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The R245X Mutation of *PCDH15* in Ashkenazi Jewish Children Diagnosed with Nonsyndromic Hearing Loss Foreshadows Retinitis Pigmentosa

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ABSTRACT

Usher syndrome is a frequent cause of the combination of deafness and blindness due to retinitis pigmentosa (RP). Five genes are known to underlie different forms of Usher syndrome type I (USH1). In the Ashkenazi Jewish population, the R245X mutation of the *PCDH15* gene may be the most common cause of USH1 (Ben-Yosef T, Ness SL, Madeo AC, Bar-Lev A, Wolfman JH, Ahmed ZM, Desnick RK, Willner JP, Avraham KB, Ostrer H, Oddoux C, Griffith AJ, Friedman TB N Engl J Med 348: 1664–1670, 2003). To estimate what percentage of Ashkenazi Jewish children born with profound hearing loss will develop RP due to R245X, we examined the prevalence of the R245X *PCDH15* mutation and its carrier rate among Ashkenazi Jews in Israel. Among probands diagnosed with nonsyndromic hearing loss not due to mutations of connexin 26 (*GJB2*) and/or connexin 30 (*GJB6*), and below the age of 10, 2 of 20 (10%) were homozygous for the R245X mutation. Among older nonsyndromic deaf individuals, no homozygotes were detected, although one individual was heterozygous for R245X. The carrier rate of the R245X mutation among the normal hearing Ashkenazi population in Israel was estimated at 1%. Ashkenazi Jewish children with profound prelingual hearing loss should be evaluated for the R245X *PCDH15* mutation and undergo ophthalmologic evaluation to determine whether they will develop RP. Rehabilitation can then begin before loss of vision. Early use of cochlear implants in such cases may rescue these individuals from a dual neurosensory deficit. **(*Pediatr Res* 55: 995–1000, 2004)**

Abbreviations

ERG, electroretinograms
SNHL, sensorineural hearing loss
RP, retinitis pigmentosa
USH1, Usher syndrome type 1
USH2, Usher syndrome type 2
USH3, Usher syndrome type 3
NSHL, nonsyndromic hearing loss
PCDH15, protocadherin 15
ASPCR, allele-specific PCR

Usher syndrome accounts for more than 50% of the deaf-blind population (1), and approaches a prevalence of 1/10,000 between the ages of 30 and 49 (2). This syndrome is defined by SNHL, vestibular dysfunction, and progressive RP, eventually leading to blindness (3). Three clinical subtypes are described: USH1 is the most severe, characterized by congenital profound deafness, onset of RP in the first decade of life and constant vestibular dysfunction. USH2 is distinguished from USH1 by a mild to severe hearing loss with a downsloping audiogram configuration and intact caloric reflexes. USH3 is distinct from the others based upon the progressive nature and delayed onset

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of the hearing loss and the occasional presence of vestibular dysfunction (4, 5).

Genes for seven USH1 loci have been mapped (4, 6) and five have been cloned (7–11). Myosin VIIA (*MYO7A)* underlies USH1B, as well as NSHL DFNA11 and what was originally described as DFNB2, but is now considered to be atypical USH1 (10, 12–15). Mutations of cadherin 23 (*CDH23*) (8, 16) are responsible for both USH1D and NSHL DFNB12. USH1C and NSHL DFNB18 are caused by allelic mutations of the *USH1C* gene, encoding harmonin (7). USH1G is caused by mutations of the *USH1G* gene, encoding SANS (11). Defects in protocadherin 15, encoded by *PCDH15*, cause USH1F and NSHL DFNB23 (17–19).

PCDH15 is located on chromosome 10q11.2-q21. Protocadherins are thought to be involved in neural development, neural circuit formation, and formation of the synapse (20). Mice carrying two mutations of *Pcdh15* show disorganization in the orientation of the stereocilia, which are finger-like projections from the apical surface of hair cells of the inner ear and are necessary for mechanosensory transduction of sound, and linear and angular acceleration of the head (21).

