Genetic hearing loss

Some clinical and genetic aspects of

the BOR syndrome, DFNA9, DFNA20/26 and DFNB1

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Contents

Chapter 1

Introduction

General introduction

In our society hearing impairment is a very common type of sensory disability. In general, half of the population at the age of 80 years[1] is hearing impaired (≥ 35 dB). As society rapidly progresses into a more communicative one, the importance of normal hearing cannot be overestimated. Therefore, hearing impairment can be a considerable professional and social burden. The aetiology of hearing loss is multifactorial and includes environmental, infectious, traumatic and genetic causes. Increasing medical knowledge and preventive and protective measures against infectious and environmental causes have diminished the number of cases that can be attributed to these agents. Proportionately, the fraction of genetic causes has therefore increased.

Historical note

The awareness that hearing loss can be inherited is not recent. Annotations dating from the 16th and 17th century suggest the notion of genetic involvement in familial hearing loss[2,3]. In the second half of the 19th century the importance of genetics in hearing loss became more and more apparent. In 1853 Sir William Wilde (1815-1876) described hereditary hearing loss in several families based on data obtained during an Irish census[4]. Arthur Hartmann presented evidence for autosomal dominant and autosomal recessive inheritance of hearing loss after studying a large Berlin population around 1880[5]. The famous otologist Adam Politzer endorsed Hartmann's view in the second edition of his manual of otology 'Lehrbuch der Ohrenheilkunde'[6] (1887), in which he distinguished direct or dominant inheritance from indirect or recessive inheritance. Since then, heredity as an aetiology of hearing loss became a generally accepted concept. Soon after, in 1900, the significance of Mendel's original work[7] (1865) was re-appreciated and provided insight into various patterns of inheritance.

Besides studies and reports on non-syndromic hearing loss at schools and in isolated populations, scientific attention was focused on inherited types of hearing loss as part of a syndrome. Syndromes in general are quite rare. However, due to the usually apparent clinical features, they are more recognisable as a separate clinical entity than isolated types of hereditary hearing loss. Therefore the amount of reported syndromes rapidly increased in the second half of the 19th

century. Among others, the first descriptions of mandibulofacial dysostosis[8] (1846), Usher syndrome[9] (1858) and Pendred syndrome[10] (1896) all date from this period. With the advent of audiometry in the 1930s, genetic hearing loss could not only be characterised by the mode of inheritance but also by audiometric configuration. Together with generally used features such as age of onset and progression, the characteristic shape of the audiogram became of great importance to characterise and subdivide genetic hearing loss. This comprised low, mid and high frequency hearing impairment as well as flat-type hearing loss. In their comprehensive work Gorlin and Koningsmark used these methods to phenotypically describe syndromic and non-syndromic types of hearing loss[11].

Genetic analysis

A new era started with the unravelling of the structure of DNA by Watson and Crick in 1953[12], which led to the development of molecular biology. By identifying many genes, this new science discovered a genetic basis for many different hereditary diseases. Until now the most widely applied technique to investigate genetic hearing loss is linkage analysis. This method is based on the principle that a mutated gene causes a specific disease. Provided that a sufficient number of affected persons, preferably ≥10, are available, a pattern of inheritance of the disease can be deduced from the pedigree. The segregation of the investigated phenotype is compared to the segregation of 300-400 polymorphic marker alleles. Based on this segregation pattern and on epidemiological features, a statistical calculation is performed to find out whether the disease and a specific marker allele co-segregate significantly and therefore show “linkage”. This calculation leads to a LOD (logarithm of odds) score whose value should be at least 3 to indicate linkage. Once linkage is found, a locus, i.e. a region on the human genome which harbours the disease-causing gene, can be assigned by testing flanking markers and its recombinations. Sometimes this candidate region can be narrowed down by analysing additional (affected) family members. Furthermore genetic database searching for a suitable candidate gene and finally mutation analysis are necessary to be able to identify the disease-causing gene. Questions remain to be answered once the gene has been identified. The characterised gene product, its expression pattern in the human body and, more specifically, within the cochlea, as well as an explanation as to why this specific protein is necessary for normal hearing still need to be unravelled.

Epidemiology and nomenclature

Congenital hearing impairment, i.e. bilateral hearing thresholds of 80dB HL or more, occurs in every 1:1000 newborns[1] and has a genetic cause in about half of the cases. Approximately 75% of these genetically caused cases of hearing impairment have a non-syndromic origin. Of these, the mode of inheritance is autosomal recessive in about 75%, autosomal dominant in approximately 25% and X-linked in less than 5% of the cases[1,13,14]. In general most of the non-syndromic forms will present with a sensorineural type of impairment[1,11,13,15]. During the early nineties of the past century, the first inherited forms of hearing loss were linked. The loci for non-syndromic forms of deafness are called DFN (DeaFNess) and are numbered in chronological order of discovery. According to the pattern of inheritance a locus is designated DFNA (autosomal dominant), DFNB (autosomal recessive) or DFN (X-linked). No specific annotation has been assigned to mitochondrially-inherited forms of hearing loss. An up-to-date overview of the currently known syndromic and non-syndromic forms of genetic hearing loss and their associated genes is given in Tables 1-5. The added year of discovery gives an impression of the progress that has been made since the first description in 1992[16]. An up-to-date overview is available on the world wide web on the “The Hereditary Hearing Loss Homepage”[17].

Table 1 Loci and genes associated with autosomal dominant, non-syndromic hearing impairment

Locus	*Localisation*	*Year*	*Gene*	*Year*
DFNA1	5q31	1992[16]	*HDIA1*	1997[18]
DFNA2	1p34	1994[19]	*GJB3 (CX31); KCNQ4*	1998[20], 1999[21]
DFNA3	13q12	1994[22]	*GJB2 (CX26); GJB6 (CX30)*	1998[23], 1999[24]
DFNA4	19q13	1995[25]		
DFNA5	7p15	1995[26]	*DFNA5*	1998[27]
DFNA6/14/38	4p16.3	1996[28], 1999[29], 2001[30]	*WFS1*	2001[31], 2001[30]
DFNA7	1q21-23	1996[32]		
DFNA8/12	11q22-24	1996[33], 1997[34]	*TECTA*	1998[35]
DFNA9	14q12-13	1996[36]	*COCH*	1998[37]
DFNA10	6q22-23	1996[38]	*EYA4*	2001[39]
DFNA11	11q12.3-21	1996[40]	*MYO7A*	1997[41]
DFNA13	6p21	1997[42]	*COL11A2*	1999[43]
DFNA15	5q31	1998[44]	*POU4F3*	1998[44]
DFNA16	2q24	1999[45]		
DFNA17	22q	1999[46]	*MYH9*	2000[47]
DFNA18	3q22	1998[48]		
DFNA19	10	1998[49]		
DFNA20	17q25	2000[50]	*ACTG1*	2003[51]
DFNA21	6p21	2000[52]		
DFNA22	6q13	2001[53]	*MYO6*	2001[53]
DFNA23	14q21-22	2000[54]		
DFNA24	4q	1999[55]		
DFNA25	12q21-24	1999[56]		
DFNA26	17q25	2000[57]	*ACTG1*	2003[51]
DFNA27	4q12	1999[58]		
DFNA28	8q22	1999[59]	*TFCP2L3*	2002[60]
DFNA29		Reserved		
DFNA30	15q26	1999[61]		
DFNA31		Reserved		
DFNA32	11p15	2000[62]		
DFNA33		Reserved		
DFNA34	1q44	2000[63]		
DFNA35		Reserved		
DFNA36	9q13-21	2000[63]	*TMC1*	2002[64]
DFNA37	1p21	2000[65]		
DFNA39	4q21.3	2001[66]	*DSPP*	2001[66]
DFNA40	16p12	Reserved		
DFNA41	12q24-qter	2002[67]		
DFNA42	4q28			
DFNA43	2p12	2003[68]		
DFNA44	3q28-29	2003[69]		
DFNA45-46		Reserved		
DFNA47	9q21-22	2003[70]		
DFNA48	12q13-14	2003[71]		
DFNA49-50		Reserved		
DFNA51	9q21			

Table 2 Loci and genes associated with autosomal recessive, non-syndromic hearing impairment

Locus	*Localisation*	*Year*	*Gene*	*Year*
DFNB1	13q12	1994[72]	*GJB2*	1997[73]
DFNB2	11q13.5	1994[74]	*MYO7A*	1997[75], 1997[76]
DFNB3	17p11.2	1995[77]	*MYO15*	1998[77]
DFNB4	7q31	1995[78]	*SLC26A4*	1998[78]
DFNB5	14q12	1995[79]		
DFNB6	3p14-21	1995[80]	*TMIE*	2002[81]
DFNB7	9q13-21	1995[82]	*TMC1*	2002[64]
DFNB8	21q22	1996[83]	*TMPRSS3*	2001[84]
DFNB9	2p22-23	1996[85]	*OTOF*	1999[86]
DFNB10	21q22.3	1996[87]	*TMPRSS3*	2001[84]
DFNB11	9q13-21	1997[88]	*TMC1*	2002[64]
DFNB12	10q21-22	1996[89]	*CDH23*	2001[90]
DFNB13	7q34-36	1998[91]		
DFNB14	7q31	1998[92]		
DFNB15	3q21-25; 19p13	1997[93]		
DFNB16	15q21-22	1997[94]	*STRC*	2001[95]
DFNB17	7q31	1998[96]		
DFNB18	11p14-15.1	1998[97]	*USH1C*	2002[98]
DFNB19	18p11	1998[99]		
DFNB20	11q25-qter	1999[100]		
DFNB21	11q	1999[101]	*TECTA*	1999[101]
DFNB22	16p12.2	2002[102]	*OTOA*	2002[102]
DFNB23	10p11.2-21	Unpublished data		
DFNB24	11q23	Unpublished data		
DFNB25	4p15.3-12	Unpublished data		
DFNB26	4q31	2000[103]		
DFNB27	2q23-31	2000[104]		
DFNB28	22q13	2000[105]		
DFNB29	21q22	2001[106]	*CLDN14*	2001[106]
DFNB30	10p12.1	2002[107]	*MYO3*	2002[107]
DFNB31	9q32-43	2002[108]		
DFNB32	1p13.3-22.1	2003[109]		
DFNB33	9q34.3	2002[110]		
DFNB34/36/38/39		Reserved		
DFNB35	14q24.1-24.3	2003[111]		
DFNB37	6q13	2003[112]	*MYO6*	2003[112]

Table 3 Loci and genes associated with X-linked, non-syndromic hearing impairment

Locus	*Localisation*	*Year*	*Gene*	*Year*
DFN1	Xq22	1995[113]	*DDP*	1996[114]
DFN2	Xq22	1996[115]		
DFN3	Xq21.1	1995[116]	*POU3F4*	1995[116]
DFN4	Xp21.2	1994[117]		
DFN5/7		Withdrawn		
DFN6	Xp22			1996[118]
DFN8		Reserved		

Table 4 Chromosomal localisations and genes associated with SOME frequent forms of syndromic hearing loss

Syndrome		*Localisation*	*Gene*	*Year*
Alport syndrome		Xq22	*COL4A5*	1990[119]
		2q36-37	*COL4A3; COL4A4*	1994[120]
Branchio-Oto-Renal syndrome	BOR I	8q13.3	*EYA1*	1997[121]
	BOR II	1q31		2000[122]
Jervell and Lange-Nielsen syndrome	JNLS1	11p15.5	*QVLQT1*	1997[123]
	JLNS2	21q22.1-22.2	*KCNE1(IsK)*	1997[115,124]
Norrie disease		Xp11.3	*Norrin*	1992[125,126]
Pendred syndrome		7q21-34	*SLC26A4*	1997[127]
Stickler syndrome	STL1	12q13.11-13.2	*COL2A1*	1996[128]
	STL2	6p21.3	*COL11A2*	1995[129]
	STL3	1p21	*COL11A1*	1996[130]
Treacher Collins syndrome	TCOF1	5q32-33.1	*TCOF1*	1996[131]
Usher syndrome	USH1A	14q32		1992[132]
	USH1B	11q13.5	*MYO7A*	1995[133]
	USH1C	11p15.1	*USH1C*	1992[134], 2000[135,136]
	USH1D	10q	*CDH32*	1996[137], 2001[90,138]
	USH1E	21q		1997[139]
	USH1F	10q21-22	*PCDH15*	2001[140,141]
	USH1G	17q24-25	*SANS*	2002[142]
	USH2A	1q41	*USH2A*	1990[143], 1998[144]
	USH2B	3p23-24.2		1999[145]
	USH2C	5q14.3-21.3		2000[146]
	USH3	3q21-25	*USH3*	1995[147], 2001[148]
Waardenburg syndrome	WSI	2q35	*PAX3*	1992[149]
	WSII	3p14.1-12.3	*MITF*	1994[150]
	WSII		*SLUG*	2002[151]
	WSIII	2q35	*PAX3*	1993[152]
	WSIV	13q22	*EDNRB*	1995[153]
		20q13.2-13.3	*EDN3*	1996[154]
	WSIV	22q13	*SOX10*	1998[155]

Table 5 Mitochondrial mutations associated with syndromic and non-syndromic hearing impairment

Syndrome	*Gene*	*Year*
MELAS (Mitochondrial Encephalopathy, Lactic Acidosis & Stroke-like episodes)	*tRNALeu*	1990[156]
MERFF (Myoclonic Epilepsy and Ragged Red Fibers)	*tRNALys*	1990[157], 1993[158]
MIDD (Maternally inherited Diabetes and Deafness)	*tRNALeu*	1992[159]
	tRNALys	1998[160]
	several	1992[161]
	tRNAGlu	1995[162]
Progressive myoclonic Epilepsy, ataxia & hearing impairment	*tRNASer*	1998[163]
KSS (Kearns-Sayre Syndrome)	*Several*	1989[164]
Non-Syndromic		
	12S rRNA	1993[165], 1997[166], 1998[167]
	tRNASer	1994[168], 1995[169,170], 1998[163,171,172], 1999[173-175]

Some genetic aspects

As is clear from the tables, many genes have been found to be involved in genetic hearing loss. The specific type of mutation, e.g. missense, nonsense, splice-site, frameshift, insertions or deletions, can have a different effect on the produced protein that is encoded by the gene. They may not only lead to (subtle) phenotypic differences within a certain type of hereditary hearing loss, but even to a different pattern of inheritance, e.g. dominant, recessive or (non-)syndromic. Besides this, the final effect of a mutated gene depends on its function, on the cellular pathways in which the produced (defective) protein is required, as well as on other genetic factors, as is shown by the discovery of a modifier locus[103] (DFNM1).

So far the type of genes involved in syndromic- and non-syndromic genetic hearing loss can be roughly divided in four categories. The first category of genes is involved in maintaining ion homeostasis for the cochlear hair cells and mainly consists of genes that encode channel (components) and ion pumps (*GJB2, GJB3, GJB6, KCNQ1, KCNQ4, KCNQE1, SCLC26A5 and ATP6B1*). Another category of genes encodes essential molecules in hair cell function that are mainly expressed near the stereocilia of the inner and outer hair cells (*MYO1A, MYO3A, MYO6, MYO7A, MYH9, MYO15, DIAPH1, OTOF, CDH23 and STRC)*. A third group of genes encodes protein components of the extracellular matrix and the tectorial membrane (*TECTA, COCH, COL2A1, COL4A3/4/5 and COL11A1/2)*. Last but not least, a group of transcription factors is involved in cochlear development (*POU3F4, POU4F3*, *EYA1, EYA4, PAX2, MITF, SOX10 and EDNRB*). Figure 1 shows a schematic representation of a transverse section of the cochlear duct with localised gene expression.

Outlining the effect of a mutated gene requires a critical and thorough clinical description, including evaluation of long-term audiometric follow-up, speech-recognition scores and vestibular function. Studies of the gene and its expression pattern combined with comprehensive phenotypic characterisation are in fact in-vivo studies on the effect of a given genetic defect.

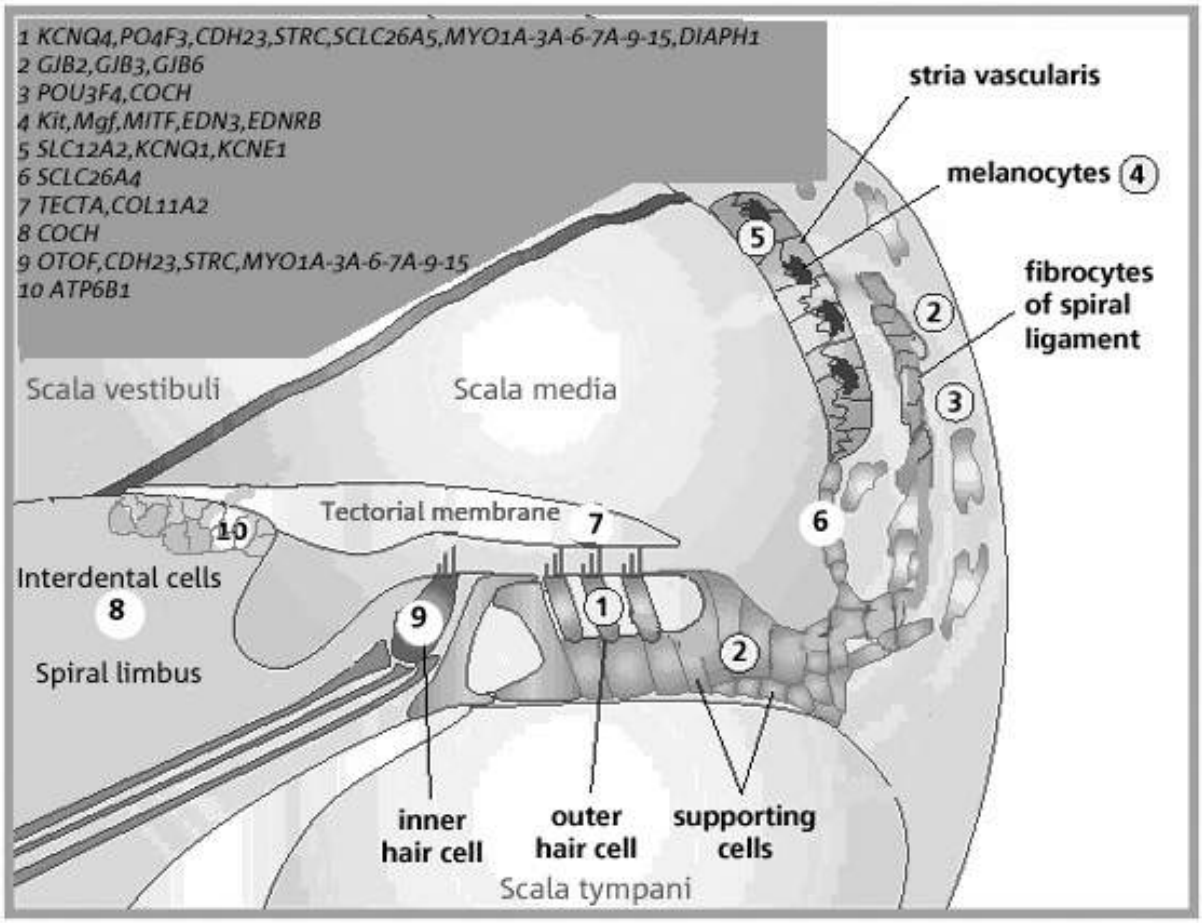

Figure 1 Localised gene expression in syndromic and non-syndromic hearing impairment (modified after Steel et al[176]

Diagnostics

Although many different DFNA and DFNB families are known these days, the actual prevalence is low. Simply testing all genes involved in hereditary hearing loss as a routine diagnostic procedure is expensive and consumes a lot of time. Therefore genetic diagnostic procedures are designed for those with a relatively high frequency in the general population. In Nijmegen for example, mutation analysis on *GJB2* (DFNB1; connexin 26) and *COCH* (DFNA9; cochlin) genes, as well as for certain syndromes, such as Wolfram, Usher (type Ib and IIa), BOR and Pendred syndrome, have become routine diagnostic procedures in general practice, providing parents and affected individuals with aetiological answers. The increasing genetic knowledge in general and specifically in this field of research provides us with insight into the molecular mechanisms underlying (ab)normal hearing and vestibular (dys)function. Altogether this knowledge will hopefully pave the way for future adequate treatment strategies.

Outline and objectives of this thesis

There has been a special interest in genetic hearing impairment at the Departments of Otorhinolaryngology and Human Genetics of the University Medical Centre Nijmegen since 1973. In line with this tradition, this thesis deals with some clinical and genetic aspects of syndromic and non-syndromic forms of genetic hearing loss. Most of the work presented in this thesis is the product of joint efforts of both departments. The research on BOR syndrome (*Chapter 2)* has been realised in collaboration with Prof. dr. W.J. Kimberling and Prof. S. Kumar working at the Center for Hereditary Communication Disorders, Boys Town, Omaha, USA. The first results of this successful collaboration date from 1987.

Chapter 2 deals with the branchio-oto-renal (BOR) syndrome. In 1864 a case report was published on a young girl presenting with mild dysplasia of the external ear, a preauricular sinus, severe hearing loss and cervical fistulae[177]. A few years later, Sir James Paget practically described the complete syndrome in two generations of one family[178]. Before Fraser et al proved that renal anomalies are also part of this syndrome[179], it was known as the earpits-deafness syndrome. Due to lack of systemic renal investigations, such anomalies were not always mentioned at that time. *Chapter 2* includes a review on BOR syndrome (*Chapter 2.1*). Since the late seventies of the past century several studies on BOR syndrome have been performed[180-185] at the Nijmegen ENT department. A clinical case report (*Chapter 2.2*) on the radiological findings and audiometric follow-up of a father and son affected by BOR syndrome initiated a series of studies on this disorder. The radiological malformations and audiometric data found in this family encouraged us to perform a comprehensive magnetic resonance imaging (MRI) study (*Chapter 2.3*) as well as a thorough audiometric follow-up study (*Chapter 2.4*) in a total of six BOR families. Gene linkage and mutation analysis studies were initiated previously in these families in Boys Town, Omaha[186-189]. The obtained results were used to detect any trends and/or existing correlations between radiology, audiometry and mutation analysis results.

Chapter 3 provides a comprehensive summary on DFNA9/*COCH* (*Chapter 3.1*) and reports on two Dutch DFNA9/*COCH* families. The discovery of the first family dates from 1998, when this family was the subject of a term paper on familial autosomal dominant sensorineural hearing loss[190]. Soon after, the underlying

disease-causing P51S mutation in the *COCH* gene was detected and published. This publication also included audiometric and vestibular data to outline clinical features (*Chapter 3.3*). *Chapter 3.2* reports on a G88E/*COCH* mutation present in a Dutch family and compares the available audiometric and vestibular (follow-up) data to those of the originally reported family from the United States of America carrying the same mutation.

In *Chapter 4* another Dutch family with autosomal dominant inherited sensorineural hearing loss is presented. Linkage analysis enabled us to localize the disease to the DFNA20/26 and delimit the critical region. Not only genetic data, but also the clinical features of both families were studied and compared to outline the phenotype as accurately as possible.

DFNB1/*GJB2* is the subject of *Chapter 5*. *GJB2* mutations, also known as connexin 26 mutations, are the most prevalent causes of autosomal recessively inherited hearing loss as well a sporadic deafness. This high prevalence has led to the implementation of *GJB2* mutation analysis as a diagnostic tool in many genetic laboratories all over the world. This also applies to the University Nijmegen Medical Centre. *Chapter 5* gives a review on connexin 26 and hearing loss and provides the first results bearing on *GJB2* mutation analyses in Nijmegen and in The Netherlands.

Finally, the conclusions of this thesis are summarised and discussed in the light of the present knowledge in *Chapter 6*.

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Chapter 2

BOR syndrome

Chapter 2.1

The Branchio-Oto-Renal syndrome

MH Kemperman

C Stinckens

S Kumar

FBM Joosten

PLM Huygen

CWRJ Cremers

Advances in Oto-Rhino-Laryngology. Genetic hearing impairment. Its clinical presentations.

Basel, Karger 2002;61:192-200

Introduction

Apart from brief descriptions dating from the 19th and the beginning of the 20th century, Melnick et al.[1] were the first to report on the clinical aspects of the branchio-oto-renal (BOR) syndrome. The autosomal dominant BOR syndrome (OMIM #113650), formerly known as the earpits-deafness syndrome, shows a wide spectrum of highly variable clinical manifestations, comprising combinations of branchial-arch, otic and renal anomalies[2]. The four most characteristic clinical symptoms are: (i), hearing loss; (ii), second-branchial arch cleft, sinus of fistulas; (iii), malformations of the auricle, the ear canal, the middle and/or inner ear including earpits, and (iv), renal anomalies, ranging from mild hypoplasia to complete agenesis[3-5]. Chronic infection of a second-branchial arch cleft, sinus of fistulas can make surgical excision necessary. The frequencies of the main features in the BOR syndrome based on a review of 184 cases from the literature are summarised in Table 1[6]. Other associated but less common features include facial/palatal abnormalities, lacrimal duct stenosis and external auditory canal stenosis[1,4,5,7]. This disorder shows almost complete penetrance, whereas its expression can be quite variable[1-3]. BOR syndrome has an estimated general prevalence of 1:40,000 and occurs in 2% of profoundly deaf children[4].

