

## BRIEF REPORT

## A Mutation of PCDH15 among Ashkenazi Jews with the Type 1 Usher Syndrome

Tamar Ben-Yosef, Ph.D., Seth L. Ness, M.D., Ph.D., Anne C. Madeo, M.S.,  
Adi Bar-Lev, M.S., Jessica H. Wolfman, Zubair M. Ahmed, Ph.D.,  
Robert J. Desnick, M.D., Ph.D., Judith P. Willner, M.D., Karen B. Avraham, Ph.D.,  
Harry Ostrer, M.D., Carole Oddoux, Ph.D., Andrew J. Griffith, M.D., Ph.D.,  
and Thomas B. Friedman, Ph.D.

From the Laboratory of Molecular Genetics, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Rockville, Md. (T.B.-Y., A.C.M., J.H.W., Z.M.A., A.J.G., T.B.F.); the Department of Human Genetics, Mount Sinai School of Medicine, New York (S.L.N., A.B.-L., R.J.D., J.P.W.); the Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel (K.B.A.); and the Human Genetics Program, New York University School of Medicine, New York (H.O., C.O.). Address reprint requests to Dr. Friedman at the Laboratory of Molecular Genetics, NIDCD, 5 Research Ct., Rm. 2A15, Rockville, MD 20850, or at [friedman@nidcd.nih.gov](mailto:friedman@nidcd.nih.gov).

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**T**HE USHER SYNDROME IS AN AUTOSOMAL RECESSIVE DISORDER characterized by bilateral sensorineural deafness and progressive loss of vision due to retinitis pigmentosa. It is the most frequent cause of deafness and concurrent blindness,<sup>1</sup> with a prevalence of 1 in 16,000 to 1 in 50,000.<sup>2</sup> The majority of cases of the Usher syndrome can be classified into one of three clinical subtypes, the most severe of which is the type 1 Usher syndrome, characterized by profound prelingual hearing loss, vestibular areflexia, and prepubertal onset of retinitis pigmentosa.<sup>2</sup> Seven loci for the type 1 Usher syndrome (*USH1A* to *USH1G*) have been mapped to distinct chromosomal regions by genetic-linkage studies,<sup>2,3</sup> and the causative genes have been identified for five of them.<sup>4-10</sup>

Several rare genetic disorders in Ashkenazi Jews are associated with prevalent founder mutations segregating in this population.<sup>11-15</sup> A reduction in the incidence of such disorders is possible through effective genetic education, screening, and counseling. We previously identified a founder mutation in the *GJB2* gene, 167delT, which is carried by 4 percent of Ashkenazi Jews and is one of the major causes of autosomal recessive nonsyndromic hearing loss in this population.<sup>16</sup> We hypothesized that, similarly, at least one founder mutation that arose in an ancestral Ashkenazi Jew is a prevalent cause of the type 1 Usher syndrome in the current population.

### METHODS

#### SUBJECTS

Ashkenazi Jewish subjects with the type 1 Usher syndrome were identified in North America through the National Institute on Deafness and Other Communication Disorders and the Mount Sinai School of Medicine and in Israel through the Sackler School of Medicine at Tel Aviv University and the Center for Deaf-Blind Persons. The study was approved by the institutional review boards, and written informed consent was obtained from all participants. The affected persons or their parents completed a questionnaire regarding their medical history, and when possible, medical records were obtained. All affected persons met the diagnostic criteria for the type 1 Usher syndrome,<sup>17</sup> including profound congenital sensorineural hearing loss and prepubertal onset of retinitis pigmentosa. Delayed attainment of motor developmental milestones was consistent with the presence of peripheral vestibular dysfunction in all subjects in whom caloric testing could not be performed to document caloric areflexia.

