ONLINE MUTATION REPORT

Sensorineural hearing loss, striate palmoplantar hyperkeratosis, and knuckle pads in a patient with a novel connexin 26 (*GJB2*) mutation

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Mutations in connexin 26 (*GJB2*, *Cx26*) cause autosomal recessive and occasionally dominant non-syndromic sensorineural hearing loss (SNHL). *Cx26* mutations have also been identified in SNHL with dermatological features of autosomal dominant diffuse palmoplantar hyperkeratosis (DPPK).¹⁻³

We describe a girl with bilateral sloping sensorineural hearing loss, striate palmoplantar hyperkeratosis (SPPK), and knuckle pads, who has a novel heterozygous missense mutation (G59R) in the connexin 26 gene (*GJB2*, *Cx26*). This mutation resides in the same codon in which a mutation was previously described for a family with dominant sensorineural hearing loss with a diffuse palmoplantar hyperkeratosis (DPPK).¹ *Cx26* mutations have not been reported previously with the striate form of palmoplantar hyperkeratosis nor with knuckle pads.

Mutations in *Cx26* have also been described in a severe condition of congenital deafness, keratopachydermia, and constrictions of fingers and toes (Vohwinkel syndrome; OMIM 124500)^{4,5} as well as in KID syndrome (OMIM 148210)⁴ with keratitis, ichthyosis, and deafness.^{6,7} To date, mutations in *Cx26* have not been associated with a striate subtype of palmoplantar hyperkeratosis (SPPK) or with knuckle pads.

CASE REPORT

We report an 8 year old girl who had congenital onset of bilateral non-progressive SNHL, mild (30–40 dB) in the low frequencies (250–500 Hz), increasing to severe (75 dB) in the higher frequencies (≥ 2000 Hz). At 6 years of age she developed striate linear lesions of keratoderma on the palms (fig 1A) and knuckle pads on the dorsum of fingers (fig 1B). Striate linear lesions on the soles and mild hyperkeratosis over one elbow were developing. She had mild hyperkeratotic lesions on the lateral aspect of her feet. She showed no evidence of restricting lesions or diffuse keratoderma. Teeth, hair, and nails were normal with no evidence of leuconychia. She was healthy with no risk factors for acquired hearing loss. Complete clinical examination identified no additional syndromic forms of hearing loss. Ultrasound of the kidneys, CT scan of the internal auditory canals, electrocardiogram, and thyroid function were normal. There were no other family members with hearing loss or dermatological conditions. Her mother showed mild thickening on the medial aspect of her index finger, but no PPK or knuckle pads. The thickening was felt to be secondary to repetitive use. The child's father and sister were healthy.

MATERIALS AND METHODS

Cx26 (*GJB2*) gene sequencing

DNA was obtained from the patient's peripheral blood leukocytes using a Qiagen QIAamp DNA mini kit. PCR

Key points

- Mutations in connexin 26 (*GJB2*, *Cx26*) cause autosomal recessive and dominant non-syndromic sensorineural hearing loss (SNHL).
- *Cx26* mutations have also been identified in SNHL with dermatological features of autosomal dominant diffuse palmoplantar hyperkeratosis (DPPK), Vohwinkel's syndrome and keratitis, ichthyosis and deafness (KID) syndrome.
- We describe a girl with bilateral sloping sensorineural hearing loss, striate palmoplantar hyperkeratosis (SPPK), and knuckle pads, who has a novel heterozygous missense mutation (G59R) in *Cx26*.
- This mutation resides in the same codon in which a mutation was previously described for a family with dominant sensorineural hearing loss with diffuse palmoplantar hyperkeratosis (DPPK).¹
- The clinical features of our case expand the phenotype of dermatological conditions that can be seen with *Cx26* mutations.

amplification of exon 2 of *Cx26* was performed using the following primer sets: CX26SEQ1L (CAAACCG–CCCAG AGTAGAAG), CX26SEQ1R (GCGGACCTTCTGGGTTTT); CX26SEQ2L (GAAGTTCATCAAGGGGGAGA), and CX26SEQ2R (CCTCATCCCTCTCATGCTGT). In each reaction, 150 ng of DNA was amplified with BRL platinum *Taq* polymerase under the following conditions: 94°C for 2 minutes, 30 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, followed by a 10 minute incubation at 72°C. The PCR products were purified using Edge BioSystems QuickStep 2 PCR purification kits. Sequencing reactions were performed using BigDye Terminator 3.0 (Applied Biosystems) according to the manufacturer's instructions. Sequencing products were cleaned using Performa Dye Terminator Removal Gel Filtration Cartridges (Edge BioSystems). Sequencing reactions were electrophoresed on a 5.3% acrylamide and 6 mol/l urea gel with an ABI 377 Gene Analyzer. To verify the connexin 26 mutation in our patient, we sequenced the *Cx26* gene using alternate primers: C261F (TATGTTCTGTGTTGTGTGCAT), C261R (TTTGATCTCTCG ATGTCCTTA); CX262F (GTGGCCTACCGGAGACAT), CX262R (CCCTCTCATGCTGTCTATTCTT).