Table 1 Frequency of the main features of the BOR syndrome in 184 patients based on a review of 184 cases from the literature (with courtesy of Stinckens et al[6])

	Reported presence/absence of features in 184 cases	*Reported presence of main features*
Malformed auricles	121	105/121 (86.8%)
Second branchial arch fistula/cyst	155	134/155 (86.5%)
Preauricular sinus	169	147/169 (87.0%)
Renal anomalies	115	67/115 (58.3%)
Stenosis of nasolacrimal duct	34	16/34 (47.0%)
Hearing impairment	153	146/153 (95.4%)

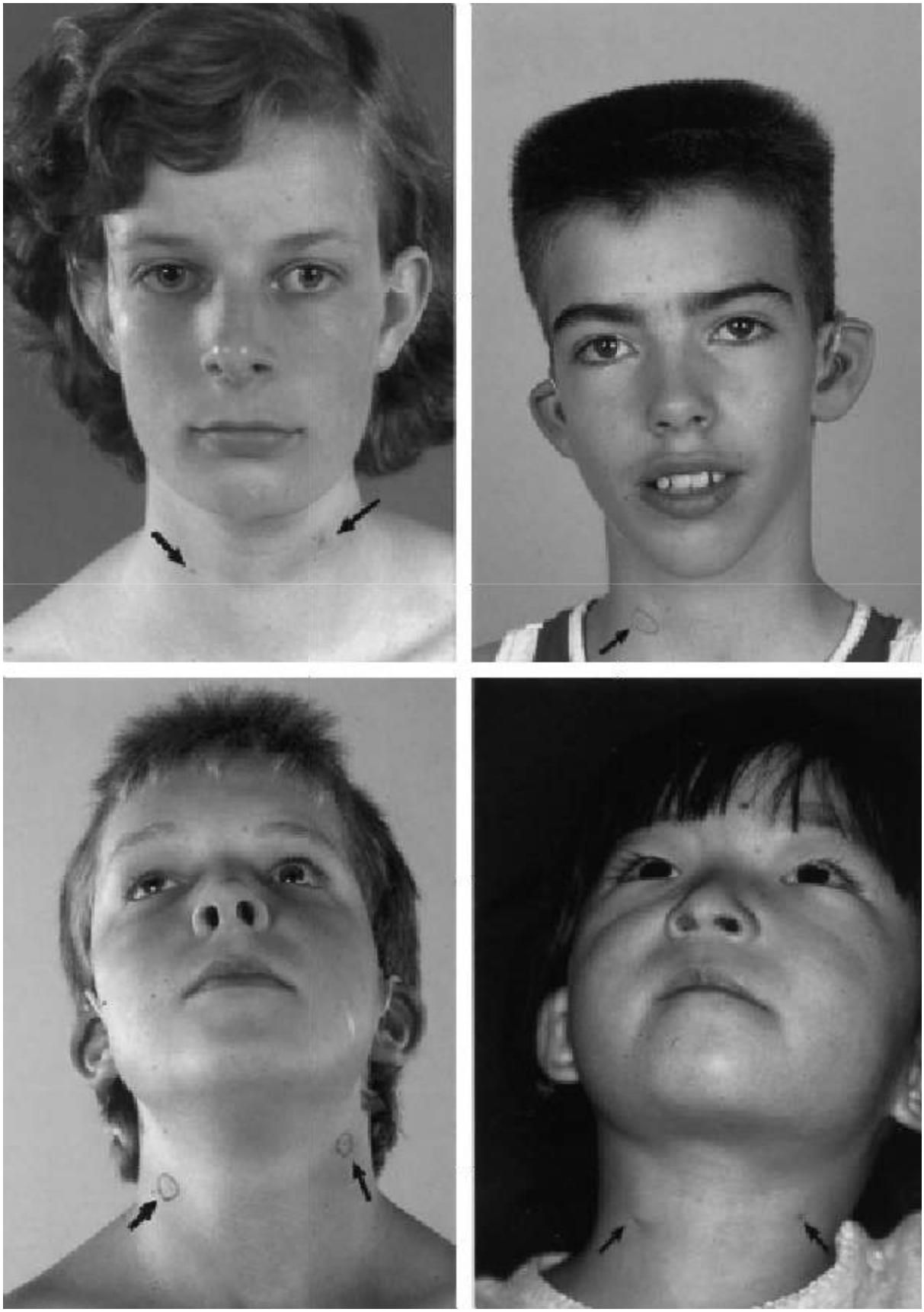

Figure 1 Pictures of typical clinical features in different BOR patients

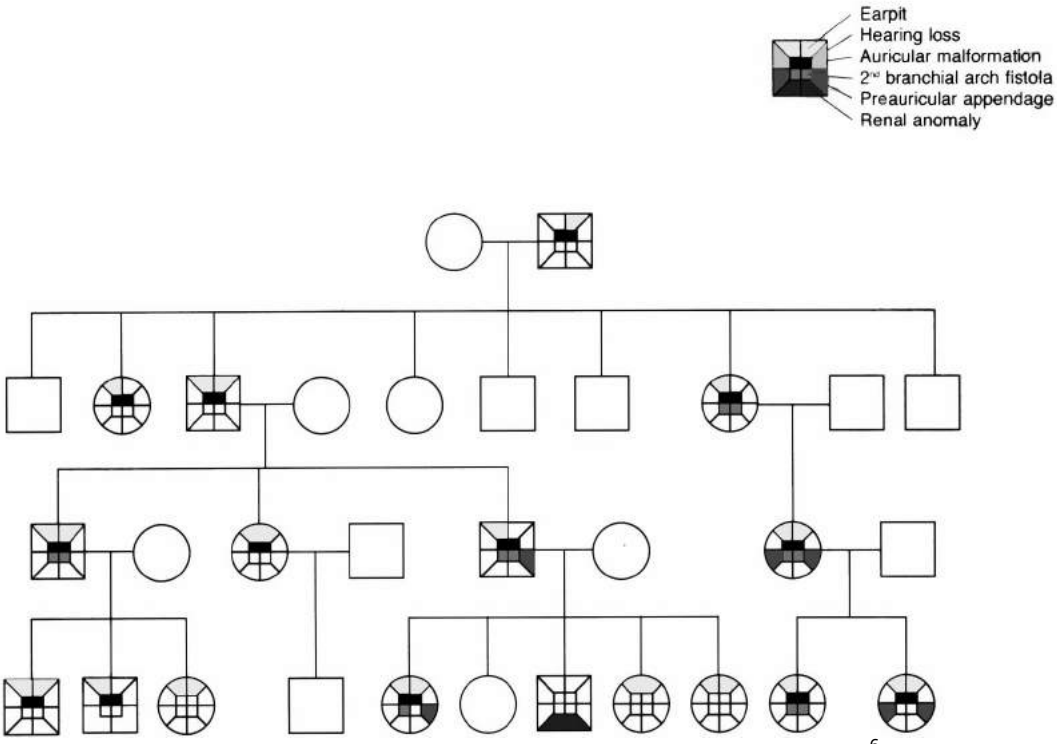

Figure 2 Example of a pedigree of a BOR family (with courtesy of Stinckens et al[6])

Hearing loss and vestibular function

The type of hearing loss can be conductive, sensorineural or mixed and was formerly considered to be stable. A few reports mentioned progressive hearing loss. A recent long-term audiometric follow-up study of a number of suitable patients disclosed that progressive fluctuant hearing loss may be a regular finding in the BOR syndrome (authors' unpublished data)[6,8] Vestibular studies are rarely reported. In one study vestibular impairment was reported to be present in about half of the affected cases (n = 13)[9].

Renal anomalies

Renal involvement in the BOR syndrome is also characterised by great variability, ranging from asymptomatic minor deformities to severe dysplastic kidneys or even kidney agenesis[3-5,10,11]. The expression of any type of renal anomaly is almost 25%. Due to its variability, many renal problems remain clinically and anamnestically undetected, whereas other patients depend on dialysis and await kidney transplantation. Especially minor renal abnormalities do not show any progressive characteristics[10]. Recent results of studies in mouse models suggest a role of the *EYA1* gene in the development of the kidney (see below).

Middle-ear and inner-ear morphology

Branchial-arch involvement of the BOR syndrome accounts for the serious involvement of the middle- and inner-ear structures. Various types of middle-ear anomalies have been documented, including (i) displacement, hypoplasia, or aplasia of middle-ear ossicles, (ii) fusion and fixation of two or more ossicles, (iii) stapes ankylosis and/or absence of oval window, and (iv) varying size and shape of the middle-ear cavity[9]. Radiological studies of the inner ear in genetic syndromes are few and mainly limited to individual cases. Both the cochlear and the vestibular partitions can be involved in inner-ear abnormalities, ranging from an enlarged vestibular aqueduct, hypo-/dysplastic cochlea, bulbous internal acoustic canals, a deep posterior fossa and acutely-angled promontories to hypoplastic vestibule and/or semicircular canals[5,9,12-16].

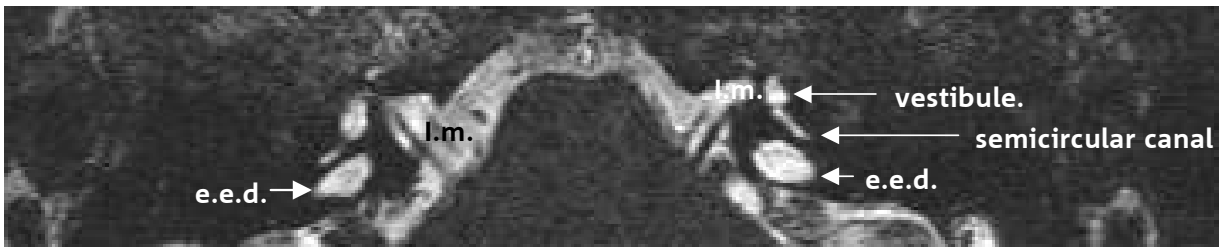

Figure 3 High resolution (CISS) heavily T2 weighted MR image of the temporal bone at the level of the internal meatus (I.m.). Typical example of the enlarged endolymphatic duct (e.e.d.) on both sides.

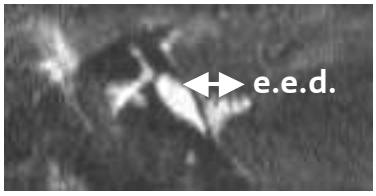

Figure 4 Multiplanar reformatted image (MPR) of the same patient (Figure 3). Semisagittal plane through the endolymphatic duct (e.e.d.) on the left side showing the course of this duct in the longitudinal direction.

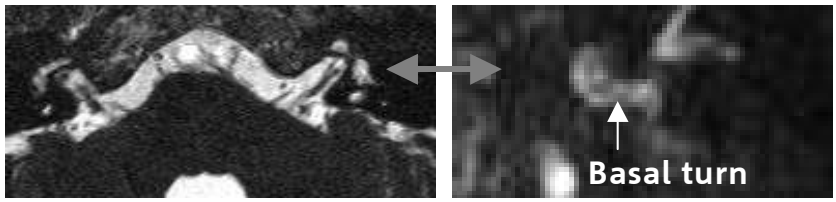

Figure 5 Another typical sign is the hypoplastic cochlea as shown here by an MPR image of an affected cochlea. Image in the axial plane at the level of the internal meatus and apex of the cochlea. Semicoronal section through the turns of the cochlea shows only 1 complete turn and no middle and apical turns.

More recently performed MR-imaging studies confirmed the frequent occurrence of such inner-ear abnormalities in 7 families affected by the BOR syndrome (authors' unpublished data)[6,8]. Apart from these anomalies, the presence of an enlarged endolymphatic duct and/or sac could also be demonstrated in some affected family members. Although long-term audiometric follow-up demonstrated the presence of progressive fluctuant hearing loss in some of the

affected BOR patients, a clear correlation between the MRI findings and this type of hearing loss could not yet be demonstrated[6,8]. However, sensorineural thresholds were significantly higher in cases with enlargement of the endolymphatic duct and/or sac (authors' unpublished data).

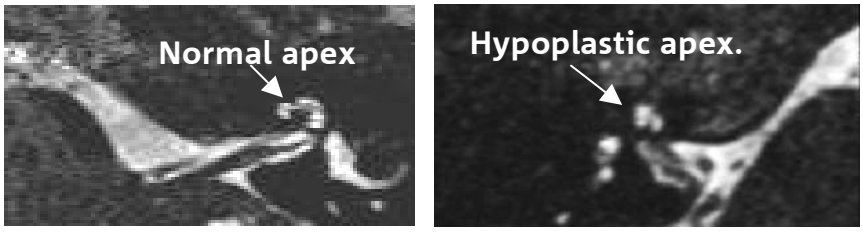

Figure 6 For comparison a normally developed cochlea (left) and an example of an affected cochlea (right) showing absent apical turns in the axial plane.

Reconstructive middle ear surgery

The conductive component in the hearing impairment is mostly due to congenital anomalies of the ossicular chain. A predisposition for otitis media with effusion might be present. As a result of the branchiogenic origin of the ossicular chain all ossicles can be anomalous. Ankylosis of the stapes footplate as well as a too short long process of the incus are frequently present. Even the malleus handle can be missing[9]. A malleovestibulopexy can be needed to reconstruct the ossicular chain functionally. The curvature of the anterior bony canal is usually so severe that a canal-plasty in the same procedure is needed to allow crimping of the stapes-incus replacing teflon-platinum prosthesis around the malleus handle[17]. Congenital anomalies of the middle ear can be severe; the round window niche can be missing and the facial nerve may cross the oval window or the promontory. Minor congenital ear anomalies causes reconstructive surgery of the ossicular chain in BOR syndrome to be less successful than usual. A preauricular sinus can be abnormally large and communicating with the middle ear cleft[18]. In case of chronic infection of a sinus excision can be necessary.

Genetics

The *EYA1* gene (OMIM #601653) has been found to underlie the BOR syndrome[19]. This is the human homologue of the drosophilia 'eyes absent' gene one (*eya1*) and is localised on human chromosome 8q13.3[19-22]. *EYA1*, consisting of 16 exons with a genomic interval of 156 kB, forms part of a gene family comprising at least 3 other isoforms (*EYA2*, *EYA3* and *EYA4*)[22]. So far three different transcripts of the *EYA1* gene have been identified to result from alternative splicing of mRNA

transcripts. The gene encodes a 559-amino acid polypeptide and contains a highly conserved region called the *eyes absent* homologous region (*eya*HR), encoded by exons 9-16, which has an essential role in normal gene function. Many different types of disease-causing mutations have been identified and most of these cluster in *eya*HR, which is therefore the region of major interest for mutation analysis of this gene.

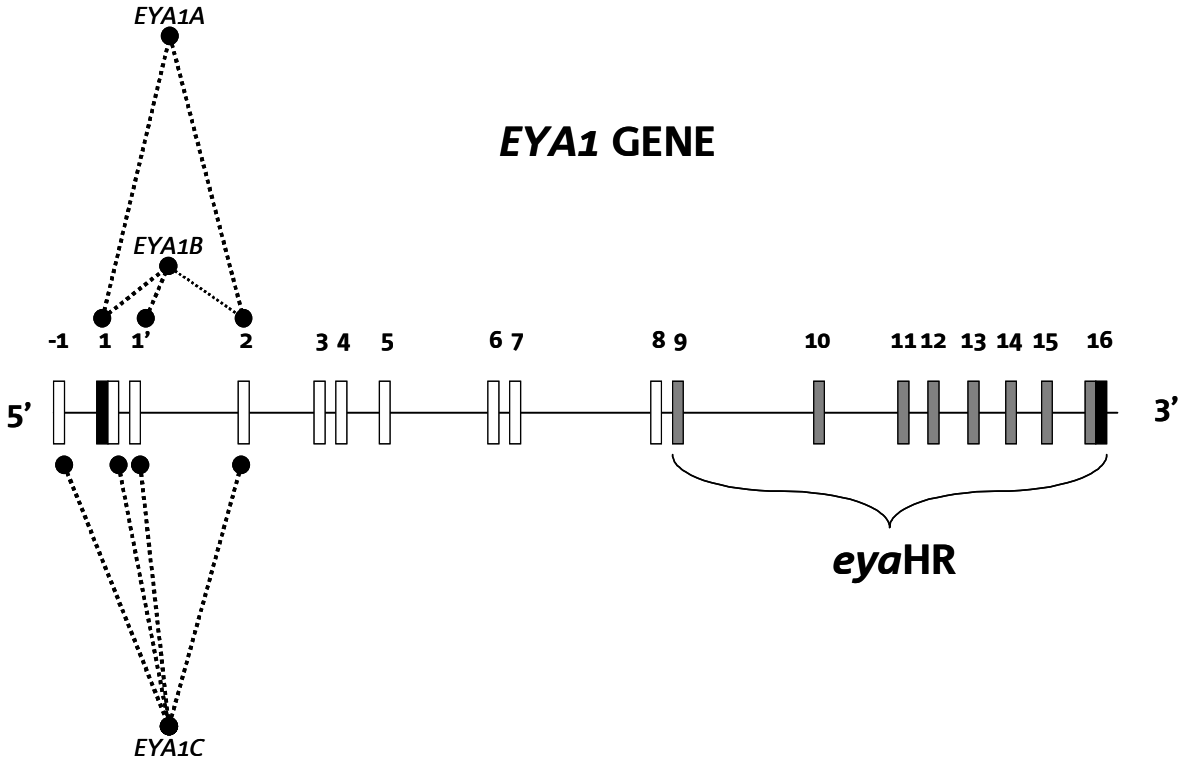

Figure 7 Schematic representation of the *EYA1* gene structure (unscaled). All boxes are coding exons except for the black-filled boxes. The grey-filled boxes indicate the *eya*-homologous region. The dotted lines indicate how the different isoforms (*EYA1A, EYA1B and EYA1C*) are built up.

In spite of positive linkage to the *EYA1* locus, mutations in this gene have been detected in only 25% of the patients with the diagnosis of BOR. This can be explained by mutations in yet unknown important structures of this gene, i.e. promoters or introns, which are not recognised with the present methods and knowledge. A second gene has recently been discovered on chromosome 1q31 in a family without signs of second-branchial arch cervical fistulas[23]. It is not yet known what proportion of BOR cases is caused by mutations in this gene. Involvement of this second gene together with the various different mutations in the *EYA1* gene is evidence of the genetic heterogeneity of BOR syndrome. Recently Rickard et al.[24] proposed to limit the screening of the *EYA1*gene to cases of classical BOR syndrome, until mutation-detection strategies yield higher detection rates. Although positive mutation analysis can provide tools to predict

the risk of recurrence in a given family, it does not allow for the prediction of phenotypic features due to the variable expressivity of the syndrome. This, together with our lack of knowledge regarding genotype-phenotype correlations, makes genetic counselling a difficult task. Further research on the BOR syndrome will have to clarify the factors and genes that influence the phenotypic variability of BOR patients.

Animal models

In Drosophila the *eya* gene is involved in the formation of the compound eye, whereas the expression pattern of the murine orthologue, *Eya1*, suggests a role in the development of major inner-ear components and metanephric cells[22]. Johnson et al.[25] described a spontaneous mutation in the *Eya1* gene causing an autosomal recessive phenotype of deafness in a mouse model with circling and head-bobbing behaviour. Subtle developmental anomalies in the superior part of the labyrinth, including foreshortening and narrowing of the lateral semicircular canals and incomplete formation of the common crus, were noted. Xu et al.[26] inactivated the *Eya1* gene in mice and reported that $Eya1^{+/-}$ heterozygotes showed conductive hearing loss associated with middle ear malformations. Similar to the BOR syndrome, these mice showed renal defects at low penetrance, including renal hypoplasia and unilateral agenesis. Inner-ear abnormalities in these heterozygotes included the vestibular labyrinth, but no specific details were given. $Eya1^{-/-}$ homozygotes lacked ears and kidneys due to defective inductive tissue interactions and apoptotic regression of the organ primordia.

Animal models provide insight in the way the genotype affects the phenotype. They enhance our understanding of the BOR syndrome and its underlying mechanism. Therefore, more well-designed animal models are needed to unravel this syndrome.

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Chapter 2.2

Progressive, fluctuant hearing loss, enlarged vestibular aqueduct and cochlear hypoplasia in the BOR syndrome

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PLM Huygen

FBM Joosten

CWRJ Cremers

Otology & Neurotology 2001;22:637-643

Abstract

Objective: To study the results of petrosal bone imaging and audiometric long-term follow-up of two patients with branchio-oto-renal (BOR) syndrome and relate them to the clinical features, including caloric responses.

Study design: Longitudinal case study.

Setting: Tertiary referral center.

Patients: A father and son with the BOR syndrome.

Main outcome measures: Both patients underwent imaging studies to detect and evaluate inner ear anomalies. Longitudinal audiometric analysis of the hearing threshold data over the previous 23 years was performed. Caloric tests were performed at various ages.

Results: The son had a short, wide internal acoustic canal, a hypoplastic cochlea, a plump vestibule and a wide vestibular aqueduct on both sides; the semicircular canals and endolymphatic sac were of normal size. He showed progressive, fluctuant sensorineural hearing loss. Caloric tests disclosed hyporeflexia on the left side. The father had a plump internal acoustic canal and hypoplastic cochlea on both sides. The left vestibule was hypoplastic and the left vestibular aqueduct was marginally enlarged. He showed severe hearing impairment, without substantial progression or fluctuation, and caloric areflexia on the left side.

Conclusion: These findings suggest a correlation between progressive, fluctuant sensorineural hearing loss with caloric hypofunction and the presence of an enlarged vestibular aqueduct in the BOR syndrome. Additional longitudinal case studies are needed to further evaluate such a correlation.

Introduction

The branchio-oto-renal (BOR) syndrome is an autosomal dominant inherited syndrome, in which affected individuals may have sensorineural, mixed or conductive hearing loss, preauricular pits and structural defects of the outer, middle and inner ear. Other features include lacrimal duct stenosis, branchial fistulas or cysts of the second branchial arch, and renal anomalies ranging from mild hypoplasia to complete agenesia. A long and narrow face with a high-arched palate and deep overbite are less frequent symptoms[1-4]. Hearing loss, branchial clefts and earpits are most frequently expressed. Hypoplasia of the cochlea is another feature of the BOR syndrome[2,5,6]. The penetrance of relevant clinical features has been reported previously[3,4]. The estimated general prevalence of the BOR syndrome is 1:40,000; in profoundly deaf children the relative prevalence is 2%[7].

The first gene underlying the BOR syndrome has been identified as the human homologue of the drosophila eyes absent gene 1 *(EYA1)*[8-10]. Expression of the murine orthologue *Eya1* occurs in all components of the inner ear and in the metanephric cells surrounding the ureteric branches, which suggests a role in the development of inner ear and kidney[9]. The BOR syndrome shares important features with other branchial arch syndromes[11,12]; it shows high penetrance but very variable expression, part of which may be explained by genetic heterogeneity[1,13-15] A second gene has been identified recently[16].

An enlarged vestibular aqueduct (VA) and a hypoplastic cochlea are common radiological findings in Pendred syndrome[17-19]. The hearing loss found in this autosomal recessive inherited syndrome varies in severity and progression[17-19]. MRI of the petrosal bones and audiometric follow-up studies showed a correlation between a widened VA and this progressive hearing loss[17,18]. Recently, several mutations have been identified in the gene underlying Pendred syndrome *(PDS)*[17,18,20,21]. Mutations in the *PDS* gene and bilaterally enlarged VAs were also found in three individuals with congenital profound non-syndromic autosomal recessive hearing loss (DFNB4)[22]. The perchlorate test was not performed on the affected members of this family and therefore Pendred syndrome has not been excluded.

An enlarged VA and hypoplasia of the cochlea have also been reported in the sensorineural deafness-oligodontia syndrome and in the BOR syndrome[1,5,23,24]. In a histopathologic study of the temporal bones of a BOR patient Fitch et al.[5] found enlarged VAs and cochlear hypoplasia. Daggilas et al.[23] and Chen et al.[1] were the first to demonstrate enlarged VAs on CT scans of BOR patients. In this study 2 BOR patients, who had already been followed-up for a long time with repeated audiometry and caloric tests, underwent imaging studies to find out whether they had similar inner ear anomalies underlying their specific functional features.

Material and methods

We investigated a 3-generation family in which three members were affected by the BOR syndrome. A mutation in the *EYA1* gene was found in these patients.(authors' unpublished data) Patient A has been previously indicated as case C-201 or C-13 [4,25-27] and patient B as case C-302 or C-14[4,25,27]. Both patients underwent high-resolution CT scanning (Siemens Somiton plus 4, Siemens, Forchheim, Germany) in the axial plane, as well as high-resolution heavily T_2 weighted 3D MR imaging of the temporal bones (Magneton Vision, 1.5 Tesla, Siemens, Erlangen, Germany). This MRI technique enables 3D reconstruction in every desirable plane to study abnormalities of the inner ear structures. Because of the presence of endolymph these structures have a high signal intensity on T_2 weighted images. A VA is considered to be widened when the middle part is wider than the posterior semicircular canal (SCC) and measures more than 1.5 mm on CT and/or MRI[28].

