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Congenital non-syndromal sensorineural hearing impairment due to connexin 26 gene mutations — molecular and audiological findings

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Abstract

We screened DNA from 72 sibships and 138 sporadically affected individuals with congenital non-syndromal sensorineural hearing impairment (NSSNHI) for mutations in the 26 (*CX26*) gene. A total of 20 (27.8%) of the sibships and 11 (7.9%) of the sporadically affected individuals were homozygous or compound heterozygotes for *CX26* mutations. A total of 11 (17.2%) of 64 individuals with severe and 30 (30%) of 100 with profound NSSNHI compared to eight (8.7%) of 92 persons with moderate and none (0%) of 19 individuals with mild hearing impairment were homozygous or compound heterozygotes for *CX26* mutations (χ^2 test, 3 df, $P = 0.000$). *CX26* mutation status had no effect on the symmetry of the hearing impairment or configuration of the audiogram. In addition, serial audiograms showed no evidence of progression of the hearing impairment or differences in the severity of the hearing impairment in affected siblings in persons whether or not due to *CX26* mutations. Sporadically affected individuals with congenital NSSNHI should be routinely tested for mutations in *CX26*, especially if the hearing impairment is severe or profound in severity, since identification of a mutation in *CX26* allows use of Mendelian recurrence risks.

Keywords: Non-syndromal sensorineural hearing impairment; Connexin 26; Mutations; Audiology

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1. Introduction

Epidemiological studies have shown that ~1 in 1000 children are born with or present in early childhood with severe or profound hearing impairment [1,2], of which approximately one-half are due to a genetic cause. In approximately one-third of these the hearing impairment occurs in association with other physical features or medical problems, i.e. is syndromal, in two-thirds the hearing impairment occurs in isolation, i.e. is non-syndromal [2–4]. Approximately three-quarters of inherited non-syndromal hearing impairment is due to an autosomal recessive gene, one-quarter due to an autosomal dominant gene and the remainder being due to X-linked genes or mitochondrial inheritance [3,5,6]. The results of segregation studies of the offspring of normal hearing parents with deaf parents and deaf parents with deaf parents have estimated that the number of genes responsible for non-syndromal inherited hearing impairment could be as few as five and as many as 100 or more [5,7].

Recent progress in the identification of genes for inherited hearing impairment has shown it to be markedly genetically heterogeneous with 21 autosomal recessive loci (DFNB) and 19 autosomal dominant loci (DFNA) reported to date [8]. In the last 2 years, significant progress has been made in the identification of six genes responsible for autosomal recessive non-syndromal hearing loss (Table 1) [9–15]. Mutations in *CX26*, MYOVIIA and TECTA can also cause dominantly inherited hearing impairment [9,16–18] while mutations in the MYOVIIA and PDS genes were originally reported as causing syndromal forms of inherited hearing impairment [19,20].

Table 1
Autosomal recessive non-syndromal sensorineural hearing impairment loci for which the gene has been cloned

Locus	Gene	Reference
DFNB1	Connexin 26 (*CX26*)	[9]
DFNB2	Myosin VIIA (*MYOVIIA*)	[10,11]
DFNB3	Myosin 15 (*MYOXV*)	[12]
DFNB4	Pendrin (*PDS*)	[13]
DFNB5	Alpha tectorin (*TECTA*)	[14]
DFNB6	Otoferlin (*OTOF*)	[15]

A number of studies have revealed that up to 60% of autosomal recessive families and up to all 40% of sporadic cases NSSNHI are caused by mutations in the *CX26* gene which codes for the gap junction beta-2 (GJB2) protein [21–25]. To date, more than 30 different mutations have been identified in the coding sequence of the *CX26* gene, including conserved amino acid substitutions, base transversions and small deletions/insertions introducing frameshifts causing premature peptide truncation, the majority being due to a single base pair deletion known as 35delG [21–32].