In the Ashkenazi Jewish population, the R245X mutation of *PCDH15* accounts for more than half (58.3%) of USH1 cases (22). The Israeli population is genetically heterogeneous and divides into three main ethnic groups, the Ashkenazi Jews originating from Europe, the Sephardic Jews from the Mediterranean Sea area, and the Oriental Jewish community, who came from the Middle East. In the Ashkenazi Jewish population, two mutant alleles of connexin 26 (*GJB2),* 35delG and 167delT, account for the majority, but not all cases of nonsyndromic recessive deafness (23–26). In addition, a deletion in the connexin 30 (*GJB6)* gene, in trans configuration with one of the *GJB2* mutations, has been shown to be associated with deafness in this population (27, 28). In the present study, we report the estimated prevalence of the R245X mutation among Ashkenazi Jewish deaf probands in Israel, and carrier rates found in the population for this mutation. We also determined that the putative M1853L mutation (22) is a nonpathogenic change in *PCDH15*. Since the R245X mutation of *PCDH15,* which is known to be involved in USH1F, is relatively common in this population (22), some children incompletely diagnosed with NSHL may have two R245X alleles. Molecular diagnosis of R245X would thus provide important presymptomatic detection of USH1, enabling optimal rehabilitation of communication in anticipation of loss of vision.

METHODS

Subjects

Hearing-impaired probands were ascertained through genetics clinics at the Haim Sheba Medical Center, Rabin Medical Center, Hadassah Medical Center, and the Wolfson Medical Center, Israel. Family histories were obtained, as well as a complete clinical history of each affected individual to ensure that no obvious environmental factors were involved in the hearing loss. Audiograms were obtained from each proband. Screening for the R245X mutation of PCDH15 was performed in 59 Ashkenazi probands that were referred with a diagnosis of congenital NSHL, including children and adults, after excluding mutations in *GJB2* and *GJB6*. Funduscopy and ERG examinations were performed in two members of one family, under the age of 10, that were homozygous for the R245X mutation, and in an additional 13-y-old proband diagnosed with USH1 based on congenital hearing loss and RP (not included among the 59 probands diagnosed with NSHL).

The project was approved by Helsinki (Institutional Review Board) Committees at Tel Aviv University, Haim Sheba Medical Center, Rabin Medical Center, Hadassah Medical Center, and the Wolfson Medical Center, Israel and by the NINDS/NIDCD institutional review board at the National Institutes of Health, Bethesda, MD, U.S.A. Blood samples were drawn after obtaining informed consent from each individual and, in case of individuals under 18 y of age, from their parents.

Anonymous DNA samples from 505 hearing Ashkenazi Jews, obtained from Sheba Medical Center and from the Rabin Medical Center, were used as controls.

Experimental Methods

Mutation exclusion of GJB2 and GJB6. The open reading frame of *GJB2* was examined for mutations as described previously (25). Briefly, primers GJB2–1F, 5'-TCTTTTCCAGAGCAAACCGC-3', and GJB2–2R, 5'-GGGCAATGCGTTAAACTGGC-3' amplified a 722 bp fragment that was sequenced and analyzed for the presence of mutations. For *GJB6*, a deletion [Δ(GJB6-D13S1830)] identified in Ashkenazi Jewish and Spanish hearing-impaired individuals (28, 29) was analyzed in our DNA samples. Primers GJB6–1R, 5'-TTTAGGGCATGATTGGGGTGATTT-3', designed 244 bp upstream of the proximal breakpoint of the deletion and GJB6-BKR-1, 5'-CACCATGCGTAGCCTTAACCATTTT-3', localized 216 bp downstream of the distal breakpoint of the deletion, amplified a 460 bp fragment encompassing the deletion breakpoint if it was present. To positively detect a wild-type product, we designed a third primer localized 681 bp downstream of the GJB6–1R primer that was included in the same reaction, GJB6-RVS2, 5'-TCATCGGGGGTGTCAACAAACA-3'. Wild-type DNA yielded a 681 bp band.