Audiograms were obtained in a sound-treated room, according to common clinical standards. Binaural caloric tests were performed with electronystagmography (eyes open in the dark) and computer analysis. Statistical analyses (Prism PC program, version 2, GraphPad, San Diego, CA, USA) comprised linear regression analysis of the longitudinal hearing threshold data and the threshold shifts between consecutive audiograms obtained for each frequency; this analysis included a runs test on the validity of the (linear) regression model. Progression was called "significant" if it could be linked to correlation coefficients that were significantly greater than zero at a sufficient number of frequencies (binomial distribution statistics). Cofluctuation analysis consisted of performing correlation analysis between any relevant pair of synchronous threshold shifts.

Cofluctuation was called "significant" if it was linked to a sufficiently high number of significant correlations between pairs of synchronous shifts. The probability level used in any test was P = 0.05.

Case studies

Patient A, a 55-year-old man, was seen for the first time in 1976 at age 33 years, because of bilateral discharging cervical fistulas and preauricular sinuses. A cleft palate had been treated surgically in childhood. On examination, bilateral cervical fistulas and preauricular sinuses were seen (Figure 1). A preauricular tag was noted in front of his left ear. Examination of the tympanic membranes showed no anatomical abnormalities. A previous intravenous pyelogram had revealed a renal malformation[27]. Bilateral mixed hearing loss of 90 dB was present[25]. Caloric tests were performed at age 32 and 44 years and disclosed vestibular areflexia on the left side. The bilateral cervical fistulas were removed surgically, as well as the preauricular fistula, which communicated with the tympanic cavity[26]. Exploratory tympanotomy was not performed.

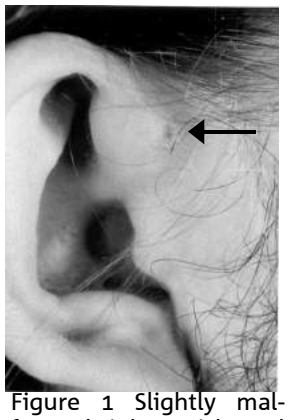

Figure 1 Slightly malformed right auricle and preauricular sinus (arrow) of patient A.

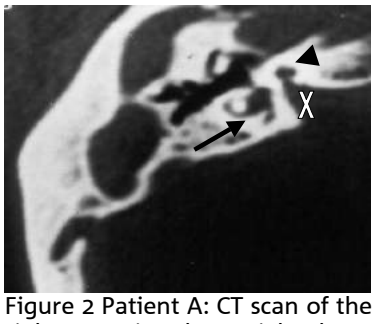

Figure 2 Patient A: CT scan of the right ear in the axial plane. Widened IAC ("X") and hypoplastic cochlea (arrowhead). The vestibule is widened, the lateral SCC is slightly too small (arrow).

Computed tomography of the temporal bones demonstrated a wide, plump internal acoustic canal (IAC) and a hypoplastic cochlea on both sides. The left vestibule appeared to be hypoplastic. The lateral SCC was slightly too small (Figure 2). MRI showed a marginally widened left VA. The right VA was not abnormally wide. No endolymphatic sac could be visualised. (Figure 3).

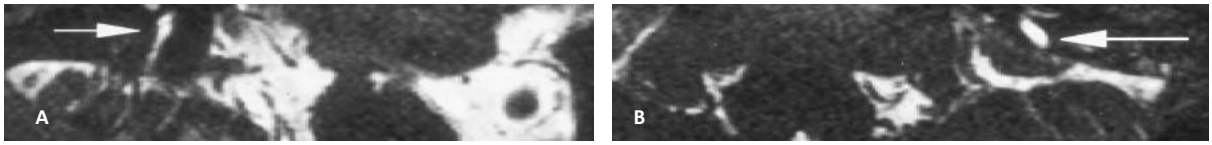

Figure 3 Patient A: Coronal reconstruction MRI through the endolymphatic ducts on the right (A) and left side (B). The left endolymphatic duct is abnormally wide, whereas the right one is of normal size (arrows). No endolymphatic sac could be visualised.

Increasing bilateral hearing loss from about 90 dB in 1976 to 100-105 dB in 1998 was evident in the 22-year audiometric follow-up data of this patient (n=6 audiograms, age 32-54 years) (Figure 4). Progression was significant at all frequencies, except at 1 and 4 kHz in both ears and at 0.25 kHz in the right ear.

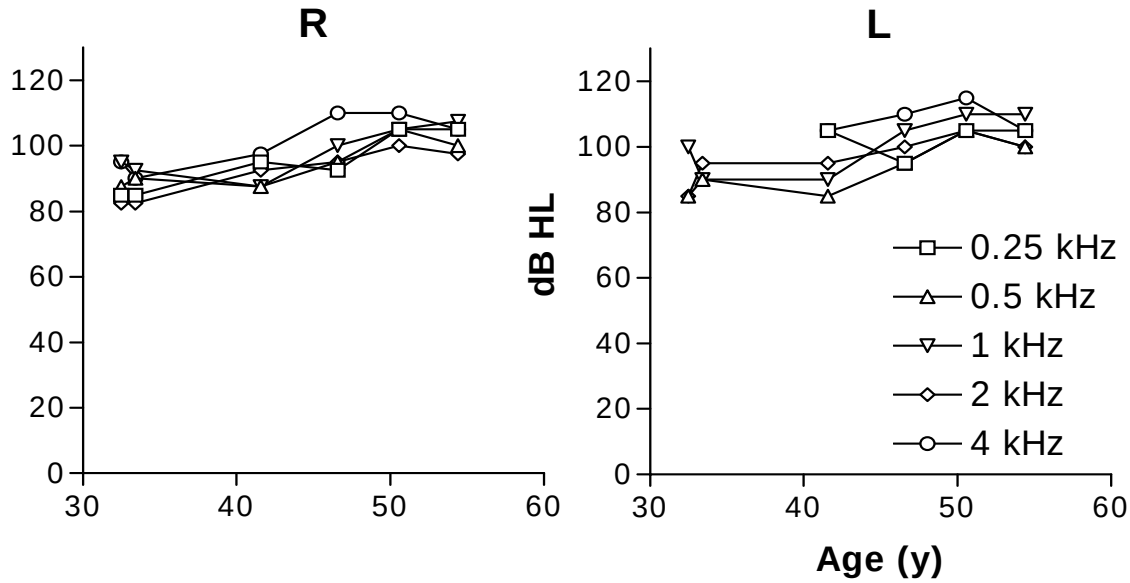

Figure 4 Patient A: air conduction thresholds plotted against age (R, right; L, left). These longitudinal threshold data show only limited progression, well within the normal limits of presbyacusis (see text). The data are not suitable for fluctuation analysis.

However, the slopes were fairly similar to those on plots that were prepared (not shown) using median presbyacusis threshold data according to ISO norms at similar ages[29]. The apparent progression vanished at all frequencies when the threshold levels were corrected for median presbyacusis. Progression could therefore be attributed to presbyacusis. Analysis of fluctuations was impossible because of an insufficient number of observations. The air-bone gap (ABG) lies between about 50 dB at 0.5 kHz and about 30 dB at 2 kHz. The bone conduction threshold increased by about 10 dB between the age of 32 and 54 years, which seems to be in line with the threshold increase associated with presbyacusis. This patient clearly stated that his hearing had been much better during childhood and adolescence. Audiograms obtained at that age could not be retrieved.

Patient B is the 30-year-old son of patient A. At his first examination (age 7 years) he was found to have a 50 to 80 dB mixed hearing loss. Physical examination revealed bilateral cervical fistulas as well as preauricular sinuses (Figure 5). There were no preauricular tags but his auricles were slightly cup-shaped. Some

retrognathia and a high-arched palate were present and otoscopy showed no abnormalities[25].

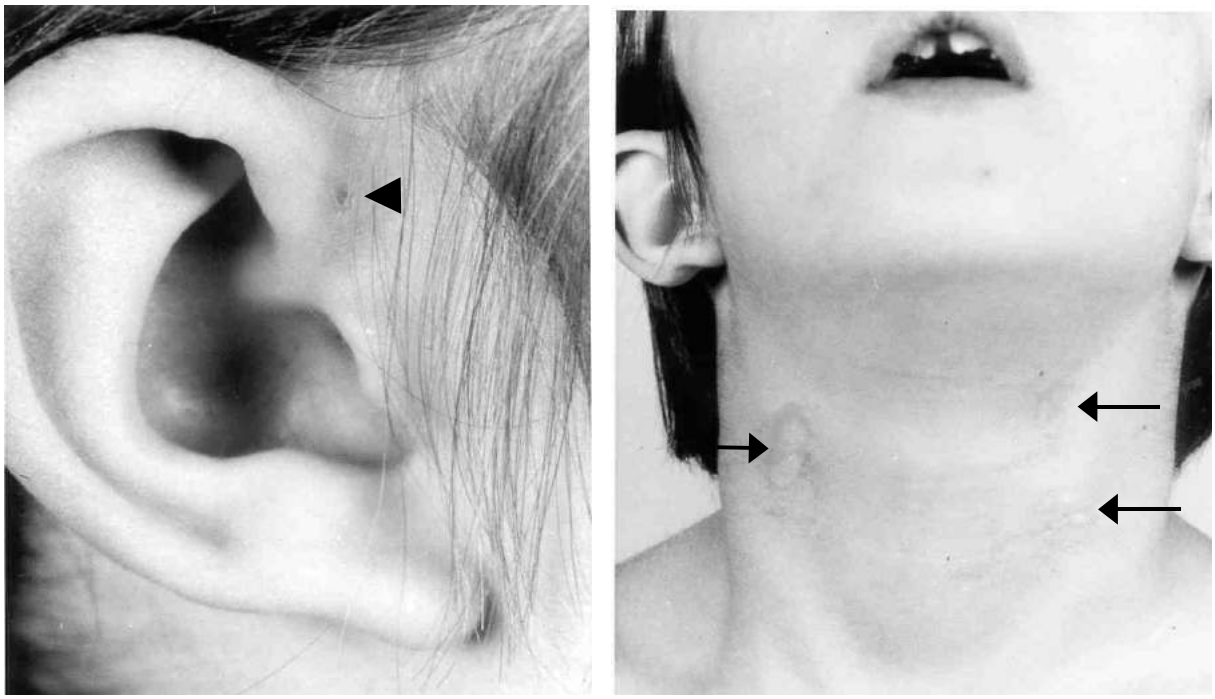

Figure 5 *Left panel*: Slightly malformed right auricle and preauricular sinus (arrowhead) of patient B at 7 years of age. *Right panel*: Bilateral second branchial arch fistulas (arrows) of patient B at 7 years of age.

In 1976, temporal bone tomography had shown scarcely pneumatised mastoids. An abnormal configuration of the ossicular chain was seen, as well as a Mondini-type cochlear dysplasia and a wide IAC bilaterally. Renal malformations were visible on a previously obtained intravenous pyelogram[27]. The cervical fistulas were bilaterally excised. Exploratory tympanotomy of the right ear revealed a dysplastic, plump long process of the incus and incomplete stapedial crurae. The oval niche could not be identified, but the niche of the round window was visible. The facial nerve was dehiscent and no ossicular chain reconstruction was performed. Five years later, grommets were placed twice in the left ear, because of recurrent otitis media with effusion. In 1990, myringoplasty of the left ear was performed to close the remaining perforation, however the perforation recurred a few years later. The patient also suffered from recurrent external otitis as a result of occlusion of the external ear canal by the mold of his hearing aid.

CT scanning of the temporal bones (Figure 6, left panel) showed a short, wide IAC and a hypoplastic cochlea on both sides. MRI of the temporal bone (Figure 6, right panel) showed a plump vestibule with normal-sized SCCs. A wide VA and normal-

sized endolymph sac were found bilaterally. Caloric tests were performed at age 10 and 16 years; they revealed hyporeflexia on the left side.

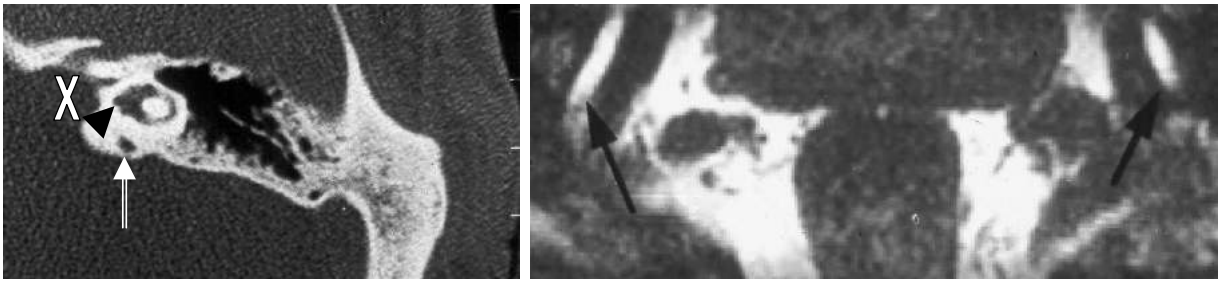

Figure 7 *Left panel*: CT scan of the left ear in the (transverse) axial plane of patient B. A widened internal meatus is visible ("X") as well as a plump vestibule (arrowhead). The vestibular aqueduct (arrow) is widened. *Right panel*: Coronal reconstruction of 3D high-resolution MRI showing an enlarged endolymphatic duct (arrows) on both sides of patient B at the age of 28 years.

The audiometric follow-up data of this patient over 23 years (29 audiograms; age range, 6-29 years) (Figure 8) demonstrated clear progression of hearing loss. This consisted of an increase in the sensorineural component first noted in the right ear, which later on also developed in the left ear. Progression may have been most prominent early in the follow-up period, especially at the lower frequencies, but such an interpretation is questionable, because it was mainly based on the earliest audiograms, obtained at the age of 6-7 years. Regression analysis (after exclusion of the first audiogram) was performed for air conduction and showed that progression was generally significant at all frequencies. However, even after exclusion of the first audiogram, progression may have been nonlinear; the runs test was significant at 0.25-2 kHz in the left ear and at 0.25 kHz in the right ear. All frequencies showed considerable threshold fluctuations (Figure 8).

Cofluctuation analysis comparing the separate frequencies in each ear showed that, with few exceptions, synchronous air conduction threshold shifts between consecutive audiograms generally covaried in the same direction. Both the right ear (positive, significant cofluctuation in 5 of 10 comparisons) and the left ear (13 of 15 comparisons showed cofluctuation, 9 of those were significant) showed significant cofluctuation of the separate frequencies. Binaural cofluctuation in air conduction threshold was observed for the frequencies 0.25, 0.5, 1, 2 and 4 kHz (significant at 0.5 and 1 kHz). Shifts in air and bone conduction thresholds demonstrated a high degree of covariation.

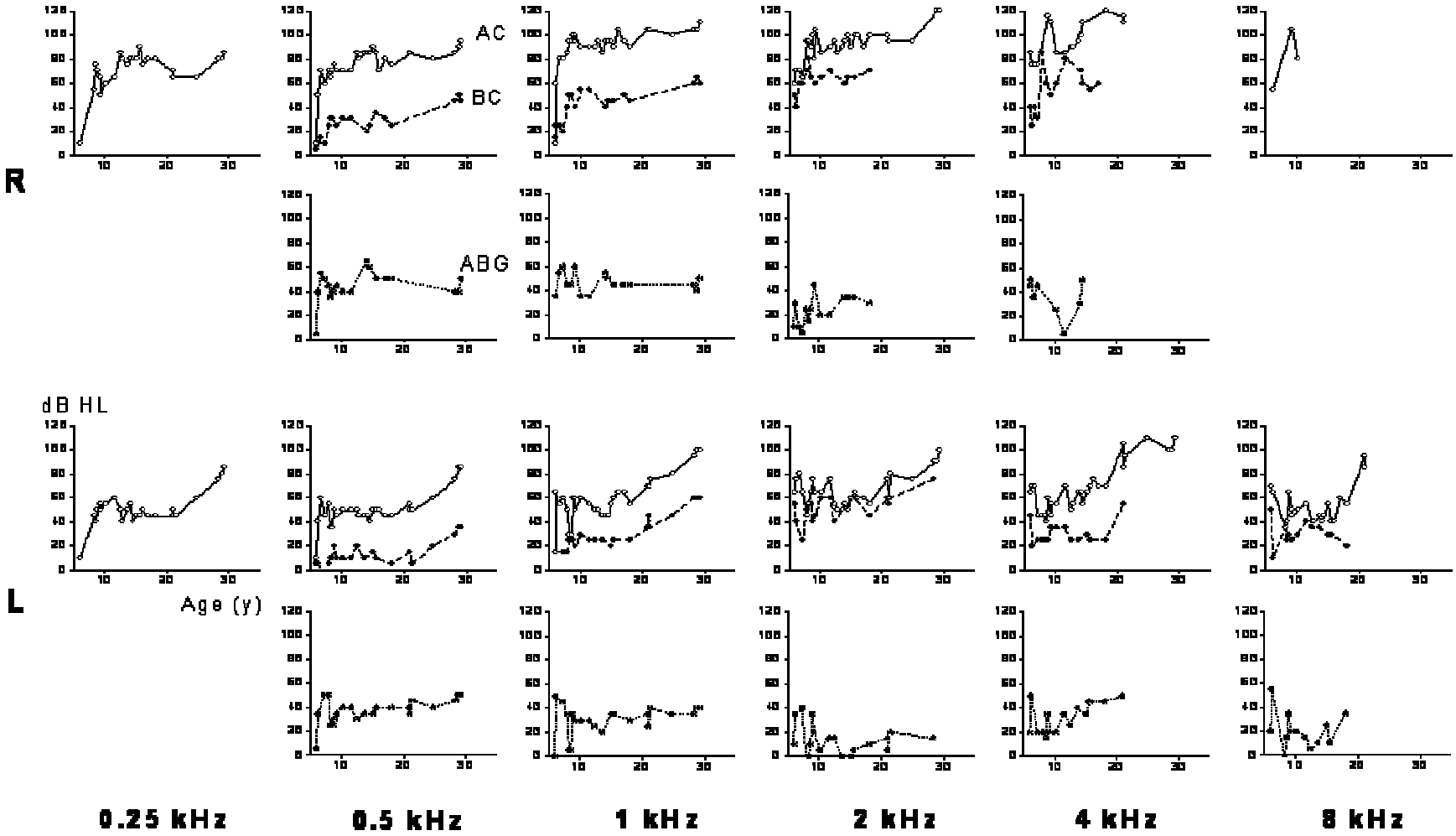

Figure 8 Patient B: Plots of 0.25-8 kHz air conduction thresholds (AC, dB HL, o-), bone conduction thresholds (BC, dB HL, ●---) and air bone gaps (ABG, *···) in the right (R) and left (L) ear against age (in years). There is clear progression in AC and BC levels at most frequencies, which is lacking in ABG. Fluctuation analysis (see text) constituted pairwise intervariable comparison (per ear) or interaural comparison (per variable) at given frequencies (vertically aligned panels).

Progression in bone conduction thresholds was significant at 0.5 and 1 kHz in both ears and at 2 kHz in the left ear. Independently of age, the ABG in both ears was 30 to 60 dB at 0.5 to 1 kHz and under 40 dB at the higher frequencies. Thus the ABG did not show any substantial progression, but it did show considerable fluctuation.

It is obvious that ABG data depend on both the air conduction and bone conduction levels. We would have liked to evaluate the relationship between these variables, but as they are not stochastically independent, regression or correlation analysis is prohibited. We therefore only inspected the synchronous consecutive shifts in bone conduction level and ABG directly at a given frequency in a given ear. We observed a remarkable counterfluctuation (data not shown). Stochastical interdependency can be avoided by replacing one of the variables involved by the corresponding variable pertaining to the other ear. Following such a replacement, we could not detect any significant correlation between any of the variables involved. These findings suggest that the observed counterfluctuation of ABG and bone conduction threshold pertaining to the same ear was a trivial phenomenon.

Discussion

More than 20 years of audiometric follow-up data and recent MRI and CT of the temporal bones were evaluated in a father and son with the BOR syndrome. The young patient (case B) showed progressive and fluctuant sensorineural hearing loss, which first started in the right ear and later affected the left ear. The older patient (case A) already had severe hearing impairment, but he clearly indicated that his hearing had been much better in the past. Therefore, we may have missed any progression and fluctuations in hearing threshold. The young patient, who showed clear progression and fluctuation, had a bilaterally wide VA and hypoplastic cochlea; caloric responses were diminished on one side only. The older patient had a hypoplastic labyrinth on one side with a marginally hypoplastic lateral SCC and a marginally wide VA. He showed caloric areflexia on that side, but hearing impairment was bilaterally severe and symmetric. Therefore, there was (incomplete) correlation between the imaging findings and functional performance in these two cases.

About 200 cases of the BOR syndrome have been reported in the literature. Only a few reports clearly described progressive hearing loss in individual cases[3,30-32]. In some cases it was recognised in childhood, while other patients reported to have had normal hearing before the age of 20. Fourman and Fourman mentioned that hearing impairment varied from mild to severe[33]. Mild head injury can lead to progression of hearing loss. This phenomenon appears to be especially related with an enlarged VA and has come to be known as the large VA syndrome (LVAS)[33-36]. The audiometric configuration in children with LVAS is usually downsloping. The LVAS was found to be an almost obligatory feature of the Pendred syndrome in recent imaging studies[17-19]; this syndrome is caused by a mutation in the *PDS* gene which encodes an chloride-iodide cotransport protein[20,37]. Recently, linkage was found to the *PDS* locus in several patients with an autosomal recessive inherited form of LVAS and no clinical evidence of the Pendred or BOR syndrome, whereas another family with the same trait had mutations in the *PDS* gene[38,39]. Although the cochlear malformation may underlie our patients' hearing impairment, it is perhaps more plausible that their progressive hearing loss, which was clearly fluctuant in one and accompanied by vestibular impairment in both of them, fits in with the LVAS.

Conclusion

Our findings suggest a correlation between progressive, fluctuant sensorineural hearing loss with caloric hypofunction, all of which constitute the LVAS as part of the BOR syndrome. Additional longitudinal case studies are needed to further evaluate such a correlation.

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Chapter 2.3

Inner ear anomalies are frequent but nonobligatory features of the Branchio-Oto-Renal syndrome

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Abstract

Objective: To summarize the syndromic features and evaluate the presence of inner-ear anomalies in 35 BOR patients from 6 families (A-F).

Design: Retrospective evaluation of magnetic resonance imaging of the temporal bones and clinical features in patients with BOR syndrome.

Setting: Tertiary referral center.

Patients: The study population comprised 35 clinically affected patients with BOR syndrome from 6 families. Most of these families were followed for over 25 years.

Main outcome measures: Twenty-four patients underwent high-resolution heavily T_2-weighted 3D MR imaging of the temporal bones for evaluation of inner-ear anomalies. Special attention was paid to the endolymphatic duct and sac.

Results: A total of 7 enlarged endolymphatic ducts and sacs (3 bilaterally, 4 unilaterally) and 5 enlarged endolymphatic ducts only (2 bilaterally, 3 unilaterally) were observed. Nine hypoplastic cochleas and 6 hypoplastic labyrinths were seen bilaterally. Eight family members had normal inner ears.

Conclusion: These findings suggest that inner ear anomalies are frequent but non-obligatory features of the BOR syndrome.

Introduction

The branchio-oto-renal (BOR) syndrome is defined as an autosomal dominant inherited disorder characterized by the following three essential clinical features: (1), hearing loss with structural defects of the external, including earpits, middle and/or inner ear;(2), second-branchial arch defects; and (3), renal anomalies, ranging from mild hypoplasia to aplasia, which can lead to varying degrees of renal failure. Accompanying features like lacrimal duct stenosis or a high-arched palate can also be present in these patients[1-4]. One gene underlying the BOR syndrome, *EYA1* (chromosome 8q13.3) has been identified[5-8]. Recent linkage analysis provided evidence for a second gene on chromosome 1q31[9]. This disorder has a high penetrance but variable clinical expression. The major clinical findings associated with BOR syndrome are branchial clefts, hearing loss and renal failure[1-4,10,11]. The general prevalence of BOR syndrome is 1 in 40,000 people, and it occurs in 2% of profoundly deaf children[12].

Radiological and histological investigations have demonstrated the presence of congenital inner ear anomalies in patients with BOR syndrome[4,13]. Enlarged vestibular aqueduct and cochlear hypoplasia have been identified and may be important findings in BOR syndrome.

The syndromic features of 35 patients with BOR syndrome from 6 families are described in the present article. Magnetic resonance imaging (MRI) of the temporal bones was performed in most of the patients to evaluate inner ear anomalies. Special attention was paid to a large endolymphatic duct and sac.

Patients and methods

We investigated 6 families with BOR syndrome (families A-F) with 35 affected family members. The family members who participated in this study were seen at the outpatient clinic of the Department of Otorhinolaryngology, University Medical Center St Radboud, Nijmegen, the Netherlands. Most of these BOR families were followed for over 25 years[3,10,14-17]. Renal function tests, intravenous pyelography and/or ultrasonography of the kidneys have been performed to record any renal involvement in most patients[11]. Pedigrees were updated (Figure

1) and the results of the otorhinolaryngological examination were evaluated (Table 1).

