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A Mutation (2314delG) in the Usher Syndrome Type IIA Gene: High Prevalence and Phenotypic Variation

To the Editor:

Usher syndrome (USH), an autosomal recessive condition, is characterized by hearing impairment associated with retinitis pigmentosa (RP). It is a clinically and genetically heterogeneous condition. Three clinical forms of USH have been described, and eight loci have been mapped (Hereditary Hearing Loss home page). USH type I (USH1) is the most severe form, manifested by profound congenital deafness, constant vestibular dysfunction, and prepubertal onset of RP. USH type II (USH2) is characterized by congenital moderate-to-severe hearing impairment, normal vestibular responses, and RP (Smith et al. 1994). USH type III (USH3) is characterized by progressive hearing loss, variable vestibular problems, and RP (Pakarinen et al. 1995). The *USH1B* gene has been identified (Weil et al. 1995), and a variety of mutations leading to USH1 have been catalogued in the *MYO7A* gene (Liu et al. 1998). USH2 is the most common of the three types of USH, accounting for more than half of all cases (Hopes et al. 1997; Rosenberg et al. 1997).

Recently, Eudy et al. (1998) reported the identification of the USH type IIA gene (*USH2A*; MIM 276901). *USH2A* encodes a putative extracellular matrix protein of 1,551 amino acids with laminin epidermal growth factor and fibronectin type II motifs. Expression of the *USH2A* gene has been detected by reverse transcriptase PCR (RT-PCR) from fetal human cochlea, eye, and adult human retina (Eudy et al. 1998). Three different mutations were identified in patients with USH2A, the most frequent of which is the 2314delG mutation. Of 96 probands tested, 21 carried the 2314delG mutation.

In the present study, we have undertaken mutational analysis of the *USH2A* gene in 23 families with USH (both USH2 and atypical USH), from the United Kingdom and China. We found that the majority of families with USH2 carry the 2314delG mutation. Surprisingly, we determined that the 2314delG mutation in the *USH2A* gene can also lead to atypical USH.

Of 23 families with USH analyzed in this study, 15 have been described elsewhere (Hopes et al. 1997). All available affected subjects were examined by one of the authors. Full clinical histories were obtained, with emphasis on any potential causes of hearing impairment. The audiovestibular and ophthalmic evaluations were done on the basis of recommendations by the Usher Syndrome Consortium (Smith et al. 1994). The details of the clinical evaluations can be found in Hopes et al. (1997). The cases studied here are classified clinically into the following two groups: (1) USH2, consisting of congenital sloping moderate/severe hearing impairment, normal vestibular function, and RP (Smith et al. 1994); and (2) atypical USH, consisting of bilateral sensorineural progressive hearing loss without other obvious factors as the cause of the progression of the hearing impairment, along with variable vestibular dysfunction and RP.

Of 23 families studied, 13 were given a diagnosis of USH2 and 10 were given a diagnosis of atypical USH. Eighteen families living in the United Kingdom and five in China were included in our analyses. Twenty-three probands with USH were screened for the 2314delG mutation by combined SSCP/heteroduplex analysis. Twelve families (52%) carried the 2314delG mutation—8 (62%) of 13 patients with typical USH2 and 4 (40%) of 10 patients with atypical USH (table 1). Of 12 families with this mutation, 7 were identified with only one affected member (sporadic cases). Of the remaining five families with more than one affected individual, two families (USH.05 and USH.10) with two affected sibs were tested for cosegregation of the disease with the *USH2A* locus (Eudy et al. 1998). The segregation analysis was consistent with linkage to *USH2A* (data not

Table 1

Summary of 2314delG Mutations Identified in the *USH2A* Gene in Families with USH