Most of the reports published to date provide limited information of the audiological characteristics of the hearing impairment due to mutations in *CX26* [21–24,26–31]. We wanted to assess in detail whether there was any evidence of particular audiological characteristics when the hearing impairment is due to mutations in *CX26*. This would be important in appropriately directing samples for mutation screening as well in recurrence risk advice in genetic counselling for families with sporadically affected children with NSSNHI.

2. Materials and methods

2.1. Individuals/families

A cohort of 284 individuals with a congenital non-syndromal sensorineural hearing impairment consisting of 146 individuals from 72 sibships (made up of 70 sib-pairs and two sibships of three affected individuals) and 138 sporadically sporadically affected persons were ascertained from a variety of sources including specialists in ENT, Audiological Medicine and Clinical Genetics, the Family Fund and the British Deaf Association Newsletter. All of the children had a congenital hearing impairment but varied in their age of diagnosis which varied from the first year to 3 years of age, often being later if the hearing impairment was mild or moderate in severity. Individuals or families were excluded from the

study if there was a family history of hearing impairment in previous generations, a recognised environmental cause was identified or if the results of physical examination or routine investigations revealed a syndromal cause.

The study was approved by the Ethical Committee of the St James's NHS University Hospital Trust. Informed consent was obtained from all subjects or the parents of children.

2.2. Laboratory methods

A total of 10 ml venous blood was collected from each individual and DNA extracted from peripheral blood lymphocytes using a standard extraction procedure. PCR amplification of the entire coding sequence of the *CX26* gene was carried out using four primer sequences: 5′-TCTTTTTCCAGAGCAAACCGC-3′ with 5′-GACACGAAGATCAGCTGCAG-3′ and 5′-GGGCAATGCGTTAAACTGGC-3′ with 5′-CCAGGCTGCAAGAACGTGTG-3′. The *CX26* 5′ non-coding exon (exon 1) and flanking donor splicing sequences were amplified using the primers PP18 (5′-TCCGTAACTTTCCCAGTC-CCGAGGGA-AGAGGG-3′) and PP21 (5′-CC-CAAGGACGTGTGTTGGTCCAGCCC-3′).
PCR reactions and sequencing of the PCR products were performed as previously described [24]. The molecular results for 13 of the affected siblings and 44 of the sporadically affected individuals have been reported previously [22,24].

2.3. Audiology

2.3.1. Pure tone audiograms

Audiological testing by pure tone audiometry was performed by standard methods [33,34]. In the younger individuals (< 4 years), assessment of their hearing impairment was determined by other means such as brain stem audiometry or behavioural/performance free field audiometry limiting the analysis to the severity of the hearing impairment.

The results of the pure tone audiograms (PTAs) were classified according to severity, asymmetry/symmetry, shape and progression defined as follows [35]: Severity — The severity of the hearing impairment was classified by the average of the best ear hearing at 500, 1000, 2000 and 4000 Hz as: Mild, > 20 dB and < 40 dB; Moderate, > 40 dB and < 70 dB; Severe, > 70 dB and < 95 dB; Profound > 95 dB. Asymmetry/Symmetry — Asymmetry was defined by > 10 dB difference between the ears at least at two frequencies with a difference in the pure tone average between the ears of > 20 dB; Shape — Sloping is defined as a > 15 dB difference between the mean of 500 and 1000 Hz and the mean of 4000 and 8000 Hz, Flat is defined as < 15 dB difference in thresholds between 125 and 8000 Hz, Mid-Frequency U-Shaped is defined as > 15 dB difference between the poorest thresholds in the mid frequencies and those at higher and lower frequencies, Low Frequency Ascending is defined as > 15 dB difference from the low frequency thresholds to the higher frequencies; Progression — A deterioration of > 15 dB in the pure tone average within a 10 year period.

