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Genetics of congenital hearing impairment: A clinical approach

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Key Words

Genetics

Hearing

Connexin 26

Neonatal hearing screening

Abbreviation

HI: Hearing impairment

Genetics of congenital hearing impairment: A clinical approach

Abstract

Hearing impairment (HI) is the most frequent sensory disorder, with a genetic etiology in >50% of all cases, due to mutations in >44 identified genes. Autosomal recessive inheritance explains the majority, with *GJB2* (connexin 26) mutations accounting for 15–50% of paediatric HI. Delayed presentation of HI to 11–60 months in cases of biallelic *GJB2* mutations is a concern, necessitating a good audiological follow-up in addition to neonatal hearing screening. Providing a genetic diagnosis in congenital HI has implications for the prognosis, the possible risk of associated medical manifestations, and precise genetic counseling of the family, and should be integrated into the medical examinations done in order to diagnose syndromic features. Large-scale mutation detection methods, such as micro arrays, are promising for wider genetic testing, but few studies on their clinical utility have been published, so far. Limitations of interpretation of genetic test results, combined with significant ethical issues, currently do not justify to institute genetic screening for *GJB2* mutations in neonates before a diagnosis of HI is established.

Sumario

La discapacidad auditiva (HI) es el trastorno sensorial más frecuente, con etiología genética en >50% de todos los casos, debido a mutaciones en >44 genes identificados. La herencia autosómica recesiva explica la mayoría de ellos, en los que las mutaciones del *GJB2* (conexina 26) son responsables del 15–50% de la HI pediátrica. La presentación tardía de la HI entre 11–60 meses en casos de mutaciones alélicas del *GJB2* es preocupante, porque además del tamiz auditivo neonatal, requiere de un buen seguimiento audiológico. Considerando que el diagnóstico genético en la HI congénita tiene implicaciones para el pronóstico y el posible riesgo de manifestaciones médicas asociadas, debe integrarse a los exámenes médicos el consejo genético preciso a la familia, con objeto de diagnosticar los rasgos sindrómicos. Son prometedores los métodos de detección de mutaciones a larga escala como los micro arreglos, para hacer evaluaciones genéticas más amplias, a pesar de lo cual se han publicado pocos trabajos sobre su utilidad clínica. Las limitaciones en la interpretación de los resultados de las pruebas genéticas, combinadas con situaciones éticas significativas, no justifican actualmente la práctica de tamiz genético para las mutaciones del *GJB2* en neonatos, antes de que se haya establecido el diagnóstico de HI.

Permanent hearing impairment (PHI) affects 1 in 1000 newborns and another 1 in 1000 becomes affected during early childhood (Parving et al, 2003; Fortnum et al, 2001). A multitude of factors may cause HI, and at least 50% are due to genetic etiology (Morton & Nance, 2006). A disruption of different classes of proteins involved in ion homeostasis, cytoskeletal and extracellular matrix components, transcription factors, receptors, cellular trafficking proteins and molecules belonging to the cadherin superfamily may all cause HI with or without associated syndromic features (Bitner-Glindzicz, 2002). Syndromic forms constitute about one third of congenital HI, and the remaining two-thirds are non-syndromic. Among the non-syndromic forms the large majority (70–80%) are autosomal recessive, often characterized by being profound and pre-lingual, whereas the 20% autosomal dominant types most often are milder, post-lingual, and progressive.

HI can also be subdivided into conductive, sensorineural, and mixed conductive-sensorineural types. A classification into pre-lingual or post-lingual and into different degrees of severity is also commonly used. The grade of HI can be defined according to hearing thresholds averaged over the frequencies 0.5–4 kHz in the better ear: mild refers to a 20–40 dB HL, moderate to 41–70 dB HL, severe to 71–95 dB HL, and profound to >95 dB HL (Mazzoli et al, 2003). Systematic description of a shared

terminology and ways of classifying genetic HI is summarized by Mazzoli et al (2003).

Early hearing detection and intervention (EHDI) programmes with goals of $\geq 90\%$ coverage in universal neonatal hearing screening and early diagnosis of HI is also recommended to include referral to genetic evaluation, but health providers have limited training and competence in providing genetic counseling and services.

How many genes are involved in congenital autosomal recessive hearing impairment?

Non-syndromic chromosomal HI loci (distinct genetic interval on a specific chromosome containing a deafness gene based on linkage analysis) have been named systematically, along with the identification of each one. Currently, there are 75 loci for autosomal recessive non-syndromic (DFNB) forms of deafness, and 57 loci for non-syndromic autosomal dominant (DFNA) forms (Petersen, 2002; Petersen & Willems, 2006) (see Figure 1A, 1B, and 1C). In addition, five loci for X-linked HI (DFN), and one Y-linked locus are reported, but only one gene has been identified (HHH; Petersen et al, 2008).