Cx30 (*GJB6*) gene dosage analysis

The patient sample was screened for dosage within the *Cx30* region previously reported to be deleted in *trans* with

Figure 1 (A) Striate palmar hyperkeratosis, well demarcated; (B) knuckle pads on the dorsum of fingers. Reproduced with permission.

mutations in *Cx26*. The reaction consisted of a multiplexed array of two quantitative reactions: a *TaqMan GJA5* gene pre-developed assay reagent (PDAR; Applied Biosystems) as a two copy control and the *Cx30* amplification, which used the following primers and probe: GJB6F (TCTAGGATGCGGGTTTCAGTTC), GJB6R (CCCATACCTGCGGTTCTC) and the *TaqMan* probe VIC (ACCCCTCAACTTGGGCMGBNFQ). All real time PCR amplifications were carried out in triplicate on an ABI Prism 7900HT sequence detection system (Applied Biosystems) using a 384 well plate. Each reaction mixture consisted of 200 nmol/l of each primer, 50 nmol/l of *TaqMan* probe, 40 ng of genomic DNA, 0.3 µl of PDAR (Applied Biosystems), and 7.5 µl of 2× *TaqMan* mix (Applied Biosystems) in a total volume of 15 µl. Thermal cycling conditions included a pre-soak for 2 minutes at 50°C and 10 minutes at 95°C. Samples were amplified for 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. Sequence detection software (SDS version 2.0; Applied Biosystems) was used to process raw fluorescence data and produce a threshold cycle number (Ct) for each sample. The Ct value was the cycle number at which the fluorescence emission of the reporter dye passed a fixed threshold on the amplification plot. The default setting for the threshold was 10 SD above the mean baseline emission. All Ct settings were manually checked and adjusted within the logarithmic curve between background and plateau levels. The ABI 7900HT data were exported to a Microsoft Excel macro in which all Ct values were normalised to the multiplexed PDAR and compared with the wild type values.

Mitochondrial testing

The entire mitochondrial genome was screened for mutations using an array of 19 amplified PCR products (MitoScreen™ assay kit; Transgenomic). The manufacturer's protocol was followed using multiplex dHPLC analysis of amplified products (WAVE® system; Transgenomic). A PCR product amplified from mitochondrial region 6318–7707 bp was also sequenced, using BigDye Terminator 3.0 (Applied Biosystems) as outlined above.

RESULTS

Sequencing of the *Cx26* (*GJB2*) gene within exon 2 identified a single de novo mutation G59R (175G→C) (fig 2). Confirmation of this mutation was achieved by amplifying and sequencing the surrounding region using independently placed primer sets (see above). The G59R mutation has not been previously described but is within the same codon (G59A) as reported in a family with diffuse PPK and SNHL.¹ The G59R mutation was not detected in either parent. No mutation was detected on the second allele. Parentage was confirmed by a genomewide multiplex analysis of 15 tetranucleotide repeat loci. The G59R mutation was not detected in 85 unrelated control subjects with SNHL who had no signs of PPK on full clinical examination.

It has been reported that some non-syndromic hearing impaired patients with only one *GJB2* mutation have in *trans* deletions within the *GJB6* gene.^{8–9} To rule out this possibility in our patient, we analysed *GJB6* gene dosage using real time PCR. There were no cycle threshold (Ct) differences between normal control samples and the patient, indicating that our patient does not have a deletion within the 5' UTR of *Cx30* (data not shown).

Similarly, screening the entire mitochondrial genome of this case using multiplex dHPLC analysis and sequencing the mitochondrial genome from 6318 to 7707 bp ruled out the presence of the serine tRNA 7445A→G mutation.^{10–12}

DISCUSSION

Our patient shows clinical features of SPPK and SNHL (OMIM 148350),⁴ which is inherited as an autosomal dominant trait. In addition, she has knuckle pads, which have been described in Bart-Pumphrey syndrome (OMIM 149200),⁴ a condition with DPPK and leuconychia. Our patient may represent a variant form of Bart-Pumphrey as not all individuals with the condition have leuconychia.¹³ To date, a gene for Bart-Pumphrey has not been reported in the literature.

The G59R mutation probably arose as a new event for our patient. Although SNHL with PPK has been described as an autosomal dominant condition, an autosomal recessive pattern of inheritance with an unidentified mutation on the opposite allele or mutations at another site cannot be excluded, nor can mutations in other genes implicated in PPK. The common region deleted in *Cx30* was not detected in our case.

Isolated striate subtype of PPK without SNHL has been caused by mutations in desmoplakin (DSP),¹⁴ keratin 1 (KRT1),¹⁵ and desmoglein 1 (DSG1).¹⁶ Non-epidermolytic

Figure 2 Sequence chromatogram of connexin 26 (*GJB2*) showing the sense (left panel) and antisense (right panel) sequences for (A) the 175G→C (G59R) mutation and (B) the wildtype sequence.

palmoplantar keratoderma without knuckle pads has been seen in association with SNHL and the mitochondrial serine tRNA 7445A→G mutation.^{10–12} Our case did not show any evidence of the 7445A→G mutation, nor any mitochondrial point mutations.

This is the first report of striate form of palmoplantar keratoderma (SPPK) with knuckle pads being associated with a Cx26 mutation. As this is a sporadic case, additional reports of PPK and SNHL with a similar mutation will be necessary to confirm the pathogenicity of the mutation and to establish that PPK and SNHL do not occur together by chance. Heathcote and colleagues¹ have described a family with diffuse PPK and SNHL at the same amino acid, lending additional support to this notion. Functional assays of connexin 26 with a G59R mutation would help to provide additional information. Individuals with striate PPK and/or knuckle pads with SNHL should have the option of Cx26 screening. Our case illustrates the variable phenotype that can be seen in individuals with Cx26 mutations.

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