3. Results

3.1. CX26 mutations

In 20 (27.8%) out of 72 sibships, the hearing impairment was due to *CX26* mutations, 12 being homozygous for 35delG mutation, one homozygous for 167delT and three compound heterozygotes for 35delG and W77R, 302del3 and 310del14 (Table 2). A further 12 (16.6%) sibships were heterozygous for a *CX26* mutation, ten for 35delG and one each for 469delG and 310del14. In 11 (7.9%) of the 138 sporadically affected individuals, the hearing impairment was due to *CX26*, nine being 35delG homozygotes and two compound heterozygotes for 35delG and 167delT. A further five (3.6%) of the sporadically affected individuals were heterozygous for the 35delG. The 35delG mutation accounted for 52 (71.2%) out of 73 of the mutated alleles detected in the *CX26* gene. The children from the single Asian sibship were homozygous for the 35delG mutation. The individuals with the 167delT mutation in the *CX26* gene were of Ashkenazi Jewish ancestry.

The results of the screening for mutations in the *CX26* gene were grouped into three categories for

Table 2
CX26 mutations identified in affected sibs and sporadically affected individuals

Sibships	*CX26* mutation status			
	+/+	+/−	−/−	Total
	35delG/35delG-16[a] 35delG/W77R-1 35delG/302del3-1 167delT/167delT-1 35delG/310del14-1	35delG/−10 469delG/−1 310del14/−1	40	
Sub-total	20	12	40	72
Sporadically Affected Individuals	35delG/35delG-9 35delG/167delT-2	35delG/−5	119	
Sub-total	11	5	122	138

[a] The single sibship of Asian origin was homozygous for the 35delG mutation.

the purpose of analysis of the audiological data: homozygotes or compound heterozygotes in which the hearing impairment was certain to be due to *CX26* = +/+, heterozygotes in which the hearing impairment could be due to *CX26* = +/− and individuals in whom no identifiable mutation in *CX26* could be detected in which the hearing impairment would be due to another cause equal to −/−.

3.2. Severity of hearing impairment

The severity of the hearing impairment could be classified in 275 persons which included 250 (90.9%) Caucasians, 23 (8.4%), Asian (Indian subcontinent) of which one sporadically affected individual was the offspring of a first cousin parents and two (0.7%) Afro-Caribbeans. There were 142 (51.6%) males and 133 (48.4%) females ranging in ages from 4 to 46 years with a mean age of 9.28 years (Table 3). The severity of the hearing impairment in individuals from the sibships did not significantly differ from that seen in the sporadically affected individuals. Data on the severity of the hearing impairment was not available for nine individuals from nine sibships.

A total of 18 (94.7%) of the 19 individuals with mild hearing impairment had no identifiable mutation in the *CX26* gene, although one (5.3%) individual was heterozygous for the 35delG mutation. Nine (9.8%) of the 92 individuals with moderate hearing impairment had two mutations in the *CX26* gene, six were homozygous for the 35delG mutation, two persons were 167delT homozygotes and one was a compound heterozygote 35delG/167delT. A further five (5.4%) of those with moderate a hearing impairment were heterozygous for a *CX26* mutation all being heterozygous for the 35delG mutation. A total of 11 (17.1%) of 64 individuals with severe hearing impairment had two mutations in the *CX26* gene with ten persons being 35delG homozygotes and one individual a compound heterozygote 35delG/167delT. A further four (6.1%) individuals with severe hearing impairment were 35delG heterozygotes. A total of 30 (30%) of 100 individuals with profound hearing impairment had two identifiable *CX26* mutations, 24 being 35delG homozygotes and six compound heterozygotes, all being heterozygous for 35delG, the second mutation 302del3 in two, W77R in two and 310del14 in two. A further 18 (18%) individuals were heterozygous for a *CX26* mutation, 14 with 35delG, two with 469delG and two with 310del14.