Forty-four genes have been identified showing DFNA genes and DFNB genes, respectively (Tables 1 and 2). Some genes are

Table 1. Overview over all non-syndromic autosomal recessive genetic loci (DFNB's) for which genes have been identified, and some clinical additional information (update February 2008).

<i>DFN</i>	<i>Locus</i>	<i>Gene</i>	<i>Protein</i>	<i>Function</i>	<i>Age of onset</i>	<i>Phenotype of HI</i>	<i>OMIM no</i>	<i>Frequency, geography</i>
DFNB1	13q11	<i>GJB2</i> <i>GJB6</i>	Connexin 26 Connexin 30	Gap-junction subunits; Potassium recycling	Prelingual	Usually severe HI	220290 604418	30–40% of genetic HI worldwide occurrence
DFNB2 (DFNA11)	11q13.5	<i>MYO7A</i>	Myosin 7A	Unconventional myosin	Prelingual	Severe-profound HI with vestibular dysfunction, allelic to USH1B	600060	Tunisia, China, USH1B worldwide
DFNB3	17p11.2	<i>MYO15A</i>	Myosin 15A	Unconventional myosin	Prelingual	Profound HI	600316	Bali, Pakistan (5%), India, Turkey
DFNB4	7q31	<i>SLC26A4</i>	Pendrin	Anion transporter	Post- or pre-lingual	Fluctuating high-frequency HI, progressive, heterozygous carriers 30 × increased risk of EVA	600791	10% of genetic HI, worldwide occurrence
DFNB6	3p21	<i>TMIE</i>	Tmie	Transmembrane protein	Prelingual	Profound HI	600971	India, Pakistan
DFNB7/11 (DFNA36)	9q13-21	<i>TMCI</i>	Tmcl	Transmembrane protein	Prelingual	Profound HI	600974	India, Pakistan (1.8%), Turkey
DFNB8/10	21q22	<i>TMPRSS3</i>	Tmprss3	Serine protease	Prelingual / 10–12 years	Profound, or moderate and progressive	601072	Pakistan, Palestine, Tunisia, Germany
DFNB9	2p22	<i>OTOF</i>	Otoferlin	Vesicle exocytosis	Prelingual	Profound; may be auditory neuropathy	601071	Spain, Lebanon, Turkey, India, Cuba
DFNB12	10q21	<i>CDH23</i>	Otocadherin	Cytoskeleton protein	Prelingual	Profound; allelic with USH1D	601386	Syria, Pakistan, India, Turkey; USH1D widespread
DFNB16	15q15	<i>STRC</i>	Stereocilin	Stereocilia protein	3–5 years/ pre-lingual	Moderate; all frequencies, or profound	603720	Pakistan, Middle East, Spain
DFNB18	11p15.1	<i>USH1C</i>	Harmonin	Cytoskeleton protein	Pre-lingual	Profound and rare- allelic with USH1C	602092	India, China, USH1C more widespread
DFNB22	16p22.2	<i>OTOA</i>	Otoancorin	Anchoring protein at apex of sensory cells	Pre-lingual	Moderate-severe	607039	Palestine
DFNB23	10q21	<i>PCDH15</i>	Protocadherin	Stereocilia protein	Pre-lingual	Profound, allelic to USH1F	609533	Pakistan; USH1F: UK, Askhneazi Jews
DFNB24	11q23	<i>RDX</i>	Radixin	Cytoskeleton protein	Pre-lingual	Profound	179410	Pakistan
DFNB28	22q13.1	<i>TRIOBP</i>	Triobp	Cytoskeleton protein	Pre-lingual	Profound	609823	Pakistan, India, Palestine
DFNB29	21q22.3	<i>CLDN14</i>	Claudin 14	Tight junction protein	Pre-lingual	Profound	605608	Pakistan
DFNB30	10p11.1	<i>MYO3A</i>	Myosin 3a	Unconventional myosin	Pre-lingual	Profound or progressive high-tone	607101	Israel
DFNB31	9q32–q34	<i>WHRN</i>	Whirlin	Cytoskeleton protein	Pre-lingual	Profound- allelic to USH2D	607084	Jordan, Tunisia

Table 1 (Continued)

