D1S229, and D1S227, relative to D1S70 and PPOL, the pair that previously had been found to flank the USH2A gene (Kimberling et al. 1991). Since the order of the four Généthon markers had been established at >1,000:1 odds, the placement of D1S70 and PPOL within that group would yield an anchor map around which other new markers could be mapped. On the basis of existing maps, it was reasonable to expect D1S70 to be at or near the most proximal Généthon marker of the set, D1S245. Therefore, the three orders—D1S70 - D1S245 - D1S237, D1S245 - D1S70 - D1S237, and D1S245–D1S237–D1S70—were tested by both multipoint analysis and meiotic mapping. Through multipoint analysis, the maximal LOD score observed for the placement of D1S70 between D1S245 and D1S237 was >3 over the maxima observed for the other two orders, thus establishing that order as D1S245 - D1S70 - D1S237. This conclusion was supported by direct gametic analysis. A similar approach was used to position PPOL within the Généthon set. The LINKAGE multipoint analysis placed PPOL distal to D1S227, with >1,000:1 odds. This finding was also supported by haplotype analysis. Thus, the order of markers on chromosome 1q was established to be D1S245 - D1S70 - D1S237 - D1S229 - D1S227 - PPOL.

Within this framework, we placed two additional markers, D1S320 and TGFB2. The results of a linkage analysis for TGFB2 and D1S320 indicated that both genes were between D1S227 and PPOL, with the location of each supported at a high degree of certainty. However, analysis of these two polymorphisms relative to each other yielded results that ordered the markers D1S227 - TGFB2 - D1S320 - PPOL at >100:1 odds. The order of TGFB2 relative to D1S320 was also supported by meiotic mapping. SSCP determination for HLX1 types was done only on families showing critical crossovers in the region; it showed no crossing-over with D1S320, and haplotype analysis placed it between TGFB2 and PPOL. HLX1 is not shown in the map order and distances given below, because only limited haplotype data were available for the analysis. Two more-proximal markers, D1S217 and D1S556, were also studied; both were found to lie between D1S70 and D1S237, at >1,000: 1 odds, and their order relative to each other is as indicated below, but with only ~100:1 certainty.

The order of the final map constructed is D1S245 - 2.2 - D1S70 - 1.9 - (D1S217 - 1.8 - D1S556) - D1S237 - 2.1 - D1S229 - 2.0 - D1S227 - 1.3 - (TGFB2 - 0.3 - D1S320) - 0.3 - PPOL, in which the sex-averaged interval distances are presented in centimorgans. Markers in parentheses are those whose location is supported by the data, but not at 1,000:1 levels. These data are the result of analyses of ~438 meioses in 74 two- and three-generation families, which includes both the 68 families with Usher type II that are reported here and 6 additional families (1 with recessive retinitis pigmentosa and 5 with atypical Usher syndrome families). The mapping results agree well with the estimates of order and distance that recently were reported by the CHLC data bank and the recent chromosome 1 workshop.

The importance of locating TGFB2 and HLX1 outside the critical region for USH2A is that both have been suggested as candidates for Usher type II (Nishimura et al. 1993). TGFB-like genes play a role in the normal development and differentiation of neural tissue, making them a logical candidate for Usher syndrome (Delannet and Duband 1992; Johnson et al. 1993). HLX1, though principally expressed in hematopoietic progenitors and activated lymphocytes, is expressed in fetal brain (Deguchi et al. 1991) and could be considered to be involved in both auditory and retinal development.