Individuals with severe or profound hearing impairment were more likely to have two identifiable mutations in the *CX26* gene rather than mild or moderate hearing impairment (χ^2 test, 3

df, $P = 0.000$). Individuals heterozygous for a *CX26* mutation showed a similar pattern of distribution of severity of their hearing impairment to those with two identifiable *CX26* mutations (χ^2 test, 3 df, $P = 0.402$) but differed significantly from those without an identifiable *CX26* mutation (χ^2 test, 3 df, $P = 0.001$).

The severity of hearing impairment in individuals homozygous for the 35delG mutation (39 persons, mean $\pm$ 1 SD = 93.19 $\pm$ 23.07) compared to those homozygous for other mutations or compound heterozygotes (nine persons, mean $\pm$ 1 SD = 88.41 $\pm$ 25.24) was not significantly different ($P = 0.585$, Student's t-test, unpaired samples).

Table 3
Severity of hearing impairment (best ear, average 500–4000 Hz) and *CX26* status

Severity	*CX26* mutation status			
	+/+	+/−	−/−	Total
Mild	0	1 35delG/−1	18	19
Moderate	9 35delG/ 35delG-6 167delT/ 167delT-2 35delG/ 167delT-1	5 35delG/−5	78	92
Severe	11 35delG/ 35delG-10 35delG/ 167delT-1	4 35delG-4	49	64
Profound	30 35delG/ 35delG-24 35delG/ 302del3-2 35delG/ W77R-2 35delG/ 310del14-2	18 35delG/−14 469delG/−2 310del14/−2	52	100
Sub-total	50	28	197	275

Table 4
Configuration of the audiogram and *CX26* mutation status

Configuration	*CX26* mutation status			
	+/+	+/−	−/−	Total
Sloping	32	12	115	159
Flat	13	8	51	72
Mid frequency U-shaped	4	6	21	31
Low frequency ascending	0	1	5	6
Total	49	27	192	268

3.3. Audiogram configuration

Determination of the configuration of the hearing impairment was not available for 16 children as pure tone audiometry could not be reliably performed because they were too young (< 4 years of age) for audiological assessment by this method. Of the 268 individuals with pure tone audiograms in which the configuration of the hearing impairment could be classified, there was no significant difference between those without and those heterozygous for *CX26* mutation (χ^2 test, 3 df, $P = 0.453$) or those with two identifiable *CX26* mutations (χ^2 test, 3 df, $P = 0.494$) (Table 4).

3.4. Asymmetry of hearing impairment

Comparison of the average difference in the hearing impairment between the ears in those with one or two identifiable *CX26* mutations and those without identifiable *CX26* mutations did not show any significant differences ($P = 0.594$ and 0.385, Student's t-test, unpaired samples) (Table 5).

Table 5
Average difference (dB $\pm$ 1 SD) in hearing impairment between ears and *CX26* mutation status

	CX26 mutation status		
	+/+	+/−	−/−
Number	45	26	175
Mean ($\pm$1 SD)	6.33 ($\pm$8.09)	6.66 ($\pm$8.05)	7.86 ($\pm$11.19)

Table 6
Average difference (dB+1 SD) in hearing impairment (best ear) between sibs and *CX26* mutation status

	CX26 mutation status		
	+/+	+/−	−/−
Number	18	11	33
Mean (±1 SD)	17.29 (±17.30)	12.04 (±15.99)	12.61 (±16.27)

3.5. Recurrence of severity of hearing impairment

In 62 sibs, the recurrence of severity of hearing impairment was not significantly different in those without and those with one ($P = 0.92$, Student's t-test, paired samples) or two ($P = 0.32$, Student's t-test, paired samples) identifiable mutations in the *CX26* gene (Table 6).