**DETECTION OF MUTATIONS**

Genomic DNA was extracted either from venous-blood samples (Puregene, Gentra Systems) or from buccal mucosal cells obtained with a swab.<sup>18</sup> DNA samples were amplified by polymerase chain reaction (PCR) with fluorescent-dye-labeled primers flanking microsatellite repeat markers for the *USH1A* to *USH1F* loci (information is available at <http://www.uia.ac.be/dnalab/hhh>). The PCR products were visualized by gel electrophoresis on an ABI-377 DNA sequencer, and the genotypes were determined by GeneScan and Genotyper software with the use of an ABI-GeneScan-350 TAMRA size standard (Applied Biosystems), and DNA from Centre d'Etude du Polymorphisme Humain family members NA06990 and NA07057 (Coriell Cell Repositories) as references for allele sizes. (Centre d'Etude du Polymorphisme Humain family members are from multigenerational anonymous white families from Utah, described by Dausset et al.<sup>19</sup>) The 32 coding exons of *PCDH15* were amplified as described previously.<sup>9</sup>

To detect the R245X mutation by allele-specific PCR,<sup>20</sup> two PCR reactions that amplify only the wild-type or only the mutant allele were performed for each DNA sample. The wild-type allele was amplified with the common primer 5'CTTTGTGTTAAAATGTATTCATACTCCCTG3' and the wild-type primer 5'AGGACCGTGCCCAAATCTGAATGAGAGCC3'. The R245X mutant allele was amplified with the common primer and the mutant primer 5'AGGACCGTGCCCAAATCTGAATGAGAGCT3'. The PCR reactions were performed in a 25- $\mu$ l volume with 50 ng of genomic DNA, 1 $\times$  PCR buffer (Applied Biosystems), 1.5 mM magnesium chloride, 0.02 U of thermostable DNA polymerase, 160  $\mu$ M of each deoxynucleotide triphosphate, 200 nM of the common primer, and 50 nM of the wild-type or mutant primer. The cycling conditions were 95°C for 5 minutes, then 35 cycles of 95°C for 30 seconds, 61°C for 30 seconds, and 72°C for 30 seconds, followed by a final step of 72°C for 10 minutes.

**RESULTS**

To identify founder mutations for the type 1 Usher syndrome in Ashkenazi Jews, we searched for a haplotype of genetic markers closely linked to any of six reported *USH1* loci (*USH1A* to *USH1F*) that was shared among persons with the type 1 Usher syndrome in four Ashkenazi families (Fig. 1A). A conserved haplotype of three polymorphic marker al-

leles (D10S2537, D10S546, and D10S2536) located within the *USH1F* gene, *PCDH15*, cosegregated with the type 1 Usher syndrome in these four families (haplotype A in Fig. 1A and 1B).

To detect mutations in the *PCDH15* gene, we determined the sequence of each of the 32 coding exons in five affected persons from three of the families. All five were found to be homozygous for the same mutation, a C-to-T transition at position 733 from the translation initiation codon (733C→T) (GenBank accession number AY029237). The 733C→T transition, located in exon 8, leads to the substitution of a translation stop codon for an arginine codon at position 245 of protocadherin 15 (R245X) (Fig. 2A). We then amplified and sequenced exon 8 of *PCDH15* in all participating family members and in eight additional persons with sporadic type 1 Usher syndrome. In each of the four originally analyzed families, the affected persons were homozygous and their parents were heterozygous for R245X (Fig. 2A). A total of 18 affected persons from 12 unrelated families were tested for R245X. In four families (33 percent), the affected persons were homozygous for the wild-type allele of *PCDH15*. In two of these persons, we detected a previously reported mutation in the *USH1B* gene, *MYO7A* (IVS18+1g→a).<sup>21</sup> In seven families (58 percent), the affected persons were homozygous for R245X. In one family (8 percent) (Family 5, Fig. 1A), the affected person was a compound heterozygote for R245X and a second putative mutation in exon 33 of *PCDH15*. An A-to-C transversion at nucleotide position 5556 (5556A→C) (Fig. 2A) leads to the substitution of leucine for methionine at residue 1853 of protocadherin 15 (M1853L).

To facilitate detection of R245X carriers and homozygotes, we developed an allele-specific PCR assay (Fig. 2B). The sensitivity and specificity of this assay were tested on multiple DNA samples with genotypes known from direct sequencing. Using this assay, we found R245X carrier frequencies of 0.79 percent (95 percent confidence interval, 0 to 1.8) and 2.48 percent (95 percent confidence interval, 0.1 to 4.9) among Ashkenazi Jews from New York and Israel, respectively (Table 1). These frequencies are not significantly different ( $P=0.19$  by the chi-square test). The combined carrier frequency among Ashkenazi Jews was 1.38 percent (95 percent confidence interval, 0.3 to 2.4). No R245X carriers were detected among 293 Jews of non-Ashkenazi background or 96 anonymous non-Jewish whites (Table 1). We found M1853L carrier frequen-

cies of 0.29 to 3.39 percent in various Ashkenazi and non-Ashkenazi Jewish populations, but not in the 93 non-Jews (Table 1). No carriers of the MYO7A mutation IVS18+1g→a were detected among 200 Israeli Ashkenazi Jews.