Twenty-four patients underwent high-resolution heavily T_2-weighted 3D MR imaging of the temporal bones (Siemens Magnetom Vision, 1.5 Tesla; Siemens, Erlangen, Germany). This MRI technique enables 3-dimensional reconstruction in any desirable plane to study abnormalities of the inner ear structures. Owing to the presence of endolymph these structures have a high-signal intensity on T_2-weighted images. Thin-section MR imaging technique enables us to visualize the, often invisible, endolymphatic duct and sac, especially if they are enlarged[18]. The endolymphatic duct is considered to be dilated when its diameter, at the midpoint between the common crus and its external aperture, is 1.5 mm or more on thin section images[19].

Linkage analysis and/or mutation analysis of the *EYA1* gene is performed in all 6 families

Results

The pedigrees of the families A through F are shown in Figure 1 and relevant clinical information is presented in Table 1 for each affected family member separately. The number of affected individuals in each generation conformed to an autosomal dominant pattern of inheritance with close to 100% penetrance in almost each family, i.e. including the ones indicated as 'affected by history', whose children were affected. The only possible exception was the second generation of family E (P= 0.04 in binomial distribution). Male-to-male transmission was documented in families A, C and D.

Clinical features present in about 32 cases (95%), were malformed auricles, preauricular sinus and/or pit, second-branchial arch fistula and hearing impairment. Renal malformation was fairly common (13 patients [37%]), whereas preauricular tags and lip pits (6 patients [17%]) were less common. The penetrance of these features did not differ significantly in any of the separate families from the average penetrance calculated for the combined families.

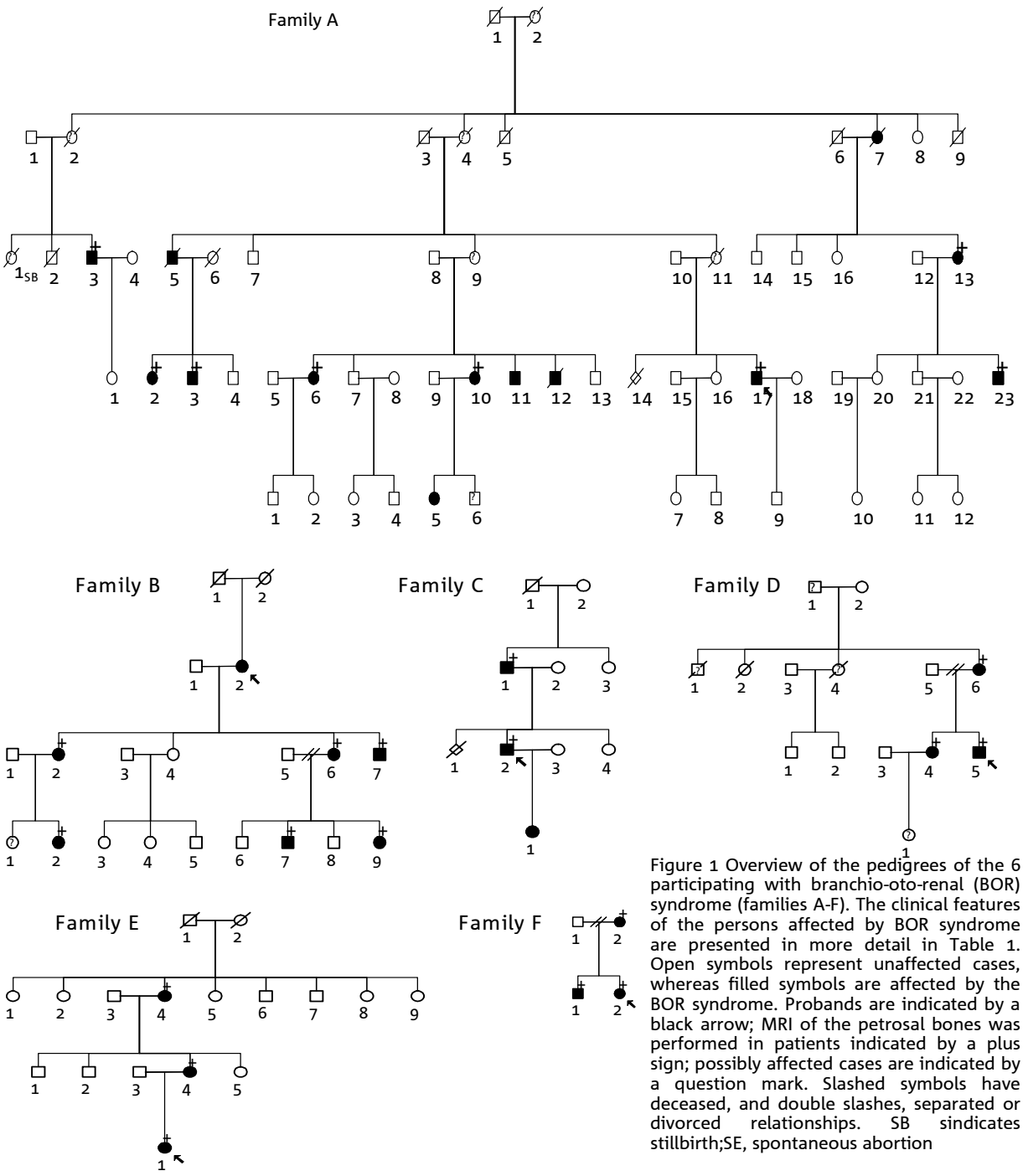

Figure 1 Overview of the pedigrees of the 6 participating with branchio-oto-renal (BOR) syndrome (families A-F). The clinical features of the persons affected by BOR syndrome are presented in more detail in Table 1. Open symbols represent unaffected cases, whereas filled symbols are affected by the BOR syndrome. Probands are indicated by a black arrow; MRI of the petrosal bones was performed in patients indicated by a plus sign; possibly affected cases are indicated by a question mark. Slashed symbols have deceased, and double slashes, separated or divorced relationships. SB sindicates stillbirth;SE, spontaneous abortion

Most of the patients had had their second branchial arch fistulas surgically removed. Preauricular sinus and/or pits or tags had only been removed incidentally. Surgical intervention is often indicated because of recurrent infection of these anatomical variations. Aesthetic surgical correction of malformed auricles has been performed in a few cases (Table 1). Nine patients (38%) required middle-ear surgery. Cremers et al.[10,14-16] and Kemperman et al.[17] described the results and details of these interventions and, based on these data, discussed the impact of syndromic diagnosis, including BOR syndrome, on the outcome of reconstructive ear surgery[20].

Table 1 Clinical features in family A-F.

Family	Case	*Malformed auricles*	*Preauricular sinus/pit/fistula*	*Sec.branchial arch fistula*	*Hearing loss*	*Renal malformation*	*Additional information*
A[3,10,11,18]	II-7	R+L	R+L	R+L	+	-	
	III-3	R+L	L	-	+	-	
	III-5	R+L	R+L	R+L	+	R+L	R preauricular tag
	III-11	na	na	na	na	na	Died from renal failure
	III-13	R+L	R	R+L*	+	-	
	IV-2	R+L	R+L	R+L+M*	+	R+L	
	IV-3	-	R+L	-	+	R+L	Severe renal failure→Kidney transplantation;
	IV-6	R+L	R+L	R+L	+	-	
	IV-10	R+L	R+L	R+L	+	-	
	IV-11	R+L	R+L	R+L*	+	-	
	IV-12	na	na	na	na	na	Died from renal failure
	IV-17	**R+L**	**L**	**-**	**+**	**+**	**R lip pit**
	IV-23	R+L	R*+L*	R+L*	+	na	Bilateral reconstruction of external auditory canal atresia
	V-5	R	R+L	R*+L*	+	L	Born with inspiratory stridor caused by bilateral recurrent nerve palsy
B[3,10]	**II-2**	**-**	**R+L**	**R*+L***	**+**	**IVP normal**	**Hearing worse after giving birth to daughter;reduced renal function**
	III-2	-	R	R*+L	+	L hypopl kidney	Diminished renal function
	III-6	+	R+L	R*+L*	+	-	Dolichocephal skull; Bilateral surgical correction of malformed auricles
	III-7	+	R+L	L*	+	-	High-arched palate
	IV-1	R+L	-	-	-	na	
	IV-2	+	R+L	R*+L*	+	na	
	IV-7	R+L	L	R*+L*	-	-	Bilateral lacrimal duct aplasia; dolichocephal skull
	IV-9	R+L	R+L	-	+	-	L lip pit; Bilateral surgical correction of malformed auricles
C[11,14,16,17]	II-1	-	R+L*	R*+L*	+	+	Normal renal function;L preauric tag*; Surgical treatment of cleft palate
	III-2	**+**	**R+L**	**R*+L***	**+**	**+**	**Myringoplasty L ear**
	IV-1	+	R+L	R+L	+	+	
D[6,16]	II-6	-	R+L	R+L	+	-	R lip pit
	III-4	+	R+L	R*	+	-	R external auditory canal atresia; L ear microtia
	III-5	**R+L**		**L**	**+**	**-**	**R lip dimple;L lippit;R+L preauric. tags*;R+L surgic correction auricles**
	IV-1	-	L	-	na	-	Small accessory ear L
E[7]	II-4	+	-	R+L	+	-	
	III-4	+	R+L	-	-	-	High-arched palate
	IV-1	**-**	**R*+L***	**R*+L***	**+**	**-**	**High-arched palate; Surgical correction of Fallot's tetralogy**
F	I-2	-	R+L	R*+L*	+	-	
	II-1	R	R*+L*	R*+L*	+	L dysplastic kidney	1. R sided hemifacial microsomia with normal facial nerve function 2. Submucosal defect of lateral R part of orbicularis oris muscle 3. Severe renal failure. Kidney transplantation planned 4. Bilateral fistulas in medial eye canthus
	II-2	**-**	**R+L**	**R***	**+**	**-**	

R, right; L, left; M, median; +, present; -, not present; na, not available; *, surgically removed; IVP, intravenous pyelography. Probands are in boldface

Table 2 MRI findings of the temporal bones and serial audiometry in 6 BOR families (A-F). Probands in bold print

Case:	*MRI*	*Relative frequencies of anomalous findings on **MRI** of:*				*Serial audiometry*
		Cochlea	*Endolymphatic duct/sac*	*Other*	*Any*	
AIII:3	Bilateral hypoplastic cochlea; LEDS left; LED right	4/7	4/7	3/7	5/7	Progressive hearing loss
AIII:5	na *					Progressive fluctuant hearing loss
AIII:13	LEDS right; mild bilateral cochlear and vestibular hypoplasia					na
AIV:2	LED bilateral					Progressive fluctuant hearing loss
AIV:3	Normal					Progressive fluctuant hearing loss
AIV:6	Normal					na
AIV:17	**Bilateral hypoplastic cochlea and dysplastic semicircular canals**					**Progressive fluctuant hearing loss**
AIV:23	LEDS bilateral; mild bilateral cochlear and vestibular hypoplasia					na
BII:2	**na ***	4/6	3/6	4/6	5/6	**Progressive fluctuant hearing loss**
BIII:2	Mild bilateral cochlear and vestibular hypoplasia					Progressive hearing loss
BIII:6	LEDS bilateral					na
BIII:7	LEDS right; mild LED left; subtle bilateral hypoplastic labyrinth					Progressive hearing loss
BIV:2	Plump internal acoustic canal (right more than left); bilateral hypoplastic cochlea and vestibule; LEDS bilateral					No progressive fluctuant hearing loss
BIV:7	Normal					Progressive fluctuant hearing loss
BIV:9	Bilateral mild hypoplastic cochlea and labyrinth					na
CII:1	LED left	0/2	2/2	1/2	2/2	No progressive fluctuant hearing loss
CIII:2	**Plump vestibule; LED bilateral**					**Progressive fluctuant hearing loss**
DII:6	Normal	0/3	0/3	1/3	1/3	na
DIII:4	Bilateral wide internal meatus					na
DIII:5	**Normal**					**Fluctuant hearing threshold**
EII:4	Plump internal acoustic canal;	0/3	0/3	1/3	1/3	na
EIII:4	Normal					na
EIV:1	**Fluid in middle ear and mastoid; normal**					**No progressive fluctuant hearing loss**
FI:2	Bilateral plump internal meatus; bilateral hypoplastic cochlea; LEDS right	2/3	1/3	1/3	2/3	No progressive fluctuant hearing loss
FII:1	Normal					na
FII:2	**Bilateral hypoplastic cochlea**					**Progressive hearing loss**

LED, large endolymphatic duct; LEDS, large endolymphatic duct and sac; LES, large endolymphatic sac; na, not available; *, CT scans performed (outside present scope)

The MRI findings in the 6 BOR syndrome families are given in detail in Table 2, together with the findings of serial audiometry, and the relative frequency of anomalous MRI findings by structure are summarized in Table 3. In 7 patients a large endolymphatic duct and sac (LEDS) (3 bilaterally; 4 unilaterally) and in 5 others a large endolymphatic duct (LED) (2 bilaterally; 3 unilaterally) were observed.

Table 3 Summary of anomalous MRI findings by structure in 6 BOR families (A-F)

Anomalous findings in	Relative frequency
Cochlea	9/24
Endolymphatic duct/sac	10/24
Other	10/24
Total	**16/24**

Nine hypoplastic cochleae and 6 bilateral hypoplastic labyrinths were present (Table 2). We did not find any congenital defects in 8 affected BOR patients.

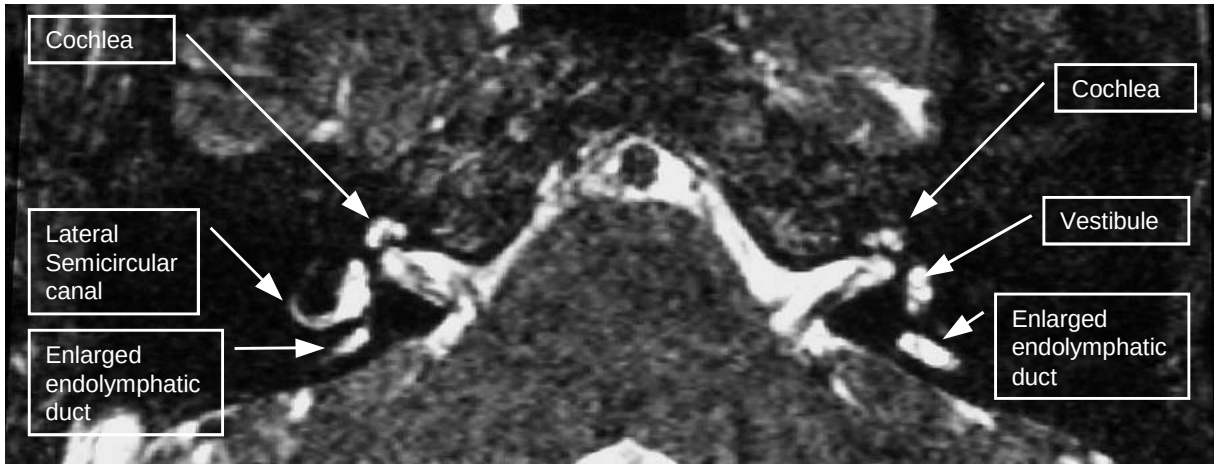

Figure 2a MRI of the temporal bones of case BIII-6 showing a bilaterally enlarged endolymphatic duct.

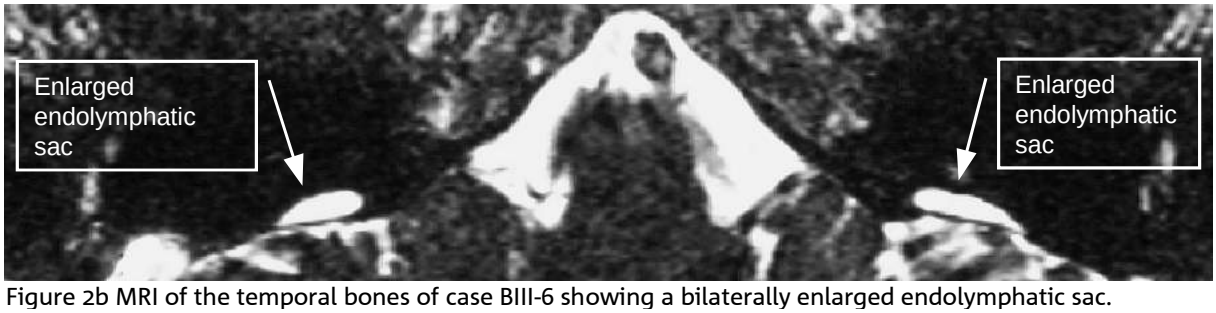

Figure 2b MRI of the temporal bones of case BIII-6 showing a bilaterally enlarged endolymphatic sac.

In most cases the type of hearing loss was mixed. Long-term audiometric follow-up analysis (threshold data not shown) demonstrated that progressive, fluctuant

sensorineural hearing loss is not uncommon in the BOR syndrome (Table 2); we were unable to confirm this feature in all BOR patients[17,21].

Although a considerable proportion of cases exhibited anomalous findings, we were unable to find a clear relationship between these and any of the features of hearing impairment (progressive and/or fluctuant).

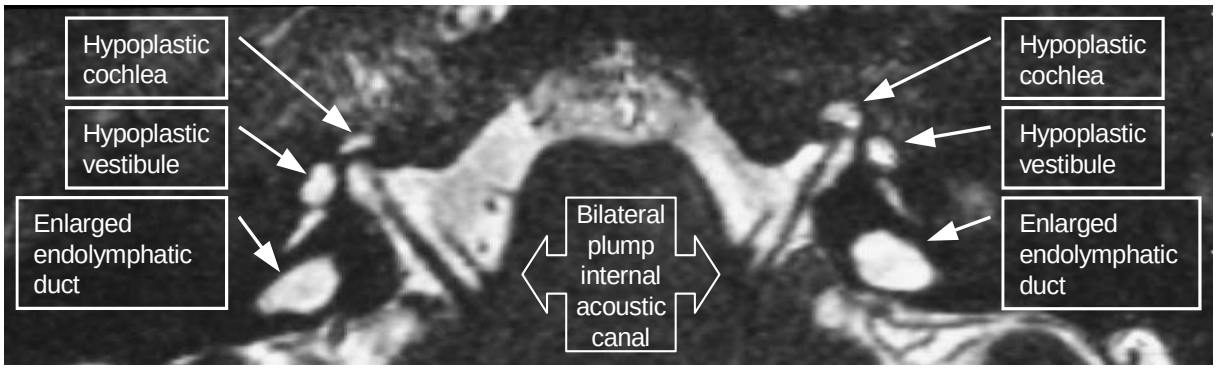

Figure 3 MRI of the temporal bones of case BIV-2 showing a bilaterally plump internal acoustic canal, bilateral hypoplastic cochleas and vestibules with an enlarged endolymphatic duct on both sides. The endolymphatic sac is also bilaterally enlarged (not shown here).

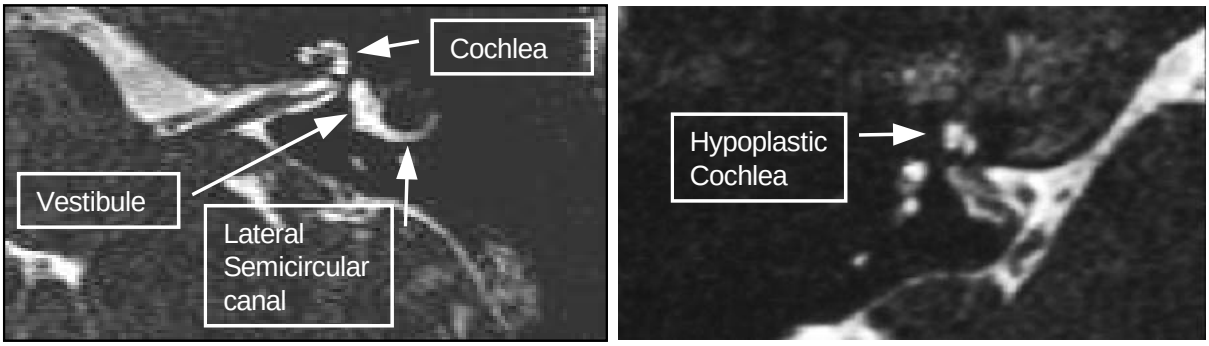

Figure 4-*left panel*: MRI of the right temporal bone of a normal individual showing the normal configuration of the cochlea, the vestibule and the lateral semicircular canal. *Right panel:* MRI of the left temporal bone of case FII-2, showing a hypoplastic cochlea.

Linkage to the *EYA1* locus was found in family A[22] and family B, however no mutations were detected by mutation analysis (author's unpublished data, 1999). In family C a mutation (IVS-9 G>C at -1) (author's unpublished data, 1999) was found which probably results in aberrant splicing of the gene. Mutation analysis in family D showed the presence of a missense mutation (T-to-C transition at position 1,360) in exon 13, resulting in a Ser454Pro substitution in the *EYA1* protein[6]. Family E has a delC at position 1592 of exon 15 causing a frameshift mutation[7]. No mutation has been detected in family F yet.

Discussion

Inner-ear abnormalities can be regarded as common findings in patients with BOR syndrome. Recently the presence of such abnormalities in combination with progressive hearing loss has been demonstrated in Pendred syndrome. In particular, the presence of an enlarged vestibular aqueduct was an almost obligatory finding in these patients[23,24]. The autosomal recessively inherited large vestibular aqueduct syndrome (LVAS) is a distinct clinical entity, although mutation analysis has shown that the LVAS and the Pendred syndrome both share mutations in the *pendrin* gene (*PDS*)[25].

The present study shows that inner-ear anomalies, such as cochlear hypoplasia and large endolymphatic duct and sac are frequent features of the BOR syndrome. This syndrome shares these features as well as progressive, fluctuant hearing loss with the Pendred syndrome. However, although such features were non-obligatory, but frequently present in our BOR patients, they were not clearly correlated to one another. The latter result was obtained by testing on possible correlations of the pooled data of all families. This procedure is, obviously, not permitted if there is genetic heterogeneity between the present families. Unfortunately we have insufficient information on form (MRI), impaired function (progressive and/or fluctuant hearing impairment) and linkage/mutation analysis to test for such a correlation in each family, even the largest ones, separately.

Because of the pathognomic presence of an enlarged vestibular aqueduct, computed tomographic scanning of the temporal bones can function as a diagnostic procedure in Pendred syndrome. We know from our experience that the branchiogenic origin of BOR syndrome can cause a wide range of anatomic malformations of the outer-, middle- and inner ear structures. Indeed, many different forms of inner-ear anomalies were present in our patients, however none of them seems to be pathognomic for BOR syndrome. Nevertheless MRI remains a useful additional tool that can visualize the neuronal tissues and the endolymphatic- and perilymphatic filled structures, such as the cochlea and endolymphatic duct. It is clear that this technique refines our knowledge of the inner-ear anatomy in general and specifically in BOR patients.

Until now many mutations in the *EYA1* gene have been described[5-7] and recent linkage analysis provided evidence of involvement of another gene underlying the

BOR syndrome[9]. No mutations are detected in the coding sequence of *EYA1* in approximately 70% of families with the BOR-phenotype. It would be interesting to perform further linkage analysis in such families. This would enable us to study the possible correlations between imaging findings, audiometrical follow-up results and linkage results.

Acknowledgements

We would like to thank the family members who participated in this study

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Chapter 2.4

Evidence of progression and fluctuation in hearing impairment in Branchio-Oto-Renal syndrome

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Int J Audiol 2004 accepted for publication

Abstract

We retrospectively analysed long-term serial audiometry data in patients with the branchio-oto-renal (BOR) syndrome to show the features of progression and fluctuation in hearing impairment and relate the findings to the patient's age and magnetic resonance imaging (MRI) findings in their petrosal bones. 32 clinically affected BOR patients from 6 Dutch families (A-F) were included. Audiograms were available in 24 cases, covering follow-up intervals of between 3 and 30 years, and suitable for individual statistical analysis in 16 of them; 14 cases also had MRI findings. Significant progression in hearing impairment was found in 10 cases, while findings of significant fluctuation were made in 7 cases. These findings did not clearly correlate with MRI findings. Substantial fluctuation occurred only in cases followed at a relatively young age. Patients with an enlarged endolymphatic duct and/or sac showed significantly higher sensorineural hearing thresholds than those with either normal MRI findings or cochlear/labyrinthine hypoplasia with or without enlarged duct or sac. We conclude that progressive, fluctuant hearing loss occurred in some of BOR patients, however, only young patients showed substantial threshold fluctuation. BOR patients with an enlarged endolymphatic duct and/or sac on MRI seemed to be predisposed to developing more severe hearing impairment.

Introduction

The autosomal dominant branchio-oto-renal (BOR) syndrome shows a broad spectrum of clinical manifestations comprising various combinations of branchial-arch, otic and renal anomalies. The four most characteristic clinical features are: (1) second-branchial arch cleft or sinus; (2) hearing loss; (3) malformations of the outer, middle and/or inner ear, including earpits; (4) renal anomalies, ranging from mild hypoplasia to complete agenesis[1-3]. Other features, such as facial and/or palatal abnormalities, have also been associated with BOR syndrome[4]. Although this disorder shows high penetrance, expression can be quite variable[1-3]. BOR syndrome, occurring in 2% of profoundly deaf children, has an estimated general prevalence of 1:40,000[5].