3.6. Progression

Serial audiograms were available for 24 persons, six persons with two identifiable *CX26* mutations from ages 3 to 17 followed up for between 1 and 10 years, seven heterozygous for a *CX26* mutation from ages 4 to 15 followed up for between 3 and 8 years and 11 with no identifiable *CX26* mutation from ages 3 to 13 followed up for between 1 and 8 years (Table 7). There was no evidence of significant progression of the hearing impairment in persons with one or two identifiable mutations in the *CX26* gene compared to those without ($P = 0.396$ and 0.444, Student's t-test, unpaired samples).

Table 7
Ages, duration of follow-up (years) and change (dB) in hearing impairment in the best ear and *CX26* mutation status

CX26 mutation status	Individual	Ages	Duration	Change
+/+	CJ	8–13	5	−1.0
	CD	6–10	4	+13.75
	DC	12–7	5	+1.25
	DW	3–12	9	+8.75
	PN	12–13	1	−1.25
	TG	4–14	10	+7.5
Mean (±1 SD)			5.66 ± 3.32	+4.83 ± 6.09
+/−	KM	7–12	5	−7.5
	JM	10–13	3	+1.75
	NB	5–8	3	+1.25
	SM	8–15	7	−1.25
	EM	5–12	7	+11.25
	DM	4–12	8	−1.25
	EC	4–5	1	+3.75
Mean (±1 SD)			4.86 ± 2.61	+1.14 ± 5.7
−/−	PA	4–9	5	+1.25
	DG	8–11	3	0
	FS	3–9	6	−4.0
	DS	3–5	2	+2.5
	PW	4–10	6	+4.75
	KW	5–7	2	+6.25
	LM	3–4	1	+8.75
	RS	4–6	2	+2.5
	JS	6–7	1	+6.25
	CM	5–13	8	+3.75
	EG	4–11	7	+1.25
Mean (±1 SD)			3.91 ± 2.55	+3.25 ± 3.49

4. Discussion

The first gene locus for autosomal recessive NSSNHI, DFNB1, was mapped to the proximal portion of the long arm of chromosome 13 (13q11-q12) in 1994 using autozygosity mapping with two complex consanguineous families from Tunisia with profound prelingual sensorineural hearing impairment [36]. Confirmation that a gene at this locus caused inherited hearing impairment was provided by reports of linkage of autosomal recessive NSSNHI in a large inbred Bedouin family with profound hearing impairment from Israel [37] and in a complex consanguineous British Pakistani family with moderate to profound hearing impairment [38].

A study of the segregation of polymorphic DNA microsatellite markers linked to the DFNB1 locus in 19 families with NSSNHI from Australia and New Zealand suggested that a gene at the DFNB1 locus made a major contribution to autosomal recessive NSSNHI [39]. A similar study of 30 Italian and 18 Spanish families with NSSNHI showed that in $\sim 80\%$, a gene at the DFNB1 locus was likely to be responsible for the hearing impairment [40].

Demonstration of mutations in connexin 26 (*CX26*) in British Pakistanis with autosomal recessive NSSNHI confirmed it as the gene responsible for inherited hearing impairment at the DFNB1 locus [9]. A deletion of a single guanine (G) residue in a stretch of six G's, at nucleotide position 30–35 (variably known as 30 or 35delG) has been found to account for 60% or more of *CX26* mutations in families with autosomal recessive NSSNHI from the Mediterranean area previously shown to be linked to DFNB1 [21]. This single base pair deletion alters the reading frame generating a stop codon at codon 13 of the *CX26* gene resulting in premature termination of translation leading to a truncated GJB2 protein at the first transmembrane domain. The 35delG mutation has also been found in $\sim 70\%$ of families with autosomal recessive NSSNHI from Tunisia, France, New Zealand and the UK [22]. To date, > 30 different mutations in the *CX26* gene which include missense, nonsense, deletions and frameshift mutations have been reported in a variety of different populations as causing autosomal recessive NSSNHI [21–32]. A number of these studies have shown that mutations in the *CX26* gene are a major cause of congenital NSSNHI being responsible for between 40 and 60% of sib-pairs or autosomal recessive families and between 1 and 10 and 1 and 3 sporadically affected individuals with NSSNHI [21,22,24,25].