<i>DFN</i>	<i>Locus</i>	<i>Gene</i>	<i>Protein</i>	<i>Function</i>	<i>Age of onset</i>	<i>Phenotype of HI</i>	<i>OMIM no</i>	<i>Frequency, geography</i>
DFNB36	1p36.1	<i>ESP</i>	Espin	Cytoskeleton protein	Pre-lingual	Profound- vestibular dysfunction	609006	Pakistan
DFNB37 (DFNA22)	6q13	<i>MYO6</i>	Myosin 6	Unconventional myosin	Pre-lingual	Profound, vestibular dysfunction, possibly RP- DFNA22: post-lingual, moderate, progressive	607821	Pakistan DFNA22: USA, Pakistan, Denmark
DFNB49	5q12-q14	<i>MARVELD2</i>	Tricellulin	Junction protein	Pre-lingual	Profound or moderate HI	610572	Pakistan
DFNB53 (DFNA13)	6p21.3	<i>COL11A2</i>	Collagen11A2	Structural protein	Pre-lingual	Profound, allelic to Spondylo-epiphysea dysplasia tarda and Stickler syndrome	120290 601868	Iran, USA, The Netherlands
DFNB59	2q31.2	<i>PJVK</i>	Pejkavin		Pre-or post-lingual	Mild- profound HI	610219	Iran (6% GJB2 negative), Turkey, Morocco
DFNB61	7q22	<i>SLC26A5</i>	Prestin	Anion transporter	Pre-lingual	Severe-profound; heterozygosity not fully penetrant	604943	USA
DFNB66/67	6p21.1	<i>TMHS</i>	Tmhs	Stereocilia protein	Pre-lingual	Profound,	610212	Tunisia, Pakistan, Turkey
	1p35	<i>GJB3</i>	Cx 31	Gap junction	Pre-lingual or early childhood	Moderate-profound, all frequencies, allelic to DFNA2, and AD erythrokeratoderma variabilis	603324	China, (Liu et al, 2000)
	6q22	<i>GJA1</i>	Cx 43	Gap junction	Pre-lingual	Profound	121014	African American, (Liu et al, 2001) questionable role in Taiwanese deaf (Yang et al, 2007)

Table 2. Overview over all non-syndromic autosomal dominant loci (DNBA's) for which genes have been identified, and some clinical information (update February 2008).

<i>DFN</i>	<i>Locus</i>	<i>Gene</i>	<i>Protein</i>	<i>Function</i>	<i>Age of onset</i>	<i>Phenotype of HI</i>	<i>OMIM no</i>	<i>Frequency, geography</i>
	16p13.11	CRYM	Crystallin	Potassium recycling(?)	Early childhood	All frequencies moderate-severe, progressive	123740	Japan
DFNA1	5q31	DIAPH1	Diaphonous	Actin polymerization	Post-lingual, 10–40 y	Low-frequency progressing to all frequencies	124900	Costa Rica
DFNA2	1p24	GJB3	Connexin 31	Gap junction protein	Post-lingual	High-frequency, allelic to erythrokeratoderma, HI + genodermatosis, peripheral neuropathy, and AR HI	600101	China, Spain China
	1p24	KCNQ4	Potassium channel		Post-lingual, 10–30 y	High-frequency,		Java, France, USA
DFNA3	13q11	GJB2	Connexin 26	Gap junction protein	Prelingual Post-lingual	High-frequency, severe mild-moderate, allelic to Palmoplantar keratoderma, Vohwinkel's syndrome, keratitis ichthyosis deafness syndrome	601544	Worldwide
		GJB6	Connexin 30	Gap junction protein	Prelingual	High-frequency, progressive, moderate-profound	604418	Worldwide
DFNA4	19q13	MYH14	Myosin 14	Unconventional myosin	Post-lingual, 20 y	Moderate-profound, progressive, fluctuating	600652	Germany, Belgium, Italy
DFNA5	7p15	ICERE-1	DFNA5	Unknown	Post-lingual, 5–15 y	Moderate-severe, high-frequency, progressive	600994	The Netherlands
DFNA6 (DFNA14) (DFNA38)	4p16	WFS1	Wolframin	Unknown	Post-lingual, pre-lingual	Low-frequency, rarely ass with optic atrophy allelic with Wolfram syndrome with deafness or high-tone HI	600965	Japan, Denmark, USA, Germany
DFNA8/12 (DFNB21)	1q22	TECTA	α -tectorin	Structural role in tectorial membrane	Prelingual-postlingual,	Moderate-severe, non-progressive, mid-frequency	601543	Austria, Belgium, Switzerland Sweden, France
DFNA9	14q12	COCH	Cochlin	Extracellular matrix protein	Post-lingual, age 20–60 y	High-frequency, progressing to all frequencies, severe-profound, vestibular dysfunction	601369	USA, Belgium, The Netherlands, Australia
DFNA10	6q22	EYA4	Eyes absent 4	Transcription activator	Post-lingual, 20–60 y	All frequencies, progressive, moderate-severe	601316	USA, Belgium,
DFNA11 (DFNB2)	11q12	MYO7A	Myosin 7A	Unconventional myosin	Post-lingual, 10–20 y	High-frequency, moderate-severe, progressive, allelic to USH1B	601317	Japan, Denmark, USA,
DFNA13 (DFNB53)	6p21	COL11A2	Collagen 11 α 2	Structural protein	Post-lingual	Mid-high frequency, moderate-severe, progressive,	601868	USA, The Netherlands
DFNA15	5q31	POU4F3	Pou domain 4F3	Transcription factor	Post-lingual, 20–40 y	Flat-sloping audiogram, moderate-severe, progressive	602459	Israel,
DFNA17	22q11	MYH9	Myosin 9	Unconventional myosin	Post-lingual, by 10 y	High-frequency, moderate-severe, progressive, allelic to May Hegglin anomaly and Fechtner/Epstein/Sebastian syndromes	603622	USA, Japan, Korea, China, Finland
DFNA20/26	17q25	ACTG1	γ -actin	Cytoskeletal protein	Post-lingual, 8–20 y	Moderate, progressive, high-frequency	604717	USA, Norway, The Netherlands