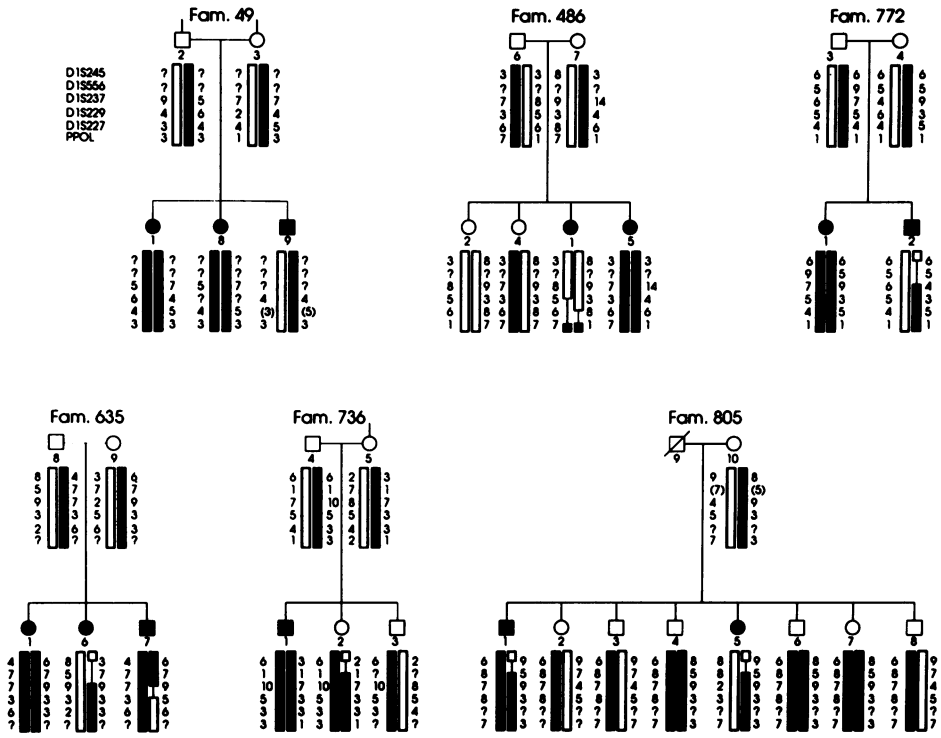

Figure 1 Pedigrees of six new Usher type II families that fail to show linkage to 1q41 markers. The pedigree of the seventh family (i.e., family 735) has been published elsewhere (Pieke Dahl et al. 1993) and is not reproduced here. Markers distal to PPOL and proximal to D1S245 have been typed but are not shown. The thin line in the haplotype bar indicates a crossover. Designation of one chromosome as black and the other as white is done arbitrarily, with the only criterion being that one of the affected offspring had a pair of "black" chromosomes. All families show at least one obligate double crossover, given localization of USH2A between D1S237 and D1S229.

Linkage of the Usher Type II Gene and Heterogeneity

There are 68 families in the sample that have well-documented Usher type II. All families not fitting our clinical criteria (Möller et al. 1989) were excluded from this analysis. It was obvious from inspection of the haplotype data that linkage heterogeneity was present in the sample. This heterogeneity had originally been noted in an American family (Pieke Dahl et al. 1993) that is part of the series of families being reported here. Since that report, we have observed six other families that also fail to show linkage; there are no clinical features that clearly let us differentiate linked from unlinked subtypes. The haplotype results for these six new families are shown in figure 1.

The multipoint analysis was done by using the interlocus distances estimated above. The origin was set arbitrarily at D1S245, and the LOD scores were calculated at ~0.3-cM intervals for each family. All sets of scores from the rolling-point multipoint analysis were used the multiple HOMOG analyses. However, only the results of the heterogeneity analysis for the LOD scores between D1S556 to D1S227 are summarized in table 2, since it was evident that the USH2a gene could not conceivably lie outside this region. Both the hypothesis of no linkage (H_0) and that of linkage with no heterogeneity (H_1) are rejected, but the hypothesis of linkage with heterogeneity (H_2) is not rejected. The best estimate of the fraction of linked families is .875. Obviously, the converse of this is that 12.5% of Usher type II families fail to show linkage with the 1q41 markers. Given this heterogeneity, the best location of the USH2 gene was calculated as being 7.5 cM from D1S245,

Table 2

Linkage Heterogeneity Analysis of Usher Type II Family Data for Chromosome 1q41 Markers