To elucidate the R245X haplotype and to identify meiotic recombination break points, we analyzed several genetic markers flanking PCDH15. The observed haplotypes revealed that all chromosomes harboring R245X had the same alleles of five markers (D10S2537, D10S546, D10S2536, D10S2522, and D10S2523), which span 415 kb flanking the R245X mutation (Fig. 1B). Most R245X-bearing chromosomes (65 percent) had an identical haplotype of marker alleles downstream and upstream from this region (haplotype A1 in Fig. 1B), although we did find evidence of historical meiotic recombinations of closely linked markers (for example, haplotypes A2 and A3 in Fig. 1B). The conserved haplotype of the region surrounding the R245X mutation might indicate that the high carrier frequency of this mutation among Ashkenazi Jews was caused by a population “bottleneck” (a large reduction in the size of the population, followed by an expansion), a founder effect, or both.<sup>22</sup> Using the degree of conservation between R245X and marker D10S2435 and the distance between the two loci (0.67 cM, assuming that 1 cM equals 10<sup>6</sup> bp), we estimated that the R245X mutation originated in the Ashkenazi population 14 generations, or approximately 350 years, ago.<sup>22</sup>

## DISCUSSION

One of the earliest written descriptions of the clinical features of the Usher syndrome was published in 1861 by a physician who observed the syndrome among Jews in Berlin.<sup>23</sup> However, there are no past or current data to suggest that this observation reflects an increased frequency of the Usher syndrome among Ashkenazi Jews. Nevertheless, we have now identified a novel PCDH15 mutation, R245X, which appears to account for a large proportion of cases of the type 1 Usher syndrome in this population. The conservation of a single haplotype of genetic marker alleles along 415 kb of DNA flanking the R245X mutation suggests a single origin for this mutant allele. The R245X carrier frequencies we observed (0.79 to 2.48 percent) are similar to the carrier frequencies of other genetic conditions for which routine screening is performed in this population, such as Tay–Sachs disease (3 to 4 percent), Gau-