Table 2 (Continued)

DFN	Locus	Gene	Protein	Function	Age of onset	Phenotype of HI	OMIM no	Frequency; geography
DFNA22 (DFNB37)	6q13	MYO6	Myosin 6	Unconventional myosin	Post-lingual, 8–10 y	Moderate-profound, all frequencies, progressive	606346	Italy, USA, Denmark
DFNA28	8q22	TFCP2L3	Tfcp2l3	Transcription factor	Post-lingual, by 5th decade	Moderate-severe, progressive associated with ARHI	608641	USA
DFNA36 (DFNB7/11)	22q1	TMC1	Tmc1	Transmembrane protein	Post-lingual, 10–30 y	Moderate-profound, high-frequency, rapidly progressive to all frequencies	606705	USA
DFNA44	3q28	CCDC50	Ymer	Epidermal growth factor regulator	Post-lingual, 6–10 y	Progressive, low-mid-frequencies, profound by 6th decade	611051	Spain
DFNA48	12q13	MYO1A	Myosin 1A	Unconventional myosin	Post-lingual, 8–10 y	Moderate-severe, all frequencies, progressive	607841	USA

a functional understanding of the individual gene products in the auditory pathways (Eisen & Ryugo, 2007). Despite the immense genetic heterogeneity of HI, surprisingly one particular gene, *GJB2* (connexin 26), turned out to be the single most frequently involved in congenital moderate-profound HI in paediatric patients. Its role in mild, progressive, and low-frequency sensorineural HI is less firmly established. The contribution of 35delG to age-related hearing impairment in a large European multicenter study found carrier frequencies between 0.4% and 5.7%, with an average of 1.3% (Van Eyken et al, 2007), but sequencing of the entire *GJB2* gene in adult patients with HI should give us more reliable figures.

Genetic testing as part of the early hearing detection and intervention process

The majority of countries in the Western part of the world have now implemented newborn hearing screening, and the average age of detection of moderate-profound hearing impairment has decreased from 2.5 years of age to 1–3 months of age (Parving et al, 2003; Parving, personal communication, 2007). An early etiological evaluation including genetic testing is thereby facilitated. Moreover, many congenitally deaf infants receive cochlear implants by an early age and undergo a systematic investigation program, including temporal bone imaging. Along these lines, the accumulating experience with genetic testing and prognosis after CI for those children having a named genetic cause of their HI established, have led to a much more pronounced parental motivation for having a genetic evaluation (Withrow et al, 2008; Kaimal et al, 2007). A recent survey of how accurately the recommendations of referrals to genetics professionals are followed, showed unfortunately that significant additional post-graduate training in providing genetic counseling and services as well as dissemination of knowledge are needed (Schimmenti et al, 2006).

Connexin 26 (Cx 26) and congenital HI

Audiological characteristics and spectrum of mutations

The mapping of DFNB1 (MIM220290) and DFNA3 (MIM601544) to chromosome 13q12 was reported in 1994, with subsequent identification of mutations in the underlying Cx 26 gene (*GJB2*) in 1997, was a turning point in the dissection of genetics of HI. Another connexin gene, *GJB6* (connexin 30) is located in close vicinity of the *GJB2* gene (Table 1 and Table 2). The *GJB2* gene encodes a gap junction protein. Connexins are integral membrane proteins with four transmembrane domains, forming aqueous gap-junction channels, allowing the transport of small molecules like potassium, calcium, and small signaling molecules (Richard, 2003). Connexin genes have a similar organization with the first exon containing most of the 5'-untranslated region and the second exon containing the complete open reading frame. Connexin 26 encodes a 208 amino acid (26-kDa) peptide. Six connexins aggregate in one connexon surrounding a central pore of 2–3 nm. Potassium, which enters the hair cells during mechano-sensory transduction of sound, is recycled to the stria vascularis via these gap-junction channels.