In the majority of the publications to date, there is limited information about the audiological characteristics of the hearing impairment due to mutations in the *CX26*. The hearing impairment is reported to be prelingual, bilaterally symmetrical usually being severe or profound, although in a minority, the hearing impairment is moderate and/or asymmetrical in its severity [21–24,26–31]. Recently, Denoyelle et al. [25] has reported the audiological clinical features of 140 children from 104 families either sporadically affected or with two or more affected siblings with pre- and post-lingual hearing impairment recruited from hospital genetic counselling services. *CX26* mutations were identified in 43 (49%) of 88 of the families with prelingual and none of the 16 families with postlingual hearing impairment. *CX26* mutations were found in 20 (51%) of the 39 families with affected siblings and 15 (31%) of the 49 families with a sporadically affected individual. The 35delG mutation constituted 67 (86%) of the 78 mutant alleles.

The results of the present study differed in revealing in a smaller proportion, 20 (27.8%) of 72 sibships and 11 (7.9%) of 138 sporadically affected individuals with congenital non-syndromal sensorineural impairment, to be due to mutations in *CX26*. Although the means of ascertainment of the families in the present study was primarily for research purposes rather than a series consecutively ascertained through a genetic counselling clinic, it is unlikely that the two different methods of ascertainment would account for the difference observed. An alternative explanation is that the variation in the population prevalence of mutations, especially the most common mutation 35delG, in the *CX26* gene could account for this difference. Previous studies have reported variation in the prevalence of the 35delG mutation in different populations [21–24]. In support

of this is that the 35delG mutation only accounted for just over 70% of the *CX26* mutations in the present study compared to 86% in the study by Denoyelle et al. [25]. In addition, the inclusion of 25 individuals of ethnic minorities of non-Caucasian origin in which only one sib-pair had identifiable connexin 26 mutations will, in part, account for the lower prevalence of connexin 26 mutations in the series as a whole.

No individuals who are compound heterozygotes for non-35delG mutations were observed in the series. If the 35delG mutation accounts for $\sim 70-80\%$ of the mutations in the *CX26* gene in our series, one would expect between 1 and 10–20 individuals with hearing impairment due to *CX26* to be compound heterozygotes for non-35delG mutations which would be between 1 and 3 individuals/sibships in our series. A possible reason no such children were seen in our series is that our mutation screening method was not robust. We believe this is unlikely since we did not rely on a single mutation screening method but also sequenced the coding region of the *CX26* gene of all individuals in the series reported. It is possible that mutations in the promoter region 5′ to the coding region of the *CX26* could account for the lack of individuals who are compound heterozygotes for non-35delG mutations. We are screening the children from the series without a second identifiable *CX26* mutations for this possibility.

The hearing impairment in those individuals with two *CX26* mutations in the report by Denoyelle et al. [25] varied from mild to profound in severity with 31 (55%) of 56 children with profound hearing impairment, 14 (48%) of 29 with severe hearing impairment, eight (42%) of 19 with moderate and one (14%) of 14 with mild hearing impairment being due to *CX26* mutations.

In the present study, mutations in the *CX26* gene were also more likely to be found in individuals with congenital non-syndromal hearing impairment which was severe (17.1%) or profound (30%) in severity although, again, they accounted for a smaller proportion of individuals in those groups. This difference could be explained, in part, by the differences in population prevalence of the 35delG mutation. Although a similar proportion of individuals with moderate hearing impairment were found to be due to *CX26* mutations, only one of the 18 individuals with mild hearing impairment was heterozygous for a *CX26* mutation compared to 1 of 7 (14%) found to be due to *CX26* mutations in the study by Denoyelle et al. [25]. This latter difference is probably a result of the relatively small numbers analysed in both studies. This is likely to be a consequence of the means of ascertainment through hospital sources which would be more likely to involve individuals with more severe hearing impairment rather than a population based series which would be more likely to include children with less severe hearing impairment. The parents of the latter group might not seek genetic counselling or could feel less motivated to participate in research studies.