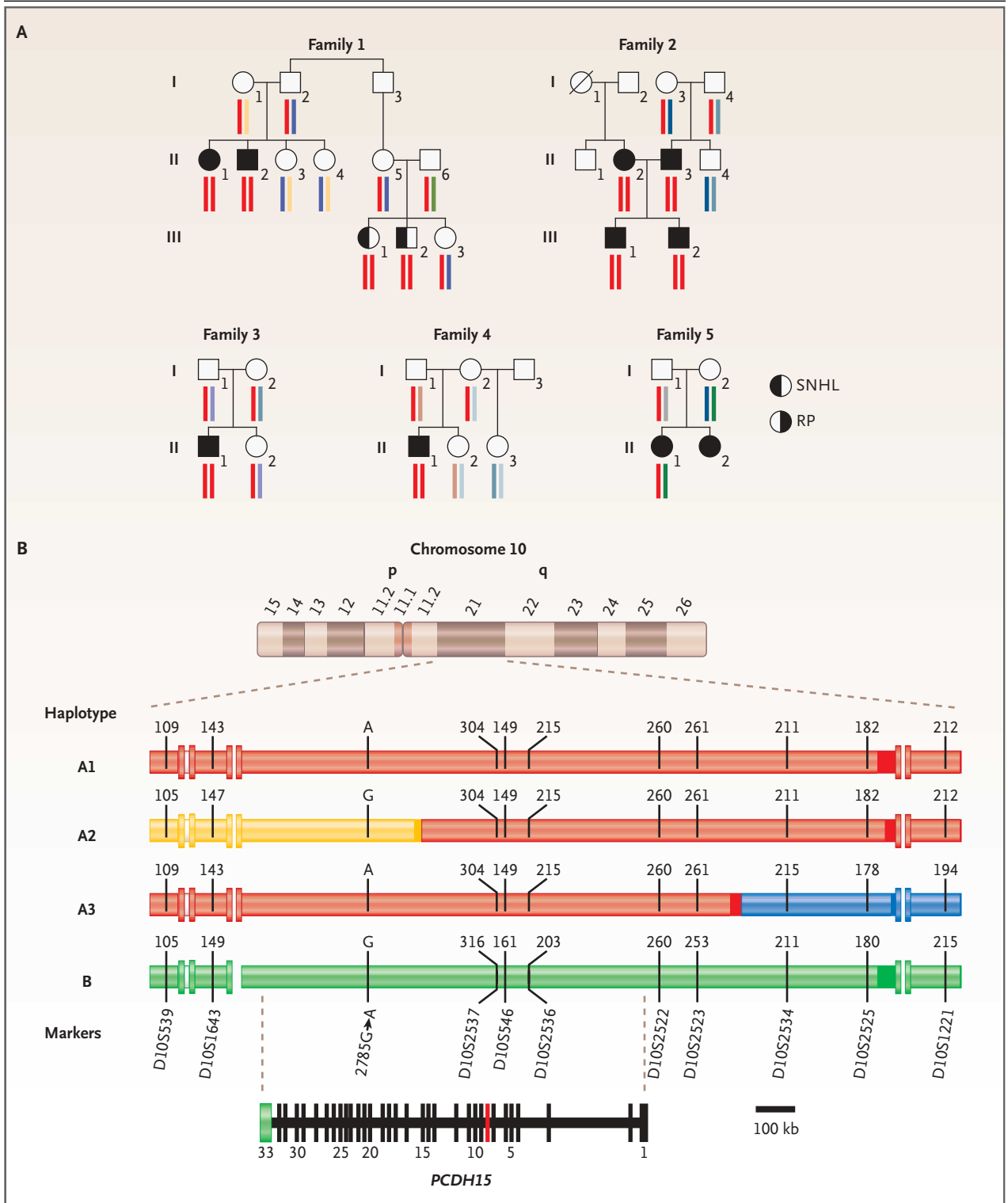
cher’s disease (4 to 6 percent), and Canavan’s disease (1 to 2 percent).<sup>13–15</sup> No R245X carriers were detected among other Jewish or non-Jewish population controls, indicating that this mutation may be unique to Ashkenazi Jews.