Altogether, more than 100 different disease mutations have been reported (The connexin-deafness homepage). In Europe,

North America, and in particular in the Mediterranean countries the most common mutation is a deletion of a single guanine nucleotide in a series of six guanines, known as 35delG (previously called 30delG). The carrier rate is 1–3%, highest in the Greek population (Petersen et al, 2006). In Ashkenazi Jews another mutation, 167delT, is prevalent with a carrier rate of 3–4%, and in the Asian population the 235delC is frequent, stemming from a founder mutation with a carrier rate of approximately 1–4.5%. The carrier rate of the V37I mutation in the Taiwanese population is 11.6%. In Ghana, the R143W mutation constituted >80% of detected disease alleles, and the absence of *GJB2* mutations in Indonesian deaf patients is noteworthy (Snoeckx et al, 2005).

Petersen and Willems (2006) reviewed the genetic epidemiological experience regarding *GJB2* mutations and found that in 44 reports from 31 different countries the percentage of individuals with biallelic *GJB2* mutations varied between 0 and 45%, and the proportion of 35delG/35delG homozygous individuals ranged from 0 to 40%. The frequency of *GJB2* mutations was particularly high in France, Spain, Italy, Greece, Slovakia, and Israel. It is difficult to compare the different studies because of considerable methodological differences (method of ascertainment of patients, audiological criteria, age groups, variable information about the family history, and mutation detection assays), but the global presence of *GJB2*-related deafness justifies sequencing of the *GJB2* gene as a first step of genetic evaluation of all HI.

It has been a puzzle why only one *GJB2* mutation in the coding exon 2 was detected in 10–40% of presumably autosomal recessive cases of non-syndromic severe hearing impairment. A mutation in the non-coding exon 1 region of the *GJB2*, IVS+1G>A, which is not routinely investigated, is fairly frequent among Czech and Dutch HI (Seeman et al, 2006; Santos et al, 2005), but seems infrequent elsewhere (Snoeckx et al, 2005).

Another explanation for monoallelic *GJB2* genotype is digenic combination with one of two recognized deletions in the neighboring *GJB6* gene: del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854), which can be detected in one diagnostic test (del Castillo et al, 2005). In affected *GJB2* heterozygotes the del(*GJB6*-D13S1854) was detected in >20% of Spanish and UK patients, but only 1% of >800 HI-patients in a USA multicenter study had the del(*GJB6*-D13S1830) mutation (Putcha et al, 2007), and in most countries, a fraction of 10–30% of patients, still, only have one mutation identified. There is some evidence for an unidentified cis-acting regulatory mechanism of *GJB2* and *GJB6*, as suggested by Wilch and co-workers (2006).

GJB2 and genotype-phenotype aspects

In a study of over 1500 patients with bi-allelic *GJB2* mutations from mostly USA and Northern Europe, the ones with 35delG in either homozygosity or compound heterozygosity with another truncating mutation had the most severe hearing impairment, whereas L90P usually gives a milder audiological deficiency (Knoexks et al, 2005). The majority of studies analysed *GJB2* mutations in patients with severe-profound HI, but there is audiological variation across all *GJB2* genotypes (Hismi et al, 2006). Mild HI was found to be associated with homozygosity for V37I mutation in Australia (Dahl et al, 2006), and this mutation was present in 11.6% of Taiwanese people,

and frequent elsewhere in Asian populations (Bason et al, 2002, Wattanasirichaigoon et al, 2004).

Are GJB2 mutations associated with unilateral progressive HI? *GJB2* mutations were found in 9/39 Italian patients with progressive HI, but only two were biallelic (Ravecca et al, 2005). Recently, eleven children from USA and Austria with biallelic *GJB2* genotypes were diagnosed with HI as late as at age 11–60 months, because of non-penetrance when they underwent neonatal hearing screening, and thereby escaped early detection (Norris et al, 2006; Ramsebner et al, 2007). The absence of *GJB2* mutations in 25 patients with unilateral sensorineural HI (Preciado et al, 2004), should be interpreted with caution, not excluding the possibility of *GJB2* being related to ‘partial penetrance’ in unilateral HI. It has great implications for continuous audiological follow-up of children who are not diagnosed neonatally with HI, if they are later suspected of having HI. *GJB2* mutations should be analysed in unilateral, and progressive HI as the first diagnostic step in genetic evaluation.