Analysis of the severity of hearing impairment with particular mutations in the *CX26* gene in the present study did not show any correlation, in contrast to the findings by Denoyelle et al. [25] where individuals who were compound heterozygotes for *CX26* were more likely to have severe hearing impairment, whereas those who were 35delG homozygotes were more likely to have moderate or profound hearing impairment. The severity of the hearing impairment between the two ears did not vary significantly whether due to *CX26* mutations or not in both the present study and that by Denoyelle et al. [25].

The audiogram in the majority of individuals with hearing impairment with *CX26* mutations in the present study was usually sloping or flat and rarely ascending or U-shaped, similar to the findings seen in persons with hearing impairment without identifiable *CX26* mutations. In contrast, in the series reported by Denoyelle et al. [25], the distribution of the audiogram configurations differed significantly in those with and without *CX26* mutations. In that study, although flat or sloping audiograms were predominant, none of the individuals with hearing impairment due to *CX26* had U-shaped or ascending shaped audiograms, whereas one of those audiogram configurations was present in ~ 1 in 5 of those without *CX26* mutations.

In the present study, we found that there was no evidence of progression of the hearing impairment but the number of individuals studied and period of follow-up was limited. A total of two out of 16 individuals with *CX26* mutations in the series reported by Denoyelle et al. [25] had significant progression of the severity of their hearing impairment. In order to reliably determine whether progression of the hearing impairment occurs with *CX26* mutations, a larger series of individuals and a longer period of follow-up is necessary.

Analysis of the recurrence of the severity of the hearing impairment in the present study was not significantly different in those with and without *CX26* mutations, unlike the series reported by Denoyelle et al. [25] in which one-half of the sibs in which the hearing impairment was due to *CX26* mutations, there was significant variation in the severity. They do not report, however, the results of the recurrence of the severity of the hearing impairment in sibs without *CX26* mutations for comparison. It is important to determine whether this difference is unique to inherited hearing impairment due to *CX26* mutations since it would be potentially useful information in recurrence risk advice when providing genetic counselling to parents of sporadically affected children with hearing impairment.

The series reported by Denoyelle et al. [25] also reported the results of high resolution computed axial tomography of the temporal bone. In 23 children with hearing impairment due to *CX26*, there were no abnormalities observed compared to seven out of 32 with hearing impairment without *CX26* mutations. Although, in most centres temporal bone imaging is not routinely obtained this difference, if substantiated in a larger series, could be a useful clinical discriminator for directing molecular analyses. Finally, although the numbers studied were small, Denoyelle et al. [25] also showed that there was no evidence of associated vestibular abnormalities in ten children from eight families with *CX26* mutations. In comparison, four out of 15 children from 15 families without *CX26* mutations showed abnormal vestibular function. Again, confirmation of these preliminary findings in a larger study is important.

In summary, the results of the present study and that of Denoyelle et al. [25] demonstrate that mutations in the *CX26*, especially the 35delG mutation, are a major cause of congenital or prelingual non-syndromal hearing impairment. The hearing impairment in children with non-syndromal sensorineural hearing impairment due to *CX26* is likely to be severe or profound, symmetrical, exhibit a sloping or flat audiogram configuration and is associated with a similar severity when it recurs in siblings. However, none of these features, especially the latter ones, can be used reliably to determine when the hearing impairment is due to *CX26*. The results of the present study, along with those Denoyelle et al. [25], support the suggestion that testing for mutations in *CX26* should be a routine investigation for sporadically affected individuals with congenital or prelingual hearing impairment, especially if severe or profound in severity. Identification of *CX26* mutations as being responsible for the hearing impairment allows the parents to be given precise Mendelian recurrence risks.

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