Family	Origin[a]	2314delG Mutation	No. of Chromosomes	Clinical Type
USH.01	UK	Homozygous	2	Atypical
USH.02	UK	Homozygous	2	II
USH.03	UK	Heterozygous	1	II
USH.04	UK	Heterozygous	1	II
USH.05	UK	Homozygous	4	Atypical
USH.06	UK	Heterozygous	1	II
USH.07	UK	Heterozygous	1	II
USH.08	UK	Heterozygous	1	Atypical
USH.09	China	Homozygous	2	Atypical
USH.10	UK	Heterozygous	2	II
USH.11	China	Heterozygous	1	II
USH.12	UK	Heterozygous	1	II
USH.13	China	None	0	II
USH.14	China	None	0	II
USH.15	UK	None	0	II
USH.16	UK	None	0	II
USH.17	UK	None	0	II
USH.18	China	None	0	Atypical
USH.19	UK	None	0	Atypical
USH.20	UK	None	0	Atypical
USH.21	UK	None	0	Atypical
USH.22	UK	None	0	Atypical
USH.23	UK	None	0	Atypical

[a] UK = United Kingdom.

shown). Examination of genotypes for markers *AFM143XF10, AFM268D1,* and *AFM144XF2* reveal that all patients with the 2314delG mutation do not share the same genotype for the three markers (data not shown).

Four affected children from three families from the United Kingdom (USH.01, USH.02, and USH.05) were identified to be homozygous for the 2314delG mutation, as well as one affected child from the Chinese family USH.09 (table 1). In addition, eight affected individuals from seven other U.K. families, as well as one additional family from China, were found to be heterozygous for this mutation (table 1). To exclude the possibility that the 2314delG mutation is simply a common polymorphism in the populations we studied, we analyzed 80 unrelated control samples (40 from the United Kingdom and 40 from China) for this mutation. No mutation was detected from this panel of control DNA samples.

Of 12 families with the 2314delG mutation, 8 families had the typical USH2 phenotype—congenital moderate-to-severe hearing impairment, normal vestibular function, and postpubertal onset of RP. However, five affected individuals from the remaining four families carrying the 2314delG mutation showed atypical USH features, with progressive hearing impairment, variable vestibular function, and RP (table 2). Two patients from an atypical USH family (USH.05) were identical twins with nonconsanguineous parents. The index twin (age 49 years) had worn hearing aids from age 14 years and remembered hearing well until age ~10 years. He believed his hearing loss had progressed since that time. His last audiogram confirmed that his hearing loss had progressed from moderate to severe. He had normal vestibular function. He had suffered from night blindness since early childhood and loss of visual field since age ~20 years. The electroretinogram was extinguished and his fundi showed typical pigmentary degeneration with bone spicule pigmentation. His twin brother had been given a diagnosis of hearing loss and had worn hearing aids at age 8 years. He did not feel that his hearing loss had progressed, but there were negative caloric responses. RP was given as a diagnosis at age 40 years, but he was symptomatic in childhood. These data indicate phenotypic variation between monozygotic twins. The isolated patient (age 39 years) from the second atypical family (USH.01) was typical of USH2 in all aspects, including nonprogressive hearing loss, but had absent vestibular function, which is a critical discriminator in clinical classification. Further details of clinical evaluation for both families have been reported by Hopes et al. (1997).

In family USH.09, originating from China, the parents were unrelated and unaffected. The patient (age 38 years) had been determined to have bilateral hearing loss at age 10 years. There are no other affected individuals in her family. She believed her hearing had deteriorated. Comparison with one available audiogram 7 years previously showed progression of hearing loss from 45 decibel-hearing level (dB HL) to 75 dB HL, across all frequencies. She had normal vestibular function. Night blindness was present from age 14 years, and RP was given as a diagnosis at age 21 years.

The patient from USH.08 is a 38-year-old woman from the United Kingdom with nonconsanguineous parents. She had been given a diagnosis of hearing loss at age 5 years. Her hearing loss had progressed to the present average of 53 dB HL (down-sloping audiogram) from 38 dB HL 10 years ago. She has normal speech and normal vestibular function. Her RP was formally confirmed at age 35 years, but she was symptomatic from age 26 years.