Cx26 and associated abnormalities and disabilities

Evidence is accumulating that biallelic *GJB2* mutations may be associated with additional disabilities from different organs. No specific causative associations, however, have emerged from two recent studies (Kenna et al, 2007; Wiley et al, 2005). From a group of 163 individuals with biallelic *GJB2* mutations and sensorineural hearing loss, 29 individuals (18%) displayed neurocognitive, major structural, genitourinary, middle/external ear, and other abnormalities. Among 108 children receiving CI, there were 44% of the *GJB2* positive and 41% of the *GJB2* negative children with additional problems, including learning disabilities, developmental delay, apraxia, and attention deficit disorder (Wiley et al, 2006). The observation that patients with *GJB2* mutations equally frequently have additional disabilities may be coincidental, but suggests that paediatric patients should have the same comprehensive clinical evaluation, including development and behavior, irrespective of their *GJB2* genotype.

Until recently, it has been reported that temporal bone abnormalities are extremely rare in patients with *GJB2* mutations (Kenna et al, 2001; Preciado et al, 2004).

One case report found associated radiographic cochlear abnormalities in a patient with HI and *GJB2* mutations (Schrijver & Chang, 2006); and a larger Canadian report found temporal bone anomalies in 55% of paediatric CI recipients with biallelic *GJB2* genotypes compared to 29% in normal hearing controls undergoing neuroimaging of the petrous bone because of trauma (Propst et al, 2006a).

The fact that four patients had heterozygous *SLC26A4* disease causing mutations in addition to the two *GJB2* mutations is of interest because of a recent report claiming a many-fold increased risk of enlarged vestibular aqueduct in the presence of heterozygosity for *SLC26A4* mutations (Pryor et al, 2005). More studies are needed, but imaging of the petrous bone is probably relevant irrespective of *GJB2* genotypes.

Connexin 26 and cochlear implant

The frequency of *GJB2* mutations in candidates for CI is variable, and was 26.9%, with 78% being 35delG, in a Canadian series of 65 paediatric patients (Propst et al, 2006b). In an American group of 77 cochlear implant recipients only 18% had

GJB2 mutations and only three patients had biallelic genotype (Lustig et al, 2004), but it is definitely recommended to perform *GJB2* sequencing in all CI recipients. The language outcome was measured in 15 Australian cochlear implanted children with two *GJB2* mutations, and compared with similar measures with a group of 37 children negative for *GJB2* mutations, carefully matching the two groups with respect to the age at which they were implanted (Dahl et al, 2003). The authors found no significant differences, indicating that the presence or absence of *GJB2* mutations should not influence the decision of offering CI (Dahl et al, 2003).

Implications of identifying two GJB2 mutations in a patient

Once two disease-causing mutations have been identified, it is recommended to investigate the parents in order to confirm the recessive inheritance. Other family members may then be investigated for their carrier risk. Two exceptional families are known where only one of the parents was *GJB2* mutation carrier and the child had two *GJB2* mutations, due to uniparental disomy of chromosome 13 (Alvarez et al, 2003). Such rare genetic events have implications for the associated genetic counseling, inclusive providing recurrence risk figures for the families. If only one *GJB2* mutation is found, and maybe of questionable functional significance (i.e. M34T, R127H), further molecular genetic analyses must be considered. This includes the two *GJB6* deletions and the intronic *GJB2* mutation, despite apparently very low frequencies in major parts of the world (Putchá et al, 2007). If the genetic conclusion remains mono-allelic *GJB2* mutation, there are definite limitations in the possibilities for testing and counseling of relatives.

Genetic screening for *GJB2* mutations in newborns?

One study has been reported from Sicily, where neonatal hearing screening is not instituted (Niceta et al, 2007). During 2005, 1050 families participated (67%). Two newborns had homozygosity for 35delG (0.19%) and were deaf. Moreover, 46 children (4.5%) were heterozygous carriers. The authors did not describe how parents were informed, nor if the results were given back to the parents, and if the families received genetic counseling after results were obtained. In particular, the demonstration of heterozygosity for a *GJB2* mutation in a deaf child does not explain the etiology. Arguments in favour of genetic screening might be the documented non-penetrance of *GJB2* mutations at neonatal hearing screening, claiming that instituting genetic screening in places without neonatal hearing screening secures a minimal delay in the diagnosis of HI in some newborns. There are several challenges connected to performing such genetic screening: (1) the necessary follow-up of homozygous individuals with normal hearing; (2) the interpretation of heterozygosity, which may mean either unaffected carrier or affected with undetected second mutation as is known for 10–30% of *GJB2* related deafness; (3) the decision if other genes and other mutations should be included in addition to *GJB2*; and (4) the lack of parental readiness to absorb the genetic diagnosis without prior diagnosis of hearing impairment in their infant. Such programs would require active follow-up of all individuals being identified with *GJB2* mutations, which would be ethically demanding and require skilled health professionals. Parental attitudes to genetic testing differ regarding motivation for such