Hypothesis	Maximum Log Likelihood	Estimation of α	Estimation of Distance from S245
H_2	99.89	.875	.075 (S237–S229)
H_1	50.68	(0)	.065 (S556–S237)
H_0	...	...	(.50)

Source	Components of χ^2: df	Components of χ^2: χ^2	Likelihood Ratio
H_2 vs. H_1 (heterogeneity)	1	98.41	2.3×10^{21}
H_1 vs. H_0 (linkage)	1	101.37	1.03×10^{22}
H_2 vs. H_0 (total)	2	199.78	2.40×10^{43}

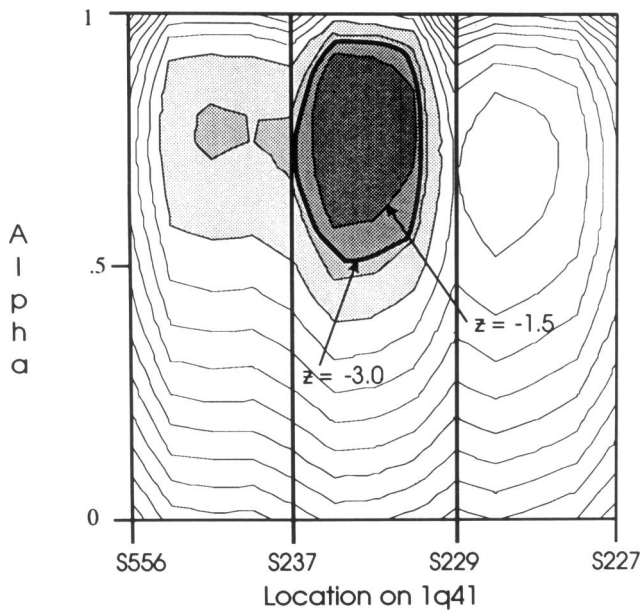

Figure 2 Linkage data graphed as isometric lines at various LOD likelihood values. LOD scores for all Usher type II families were calculated using the multipoint option of LINKAGE for the markers D1S556, D12S237, D1S229, and D1S227 and input into the HOMOG program. The values graphed here were calculated by HOMOG for values of α between 0 and 1.0, at .1 increments. The thicker solid line, which corresponds to the isometric at a LOD of −3 below the maximal LOD score, is totally contained within the brackets for D1S237–D1S229, illustrating that the USH2 gene on chromosome 1q can be placed between these markers, with >1,000:1 odds of confidence. Thus, the placement of the USH2A gene can be made with confidence, despite the fact that considerable genetic heterogeneity is present in the sample of families analyzed.

between the markers D1S237 and D1S229. Note, however, that the best position of the USH2A gene shifts to between D1S556 and D1S237 if heterogeneity is rejected. This raised the issue of the degree of confidence with which the USH2A gene can be placed into the D1S237–D1S229 segment. To address this, we plotted the LOD score distribution calculated by HOMOG, in an isobar format as shown in figure 2.

The x-axis and y-axis are, respectively, the location on chromosome 1q (shown here only for a limited location, namely, that between D1S556 and D1S227) and α, the proportion of linked families. The isobar lines represent constant levels of LOD scores as a function of both position and proportion of linked families. The maximum LOD value was observed to be 98.49 and occurred between D1S237 and D1S229. The maximum LOD score for the surface between D1S556 and D1S237 is 95.30, a value 3.19 LOD units lower than the maximum value calculated between D1S237 and D1S229. The isometric for −3 LOD, which is shown as the more-solid line, does not infringe into either the D1S556–D1S237 segment or the D1S229–D1S227 segment. Thus, it is reasonable to conclude that the USH2A gene lies between the markers D1S237 and D1S229 and that this placement can be made with a high degree of confidence, despite the complication of linkage heterogeneity.