Mutation detection assays for R245X. To detect the R245X mutation by allele-specific PCR (ASPCR), two PCR reactions were performed for each DNA sample, as described by Ben-Yosef *et al.* (22). We also used a restriction enzyme digestion assay because the R245X mutation creates an *Hph*I restriction enzyme site. A 553 bp segment of *PCDH15* exon 8 was PCR-amplified by the primers 361F (5'-ATA ACC ATG TTG GAC TGTTGTTTC-3') and 914R (5'-ATGTTTGCCAGGCTGGTATCAAAC-3'). PCR cycling conditions were 95°C for 5 min, followed by a touchdown program of 60°C to 56°C and 30 cycles of 95°C for 45 s, 56°C for 30 s, and 72°C for 30 s, followed by two steps of 72°C and 4°C for 10 min each. The PCR product was digested with *Hph*I (37°C for 10 h) and separated by electrophoresis on a 2% agarose gel. Expected product sizes are 553 bp for the wild-type allele and 372 bp and 181 bp for the mutant allele.

For sequence analysis, a 367 bp segment of exon 8 was amplified by PCR using primers F (5'-TGCCTAATTTC-

TATAAACTACCTGTTG-3') and R (5'-CCCTGA AAATA-ATTTCGGACA-3'). The PCR products were purified by QIAquick PCR Purification Kit (QIAGEN, Valencia, CA, U.S.A.). Sequencing was performed using the same primers as those used for PCR, the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, U.S.A.) and an ABI 377 DNA sequencer. Mutations were confirmed by sequencing in both directions or by using at least two assays described above.

Detection of M1853L. A restriction enzyme digestion assay was performed for detection of the 5556A→C transversion that leads to M1853L. A 553 bp segment of *PCDH15* exon 33 was PCR-amplified by the primers F (5'-CTACCTCCATTTC-CAACTCCTCT-3') and R (5'-ATTTCATTGAATTTGGGG-TAAAAT-3'). PCR conditions were as described above, with touchdown annealing temperatures of 56°C to 51°C and 45-s denaturation, annealing, and elongation times. The PCR product was digested by *Tse*I (65°C for 10 h) and separated by electrophoresis on a 2% agarose gel. A TseI site is inserted due to the mutation. Expected product sizes are 553 bp for the wild-type allele, and 427 bp and 126 bp for the mutant allele.

RESULTS

R245X mutation detection. The *PCDH15* gene contains 33 exons and is predicted to encode a protein of 1955 amino acids (18). The R245X mutation is caused by a C to T transition at position 733 in exon 8 of the *PCDH15* gene, and leads to the substitution of an arginine codon by a stop codon (GenBank accession number AY029237). Because this mutation was reported only among Ashkenazi Jews, and was not detected in other Jewish ethnic groups (22), we ascertained 59 Ashkenazi Jewish individuals diagnosed with congenital NSHL who did not have mutations in *GJB2* or in *GJB6*. Twenty of the probands were under the age of 10, and 39 were over 10 y old. We chose this age for stratification because this is typically the age of onset of loss of vision for individuals with USH1 (4). In the first group of probands under 10, 18 of 20 were found to have two wild-type *PCDH15* alleles, whereas 2 of 20 (10%) were homozygous for R245X. In the older group, no homozygotes for R245X were detected; 38 of 39 were wild type and 1 of 39 (2.6%) was heterozygous (carrier) for R245X (Table 1). The deaf individual heterozygous for the R245X mutation did not carry any other *PCDH15* mutations in the reported 33 exons of *PCDH15*, either suggesting that we have not yet found the partnering mutant allele of *PCDH15* or this person is a coincidental carrier of R245X. At least two different assays were performed to confirm the presence of R245X (data not shown).

The family members of the two homozygous R245X young probands were also screened for R245X. Family T292 (Fig. 1*A*) includes parents, two deaf children, 4 and 7 y of age, both with cochlear implants, and two unaffected children. Both parents were heterozygous for the R245X mutation, the two deaf children were homozygous for the mutation, one unaffected child was heterozygous and in the other unaffected child R245X was not detected. The older deaf child manifested a delay in motor development and walked independently at 2 y, which is consistent with the USH1 phenotype (3). Hearing loss was detected at 10 mo, and was found to be profound, with an unaided corner audiogram (thresholds of 100 dBHL at 250 Hz and 110 dBHL at 500 Hz). Aided thresholds were 60 dBHL at 250 Hz and 90 dBHL at 500 Hz, with no response at higher frequencies. The child received a cochlear implant at the age of 3 y and 4 mo. He has used the implant for 4 y, with very good results in open set tests (60% in one-syllable-word identification and 80% in both two-syllable-word identification and in words in sentences). He uses oral communication.