testing and the timing of it (Withrow et al, 2007), and these differences do not seem to vanish within one year after genetic testing performed after HI was diagnosed neonatally (Kaimal et al, 2007). The many pros and cons for different time points of performing genetic evaluation of HI are comprehensively discussed by Schimmenti and co-workers (2004). Personally, I would only be in favour of initiating genetic testing prior to documented HI within carefully planned research programs currently.

The role of other connexins in non-syndromic hearing impairment

The human family of connexin genes consists of 20 distinct peptides with different molecular mass, explaining their names (Richard, 2003). Mutations in five members of the connexin gene family have been shown to underlie distinct genetic forms of syndromic and non-syndromic deafness: *GJB2* (connexin 26), *GJB3* (connexin 31), *GJB6* (connexin 30), *GJB1* (connexin 32), and *GJA1* (connexin 43) (Richard, 2003; Liu et al, 2001). *GJB1* mutations are associated with an X-linked neurological disease, Charcot-Marie-Tooth associated with hearing loss, and *GJA1* mutations were found in autosomal recessive non-syndromic HI in four African American families (Liu et al, 2001). A study of 260 deaf individuals from Taiwan found a frequent one base pair deletion, 932delC, in 3% of disease chromosomes, but in various combinations with biallelic *GJB2* mutations as well as other genotypes, and it was not possible to draw firm conclusions about the role of *GJA1* in that study (Yang et al, 2007). *GJB3* is known as the underlying gene for non-syndromic DFNA2, autosomal recessive non-syndromic HI (Liu et al, 2000), and syndromic forms of HI with associated skin or neurological symptoms in families from China and Spain (Table 1 and Table 2). Studies from Austria, Brazil, and Taiwan revealed either no disease mutations or rare polymorphisms with unresolved pathogenic role in causing deafness (Oliveira et al, 2007; Frei et al, 2004; Yang et al, 2007). Our current knowledge justifies diagnostic genetic testing of *GJB2* and *GJB6*, but not of *GJB1*, *GJB3*, or *GJA*.

The role of unconventional myosins in deafness

Like the connexin gene family, the gene family of unconventional myosins plays an important causative role in genetic hearing impairment. Currently, seven different unconventional myosins are involved in ten different types of syndromic and non-syndromic hearing impairment with different patterns of inheritance: *MYO7A* in DFNA11/DFNB2/Ush1B, *MYH9* in DFNA17, *MYO6* in DFNA22/DFNB37, *MYO3A* in DFNB30, *MYO1A* in DFNA48, and *MYO15A* in DFNB3 (Table 1 and Table 2). Identification of such gene families have positive implications for identifying candidate genes in linkage studies, for the understanding of molecular interaction in the inner ear, and eventually for improved diagnosis and development of therapy to prevent progression of HI.

Genetic evaluation of a child with HI

Aetiological investigations of children with hearing impairment should be extensive in order to try to make a specific diagnosis

and to find out if the hearing impairment is non-syndromic or part of a syndrome with manifestations from other organs, which may have great impact on the follow-up. An investigative protocol is outlined by Bamiou and co-workers (2000). These recommendations include considering referral to a geneticist, who should be part of the team of professionals, who is taking care of the etiological evaluation.

Genetic evaluation and counseling should take place in the context of neonatal or later detection of HI. Recommendations for such procedures have recently been updated by the joint committee on infant hearing (2007). Several publications deal with the genetic approach to the child with sensorineural hearing impairment (Robin et al, 2005a; Preciado et al, 2004; Rehm, 2005; Pandya et al, 2006; Genetic evaluation of congenital hearing loss expert panel, 2002). It has previously been claimed as a benefit of genetic testing of patients with HI that costly advanced examinations might be avoided in case of detecting e.g. *GJB2* mutations (Robin et al, 2005b), but recent reports seem to find higher frequencies of additional disabilities than previously thought in children diagnosed with *GJB2* mutations (Wiley et al, 2006; Propst et al, 2006).