Mutations in the *EYA1* gene (8q13.3) were found to underlie this classical syndrome[6-9]. *EYA* genes form a family of transcription activators that interact with other proteins to regulate early embryonic development[10,11]. Interestingly, in approximately 70% of families with the BOR phenotype no mutations are detected in the coding sequence of *EYA1*[4,12]. In a large family with an almost similar clinical syndrome Kumar et al.[13] identified linkage to chromosome 1p31 using a genome wide search.

The type of hearing loss associated with the BOR syndrome can be conductive, sensorineural or mixed. Until recently, hearing impairment has generally been assumed to be stable; progressive hearing loss has only been reported in a few cases, without specific details[14-16]. Recent long-term serial audiometry and magnetic resonance imaging (MRI) of the inner ears in BOR patients demonstrated the presence of progressive sensorineural hearing loss and inner ear anomalies, including an enlarged vestibular aqueduct, suggesting that these findings have a causative relationship[17,18]. The combination of progressive, fluctuant sensorineural hearing loss and a wide vestibular aqueduct has already been demonstrated in Pendred's and the enlarged vestibular aqueduct (EVA) syndrome[19,20].

In this study we present the results of an audiometrical long-term follow-up analysis performed in 32 BOR patients from 6 Dutch BOR families (A-F), especially focusing on the features of fluctuation and progression and their possible relationship to

inner-ear morphology. The patient's age during follow up and the various types of MRI findings were also related to the sensorineural hearing threshold. MRI of the temporal bones in 24 affected family members from the same families demonstrated that inner ear anomalies are frequent, but non-obligatory features of the BOR syndrome[21].

Patients and methods

We included 32 BOR patients from 6 Dutch families (A-F) in this study. All patients participating in this study underwent ORL examination. Anamnestically special attention was paid to exclude other possible reasons for hearing impairment. Clinical features, details on middle-ear surgery, inner-ear findings obtained with MRI as well as some genetic analysis results have been reported in detail previously[3,7,8,18,21-24]. Collected blood samples were sent to Boys Town National Research Hospital for genetic evaluation, which involved linkage analysis to the *EYA1* locus. Mutation analysis of the *EYA1* gene was performed when positive linkage was found. Previously obtained audiograms were retrieved for dedicated statistical analysis. Special attention was paid to the features of progression, i.e. threshold increase, and fluctuation, i.e. cyclic threshold changes. Comparing any two modulating thresholds at a given frequency, the feature of simultaneous in-phase changes (both either deteriorating or improving) was called cofluctuation, whereas the feature of simultaneous changes in counterphase (one deteriorating, the other improving) was called counterfluctuation.

Statistical analysis

Individual serial audiograms were used to plot threshold (dB hearing level = HL) against age for air conduction (AC), bone conduction (BC), as well as air-bone gap. Statistical analysis was performed using the Prism program (PC version 3.02, GraphPad, San Diego, CA, USA). Linear regression analysis was employed to evaluate any age trend in hearing impairment in unoperated ears. Progression was called significant if a significant positive regression coefficient ($P < 0.025$) was found. Systematic significant progression was concluded to exist if the relative frequency of significant findings in a given data subset, i.e. the relative number of "hits" was high enough to show significantly low tail probability ($P < 0.05$) in the corresponding binomial distribution. Cofluctuation was evaluated by analysing the residues after linear regression in pairwise comparisons and establishing the correlation matrix for

all the relevant parameters (AC or BC in the R or L ear) pertaining to the separate octave frequencies 0.25-8 kHz. Pairwise comparisons involving a set (or sets) of residues with insufficient data (id) or residues at error level (el) were not tested (nt) on significant correlation. Significant positive correlation coefficients were related to eight different categories of pairwise comparisons involving: (1, 2) ipsilateral (R or L) AC thresholds; (3) contralateral (R and L) AC thresholds; (4, 5) ipsilateral (R or L) AC and BC thresholds; (6, 7) ipsilateral (R or L) BC thresholds; (8) contralateral (R and L) BC thresholds. Cofluctuation was concluded to exist if the finding of a significant positive correlation coefficient occurred significantly more often ($P < 0.05$) among the relevant tests than predicted on the basis of the appropriate binomial distribution statistics, provided that all data pertained to substantial fluctuation, i.e. the presence of residues > 5 dB in the regression analyses. Similarly, a significant high prevalence of the finding of a significant negative correlation coefficient was taken to substantiate the phenomenon of counterfluctuation. MRI findings and variables or features such as the patient's family, age, sensorineural hearing (BC) threshold, progression and fluctuation in threshold were evaluated for possible intercorrelations. The possible correlation between family, BC threshold, MRI findings and progression and/or cofluctuation was evaluated using contingency tables including all relevant findings in the available cases; tables were reduced to 2x2 tables where appropriate and Fisher's exact probability test was applied. Age was taken into account, as illustrated below (Figures 3 and 5), if these tests involved age-dependent variables, such as the BC threshold. Student's t test was used for comparisons between (sub)groups and included Welch's correction if Bartlett's test detected unequal variances.

Results

The pedigrees of the participating families (A-F) are shown in Figure 1. Although positive linkage to the *EYA1* locus was found in all 6 families, mutation analysis so far identified *EYA1* mutations in the families C (IVS-9 G>C at -1) (authors' unpublished data), D (Ser454Pro)[7] and E (1592delC)[8]. Serial audiograms of 24 affected BOR patients were available in these families, however, only 16 cases were suitable for longitudinal analysis (Tables 1 and 2). We analysed progression (BC) and fluctuation in hearing (AC and BC) threshold in these cases and were able to demonstrate significant progression in 10 out of 16 testable cases.

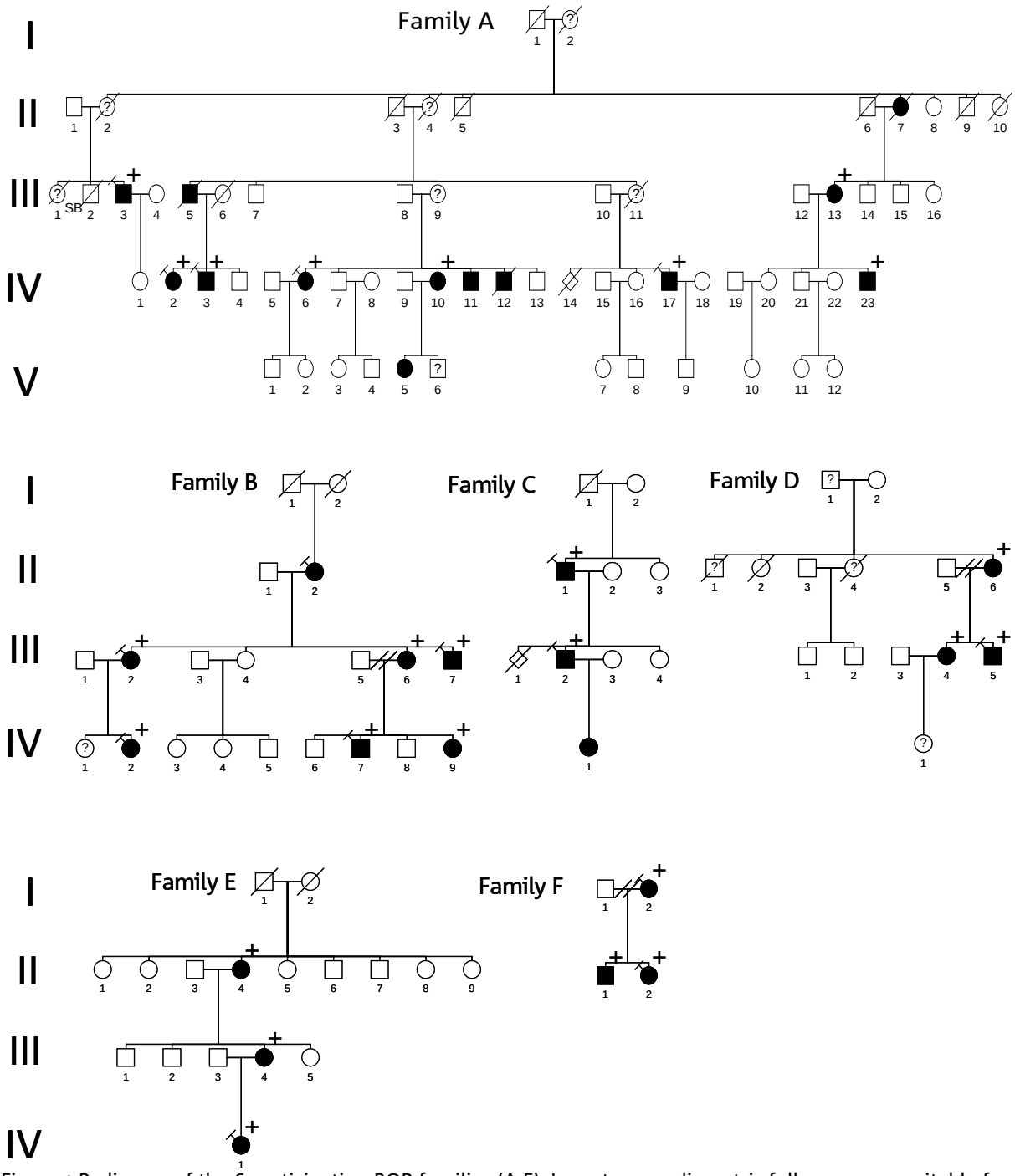

Figure 1 Pedigrees of the 6 participating BOR families (A-F). Long-term audiometric follow up was suitable for analysis in persons indicated by a side cross (↙); □ man; ○, woman; affected persons in black (■,●); +, MRI performed; ?, affected by history; SB, stillbirth; ◇, sex unknown.

The presence or absence of progression is shown together with the outcome of MRI of the inner ears in Table 1. There was no significant correlation between the absence/presence of progression and the type of MRI findings.

Table 1 Threshold progression and detailed MRI findings in 16 affected patients

Fam	Case	Follow-up interval (age in years)	N	Significant progression		MRI findings
				R	L	
A	III-3	35-57	4-5	yes	yes	Hypo cochlea R/L; LEDS L; LED R
	III-5	54-73	4-10	yes	na	na*
	IV-2	17-39	6-23	na	yes	LED R/L
	IV-3	14-37	6-15	no	no	Normal
	IV-17	6-35	8-22	yes	na	hypo cochlea R/L; dysplastic SCCs R/L
B	II-2	49-79	3-12	no	yes	na*
	III-2	18-47	5	no	no	hypo cochlea, vestibule R/L
	III-7	11-40	3-12	yes	na	LEDS R; mild LED L; subtle hypo labyrinth R/L
	IV-2	6-17	3-8	no	no	Plump IAC (R>L);hypo cochlea R/L;hypo vestibule R/L;LEDS R/L
	IV-7	3-19	4-24	yes	yes	Normal
C	II-1	32-55	4-6	no	no	LED L
	III-2	6-29	3-29	yes	yes	Plump vestibule; LED R/L
D	III-5	15-27	4-12	na	na	Normal
E	IV-1	13-16	4-8	no	no	Normal
F	I-2	35-47	3-4	yes	yes	Plump IAC R/L; hypo cochlea R/L; LEDS R
	II-2	4-21	6-16	no	yes	Hypo cochlea R/L

n, number of available longitudinal measurements (variable across frequency); hypo, hypoplastic; IAC, internal acoustic canal; L, left; LED(S), large endolymphatic duct (and sac); R, right; SCCs, semi-circular canals; na, not available; *, CT scans performed (beyond present scope)

Table 2 Relative frequency of pairwise threshold residue comparisons showing significant positive correlation indicating cofluctuation, shown with overall MRI findings. Studied cases with centre age < 25 in bold. Significantly high relative frequencies in italics. Relative frequencies of comparisons with significant negative correlation indicating counterfluctuation are also included

Family→Case		***Centre age[a] (y)***	***Fluctuation findings***								***MRI findings***	
			Pairwise comparison by AC threshold			*Pairwise comparison by AC & BC thresholds*		*Pairwise comparison by BC threshold*				
			Ipsilateral		Contralateral	Ipsilateral		Ipsilateral		contralateral		
			R (1)*	L (2)	(3)	R (4)	L (5)	R (6)*	L (7)	(8)	R	L
A→	III-3	46	id	id	nt	nt	na	id	na	na	abn	abn
	III-5	63.5	el	na	nt	nt	na	el	na	na	na	na
	IV-2	28	na	*4/15* [b]	na	na	*3/30*	na	*2/10*	na	abn	abn
	IV-3	25.5	1/15	*5/15*	*5/36*	nt	0/30	el	*2/10*	nt	norm	norm
	IV-17	**20.5**	***10/15***	**na**	**na**	***2/30***	**na**	***7/10***	**na**	**na**	**abn**	**abn**
B→	II-2	64	el	el	nt	nt	nt	id	el	nt	na	na
	III-2	32.5	el	el	nt	nt	nt	el	id	nt	abn	abn
	III-7	25.5	el	na	na	nt	na	id	na	na	abn	abn
	IV-2	**11.5**	***3/15***	***7/15***	***12/36***	**0/30**	**nt**	***3/10***	**id**	**nt**	**abn**	**abn**
	IV-7	**11**	***11/15***	***7/15|3/15*** [c]	**2/36**	***5/36***	**1/36**	***3/14***	**1/14**	**1/16**	**norm**	**norm**
C→	II-1	43.5	el	el	nt	na	na	na	na	na	norm	abn
	III-2	***17.5***	***7/10***	***8/15***	***5/30|7/30*** [c]	***6/20***	**2/18**	***5/6***	**1/6**	**2/16**	**abn**	**abn**
D→	III-5	21	na	na	na	na	na	na	na	na	norm	norm
E→	*IV-1*	*14.5*	id	id	nt	nt	nt	id	na	na	norm	norm
F→	I-2	41	el	el	nt	nt	nt	el	el	nt	abn	abn
	II-2	***12.5***	***15/15***	***15/15***	***30/36***	**nt**	**nt**	**el**	**el**	**nt**	**abn**	**abn**
Relative freq of tested cases with S fluctuation												
Cofluctuation			*5/6*	*6/6*	*4/5*	*2/4*	1/4	*4/4*	*2/4*	0/2		
Counterfluctuation				1/6	1/5							

AC, air conduction (threshold); BC, bone conduction (threshold); el, error level; id, insufficient data; na, not available; nt, not tested; [a], mean of minimum and maximum age during follow up;[b], n_1/n_2 (relative frequency) indicates n_1 pairwise comparisons showing significant correlation among n_2 pairwise comparisons; [c], relating to significant negative correlation indicating counterfluctuation (AC); S, significant; *, numbering of pairwise comparisons presented in Patients and Methods

Figure 2 illustrates the clear fluctuations in case FII-2 with a series of the most elaborate audiograms. The fluctuations can be appreciated from this figure by looking at the striking consecutive changes in (AC and BC) threshold configurations and the associated changes in the extent and pattern of the air-

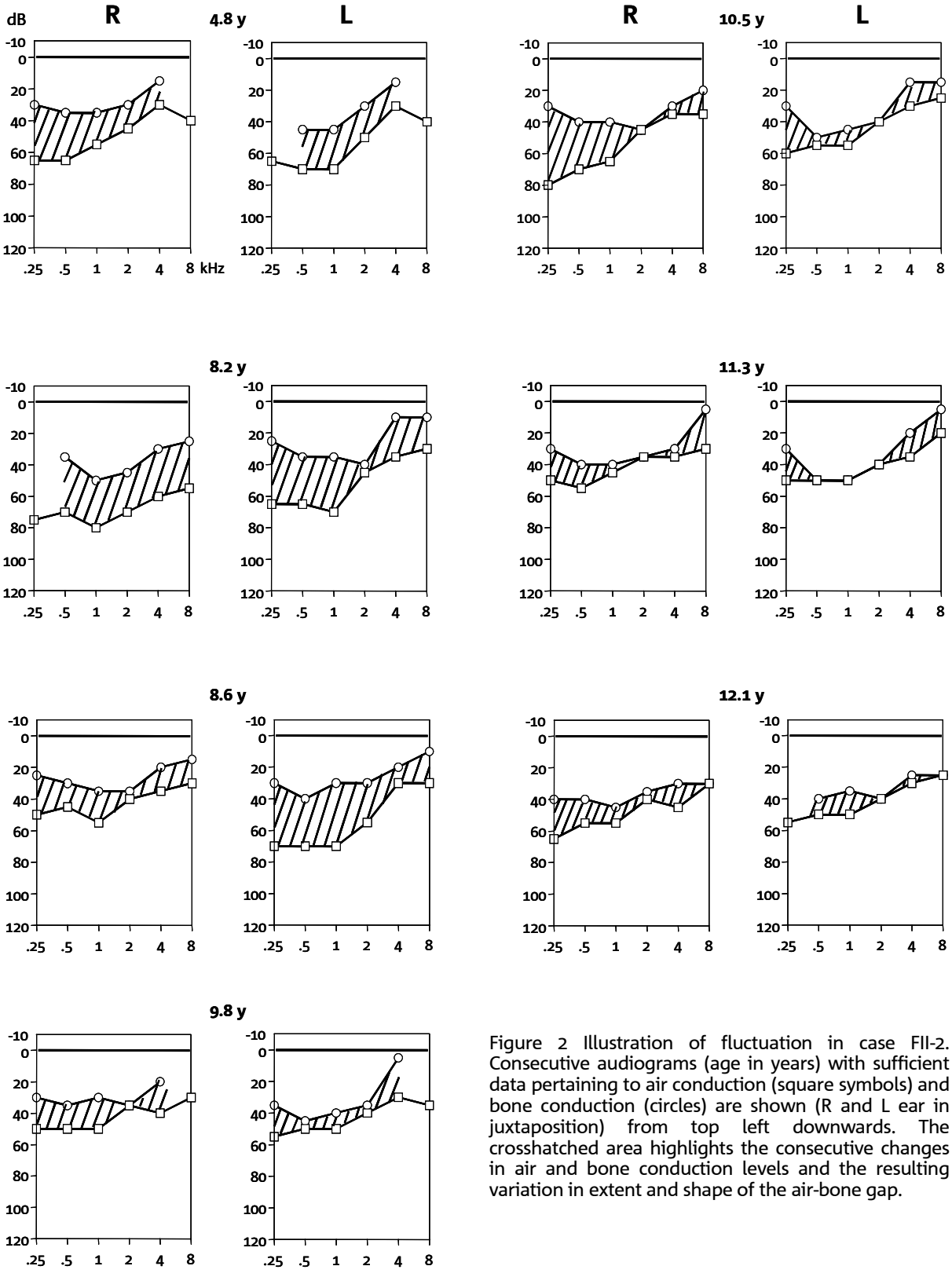

Figure 2 Illustration of fluctuation in case FII-2. Consecutive audiograms (age in years) with sufficient data pertaining to air conduction (square symbols) and bone conduction (circles) are shown (R and L ear in juxtaposition) from top left downwards. The crosshatched area highlights the consecutive changes in air and bone conduction levels and the resulting variation in extent and shape of the air-bone gap.

bone gap (Figure 2, crosshatched areas). Large longitudinal variations occurred in either ear, anywhere within the audio frequency range. Similar such variations were observed in all our follow-up cases. Remarkably, the extent of the air-bone gap during follow up varied between 0 and about 30-60 dB; it did not show a consistent, significant age-related trend (data not shown). There was a clear, significant relationship between age during follow up and the finding of significant cofluctuation (Table 2). The 8 studied cases with evaluable fluctuation and a centre age, i.e. the mean of minimum and maximum age during follow up, of > 25 years bilaterally showed fluctuations only at error level, i.e. about ±5 dB, with few exceptions, and could not be called substantial. MRI findings obtained in 6 of these cases were normal in only one of them. The 5 cases with centre age < 25 years (highlighted in bold in Table 2) showed significant cofluctuation and abnormal MRI findings, except for one case. This is illustrated in Figure 3 with data pertaining to the right ear; it was checked that similar findings were obtained for the BC thresholds pertaining to the left ear. "Fuzzy" and "noisy" superposition plots in Figure 4 are associated with a lack of cofluctuation in most cases. It can also be noted that the non-significant fluctuation in left BC threshold (comparison (7) in Table 2, relative frequency $^1/_6$) makes the other pairwise comparisons involving the left BC threshold, i.e. (5) and (8) also non-significant. The same applies to patient BIV-7 (Table 2).

In some cases, fuzziness may be due to the combination of significant cofluctuation and counterfluctuation (Table 2 and Figure 4). Significant

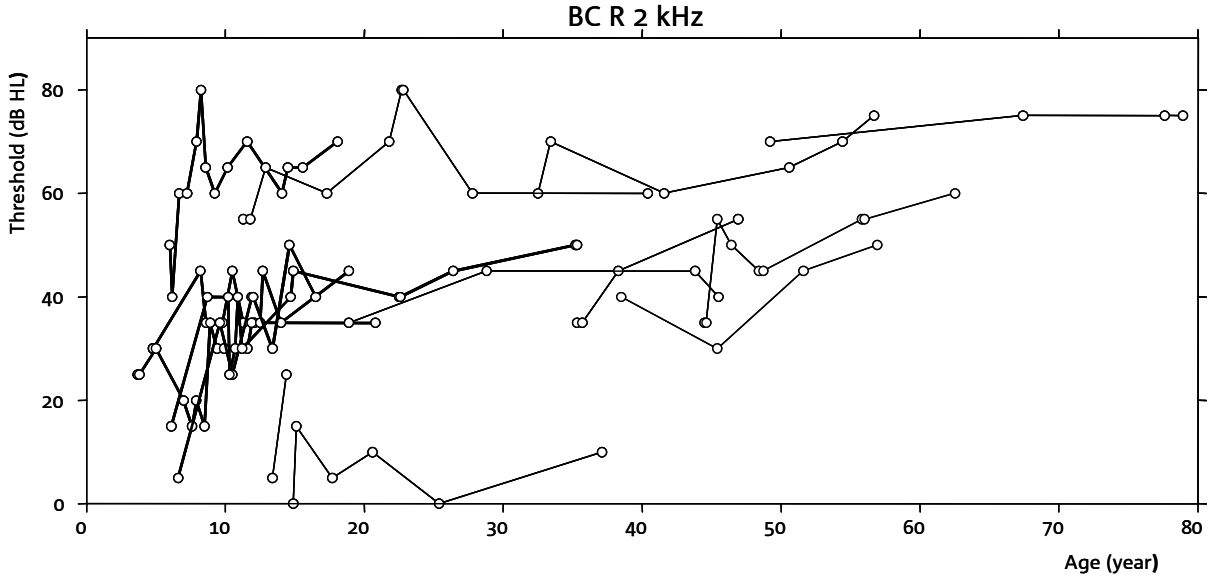

Figure 3 BC threshold (R ear) at 2 kHz plotted against age for the right ear in all cases, illustrating the finding that significant fluctuation above error level (highlighted by bold lines) only occurred at age < 25

counterfluctuation did not occur sufficiently more often among the cases than could be expected on the basis of chance alone according to binomial distribution statistics (Table 2, bottom row). This also applies to contralateral cofluctuation in bone conduction threshold and cofluctuation of the air and bone conduction threshold ("AC & BC") in the left ear. All the other types of cofluctuation indicated could be called substantial (Table 2). Remarkably, these included contralateral cofluctuation of air conduction thresholds.

The features of cofluctuation and type of MRI finding (alone or in any combination) did not show any significant correlation. Figure 5 shows clear threshold separation at 1-4 kHz between the cases with enlarged endolymphatic duct (LED) and/or sac (LEDS) alone on MRI and the others. The finding of 4 such cases, all showing relatively poor thresholds, compared to 9 other age-matched cases with different MRI findings, all showing relatively more favourable thresholds, was significant (Fisher's exact probability test). Similar plots as shown in Figure 5 were prepared highlighting the different families. The only significant family-related finding (taking age into account) was that family A showed remarkably favourable BC thresholds. The two family C cases showed remarkably high thresholds; both these cases pertain to the LED(S) category.