The mutations described here, particularly the occurrence of a frequent 2314delG mutation in familial and sporadic USH families, support the recent observation that the *USH2A* gene is responsible for USH2 (Eudy et al. 1998). Eudy and colleagues found three frameshift mutations, including 2314delG, 2913delG, and 4353-54delCT, in the *USH2A* gene. The 4353-54delCT mutation was found in a heterozygous patient with USH2A in the Louisiana Acadian population. Eudy et al. (1998) found that one of the mutations, 2314delG, occurred at a high frequency (22%) in 96 probands. Most patients

Table 2

Phenotypic Evaluation of Individuals Carrying the 2314delG Mutation

Family and No. of Affecteds	Age at Diagnosis of Deafness	Audiogram[a]	Vestibular Function[b]	Age at Diagnosis of RP (Years)	Age at Onset of Visual Symptoms (Years)	Pigmentary Degeneration
USH.01:						
1	6 years	Sloping, mod/sev	-	21	10	Moderate
USH.02:						
1	6 years	Sloping, mod/sev	+	17	17	Moderate
USH.03:						
1	15 mo	Sloping, mod/sev	+	30	20	Moderate
USH.04:						
1	Infant	Sloping, mod/sev	+	58	54	Moderate
USH.05:						
1	14 years	Progressive sloping, mod/sev	+	40	10	Moderate
1	8 years	Sloping, mod/sev	−	40	10	Moderate
USH.06:						
1	18 mo	Sloping, mod/sev	+	35	32	Moderate
USH.07:						
1	2 years	Profound	+	17	15	Moderate
USH.08:						
1	5 years	Progressive sloping, mod/sev	+	35	26	Mild
USH.09:						
1	10 years	Progressive mod/sev	+	21	14	Moderate
USH.10:						
1	5 years	Sloping, mod/sev	+	14	22	Moderate
1	2 years	Sloping, mod/sev	+	Unknown	20	Mild
USH.11:						
1	3 years	Sloping, mod/sev	+	22	18	Moderate
USH.12:						
1	2 years	Sloping, mod/sev	+	43	43	Mild

[a] "Sloping" denotes worse thresholds in the high frequencies; "mod/sev" denotes moderate to severe hearing impairment.
[b] A plus sign (+) indicates normal; a minus sign (−) indicates abnormal.

with the 2314delG mutation had northern European ancestry, but two homozygous patients were of Spanish or African origin. In the present study, we have also found that the 2314delG mutation is particularly frequent in patients with typical USH2. We found this mutation in 62% of our patients with USH2. The mutation 2314delG lies within a crucial region in the laminin epidermal growth factor motif of *USH2A* and results in a frameshift at codon 772 and a premature stop codon 20 amino acids downstream, presumably leading to a truncated protein and loss of function. It seems likely that those patients who are heterozygous for this mutation are compound heterozygotes, and identification of the other mutant alleles remains to be done. Until the entire *USH2A* gene has been screened for mutations, the relative frequency of this particular mutation cannot be accurately assessed. The families we have studied carrying this frequent mutation originate from the United Kingdom and China. The identification of the 2314delG mutation in four widely dispersed populations (northern Europe, Spain, Africa, and China) suggests that a founder effect was not involved in propagating the mutation. Rather, the 2314delG mutation may represent a mutational hot spot within the *USH2A* gene. Haplotype analysis of 2314delG in different populations could be used to address this question definitively.

The existence of two forms of USH—USH1 and USH2—has achieved a general acceptance among clinicians in the past two decades. Both types can be distinguished by the severity and the presence of vestibular dysfunction. A third group of patients that present with progressive hearing loss and variable vestibular problems along with RP. Patients in this class have been described as affected with "USH3" (Pakarinen et al. 1995), although some families, in the absence of any clear genetic distinction with other USH classes, may be best classified as having an atypical USH phenotype. However, USH3, as a clinical entity distinct from USH2, has been accepted only recently, on the basis of a genetic linkage analysis of Finnish families that demonstrated linkage to a locus on 3q not previously implicated in either USH1 or USH2 (Sankila et al. 1995).

In the present study, all but four of families with a 2314delG mutation showed typical clinical character-

istics of USH2, as determined by clinical criteria (Smith et al. 1994). The phenotypic features in the families USH.05, USH.08, and USH.09 are quite similar to the Finnish USH3 families (Sankila et al. 1995). Mutational analysis of four families with atypical USH demonstrated that four affected individuals are homozygous and one is heterozygous for the 2314delG mutation in the *USH2A* gene (table 1). This indicates that mutations in the *USH2A* gene can give rise to atypical USH, as well as to USH2.