As part of referral to a geneticist, a three-generation family history will be taken, before the consultation. Audiological affection in relatives will be compared to the proband's audiological history and validated. In cases of no affected sibs, parents or more remote relatives, it is virtually impossible to distinguish between a sporadic or autosomal recessive genetic aetiology or HI related to environmental factors. If a child in addition has developmental delay, dysmorphic features, or behavioral abnormalities, a chromosome analysis will be relevant in order to rule out or identify a chromosomal anomaly.

Genetic evaluation and counseling typically take place in one session with the family, and comprises much more than establishing recurrence risk figures for another hearing-impaired child. A component of the genetic evaluation is to establish or rule out syndromic forms of hearing impairment. It is important to be aware of parental guilt and anxiety, and deal with the emotional aspects in the family. For families with a positive result (i.e. the identification of a molecular genetic cause) recurrence risks must be explained, and prenatal diagnostic options must be clarified. It is recommended to do genetic testing of both parents in order to confirm the inheritance pattern, e.g. in case of two *GJB2* mutations identified in the child. The family history may look dominant due to assortive mating between deaf parents and/or a high population carrier frequency for disease mutations in the relevant gene, which would require careful explanation to the family. Genetic testing for prenatal detection of hearing impairment in future children raises many ethical issues for the families. The establishment of a specific genetic diagnosis makes it possible to inform the family of the natural history of HI, and about possibly associated medical symptoms, and therefore is of prognostic relevance. The follow up of the child could vary depending on the diagnosis. Although many medical professionals recognize that a negative *GJB2* testing does not rule out another genetic etiology, as yet unidentified, this is often very difficult for families to understand. Recruitment into research studies to identify new genes in families, where *GJB2* testing was negative, often takes place in the context of clinical genetics. It is very important to give realistic expectations about the likelihood and the extended time

frames needed for such additional investigations. Even when it is obvious to the family that there is a genetic etiology by the presence of several sibs or generations being hearing impaired, genetic research may take several months or years to obtain results. The family should be encouraged to seek new information with regular long time intervals, in order to take advantage of new testing possibilities with time. Some syndromic features develop long after the neonatal period.

What to do when *GJB2* testing is negative?

The genetic testing options are often limited if *GJB2* testing was negative, however, mutations in some genes seem to be particularly frequent in some geographic regions. The existing figures are extremely preliminary and are based on very different groups of patients/families tested. As indicated in Tables 1 and 2, (DFNB/DFNA), *MYO15* and *TMC1* may be involved in about 5% of prelingual deafness in Pakistan and India, *PVJK* mutations were found in >6.7% of *GJB2* negative Iranian deaf individuals, and *OTOF* mutations may constitute about 3% of genetic deafness in Spain. *STRC* mutations amount to about 5% in Middle East deaf patents, and all other DFNB genes are thought to each contribute with <1% of all deafness (Table 2).

Application of multigene mutation detection methods

The application of micro-array technology to investigate a large number of already identified mutations in several genes is promising (Gardner et al, 2006), but the clinical utility is crucially correlated to the degree with which the panel of mutations tested for, reflect the spectrum of mutations in the underlying population.

Practical genetics for evaluating HI in children

- Children with HI diagnosed after normal neonatal hearing screening should be tested for *GJB2* mutations by means of sequencing.
- Carry out systematic etiological evaluation as part of the follow up after the diagnosis of HI, in order to clarify the possibility of a syndromic form of HI.
- Genetic evaluation of etiology with sequencing of the *GJB2* gene should be the first step in all cases of all degrees of HI in a newborn, irrespective of the presence of additional disabilities (neurocognitive, neuroradiological, urologic).
- *GJB2* should be sequenced in cases of unilateral HI of stable or progressive nature HI.
- Establishment of a genetic diagnosis often provides information about the natural history of the HI, and is in general of prognostic relevance for cochlear implantation.
- Patients with heterozygosity for *GJB2* mutations should be tested for an intronic mutation in exon 1 of *GJB2*, and for the two *GJB6* deletions.
- Current evidence does not support mutation testing of *GJB1*, *GJA1*, or *GJB3* outside research programs.
- Advanced multigene mutation micro-array analyses are promising, but the clinical utility depends on how representative the testing panel is for the respective country/ ethnic group.

- Audiological follow-up programs must address the observation of delayed presentation of HI to 11–60 months in children with biallelic *GJB2* mutations, who passed neonatal hearing screening by renewed audiological examinations, when there is clinical suspicion of HI.
- Additional genetic testing should be decided, based upon audiological and genetic epidemiological information, as well as a three-generation family history.
- Close interaction between EHDI and genetics provides a good platform for research in gaining better knowledge for genetics of HI.
- It is premature to institute genetic *GJB2* screening of neonates at the current time.

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