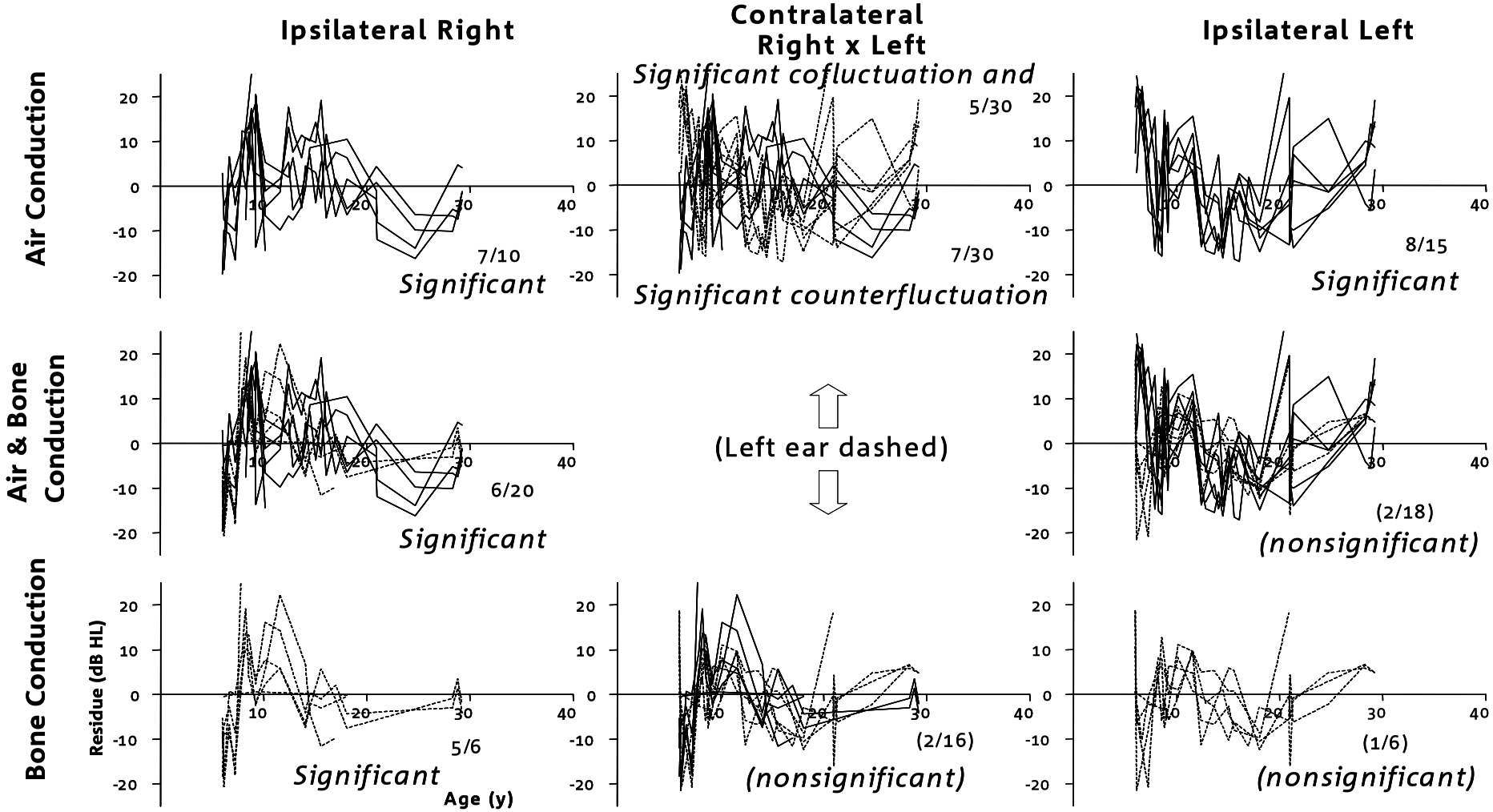

Figure 4 Illustration of formal fluctuation analysis. Eight different superposition plots for the residues after linear regression of threshold on age in case CIII-2. The eight different panels correspond to the pairwise comparisons (1) through (8) mentioned in Patients and Methods. Results of statistical tests (Table 2) are included.

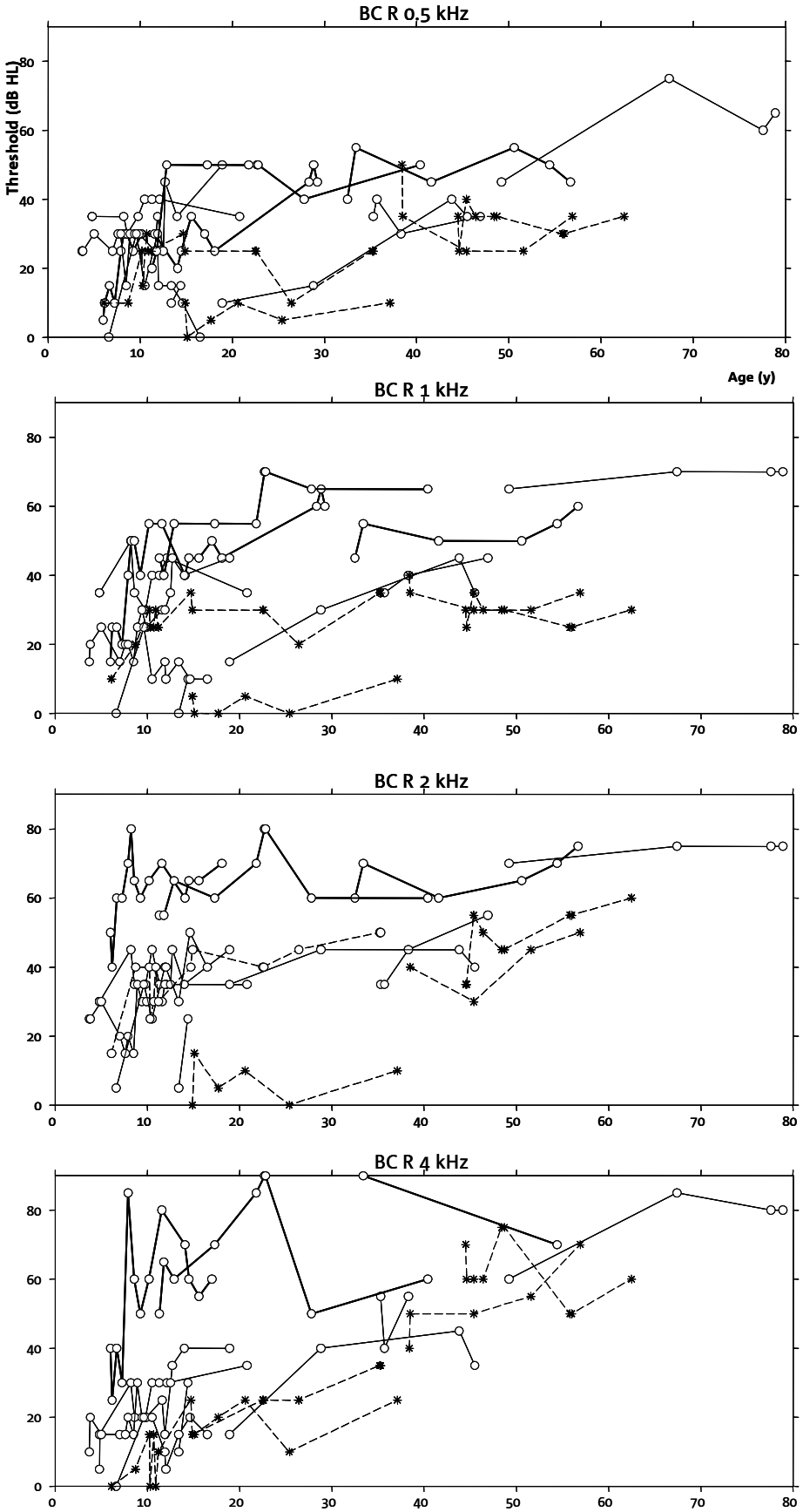

Figure 5 BC threshold (R ear) plotted against age for the 0.5-4 kHz frequencies. The other frequencies have not been consistently measured in all cases. The highlighted cases (bold lines) are those showing only LED(S) on MRI. Family A is indicated by asterisks and dashed lines.

Discussion

The analyses performed in this study unequivocally substantiated progression in a number of BOR cases that could be evaluated. Substantial cofluctuation was found in a number of cases, mainly the younger ones (< 25 years). However, there was an obvious bias present: Figure 3 shows a much higher sampling rate for the cases aged <25 years than for the older ones. Fluctuations at a more advanced age may have been missed by undersampling. It is possible that fluctuations tend to occur predominantly in the first decades of life, but this issue can only be settled by performing uniform sampling over the whole age range at a sufficiently high rate.
There was no significant correlation between progression and age or MRI findings, or cofluctuation and MRI findings. Remarkably, patients with LED(S) alone on MRI showed significantly poorer thresholds in appropriate comparisons than patients with different MRI findings. This finding suggests that an enlarged endolymphatic duct and/or sac predispose towards more severe hearing impairment in BOR syndrome. However, we have no explanation for the present observations that patients having combinations of cochlear/labyrinthine hypoplasia and LED(S) did not show significantly poorer thresholds than either patients with normal MRI findings or hypoplasia alone, and better thresholds than patients with LED(S) alone. It should be emphasised that significant progression and/or cofluctuation was also found in cases with only cochlear hypoplasia or even normal MRI findings.

According to a literature review concerning the degree of hearing impairment in 82 BOR patients performed by Stinckens et al.[17] the median values for air conduction threshold, bone conduction threshold and air-bone gap were 50 dB, 30 dB and 20 dB, respectively. These findings seem to be in line with those of our families, except for our LED(S) cases who showed greater impairment at most frequencies (Figure 5). A substantial air-bone gap may be also present in patients with large vestibular aqueduct syndrome (LVAS)[25]. We have no idea about the possible pathophysiologic mechanism underlying the longitudinal individual variations in air-bone gap. Xu et al.[11] inactivated the *Eya1* gene in mice and reported that *Eya1*$^{+/-}$ heterozygotes showed conductive hearing loss, with a remarkable amount of variation, associated with middle-ear malformations. These malformations comprised ossicular chain anomalies, including discontinuity. However, we are not aware of middle-ear malformations being associated with fluctuations in conductive hearing impairment.

The present study demonstrated significant fluctuation (at least in the young BOR patients). A great variation in audiogram configuration has been noted to exist between different BOR patients in several studies involving either cross-sectional analysis or presentation of selected audiograms[26]. In light of the present findings, it should be realised that individual longitudinal threshold data may entail such large fluctuations that the validity of "snapshot" intersubject threshold comparisons can be questioned. Besides this, Anderson et al.[27] described congenitally hearing impaired children with pseudo-mixed hearing loss mainly present at 0.25 – 2 kHz. He proposed that the observed air-bone gap was an artifact of measurement due to subtle congenital malformations of the ossicular chain, subsequently changing its inertial mass without restricting its transmission function. The air-bone gap is thus regarded as a manifestation of an increased inertia component of the bone-conduction apparatus[27]. Perhaps in the line of this, concern has recently been expressed about systematic errors in BC conduction audiometry, especially in case of conductive loss, resulting in a pattern of 'notching' at 2 kHz[28]. As fluctuations in our patients not only aff ected this frequency, but also other frequencies, as well as the AC threshold, these effects cannot explain all the present findings.

It might seem possible that the air-bone gap and threshold fluctuations findings are related to otitis media with effusion (OME). OME certainty forms part of the clinical picture of branchio-oto-renal syndrome in many cases. We have retrospectively screened the clinical notes related to the occurrence of OME and tympanometric data, especially in the patients with clear fluctuations. Owing to the fact that only incidental observations and measurements were found, we were unable to evaluate the possible effects of OME. However, it is very difficult to reconcile OME with finding such as contralateral fluctuations as well as fluctuations in bone conduction thresholds.

Apart from BOR syndrome and the autosomal recessive LVAS, progression of sensorineural hearing loss combined with the presence of an enlarged endolymphatic duct/enlarged vestibular aqueduct has also been demonstrated in Pendred's syndrome[20]. In comparison to BOR syndrome, however more rapid progression at a younger age ultimately leads to higher thresholds in the Pendred syndrome[19]. Mutation analysis of the involved *SLC26A4* gene in such patients produced evidence that the LVAS is a clinical variant of the classical Pendred

syndrome and therefore the correlation between progressive hearing loss and the inner-ear anomalies are thought to have the same molecular-based aetiology[29]. The *Eya1* gene shows expression in early stages of the murine otic vesicle and later on in the floor of the cochlear duct, the area that gives rise to the organ of Corti[10]. It may therefore be an attractive hypothesis that congenital defects in man are caused by *EYA1* mutations that predispose towards increased cochlear vulnerability to damage later in life. Lacking mutation data and the still relatively small numbers of observations in our BOR families however, prohibit a major genotype-phenotype correlation analysis to be performed.

The association of mild head injury and worsening of hearing impairment, which has often been observed in LVAS and BOR syndrome[30-33] emerged from the medical history in only one of our patients. Unfortunately we did not perform a formal enquiry to address this issue.

We have no plausible explanation for the intriguing finding of contralateral cofluctuation. Neither of the findings of substantial cofluctuation and progression was related to the MRI findings. It can be speculated that (endocrine?) homeostatic control factors have a synchronous, bilateral deleterious effect on the function and/or morphology of the membranous labyrinth in BOR. If labyrinthine morphology, indeed, is involved, it may have escaped detection by MRI. Such a possibility is inherent to any method with given limitations, including MRI; an unrelated example has been reported recently[34]. Hopefully, new developments in imaging methods may favour our understanding of mechanisms underlying the intriguing features of auditory threshold progression and fluctuation. We feel that the present study has succeeded in substantiating such features of hearing impairment. However given the small numbers of observations within each category of consistent MRI findings, it was not possible to pinpoint any significant correlations between these findings and the hearing impairment features of progression and fluctuation. Therefore careful collection of suitable families and cases is certainly needed for further study, including substantiation in all relevant details.

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We wish to thank the families that participated in this study.

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Chapter 3

DFNA9/*COCH*

Chapter 3.1

DFNA9/*COCH* and its phenotype

MH Kemperman

SJH Bom

FX Lemaire

WIM Verhagen

PLM Huygen

CWRJ Cremers

Advances in Oto-Rhino-Laryngology. Genetic hearing impairment. Its clinical presentations.

Basel, Karger 2002;61:66-72

Introduction

The cochlear and vestibular structures are embryologically, anatomically and functionally closely related. The number of hereditary disorders that affect both cochlear as well as the vestibular function is very small, contrary to the large variety of hereditary cochleovestibular disorders in mice. DFNA9 (OMIM #601369) is until now the only autosomal dominant type of non-syndromic sensorineural hearing impairment (SNHI) in human, exhibiting concomitant vestibular dysfunction[1]. Linkage analysis in an American family mapped the DFNA9 locus to chromosome 14q12-q13 in 1996[2] Histopathological temporal-bone studies of affected persons revealed characteristic depositions throughout the labyrinth with concomitant degeneration of cochlear and vestibular sensory structures. These depositions, corresponding to the expression pattern of an inner ear-specific gene in chicken, have helped to identify the disease-causing *COCH* gene in 1998. Since then three different mutations were found in all three American families, as well as one specific mutation in fifteen Dutch and Belgian families, most of which were shown to have a common founder[3,4]. The latest report on a new DFNA9 family harbouring a novel mutation in the *COCH* gene originates from Australia[5].

Audiometry

Affected American individuals suffered from progressive high-frequency hearing loss, with an average age of onset of 20 years in two and 40 years in one family, leading to profound deafness in a period of 20-30 years time[6]. The low and the mid frequencies followed the first drop at the high frequencies, resulting in an overall picture of a flat progressive loss in the low to mid frequencies coupled with a high-frequency slope[6,7.] Three different disease-causing mutations in the *COCH* gene were identified in these families[3] (see below)

Dutch and Belgian DFNA9 families all carry a specific mutation of the *COCH* gene[8] (see below). After the first clinical description of a Dutch family (OMIM #193005) in 1988, a number of additional families with a similar type of impairment have been identified[8-18]. Hearing loss, progressing to profound deafness in the 6th-7th decade, predominantly involved the high frequencies. The age of onset was determined at ~40 years in all of these families[10-20] and a particularly high prevalence of vascular disorders was noted in two Dutch families[10,14]. The natural history could be further outlined by performing extensive genotyping and

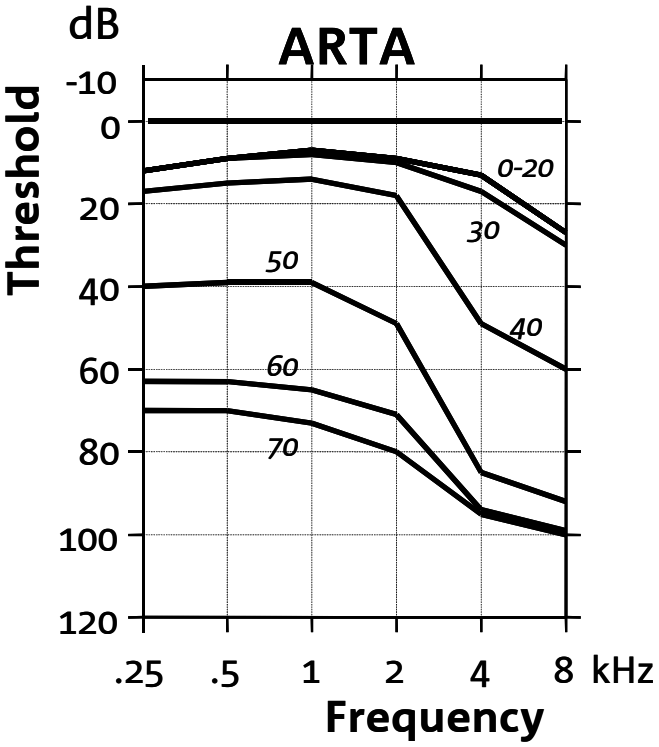

Figure 1 Age-Related typical audiograms (ARTA) of a large Dutch DFNA9 family. Italics indicate age in years

longitudinal as well as cross-sectional threshold-on-age regression analyses in a large Dutch family[10,11,19].

It appeared that significant sensorineural hearing impairment (SNHI) at 4 and 8 kHz was already present at a very young age and might have been congenital. Significant progression in SNHI did not start before the age of ~40 years. Progression of hearing impairment of ~3dB/year appeared to be concentrated in a relatively short period of time of ~20-25 years. Anamnestically a high prevalence of Ménière-like symptoms was noted in about one third of the patients[4,12,18,20]. SNHI in affected individuals from the Australian DFNA9 family initially starts with high-frequency loss in the 2nd-3rd decade and progressed to severe/profound loss across all frequencies in the 6th-7th decade[5]. Cochlear implantation has been successful in some American and European patients[7,21].

Speech recognition scores in an American family showed generally good results until the age of ~35 years, when a fairly rapid decline sets in that was supposed to be excessive given the pure-tone thresholds[7]. The benefit from using hearing aids was reported to be unsatisfactory in this stage of the disease. Forty-two affected individuals from several Dutch DFNA9 families, showed relatively poor speech recognition scores compared to age and hearing level, contrary to DFNA2 patients in the same study showing relatively good speech recognition scores[22]. Speech recognition scores in the Australian family are not available[5].

Detection of vestibular impairment requires specific vestibular function tests, otherwise it may go clinically undetected due to compensation by other systems involved in maintaining balance. So far only two forms of hereditary purely

vestibular impairment, i.e. with normal hearing, have been identified[23,24]. Uniquely, vestibular symptoms in DFNA9 developed simultaneously with hearing deterioration. Initially progressive vestibular failure was demonstrated in one American family member[25]. More recently vestibular impairment has been reported in three additional American patients and a comprehensive audiovestibular questionnaire disclosed vestibular symptoms in a few more of them[26]. All Dutch and Belgian families and the Australian family showed a fairly similar type of vestibular impairment, including failure of otolith reflexes[16], progressing to vestibular areflexia[4,5,10-18,20]. Simultaneous fluctuation in hearing thresholds and vestibular impairment were associated with Ménière-like symptoms in some European patients[4,12,17,18,20].

Genetics

The *COCH* gene (OMIM #603196), formerly known as *hCoch-5B2*, was found and characterised with the use of a cDNA library, which contained transcripts of genes expressed specifically within the fetal cochlea. *COCH* resides within the locus for DFNA9 on human chromosome 14 (14q11.2-q13)[27,28.] This gene, consisting of 12 exons, is strongly expressed in the cochlear and vestibular labyrinthine compartments, supporting structures and neural channels surrounding the inner ear and encodes a protein named cochlin. Three characteristic domains can be identified in this protein: (1), a signal peptide; (2), a cysteine-rich domain with homology to the factor C domain of the horseshoe crab Limulus (FCH domain) and (3), two regions with homology to the von Willebrand factor A (vWFA) domains. The latter are present in many extracellular matrix components and secreted proteins involved in various host-defense systems, such as haemostasis, the complement system and the immune system[3]. The true function of the gene and its protein is still unknown.

Three mutations were found in the American families, e.g. V66G, G88E, and W117R, one specific P51S mutation in all Dutch/Belgian families and a novel I109N mutation in an Australian family[4,5,11,29-32]. All reported mutations occur in the FCH domain of the *COCH* gene. Haplotype analysis in the Dutch and Belgian families revealed the presence of a common founder in this part of Europe[8]. Interestingly, one patient presenting with a homozygous P51S mutation demonstrated an

earlier onset (at 25 years) and more rapid progression than the heterozygous mutation carriers[4] (Table 1).

Table 1 overview of DFNA9/*COCH* families reported in literature

Family	*Type of mutation*	*Exon*	*Reference*
3 US families	1 missense mutation V66G[30] 1 missense mutation G88E[31] 1 missense mutation W117R[32]	4 5 5	Manolis et al[2]; Robertson et al[3]; Ketharpal et al[6,25,26]; Halpin et al[7]
15 Dutch/Belgian families	1 missense mutation P51S[29]	4	Verhagen et al[13-18]; De Kok et al[11]; Bom et al[10,19,22]; Fransen et al[4,8], Lemaire et al[12]
1 Australian family	1 missense mutation I109N	5	Kamarinos et al[5]

Histopathology and pathogenesis

Histopathological temporal bone studies showed peculiar, specific acidophilic deposits in the cochleas, maculas and cristas of DFNA9/*COCH* patients with severe degeneration of vestibular and cochlear sensory elements and dendrites[6,25]. Very recently a highly branched non-banded microfibrillar substance decorated with glycosaminoglycan-like granules was identified with electron microscopy[26]. These findings were thought to be typical of the deposits anywhere within the labyrinth. The type II collagen bundles, that are normally abundant in the spiral ligament, were conspicuously absent[26].

Cochlin expression at fairly similar sites in the chicken inner ear have lead to the hypothesis of "strangulation" of cochlear and vestibular nerve endings by the deposited substance[3,4,8]. Consistent with the observations that various *COCH* mutations cause misfolding of cochlin, which may lead to deposition of this protein, the possibility was suggested that normal fibrillogenesis is disrupted by an excess of microfibrillar substance, resulting in degradation of collagens and extracellular matrix components[6,26]. In addition, it was postulated that expression of the *COCH* gene in the stroma underlying the sensory structures of the inner ear may indicate a possible role of this gene in ion homeostasis[4]. The special vulnerability of hair cells in the basal turn might be explained by the relatively high levels of ion flux required in this part of the cochlear duct. Failure in ion homeostasis might be an appealing hypothesis because of the Ménière-like symptoms experienced by some of the *COCH* patients. Furthermore, based on the

structure of cochlin, *COCH* may even be involved in a host-defensive, rather than an architectural role, making DFNA9 patients more vulnerable to infection and/or cardiovascular disease[6,33]. However, the specific function of *COCH* and the pathogenesis of DFNA9 still needs to be further unravelled.

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Chapter 3.2

Audiometric, vestibular and genetic aspects of a DFNA9 family with a G88E *COCH* mutation

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Abstract

Objective: To perform genetic analysis and to analyse cochleovestibular impairment features in a newly identified Dutch family with DFNA9.

Design: Genetic analysis was performed using microsatellite markers and SNPs. Audiometric data were collected and analyzed longitudinally. Results were compared to those obtained in previously identified P51S *COCH* mutation carriers (n = 74). Special attention was also given to a comparison of age-related features such as progressive hearing and vestibular impairment.

Subjects: G88E *COCH* mutation carriers from a Dutch family.

Results: Pure tone thresholds, phoneme recognition scores and vestibular responses of the G88E mutation carriers were essentially similar to those previously established in the P51S mutation carriers. Hearing started to deteriorate in G88E mutation carriers from age 46-49 years onwards, whereas deterioration of vestibular function started from about age 46. In the P51S mutation carriers vestibular impairment started earlier, at about age 34 years. However, the difference in age of onset with the G88E mutation carriers was not significant. Remarkably, the proportion of patients who developed complete vestibular areflexia within the age range of 40-56 years was significantly lower for the G88E mutation carriers than for the P51S mutation carriers.

Conclusions: Apart from a significantly lower frequency of vestibular areflexia between the age of 40-56 years, there are no phenotypic differences between carriers of the G88E and P51S mutations in the *COCH* gene.

Introduction

Since the early nineties of the past century, 51 different loci and 18 different genes have been identified to be associated with non-syndromic autosomal dominant hearing impairment (DFNA)[1]. DFNA9 is the ninth discovered non-syndromic form of autosomal dominant hearing loss, which was linked to 14q12-q13 in 1996[2]. Remarkably, DFNA9 is, apart from DFNA11[3], the only type of DFNA exhibiting concomitant vestibular dysfunction. Soon after genetic linkage was established the underlying disease-causing gene was identified as *COCH*[4]. This gene contains 12 exons encoding a protein named cochlin. It is strongly expressed in the human fetal cochlea and the vestibular labyrinth[5]. The function of the protein is still unknown. So far six different mutations have been identified. Three of them originate from North America (V66G, G88E and W117R)[4], one specific founder mutation (P51S) is present in many Dutch/Belgian families[6,7],in Australia an I109N mutation has been identified[8] and recently an A119T mutation was reported in Japan[9].