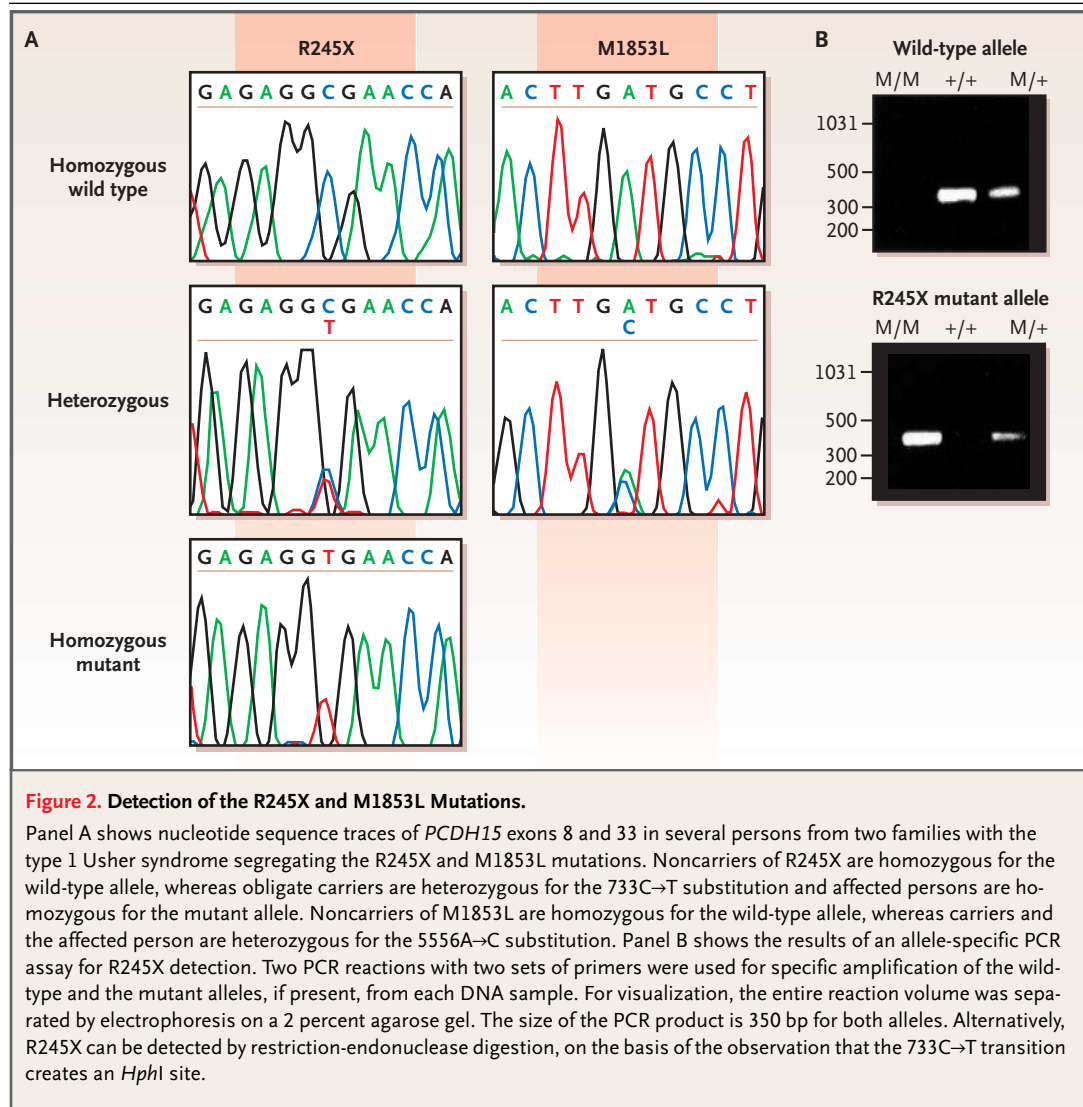
We found a difference in the carrier frequency of R245X between Ashkenazi Jews from Israel (2.48 percent) and those from New York (0.79 percent) that is not statistically significant. Within these Ashkenazi subpopulations, differences in carrier frequencies have been reported for other disease alleles as well, including the E285A mutation in the Canavan’s disease gene, ASPA,<sup>14</sup> and the nonsyndromic deafness GJB2 mutation 167delT.<sup>16,24</sup> Our data may reflect real differences in carrier frequencies between Ashkenazi subpopulations, or they may result from coincidental differences between the control groups we used.

R245X was detected among a large proportion (64 percent) of chromosomes bearing the type 1

### Figure 1 (facing page). Pedigrees, Haplotype Analysis, and Mutation-Bearing Haplotypes at the USH1F Locus.

Panel A shows the pedigrees used for haplotype analysis. Subjects III-1 and III-2 from Family 1 have profound congenital sensorineural hearing loss (SNHL). Their status with regard to retinitis pigmentosa (RP) is unknown. Haplotypes at the USH1F locus are represented by vertical colored bars. The common USH1F haplotype (haplotype A) is shown in red. Mutation-bearing haplotypes at the USH1F locus are shown in Panel B. The haplotypes, represented by horizontal colored bars, are composed of several polymorphic markers. Sequences of PCR primers used for amplification of these markers are available at the Genome Database (<http://gdbwww.gdb.org>). The locations of the polymorphic markers relative to the PCDH15 gene are marked by vertical black bars. Allele sizes are shown in base pairs above the bars. D10S539 and D10S1221 are located at 72.9 cM and 75.5 cM, respectively, on the Marshfield genetic map ([http://research.marshfieldclinic.org/genetics/Map\\_Markers/maps/IndexMapFrames.html](http://research.marshfieldclinic.org/genetics/Map_Markers/maps/IndexMapFrames.html)). In the schematic representation of the PCDH15 gene, the exons are shown as vertical bars and the exon numbers are written below them. The R245X mutation is located in exon 8, marked in red. The M1853L mutation is located in exon 33, marked in green. Most R245X-bearing chromosomes (24 of 26) had an additional sequence change, 2785G→A in exon 21, which leads to the substitution of glycine for arginine at position 1061. Sequence analysis of 402 chromosomes from Israeli Ashkenazi Jews revealed that 2785G→A is a common single-nucleotide polymorphism in this population, with the A allele found at a frequency of approximately 14 percent. We identified several unaffected persons who were homozygous for this change, confirming that it is not pathogenic.