The phenotypic variation observed in individuals with the 2314delG mutation suggests that environmental or genetic factors affect expression of *USH2A*. The same mutation is expected to be associated with a uniform clinical picture, but there are many instances in which identical mutations in a gene can result in phenotypic variation (Romeo and McKusick 1994; Wolf 1997). Genetic factors that could account for the phenotypic variability could include effects of the genetic background, including modifier genes, and even additional mutations in the *USH2A* gene modifying the phenotype. Our observations on the disconcordant phenotype of the monozygotic twins carrying the same mutation suggest that variation in the expression of the *USH2A* gene is not determined simply by genetic factors (i.e., modifier genes). We have shown that different mutations in *MYO7A* can lead to a wide range of phenotypes, including USH type IB, autosomal recessive nonsyndromic deafness, and autosomal dominant nonsyndromic deafness (Liu et al. 1997*a*, 1997*b*). Recently, we reported that mutations in the *MYO7A* gene can also lead to atypical USH (Liu et al. 1998). Including the present data, we have shown that atypical USH and USH3 are apparently genetically heterogeneous, with mutations at different loci on 3q, 11q, and 1q in different families. Most important, it is also clear that the same mutation at the *USH2A* locus can give rise to USH2 as well as to atypical USH. Overall, the present data underline both the genetic and phenotypic heterogeneity that is observed in USH and the difficulties that will face us in using molecular criteria for diagnosis and management.

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Electronic-Database Information

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Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for USH2A [MIM 276901])

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Different Functional Outcome of RetGC1 and RPE65 Gene Mutations in Leber Congenital Amaurosis

To the Editor:

Leber congenital amaurosis (LCA) has the earliest age at onset and is the most severe of the inherited retinal dystrophies (Leber 1869). It accounts for ⩾5% of all retinal dystrophies and probably accounts for a higher percentage in countries with a high rate of consanguinity (Foxman et al. 1985; Kaplan et al. 1990). LCA is an autosomal recessive condition, distinct from all other retinopathies in that the visual disorder is diagnosed at birth or during the first months of life in an infant with total blindness or greatly impaired vision, normal fundus, and extinguished electroretinogram (ERG; Franceschetti and Dieterle 1954). Although major visual impairment is easily recognizable at birth, LCA was largely underdiagnosed until ERG performed on infants showed that the condition is not uncommon. A certain degree of clinical heterogeneity has long been recognized in LCA (Merin 1991; Trabousli et al. 1995); however, genetic heterogeneity of LCA has been suspected since the report by Waardenburg et al. (1963) of normal-sighted children born to parents who were both affected with LCA.

We mapped the first LCA gene (LCA1) on the short arm of chromosome 17p13.1 and confirmed the genetic heterogeneity of the disease (Camuzat et al. 1995, 1996). Perrault et al. (1996) ascribed LCA1 to mutations in the photoreceptor-specific guanylate cyclase gene, RetGC1, which catalyses the conversion of guanine triphosphate to cyclic guanine monophosphate (cGMP) in the retina. To date, a total of 18 different mutations have been found in 20 unrelated families originating from various countries across the world, especially the Mediterranean region. The observation of missense and frameshift RetGC1 mutations suggests that the cGMP production in photoreceptor cells is markedly reduced or abolished in LCA (Perrault et al. 1996). As a consequence, the excitation process of rod and cone photoreceptors would be markedly impaired because of constant closure of cGMP-gated cation channels, with hyperpolarization of

Table 1

***RPE65* Gene Mutations in Patients in the LCA2 Group**

Family Number and Location of Mutation	Base Change	Amino Acid Change	Comment	Conservation across Mouse and Human
1:				
Exon 9	G1043A	C330Y	Homozygous	+
2:				
Intron 1	G/A	65+5G/A	Homozygous	−
3:				
Exon 3	C244T	Q64X	Homozygous	+
4:				
Intron 8	G/A	912+1G/A		−
5:				
Exon 12	C1355T	A434V		+
6:				
Exon 10	DelA	1114DelA		+
Exon 7	C754T	R234X		+
7:				
Exon 10	C1141A	P363T		+
Exon 13	T1472A	V473N		+