Ophthalmologic examination at age 7 revealed a corrected visual acuity of 6/9 in each eye. The ocular media was transparent by slit lamp biomicroscopy and the intraocular pressure was 12 mm mercury in both eyes. Color vision examined by Ishihara pseudochromatic plates showed normal results, whereas the tangent screen visual field test with a 5 mm test object indicated severe peripheral visual field construction to about 20 degrees diameter for each eye. The retinal examination through the slit lamp in conjunction with a Volk aspheric +78 diopter lens revealed narrow retinal vessels with very fine pigmentary stippling in the retinal periphery up to the vascular arcade. ERG was performed with a Nicolet CA1000 instrument with Henkes type contact lens applied on the patient's anesthetized cornea after maximal pupillary dilatation. Standard full-field flash eye stimulation under light and dark adapting conditions revealed severely reduced photopic responses and nearly absent scotopic responses, consistent with Usher syndrome.

In the 4-y-old child in Family T292, hearing loss was detected at 4 mo and was profound. He received a cochlear implant when he was 1 y and 3 mo old. He has used the implant for 2 y and is able to identify two-syllable words only in closed set tests. Results of a funduscopic examination performed at the age of 4 were normal in this child. An ERG was not available.

In Family T282 (Fig. 1*A*), the deaf children were 4 and 5 y old and both underwent cochlear implantation. Both deaf children were homozygous for R245X. The two deaf children had delays in motor development and were reported by the

Table 1. *R245X mutation frequencies among Ashkenazi Jewish probands in Israel diagnosed with deafness and carrier rate of this mutation in a control group*

Probands	Number tested	Wild type	Heterozygous	Homozygous
Congenital deafness, under 10 y old	20	18	—	2† (10%)
Congenital deafness, 10 y old and above	39	38	1* (2.6%)	—
Hearing individuals	505	500	5 (0.99%)	—

* The DNA from this deaf individual was sequenced for the reported coding exons of *PCDH15* and a second mutation was not detected.
† Probands from families T282 and T292 (see Fig. 1).

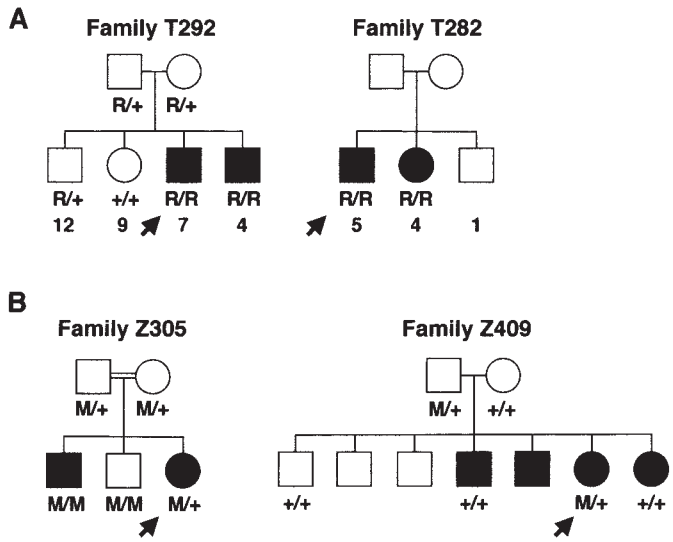

Figure 1. Pedigrees of families analyzed for mutations in *PCDH15*. Probands are indicated by arrows. (*A*) Families in which probands are homozygote for the R245X mutation of *PCDH15*. *R* refers to R245X mutation and + refers to the wild-type *PCDH15* allele. Numbers refer to ages of each child. (*B*) Families in which probands are heterozygous for the M1853L polymorphism of *PCDH15*. *M* refers to the rare M1853L polymorphism and + refers to wild-type *PCDH15* allele.

parents to have balance difficulties. In the older child, hearing loss was detected at 1 y and was profound. He received a cochlear implant when he was 1 y and 10 mo old and has used the implant for 3 y and 5 mo. He has open set recognition for familiar words.