Usher syndrome from our Ashkenazi patients, but not all. We did not identify *PCDH15* mutations in four unrelated patients who had the Usher syndrome from other genetic causes.<sup>2</sup> An additional putative *PCDH15* mutation, M1853L, was detected in compound heterozygosity in only 1 of 18 patients with the type 1 Usher syndrome. In the absence of a large family with several affected members who are homozygous for M1853L, we cannot definitively conclude that this is a pathogenic allele.

Although persons with the type 1 Usher syndrome are congenitally deaf, the loss of vision is delayed in onset and progressive. Without a high degree of clinical suspicion for the Usher syndrome, a prelingually deaf child with the type 1 Usher syn-

drome might receive an incomplete diagnosis of nonsyndromic deafness. Most participants (15 to 63 years old) in the present study had a diagnosis of the type 1 Usher syndrome that antedated their participation. An exception occurred in Family 1 in Figure 1A, in which Subjects III-1 and III-2 were six and nine years old, respectively, and appeared to have nonsyndromic deafness. However, another sibship in this family included two persons (Subjects II-1 and II-2, 24 and 32 years old, respectively) with the type 1 Usher syndrome who were found to be homozygous for R245X. Our molecular testing revealed that Subjects III-1 and III-2 are also homozygous for R245X and therefore are at risk for retinitis pigmentosa.

**Table 1. Frequencies of Carriers of the R245X and M1853L Mutations of the *PCDH15* Gene.**

Population	R245X			M1853L		
	No. of Subjects Tested	No. of Carriers	Carrier Frequency	No. of Subjects Tested	No. of Carriers	Carrier Frequency
			%			%
Ashkenazi Jewish	581	8	1.38	561	2	0.36
New York*	379	3	0.79	346	1	0.29
Israel†	202	5	2.48	215	1	0.47
Sephardi Jewish, Israel†	134	0	0	134	1	0.75
Moroccan Jewish, Israel†	98	0	0	103	2	1.94
Iraqi Jewish, Israel†	61	0	0	59	2	3.39
Anonymous non-Jewish whites‡	96	0	0	93	0	0

\* The data are from Ashkenazi Jews presenting for carrier screening for Tay–Sachs disease in the New York area who consented to the use of their DNA samples in additional genetic studies.

† Samples were obtained through the National Laboratory for the Genetics of Israeli Populations at Tel Aviv University.

‡ A panel of 96 DNA samples from non-Jewish whites (HD100CAU) was obtained from the Coriell Cell Repositories, Camden, N.J.

The carrier frequency of R245X in Ashkenazi Jews suggests an incidence of the type 1 Usher syndrome of 0.15 to 1.5 per 10,000 on the basis of random mating and complete penetrance. Since R245X was found in only 64 percent of chromosomes from our Ashkenazi Jewish patients bearing the type 1 Usher syndrome, the actual risk of the syndrome may be somewhat higher. The incidence of profound congenital hereditary deafness is approximately 5 in 10,000,<sup>25</sup> and thus, in the Ashkenazi Jewish population, mutations causing the type 1 Usher syndrome (including R245X) might account for up to 30 percent of these cases.

The identification of the R245X mutation as a significant cause of the type 1 Usher syndrome in Ashkenazi Jews and the specific detection assays we developed should facilitate molecular diagnosis, carrier screening, and genetic counseling in this population. Two mutations in the *GJB2* gene account for a high percentage of nonsyndromic recessive deafness in Ashkenazi Jews.<sup>16,24</sup> According to our findings, Ashkenazi children with profound prelingual deafness that is not associated with *GJB2* mutations should be tested for R245X and undergo ophthalmologic evaluation, including fundoscopic examination and electroretinography, to detect pre-

symptomatic retinitis pigmentosa. An early diagnosis of the type 1 Usher syndrome should direct anticipatory intervention to prepare for the progressive loss of vision, which eventually negates the usefulness of visual sign language as a mode of communication. Although conventional amplification is inadequate to rehabilitate the profound level of hearing impairment satisfactorily, cochlear implantation can be more effective.<sup>26</sup> The ability to see and read lips is a critical component of speech and hearing rehabilitation after cochlear implantation.<sup>26,27</sup> Thus, improved outcomes in communication skills may be expected in these patients if the procedure is performed before substantial loss of sight occurs.

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