In this report we describe the phenotype of the first Dutch DFNA9 family with hearing loss and vestibular impairment caused by a G-to-A transition at nucleotide 319 in exon 5 of the *COCH* gene, resulting in a change of codon 88 from GGA (gly) to GAA (glu). The clinical features are compared with the available data of the American DFNA9 family carrying the same mutation and to those established in patients carrying the P51S founder mutation from a number of previously described Dutch families.

Patients and methods

In 1999 we ascertained and investigated a Dutch family (W99-101) with a pedigree spanning five generations. Written informed consent was obtained to perform this study. The study was approved by the local medical ethical committee. Thirteen family members were affected (by history) by progressive hearing loss with, in some cases, concomitant vestibular impairment. Medical history was taken and anamnestically non-hereditary causes of hearing loss were excluded. Participating individuals underwent otological examination, paying special attention to the presence of any syndromic features, pure tone audiometry and in some cases speech audiometry. Vestibular function was tested in 10 patients. One

affected person underwent computerized tomography (CT) of the temporal bones. Blood samples were collected from six presumably affected and 36 presumably unaffected persons for the purpose of linkage and mutation analyses.

Genetic analysis

DNA was isolated from peripheral blood according to Miller et al.[10]. Because of the apparent similarity of the present type of hearing loss to DFNA9 we first searched for mutations in the COCH gene, with special attention for the known mutations.

Sequence analysis

We used primers, designed to flank the exons of the COCH gene[6] to amplify and sequence exons 2-12. Prior to sequencing, PCR fragments were purified using the Qiaquick gel extraction kit (Qiagen, Hilden, Germany). Sequence analysis of PCR-amplified exons 2-12 was performed using DNA from two family members (II-13 and II-14) and an unaffected unrelated control individual (ABI PRISM Big Dye Terminator cycle sequencing V2.0 ready reaction kit and an ABI PRISM 3700 DNA analyser (Applied Biosystems)). For testing the segregation of the detected mutation in family W99-101, exon 5 was amplified with the primers 5´ TCTTTAGATGACTTCCCTGATGAG-3´and 5´-TCACAGGTTTTTCCATCAAGGTTA-3´. PCR products were digested with AvaII (New England Biolabs), which cuts the wild-type PCR product into fragments of 154 and 214 bp. After digestion, DNA fragments were separated on a 2% agarose gel and stained with ethidium bromide.

Haplotype analysis

We studied the possible presence of a common ancestor for both the original American family and the present family using haplotype analysis. For this purpose we used polymorphic markers flanking the COCH locus (D14S262, D14S975, D14S1021, D14S257 and D14S1040) and single nucleotide polymorphisms (SNPs) present within the COCH gene (RS#2239581, RS#2239580 and RS#2295127). Analysis of the microsatellite markers was performed as described by Kremer et al.[11] The SNPs were analysed by sequencing.

Audiometry and data analysis

Pure tone audiometry was performed in a sound treated room, conforming to the International Standards Organisation (ISO)[12,13]. The individual 95th percentile threshold values of presbyacusis (P_{95}) in relation to the patient's sex and age were derived for each frequency using the ISO 7029 method[14]. Persons were considered affected if the best hearing ear showed thresholds beyond the P_{95}. Similar to the previously reported analysis of the P51S COCH mutation carriers[15], cross-sectional binaurally averaged threshold data (air conduction level in dB HL) were plotted against age for each frequency and analyzed using nonlinear regression analysis (threshold on age) fitting a sigmoidal response curve with a variable slope (Prism 3, GraphPad, San Diego, CA, USA). Student's t test was used to compare between the associated parameter values fitted for the P51S mutation and the present G88E mutation. This test included Welch's correction if Bartlett's test identified unequal variances. The level of significance used in all tests was P = 0.05. The fitted curves were used to construct age-related typical audiograms (ARTA). According to a previously described method[16], the ARTA for the present G88E mutation carriers and previously described P51S mutation carriers were compared.

Speech audiometry was performed under the above-mentioned conditions using phonetically balanced standard Dutch consonant-vocal-consonant word lists. The maximum phoneme recognition score (%Correct, mean for both ears) was obtained from monaural performance-intensity curves and was analyzed in relation to age and pure tone average (mean for both ears) at the frequencies 1, 2 and 4 kHz ($PTA_{1,2,4\ kHz}$). Linear regression analysis was performed to fit individual longitudinal scores for the present G88E carriers. These scores were compared to those used for a previously performed similar cross-sectional analysis of P51S mutation carriers (scores for right ear) from seven different families, to which a sigmoidal curve with variable slope had been fitted[17].

Vestibulo-ocular examination, data analysis and imaging techniques

Eleven affected family members and mutation carriers underwent vestibular and ocular motor tests. These included evaluation of the vestibulo-ocular reflex (VOR), using electronystagmography with computer analysis and saccadic, smooth

pursuit and optokinetic nystagmus responses. Vestibular stimulation comprised rotatory and caloric tests. Details and normal values have been previously described[18]. The analysis of the VOR focused on the time constant (T, in seconds) derived from (90°/s) velocity-step test responses in either direction. As this parameter shows a log normal distribution[19], the geometric mean for both nystagmus directions was used for further evaluation; the 90% (P5-P95) confidence interval for T was 13-23 seconds. An arbitrary zero (T = 0) was assigned to rotatory responses showing vestibular areflexia with no or just a few nystagmus beats. The above-mentioned procedure for fitting a sigmoidal curve was also applied to the T vs. age data. For comparison, we retrieved similar data from 74 P51S mutation carriers from eight previously reported different Dutch families, as well as from a recently studied, newly identified DFNA9 family[20]. Comparisons between fitted sigmoidal curves were performed as described above by applying Student's t test to the fitted parameter values. Fisher's exact probability test was used to compare the relative frequency of complete vestibular areflexia (T = 0-3 sec) between the groups of G88E and P51S mutation carriers, as well as between subgroups of the respective mutation carriers within various age classes.

A CT scan of the petrosal bones of patient III-26 was performed (Siemens Somatom Plus 4, Siemens, Forchheim, Germany). Magnetic resonance imaging (MRI) results of the posterior fossa of patient III-8 in 1998 were retrieved from elsewhere.

Results

For the sake of clarity and privacy matters a pedigree containing three generations was deduced from the original pedigree with five generations (Figure 1). Thirteen persons were affected by history. Six of them were alive. Blood samples were obtained from 42 individuals for genetic analysis (see below). Audiometry was performed and/or could be retrieved from elsewhere in 42 individuals, some of which concerned presumably affected individuals who already had deceased prior to the start of this study. An autosomal dominant pattern of inheritance is apparent, especially from the oldest generations of the pedigree. The case histories and physical examinations excluded syndromic involvement. All clinically affected individuals reported bilateral slowly

progressive hearing loss with onset age in the range of 40 – 68 years. Varying vestibular symptoms were noted in six of them (II-13, II-14, II-16, III-8, III-19, III-26), including instability in the dark, vertigo and a tendency to fall. We did not note a high incidence of cardiovascular disease or Ménière-like symptoms.

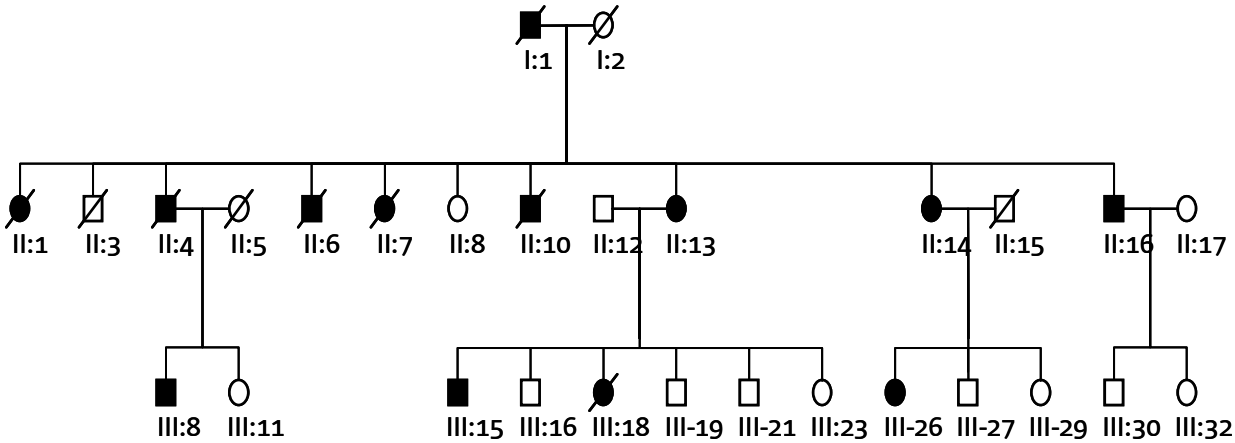

Figure 1 Simplified pedigree of family W99-101 with non-syndromic autosomal dominant hearing loss and vestibular impairment. Open symbols, anamnestically unaffected; solid symbols, anamnestically affected; deceased individuals are slashed

Genetic analysis

Sequence analysis

The pro51ser (P51S) mutation (exon 4) was not present. Further DNA sequence analysis of the *COCH* gene in cases II-13 and II-14 revealed a heterozygous guanine (G) to adenine (A) alteration at nucleotide position 263 of the protein coding region (C.263G>A) in exon 5. This missense mutation results in the substitution of a glutamic acid for a glycine at amino acid residue 88 of cochlin (G88E). No other mutations were identified in the protein coding sequences of exons 2-4 and 6-12. This G to A mutation destroys an *Ava*II restriction site, which was used to analyze co-segregation of this mutation within this family. The wild-type allele, without a G to A mutation, will be digested into two fragments of 154 and 214 bp, as illustrated for part of the digested samples in Figure 2.

Based on this restriction enzyme analysis 16 mutation carriers were identified, which included all six clinically affected individuals from the 2nd and 3rd generation with ages varying from 51-82 years (solid figures in Figure 1). Six unaffected individuals from the 3rd generation (ages from 39-53 years) and four unaffected family members in their offspring (ages from 22-29 years) turned out to be mutation carriers as well. For privacy reasons we did not include the pedigree

numbers of these unaffected cases. All other family members were homozygous for the wild-type allele. Restriction analyses of genomic DNAs from 100 healthy unrelated control individuals did not reveal this mutation (data not shown).

Haplotype analysis

Using D14S262, D14S975, D14S257 and D14S1040 did not reveal a common disease-associated haplotype in the Dutch and the American family. Only for the polymorphic marker D14S1021 there was a common allele, however this allele is too frequent to draw any conclusions. Through testing of the SNP RS#2239581 which is located very close to the mutation, a new polymorphism was detected (IVS4+80delT). In the Dutch and American families the disease-associated haplotype differed with regard to this polymorphism. Alleles of all three other SNPs in the *COCH* gene are the same for both families. However, again the most common alleles are associated with the mutation. Based on these results (data not shown) a common origin of the mutations seems unlikely, but could not totally be excluded.

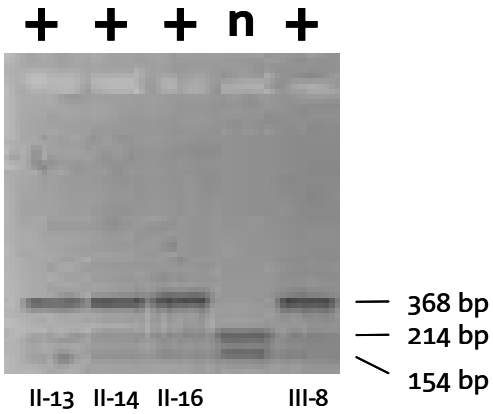

Figure 2 Part of the *Ava*II restriction analysis of PCR-amplified exon 5 of the *COCH* gene of family W99-101. Normally, digestion of exon 5 results in two DNA fragments of 154 and 214 bp; digestion of exon 5 containing the G>A alteration prevents this restriction and yields a single fragment of 368 bp. Partial *Ava*II digestion is present in the cases indicated with a "+", identifying them as heterozygous mutation carriers. n, digested DNA sample from an unaffected family member. Bp, basepairs.

Audiometry

Pure tone thresholds related to age

Figure 3 shows the cross-sectional analysis of the threshold against age data for the G88E mutation carriers. The threshold data included in the nonlinear regression analysis represent either a single snapshot measurement or, if serial audiometry was available -which was the case in seven mutation carriers- the last

visit at which a reasonable number of thresholds was still measurable. Individual longitudinal threshold measurements are also included; they seemed to conform reasonably well to the fitted sigmoidal curves, except at 8 kHz and possibly also at

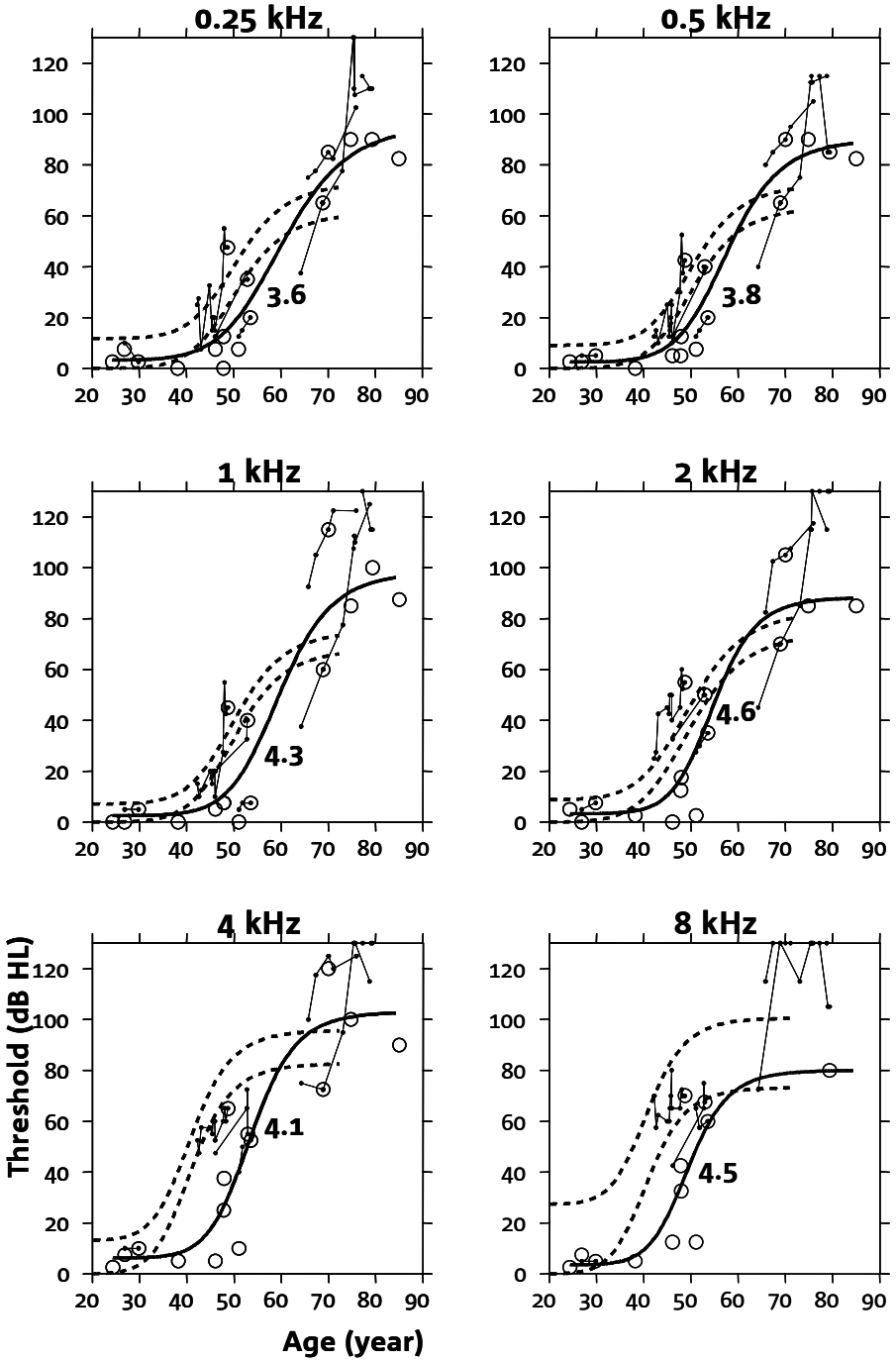

Figure 3 Cross-sectional analysis (binaural mean air conduction threshold) for the present family. Threshold data (unconnected open circles, last measurable threshold in dB HL) vs. age (in years) for all frequencies in 16 G88E mutation carriers. Longitudinal measurements of seven of these mutation carriers (small dots with connecting hairlines) are included in the plots; these data, except one in each case (open circle), were not included in the fitting procedure. The curve in bold is the fitted sigmoidal dose-response curve with variable slope, whose maximum slope is included (bold figure) as an indication of ATD. The two dashed curves in bold are shown for comparison: these sigmoidal curves are the same as those previously fitted to cross-sectional data of carriers of the P51S mutation in a single, large family[15] (uppermost dashed curve), and the same curve corrected for zero offset threshold at age zero (lowermost dashed curve)

4 kHz. It should be realised that for the longitudinal data the threshold was fixed at 130 dB (before averaging) for out-of-scale thresholds, whereas such thresholds had been excluded for the fitting procedure. For this reason, especially the curves fitted for 4 and 8 kHz were certainly biased in a downward direction, which presumably has influenced especially the fitted top level and perhaps also the maximum slope of the curve and apparent onset age.

Maximum slope of the sigmoidal curve (Figure 3, bold line in each panel) was visually estimated by drawing a tangent through the inclination point of the curve; this is an indication of maximum annual threshold deterioration (ATD, in dB/year). ATD was in the range of 3.6-4.6 dB/year. Onset age (X_{10}[15]) was estimated at 46-49 years only at 0.25-2 kHz; we did not include 4 and 8 kHz because of the presumed bias. For the sake of comparison, the fitted sigmoidal curves of P51S[15] are included without any change, as well as corrected for presumed zero offset at age zero (dashed curves). The latter was closer to the presently fitted (continuous) curve at each frequency. Student's t test comparing between the parameter values fitted for the respective sigmoidal curves only detected a significant difference between the uppermost dashed curve and the continuous one at 4 and 8 kHz: at 4 kHz the ages of onset and at 8 kHz the bottom levels differed significantly.

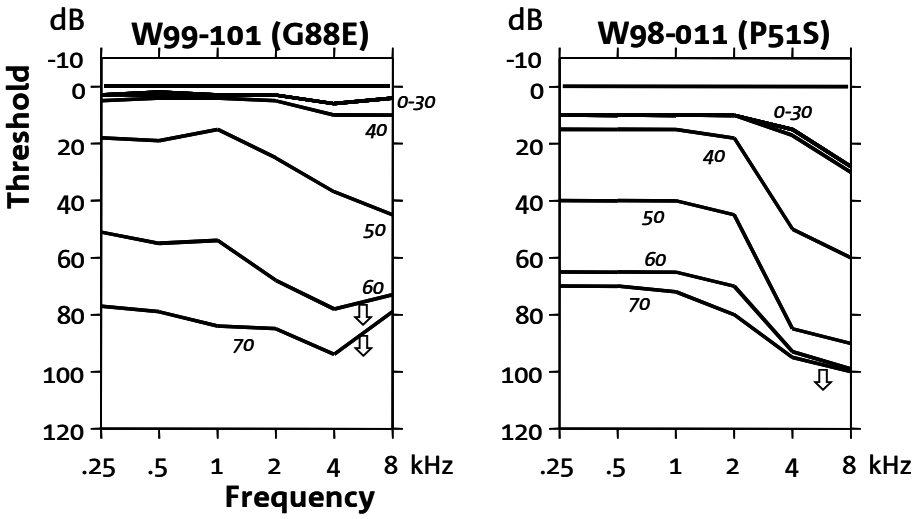

Figure 4 ARTA of the phenotypically and genotypically affected family members of family W99-101 (left panel) and affected Dutch DFNA9 family members (W98-011) carrying the P51S mutation (right panel). Italics indicate age in years. The downward arrows indicate that the thresholds at 4-8 kHz for ages 60-70 years have been underestimated (Patient and Methods)

Figure 4 shows the ARTA derived for the present mutation carriers and those previously derived for the P51S mutation carriers in juxtaposition. Using the method described elsewhere[16], we did not find any significant difference in ARTA between the two groups of COCH mutation carriers.

Speech recognition scores related to age

Figure 5 shows the single snapshot measurements and the longitudinal measurements of phoneme scores in the present G88E mutation carriers. These scores seemed to compare reasonably well with the scores previously measured for monaural presentation to the right ear in P51S mutation carriers from different families[17] to which the sigmoidal curves had been fitted (in cross-sectional analysis) as far as the impairment-performance plot (Figure 5B) was concerned. In the age-performance plot (Figure 5A) it would seem that the G88E mutation carriers had a tendency to show relatively high scores in the age range of 40-60 years, which might be associated with a higher age of onset. It should be kept in mind, however, that the present scores were averaged for the monaural responses in two ears and that many of the scores pertained to longitudinal measurements, which prohibited statistical testing of these scores against the previous cross-sectional, monaural measurements.

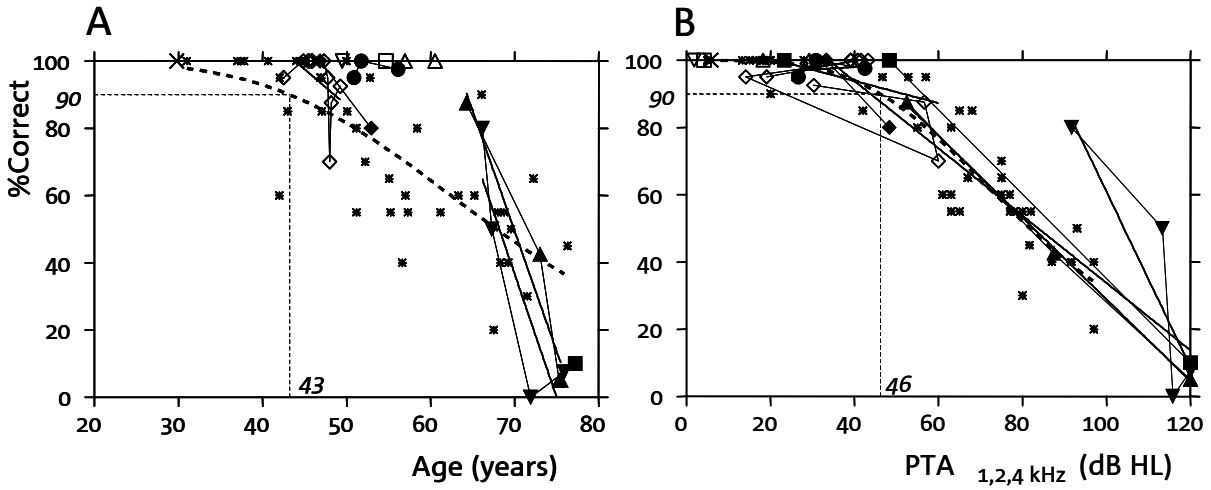

Figure 5 Mixed single snapshot and longitudinal individual measurements (large symbols connected by hairlines) of binaural mean speech recognition scores at last visit against age (A) and against binaural mean PTA $_{1,\,2,\,4\ kHz}$ (B) in 10 G88E mutation carriers. Different symbols pertain to different carriers. Straight lines are individual linear regression lines. For the sake of comparison, the cross-sectional data of P51S mutation carriers (monaural score for right ear) from 7 different families are included (small asterisks), along with the bold, dashed sigmoidal curve that was fitted to those scores; horizontal and vertical dashed hairlines and italic figures indicate onset age (A) and onset level (B) fitted for those data[17]

Vestibulo-ocular examination and imaging

Two mutation carriers showed vestibular areflexia at age 75 and 49 years, respectively. The youngest of them, who had no significant hearing loss at that age, exhibited an enhanced cervico-ocular reflex. One hearing impaired individual (III-8) revealed bilateral caloric weakness, whereas patient III-15 exhibited asymmetrical responses to caloric testing (left areflexia, right hyporeflexia). Vestibular testing in six other clinically unaffected mutation carriers at ages between 24 and 55 years, revealed no abnormalities.

Figure 6 shows the cross-sectional analysis of mean T in relation to age for the G88E mutation carriers (asterisks and bold curve), compared to a similar analysis for the P51S mutation carriers (open circles and thin curve).