In the younger child, hearing loss was detected at 3 mo and was profound. She received a cochlear implant when she was 1 y and 6 mo old and has used her implant for 2 1/2 y and has very good open set results. Ophthalmologic examinations have not been performed in members of Family T282.

One 13-y-old proband already diagnosed with USH1 based on ophthalmologic examinations, including an ERG, was found to be homozygous for the R245X mutation. This finding further confirms that the R245X mutation is associated with USH1.

R245X carrier frequency. Anonymous DNA samples (n = 505) of hearing Ashkenazi Jews were screened for the R245X mutation by the ASPCR assay and if the mutation was detected, it was confirmed by sequencing exon 8 of *PCDH15*. Five out of 505 (0.99%; 95% confidence interval, 0–2%) representative Ashkenazi Jews were heterozygous for R245X (Table 1). Because R245X was not detected among 293 non-Ashkenazi Jews screened elsewhere, and the mutation is assumed to result from a founder effect in the Ashkenazi group (22), no other ethnic Jewish groups were screened.

Screening for M1853L. A putative *PCDH15* mutation, M1853L, was previously found in compound heterozygosity in one Ashkenazi Jewish USH1 patient (22). Because carriers for M1853L were found for both Ashkenazi and non-Ashkenazi Jews (with carrier rates higher in the latter group) (22), we screened non-Ashkenazi deaf probands (n = 90) for the M1853L putative mutation of *PCDH15*. Eighty-eight were wild type and two were heterozygous for M1853L; one originated from Iraq (Oriental; Family Z305) and the second from Morocco (Sephardic; Family Z409). The parents in Family Z305 were heterozygous, and one deaf brother and one unaffected brother were homozygous for M1853L (Fig. 1*B*). In Family Z409, the father was heterozygous for M1853L but the mother was wild type. Two deaf and one hearing sibling were wild type (Fig. 1*B*). Therefore, based on our data, M1853L appears to be a benign polymorphism (*i.e.* not a pathogenic mutation of *PCDH15*).

DISCUSSION

Our results confirmed the prevalence of the R245X mutation, associated with USH1 in the Ashkenazi Jewish population, as reported by Ben-Yosef *et al.* (22). The R245X mutation generates a protein translation stop codon in exon 8 of *PCDH15*, the gene encoding protocadherin 15, and presumably results in a truncated form of protocadherin 15. Alternatively, there may be no protein formed due to nonsense-mediated decay (30). Most significantly, we report that this mutation was found in children under 10 y of age who were inadvertently diagnosed with NSHL, where hearing loss is the only symptom, before the age that RP develops. Furthermore, we tested a cohort of 59 individuals that included 39 over the age of 10 to exclude involvement of the R245X mutation in NSHL. We did not discover a homozygote R245X mutation in any individuals diagnosed with NSHL over the age of 10, further demonstrating that this mutant allele of *PCDH15* is associated with RP in late childhood as well as hearing loss (7, 17, 22).

Among the 20 probands under the age of 10 y having no mutations in *GJB2* and *GJB6*, we identified two (10%) that were homozygous for the R245X mutation. The two probands homozygous for the R245X mutation manifested delays in motor development, which is characteristic of vestibular dysfunction. One 7-y-old proband who underwent ophthalmologic consultation showed an abnormal fundus and ERG, including diffuse retinal dystrophy and visual field constriction, whereas his 4-y-old brother's results (homozygous for the R245X mutation) were within normal limits according to the funduscopy. Because onset of RP in USH1 is prepubertal, visual anomalies in the younger child may develop later.

In addition to the prevalence of the R245X mutation among children diagnosed with NSHL, we found a carrier rate of 1% among the Ashkenazi Jewish population examined in Israel, which is comparable to 0.79–2.47% among Ashkenazi Jews, reported by Ben-Yosef *et al.* (22). This has led us to propose that all Ashkenazi probands with NSHL under the age of 10 in whom *GJB2* and/or *GJB6* mutations have been excluded as the reason for the hearing loss should be screened for the R245X mutation to enable presymptomatic diagnosis of USH1. Because a *GJB2* mutant allele, 167delT, is carried by approximately 4% of the Ashkenazi Jewish population and another allele, 35delG, is carried by 0.21–1.1% of this population (23–26), individuals homozygous for the R245X mutation of *PCDH15* might also be coincidental carriers of one of these *GJB2* mutations. The presence of only one *GJB2* mutant allele should not preclude screening for R245X in a deaf Ashkenazi Jewish child.