This conclusion is also supported by haplotype analysis. Four critical families are presented in figure 3. Two families merit discussion. The first is family 652, whose parents are second cousins. Their only affected child is heterozygous for D1S237, but the expectation is that any affected child would be homozygous for all near linked genes. Consequently, it is reasonable to explain this occurrence as due to a crossover between D1S237 and USH2A and to exclude the USH2A gene from the region centromeric to D1S237. An alternative explanation is that the Usher type II gene in the family is not linked to 1q41. This possibility is reflected in the probability of linkage, which was calculated by HOMOG to be .70. Such a low probability of linkage is not unexpected for families showing a critical single crossover with either of the flanking markers. This poses a problem, since a crossover with D1S237 and not with D1S229 suggests placement of the USH2A gene telomeric to D1S237 and is thus a significant observation pointing to the distal side of D1S237 as the target region for future genetic and physical mapping. Unfortunately, the crossover with D1S237 also reduces the probability that the family is Usher type IIa and compromises the reliability of this family's data as mapping information. Fortunately, a large family (453) was ascertained that consisted of two genetically independent sibships presumed to have the same Usher type II subtype because two of their members married and have had an affected child. Although a critical crossover occurred in one person, there is sufficient linkage data to keep the probability of linkage high (>.99), so that the exclusion of the USH2A gene from the centromeric side of D1S237 can be made with good confidence. On the basis of this analysis, the distribution of families, by probability of linkage, is <.01, 7 families; .70–.90, 3 families; .90–.95, 16 families; and >.95, 42 families.

Cytogenetic Results

One member of each of 38 Usher type II families showing linkage to 1q41 markers has been karyotyped, in a effort to detect one or more patients with a cytogenetic abnormality. The average band number seen was 525. The region specifically around 1q41 was examined in detail, and no deletions, inversions, or translocations were seen.

Discussion

The map of the region around 1q41 has been updated. Data are presented that combine the Généthon map (Weissenbach et al. 1992) markers with the map of framework markers that had been previously published through the CEPH collaboration (Dracopoli et al. 1991; NIH/CEPH Mapping Group 1992). The order and distances determined in the present study agree well with that given in the Genome Data Base.

The major problem in beginning with fine gene mapping

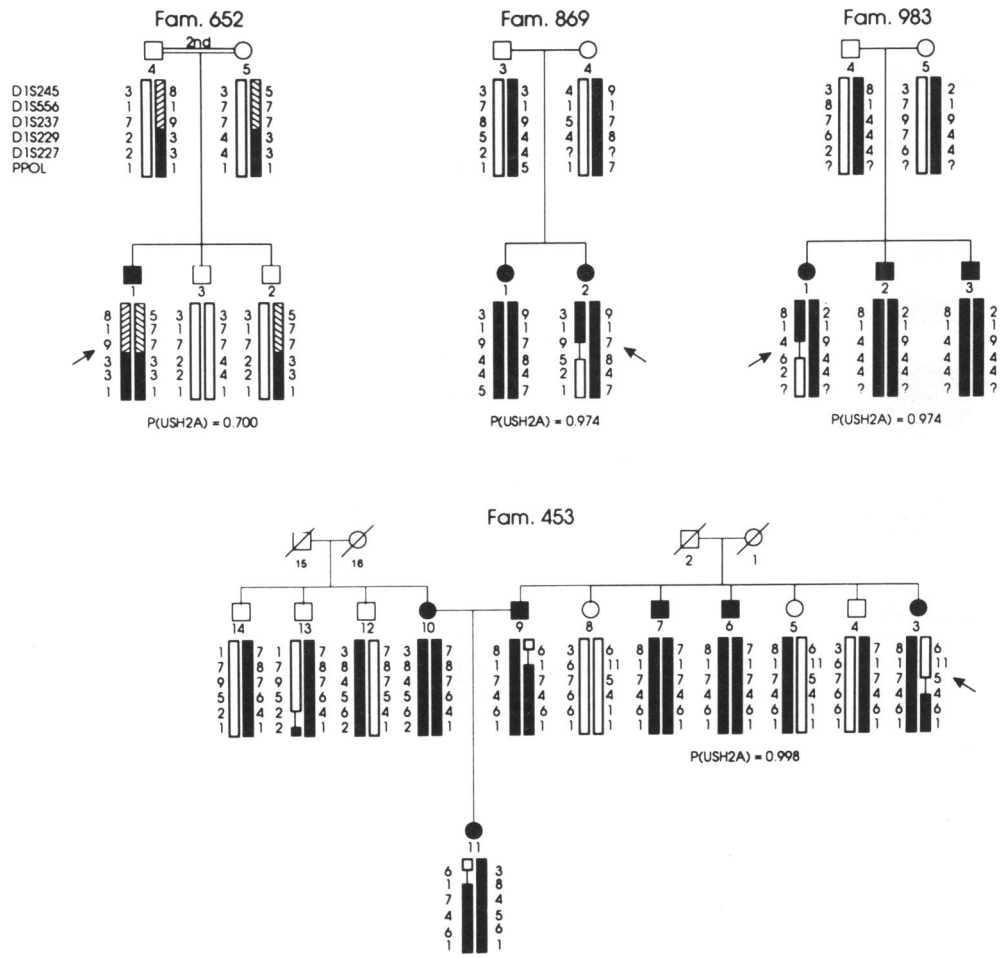

Figure 3 Pedigrees of four critical families showing probable exclusion of the USH2A gene from the region proximal to D1S237 and distal to D1S229. The posterior probability of linkage calculated by HOMOG is also given for each family. Note that family 453 has a high probability of 1q41 linkage and that it supports exclusion of USH2A from the region proximal to D1S237. Two families (869 and 983) show that the USH2A gene cannot be distal to D1S229, and thus it does not lie in the same region as do TGFB2 and HLX1. Family 652 is consanguineous and supports the exclusion of USH2a centromeric to D1S237, by virtue of heterozygosity at that marker and its proximal neighbors.

of the USH2A gene is its genetic heterogeneity. It is estimated that ~12.5% of families fail to show linkage with 1q41. This fact complicates the search for the USH2A gene because families showing critical crossovers cannot be accepted with total confidence. Consequently, physical mapping strategies are employed with some hesitation unless other factors are present that enhance the likelihood of localization. One method of circumventing the problem of heterogeneity is to use only data from a population isolate. Unfortunately that cannot be the case here, since no isolate with Usher type IIa is known. As an alternative, we have elected to increase our sample size as much as possible, with the belief that the likelihood distribution calculated from a large sample would be less sensitive to effects of heterogeneity. Furthermore, a large sample presents greater opportunities for a serendipitous observation that can be helpful in fine gene localization. Both factors were present in this study. The linkage analysis places the gene for Usher type IIa within an ~2.1-cM region flanked by the markers D1S237 and D1S229, with a high degree of confidence. While a small probability exists that the gene could be between D1S556 and D1S237, this location is contraindicated by haplotype analysis of at least one family. The data certainly rule out both TGFB2 and HLX1 as candidate genes, since TGFB2 is placed between D1S320 and PPOL and HLXI is distal to TGFB2. The proposed location of USH2A is shown graphically in the ideogram for chromosome 1q shown in figure 4.

The genetic heterogeneity of Usher type II raises certain important scientific and clinical questions. For the purposes of clarity, the Usher type II gene linked to 1q41 is designated *USH2A*, and the unlinked one has been labeled *USH2B*. A perusal of the clinical findings does not suggest any immediately obvious clinical symptomatology that would allow for a distinction of the two subgroups. It is tempting to speculate that the unlinked USH2B may be the gene responsible for Usher type III, commonly thought of as Usher syndrome with progressive hearing loss (Merin