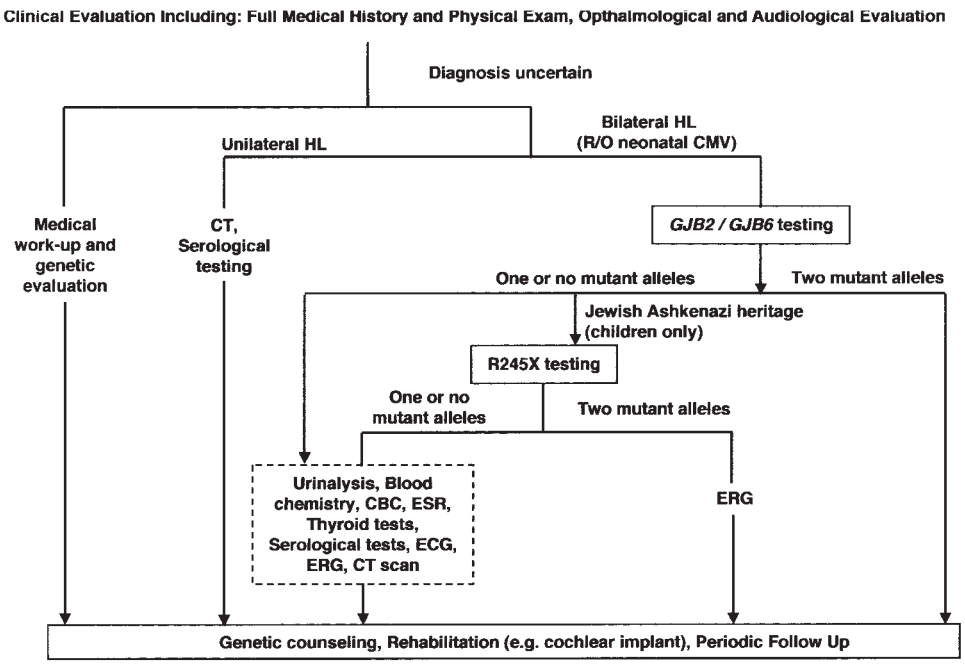

Figure 2. Diagnostic algorithm for sporadic or recessively inherited SNHL with R245X testing for Ashkenazi Jewish children. If there is a family history of hearing loss, genetic evaluation should be performed; for sporadic cases, it is more cost-efficient to start with genetic testing and then extend the analysis only if no mutations are found. *HL*, hearing loss; *SHL*, syndromic hearing loss; *CT*, computed tomography; *ESR*, erythrocyte sedimentation rate; *CBC*, complete blood count.

Early diagnosis is especially crucial when deaf children will develop blindness as well. In such cases, oral language may be the optimal mode of communication inasmuch as sign language and lip reading are both visual. There is a need to provide these children with the best hearing amplification available, with a preference for cochlear implantation if possible, accompanied by intensive training and habilitation. A recent study of Usher syndrome suggests that auditory-oral communication is more successful if cochlear implants are implemented before the onset of retinal degeneration, in conjunction with speech therapy (31). The earlier the diagnosis is made, the better chance these children have for being able to communicate optimally in society, even after they have lost a portion or all of their vision as well. In addition, early diagnosis of Usher syndrome might allow for an enhanced social and emotional adjustment for the family, recurrence risk counseling of at-risk couples, and the option for prenatal diagnosis for at-risk couples.

We suggest a diagnostic algorithm for children presenting with sporadic or recessively inherited SNHL, taking the American College of Medical Genetics statement into consideration (32) (Fig. 2). The routine implementation of comprehensive tests, such as brain computed tomography, is cost ineffective and may be circumvented in many cases by less costly molecular diagnostic tests (33). In addition, based on our data, we recommend testing for R245X in children under the age of 10 of Ashkenazi Jewish descent with little or no family history of NSHL who are heterozygous or negative for a mutation of either *GJB2* or *GJB6*. This molecular test may identify impending RP before detection by ERG, and permit timely rehabilitation and genetic counseling for the parents.

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