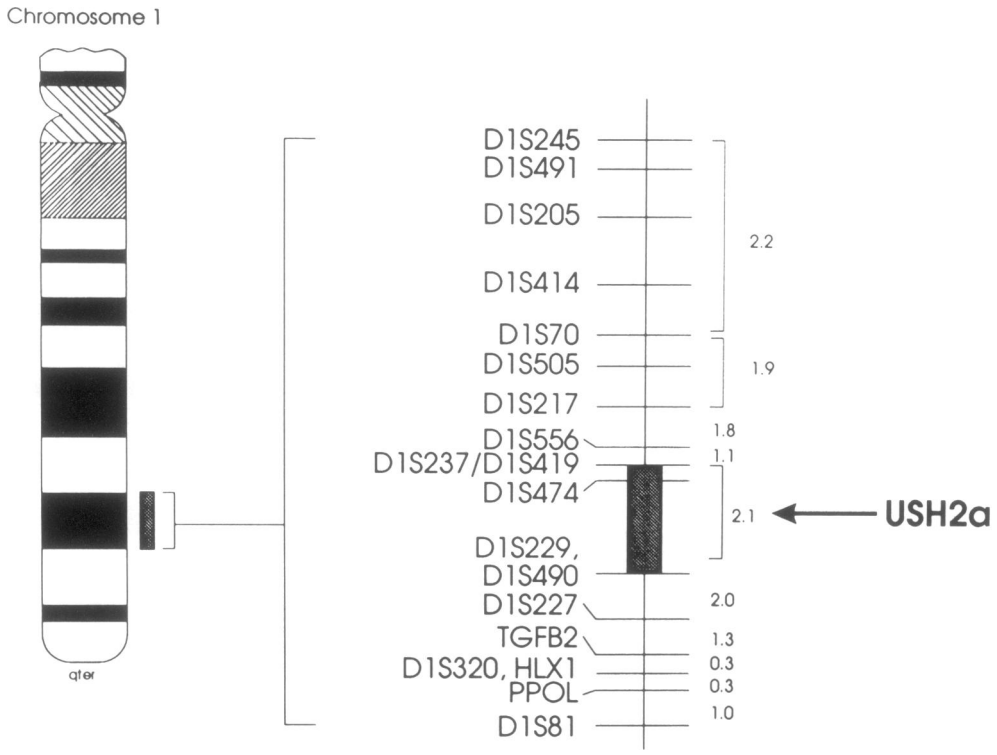

Figure 4 Location of the markers on an ideogram of chromosome 1q, with the placement of the USH2A gene indicated by the thick gray box. Three new markers — D1S419, D1S490, and D1S474 — have not yet been typed in these families but could provide evidence for further sublocalization.

et al. 1974; Karjalainen et al. 1989). How the Usher type III phenotype relates to the Usher type II genes remains to be determined, and Usher type III may represent yet another gene. Audiologic and vestibular phenotypes are both relatively unsubtle and provide little opportunity for differentiation of the Usher type II subtypes. It may be, however, that ophthalmologic psychophysical measures taken from either affected individuals or heterozygotes might provide clues to important phenotypic differences between the genes. Study of the effect in the heterozygotes is an important consideration because the disruption of retinal function may be too severe to allow for reliable differentiation in affected homozygotes. Differentiation of the subtypes by phenotypic examination may be critical in the localization of the USH2b gene, since now the only means of identifying such families is by virtue of the lack of linkage with chromosome 1q41 markers, and more families will be needed if the gene is to be mapped to a segment as small as that to which USH2A has been mapped. A recognized phenotypic difference would also give greater assurance of correctly including critical Usher type II families in the fine mapping protocol for USH2A.

None of the unlinked cases appear to come from a unique population. Three are from Sweden, one is from Spain, and the remainder are from the United States. Preliminary data (not shown) do not indicate linkage with the known location for Usher type I — namely, chromosomes 11q, 11p, or 14q — and a genome search is underway.

It has been surprising to find that Usher syndrome is the result of mutations in at least five different genes. These genes may be related and represent different forms of a gene family whose function is yet to be determined.

The findings presented here have important implications for the positional cloning of the Usher type IIa gene and focus our attention on D1S237 and D1S229 as initiation points for physical mapping experiments. YACs for D1S237 and D1S229 have been isolated, and the work to construct a contig for this region has begun (J. Sumegi, unpublished data).

Note added in proof.—The gene for Usher syndrome III has been recently mapped to chromosome 3q (Sankila et al., in press). The seven unlinked families presented here were typed for the same 3q markers, and five were found to be linked and two were unlinked. Of the five linked to 3q, four were from Sweden.

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