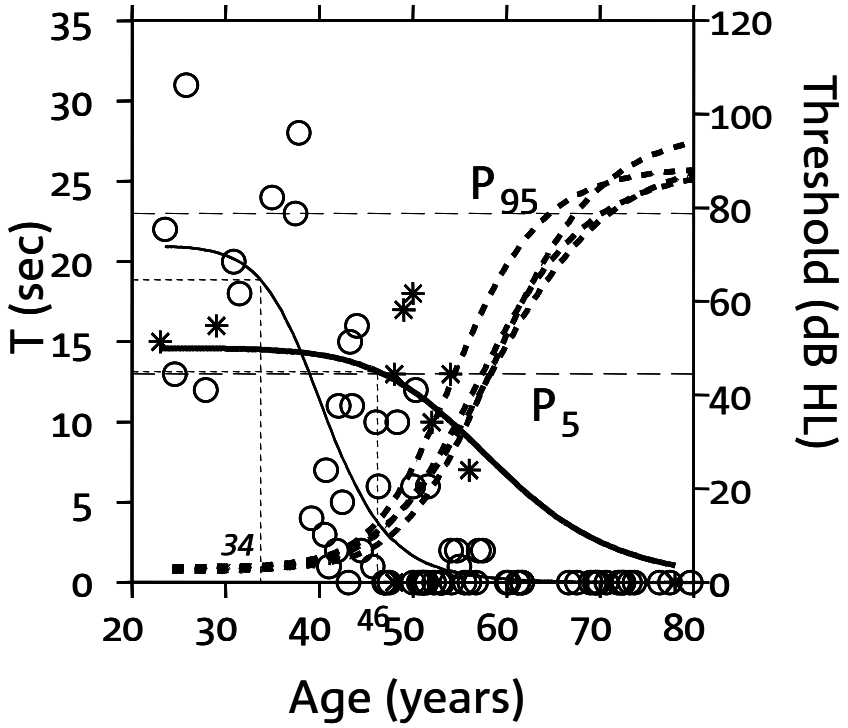

Figure 6 Mean VOR time constant (T in sec, left axis) plotted against age (years) for 10 of the present G88E mutation carriers (asterisks and bold sigmoidal curve) and 74 P51S mutation carriers[20] (open circles and thin curve). Complete vestibular areflexia (found in 2 patients) is represented by an arbitrary zero. The 5th and 95th normal percentile values are shown as dashed horizontal lines. Dashed vertical hairlines indicate onset age (X_{90}, years) for G88E (bold figure) and P51S (italic figure). For comparison, the dashed sigmoidal curves (threshold level, right axis) are included, which are the same curves as the continuous ones in Figure 3 fitted to the present threshold data at 0.25-2 kHz

Although the G88S mutation carriers seemed to have later onset (age 46) than the P51S carriers (age 34), we could not find any significant difference between corresponding parameter values fitted for the two groups of mutation carriers.

There was, however, a striking, significant difference between the two groups of mutation carriers: only two of the ten G88E mutation carriers showed complete vestibular areflexia (T = 0-3 sec), whereas 52 of the 74 P51S mutation carriers showed this feature (P = 0.0033). Restricting the comparison to mutation carriers aged 40 years and over, the significant difference was even more clear: two of eight *vs.* 52 of 64 (P = 0.0024). As the group of G88E mutation carriers included only one patient aged > 56, it might have been that the significant difference was caused by differences in age distribution between the groups of mutation carriers (Figure 6). However, there was also a significant difference found within the age class of 40-56 years: one of six *vs.* 25 of 37 (P = 0.013). Of course, this does not exclude the possibility that all or most of the G88E mutation carriers develop vestibular areflexia at ages of > 56 years. Within the present group of G88E mutation carriers, it was tested as to whether there was a significant difference in age of onset estimated (fitted) for progressive deterioration of vestibular function (i.e. shortening of T, Figure 6, left axis) and hearing threshold (Figure 6, right axis). No significant difference was detected; as indicated above, age of onset of hearing deterioration was most reliably estimated at 0.25-2 kHz, where it was in the range of 46 - 49 years.

The middle and inner ear structures of two hearing impaired family members (III-8 and II-13) had normal appearances on MRI and CT scanning

Discussion

There are many reports on DFNA9[7-9,15,21-25]. The P51S mutation is the only known *COCH* mutation encountered so far[7] in the Netherlands and is considered to be a founder mutation. A Dutch DFNA9 family carrying a G88E mutation is therefore quite remarkable. Previous reports on a family carrying this latter mutation originate from the US. This family was designated the 1Su family[26,27] and these reports also included histopathology of temporal bones of affected individuals. By giving a thorough description of the phenotype associated with the G88E mutation of the *COCH* gene, this report extends the available clinical data on the DNFA9 phenotype.

Some remarks can be made on the pattern of inheritance in this family. A clear autosomal dominant pattern of inheritance with complete penetrance based on

anamnestic and audiometric data is not present in the pedigree. There is no doubt that this finding relates to the fact that the complete phenotype only develops at a more advanced age. Most of the participating family members who turned out to be mutation carriers, were still too young (maximum age 53 years of age) to clearly exhibit clinical features. Assuming that the G88E mutation is pathogenetic, it will be interesting to see whether the present non-affected mutation carriers in this Dutch family will develop the characteristic DFNA9 symptoms.

The reported mean age of onset of hearing loss in family W99-101 (5^{th}-6^{th} decade) was comparable to that reported for the original DFNA9/1Su (G88E) kindred and the DFNA9 (P51S) families[27]. The other American DFNA9 families (1W, 1St) showed an earlier age of onset (2^{nd}-3^{th} decade)[27,28]. In general, hearing loss in all DFNA9 families first affects the high frequencies, later followed by the mid and low frequencies, resulting in progressive loss in the low to mid frequencies coupled with a high-frequency slope[15,25]. The annual threshold deterioration was similar in all DFNA9 families studied so far and this includes the present family. The audiometric characteristics of the present family are fairly consistent with those found in the original DFNA9/G88E (1Su) and the previously reported DFNA9/P51S families.

Symptoms indicating vestibular dysfunction, for example dizziness, balance problems and oscillopsia, have been described for the American kindreds 1Su and 1W, but not for 1St[25-28]. Khetarpal et al[27] reported that about 50% of affected members of family 1Su complained of vertigo or dizziness. In a later report, Khetarpal specified the symptoms encountered in the American family 1W carrying the V66G mutation[29]. Vestibular dysfunction and Ménière-like symptoms are frequently seen in DFNA9 patients carrying the P51S mutation[21]. In the present family, anamnestically, vestibular symptoms were noted in six clinically affected cases as mentioned above. Almost all tested patients with obvious hearing impairment showed loss or lack of vestibular function. Vestibular testing in the asymptomatic mutation carriers revealed no abnormalities. Furthermore a high prevalence of Ménière-like symptoms and of cardiovascular disorders have been noted in the past in *COCH*/P51S mutation carriers[21,22,24], however we could not establish this association in the present family.

Special attention was also given in the present study to a comparison of age-related features between progressive hearing and vestibular impairment. Hearing started to deteriorate in all G88E mutation carriers from age 46-49 years onwards, whereas deterioration of vestibular function started from about age 46, i.e. at about the same age. Vestibular impairment started earlier, at about age 34 years, in the P51S mutation carriers, i.e. at a significantly younger age in those patients than the apparent age of onset found for their progression in hearing impairment20. So it would seem that the G88E mutation carriers showed simultaneous progression of vestibular and hearing impairment, whereas the P51S mutation carriers, who showed progression of hearing impairment at about the same ages, exhibited earlier progression of vestibular impairment. Although there was no significant difference in the age of onset of vestibular impairment detected between the G88E and P51S mutation carriers, it was remarkable that a significantly smaller proportion of the G88E mutation carriers developed complete vestibular areflexia at ages in the range of 40-56 years. It is therefore possible that the G88E mutation carriers either showed more limited expression of complete vestibular areflexia or developed this feature at an older age (> 56 years) than the P51S mutation carriers. If this is true, it might explain why in the original American family vestibular impairment among the G88E mutation carriers may have been a less prominent finding than in the Dutch P51S mutation carriers.

Recently Grabski et al[29] reported that different *COCH* mutations vary in the amount and pattern of cochlin deposition in the extracellular matrix. One of the mutations that resulted in lack of deposition is the G88E mutation, whereas the P51S mutation led to extracellular depositions indistinguishable from wild-type cochlin. One could speculate that these diminished or absent depositions in this in vitro study cause the less severe development of vestibular symptoms. Since G88E-associated hearing loss shows a similar pattern as that associated with the P51S mutation might indicate that the difference in effect of the mutations does not occur in the cochlea.

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Chapter 3.3

Progressive cochleovestibular impairment caused by a point mutation in the *COCH* gene at DFNA9

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Laryngoscope 1999;109:1525-1530

Abstract

Objectives: analysis of phenotype-genotype correlation.

Study design: family study.

Methods: auditory and vestibulo-ocular functions were examined in a Dutch family with autosomal dominantly inherited sensorineural hearing impairment, caused by a 208C>T mutation in the *COCH* gene, located in chromosome 14q12-13 (DFNA9). Linear regression analysis of individual longitudinal hearing threshold data (n=11) on age was performed.

Results: fifteen of the sixteen genetically affected persons could be evaluated. They all developed hearing and vestibular impairment symptoms - and in many cases also cardiovascular disease - in the fourth to fifth decade. At the low frequencies (0.25-2 kHz) hearing impairment started at the age of about 40 years and showed an average annual progression of approximately 3 dB, finally resulting in profound hearing losses. In two exceptional cases, annual progression attained levels of up to 24 dB. At the high frequencies (4-8 kHz), the average threshold deteriorated from about 50 dB at the age of 35 years to about 120 dB at the age of 75 years (which amounts to 1.8 dB annual threshold increase). All affected individuals tested showed normal ocular motor functions. The patients older than 46 years generally showed absence of the vestibulo-ocular reflex, but their cervico-ocular reflex was enhanced compared with normal subjects, whereas those aged 40-46 years showed either severe vestibular hyporeflexia or unilateral caloric areflexia.

Conclusion: these findings suggest a gradual development of cochleovestibular impairment caused by the new mutation found.

Introduction

Inherited sensorineural hearing impairment can be classified into two forms: syndromic and nonsyndromic. The latter form accounts for about 75%[1,2] of all cases of congenital hearing impairment and is highly heterogeneous; nearly each family maps to a unique chromosomal locus. Prelingual nonsyndromic hereditary hearing impairment is diagnosed in approximately 1 in 700 newborns,[3] of which 75% is autosomal recessive, 20% to 25% autosomal dominant and up to 5% X-linked or mitochondrial[2].

Thorough genotypical and phenotypical analysis can provide the necessary definition and delineation of types of monogenic hearing impairment[4]. More than 40 human chromosomal loci associated with nonsyndromic hearing impairment have been reported in recent years, including more than 15 autosomal dominant (DFNA) loci[5]. More than eight different genes have been identified since 1997, including late-onset progressive types with high-frequency involvement. In some studies moderate or subclinical hearing impairment was detected in individuals heterozygous for autosomal recessive gene defects[6,7,8]. It is possible that heterozygous recessive gene defects or DNA variants in dominant deafness genes are risk factors for presbyacusis.

A large multigenerational nonconsanguineous Dutch kindred is presented, showing progressive hearing and vestibular impairment with a relatively late onset, ultimately leading to profound deafness and vestibular areflexia.

Materials and methods

We examined auditory and vestibulo-ocular functions in 16 affected members of a Dutch family of about 200 members (family W98-011, Figure 1). In addition, six deceased individuals seemed to be affected. Thus a total of 22 affected individuals were identified. The pattern of inheritance was autosomal dominant with apparently full penetrance.

Possible exogenic, nonhereditary causes of hearing and vestibular impairment were excluded. Family history data were obtained and the pedigree was drawn. Prior written consent was obtained to retrieve previous audiograms and relevant medical information. All 119 persons participating in this study underwent otoscopy. Special

attention was paid to vestibular impairment symptoms, as well as possible syndromic features. Seven persons with vestibular areflexia were also examined by a neurologist.

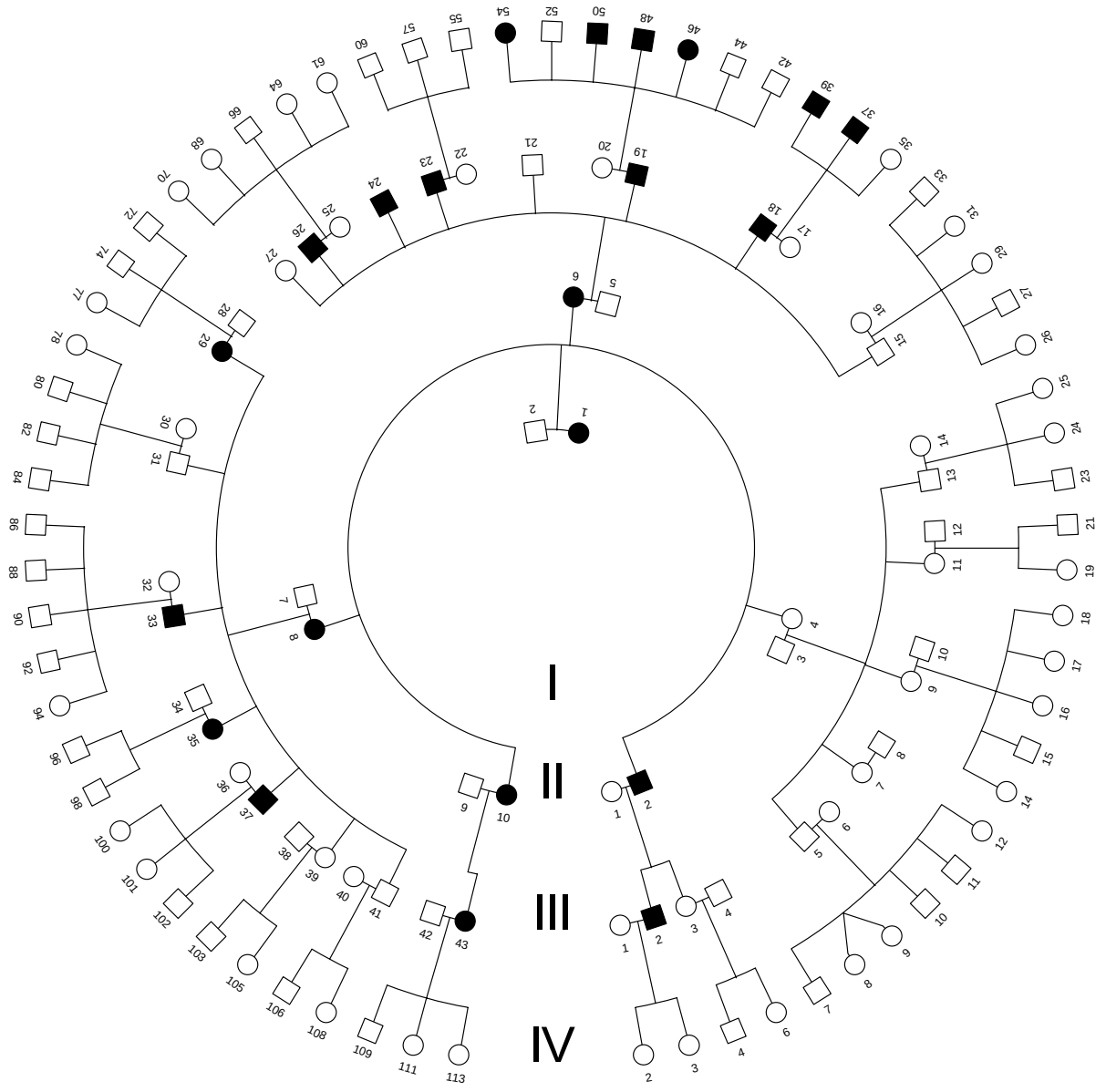

Figure 1 Pedigree of family W98-011. Solid symbols mark affected individuals, amongst whom are several deceased individuals (I-1, II-2, II-6, II-8, II-10 and III-19)

Blood samples for genetic linkage analysis were collected from 16 presumably affected individuals and 103 presumably unaffected individuals, in which chromosomal markers for the 15 known loci for nonsyndromic autosomal dominant hearing impairment were screened first, guided by the pattern of inheritance in the pedigree. Linkage to the DFNA9 locus was found and mutation analysis was performed[9].

Audiograms were obtained from all individuals, according to International Standards Organization (ISO) standards[10,11]. including air-conduction and bone-conduction levels. The method of ISO 7029[12] was followed to calculate for each patient individually the 95th percentile (P_{95}) threshold values for presbyacusis at each frequency in relation to the patient's age and sex. Because of lack of other known causes, thresholds above the P_{95} value in the better ear were initially considered as inherited sensorineural hearing impairment. The final selection comprised the mutation carriers only. Previous audiograms were retrieved from elsewhere, including affirmative audiograms from three deceased (presumably affected) individuals.

Linear regression analysis was performed in those cases where a sufficient number of serial audiograms (covering at least three years) was available. It was tested whether there was significant progression (that is, where the regression coefficient [in this report called the annual threshold deterioration, ATD, and expressed in decibels per year] was significant). The Y intercept was called the offset (threshold) and, in case of this being a negative value, an onset age was calculated (X intercept). Where appropriate, regression lines were compared using the F test (one-way ANOVA options included in the Prism computer program, PC version 2.0, GraphPad, San Diego, CA) to detect any significant differences. The Prism program tests the slopes first and subsequently the intercepts, but it abandons testing as soon as a significant difference is detected; pooled values are calculated where possible. The level of significance used was 0.05.

Gaze positions were tested to investigate whether there was any gaze-evoked nystagmus. Saccades, smooth pursuit and horizontal optokinetic nystagmus responses were elicited and analyzed as reported earlier[13]. Vestibular tests (velocity-step tests and caloric tests) were conducted with the patient in the dark with the eyes open. It was evaluated whether there was any spontaneous nystagmus. Velocity-step tests were performed with a rotatory chair (Toennies GmbH, Freiburg im Breisgau, Germany). The postrotatory nystagmus response was analysed with a computer method. Only the time constant is specified as a key response parameter in the cases with severe hyporeflexia (P_5-P_{95}, 11-26 s). The

cervico-ocular reflex was elicited in the dark by applying sinusoidal stimulation to the body with the head fixed in space[14].

Results

Affected persons reported an age of onset for hearing impairment symptoms ranging from 36 to 63 years. Hearing impairment was characterized by variable - in most cases rapid - progression to profound sensorineural impairment (Figure 2). Affected individuals showed no evidence for other factors predisposing toward hearing impairment, except for case IV- 37, where ear surgery had taken place

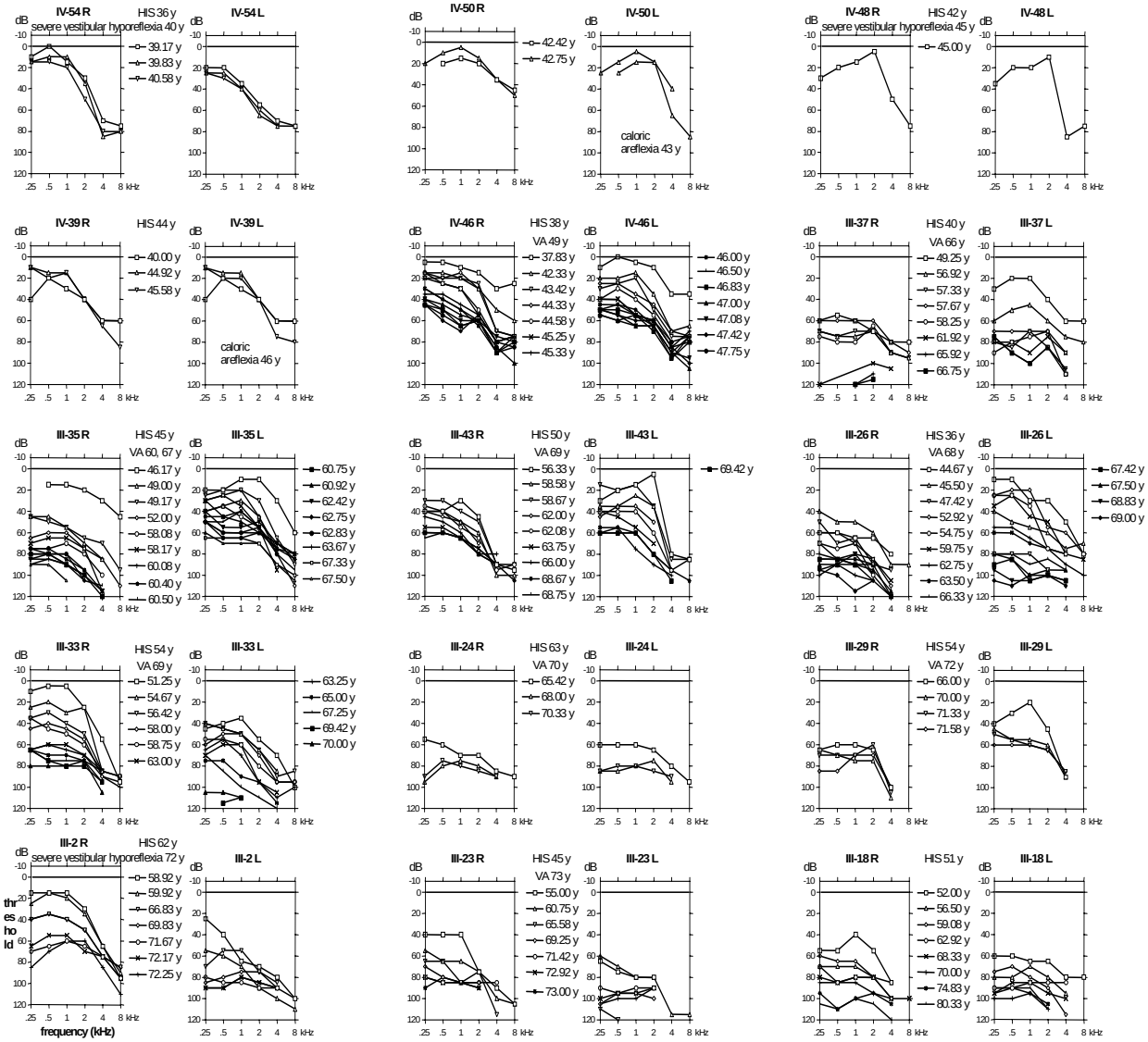

Figure 2 Individual audiograms (R, right ear; L, left ear) of 15 affected family members, ordered by the age at which the most recent audiogram was obtained (see key to symbols next to each graph). Air-conduction threshold in decibels hearing level (dB HL). HIS, hearing impairment symptoms (age, y); VA, vestibular areflexia (age, y).

previously. In most individuals, vestibular symptoms and hearing impairment symptoms started simultaneously.

The former symptoms consisted of head-movement-dependent oscillopsia and imbalance when walking in the dark; patients also reported having difficulties when riding a bicycle, especially when attempting to look over their shoulders. With their eyes closed, they generally exhibited a broad-based gait. Several affected individuals had a history of hypertension (cases III-23, III-24, III-26, III-37, IV-46, IV-48 and IV-50), stroke (cases III-18, III-19, III-24 and III-26), or ischaemic heart disease (cases III-23, III-35 and III-37). Hence 10 out of 15 individuals exhibited cardiovascular disease. One individual (case III-24) had recently experienced an episode of glossopharyngeal neuralgia. One individual (case III-2) exhibited mild symmetric distal polyneuropathy. One individual (caseIV-46) had a mild cervical myelopathy. Apart from these incidental findings, no consistent neurological deficits other than vestibular impairment were found.

Linkage analysis mapped the gene defect underlying cochleovestibular impairment in this family to an 11.0-centiMorgan (cM) region overlapping the DFNA9 interval[15] on chromosome 14q12-13. Sequence analysis revealed a 208C→T mutation (exon 4) in the *COCH* gene, resulting in a P51S substitution in the predicted protein in all affected individuals of the family; this mutation was not found in unaffected family members or in 200 control individuals[9].

All audiograms obtained from the genetically affected family members included in this study are presented in Figure 2. Case IV-37 was excluded because of previous ear surgery. All identified carriers of the mutation demonstrated sensorineural hearing impairment. At a relatively young age (generation IV; note that in Figure 2 cases are ordered by the age at which the most recent audiogram was obtained) considerable sensorineural hearing impairment at the highest frequencies was already present; audiograms showed downsloping hearing impairment in most cases. In addition, sensorineural hearing impairment at the lower frequencies had developed at a more advanced age. The patients in generation III showed moderate to severe hearing impairment with (eventually) an almost flat threshold or residual hearing (that is, functional hearing only at the low frequencies). In three of the six probably affected, deceased individuals

audiograms could be retrieved; they all exhibited severe losses typical of the general audiometrical picture in this family. Audiometrically, remarkable variability and asymmetry was seen in the progression of hearing impairment with increasing age (Figure 2).
Regression analyses of longitudinal threshold-on-age data were performed only for those age intervals where (approximately) linear homogeneous progression occurred. Relevant parameter values are presented in the Table. The estimates obtained for the longitudinal annual threshold deterioration (ATD) at the frequencies 0.25 to 2 kHz varied from 2 to 4 dB in most instances. In two exceptional cases (III-26 and IV-46) ATDs of up to 24 dB were found. ATDs for the frequencies 4 to 8 kHz were smaller in all cases in which those increases could be evaluated. Estimates for onset age could be obtained only for the low frequencies in most cases; estimates were in the range of approximately 35 to 50 years, except in case III-23, in which the extrapolated onset age was about 18 years. Case IV-46 exhibited an apparent onset age of between 25 and 40 years at all frequencies. In case III-35, an onset age of 0 years was extrapolated for the high frequencies, but the raw data (not shown) clearly indicated nonlinear, increased progression following onset at about 45 years of age.

In Case III-37 extrapolation produced an apparent onset age of about 20 years for hearing impairment at 4 to 8 kHz, but given the amount of scatter in the raw data, this estimate seems inaccurate.In five cases backward extrapolation of the 4 to 8 kHz regression lines (that is, toward the Y axis) yielded an offset threshold (positive intercept) in at least one ear, for one of these frequencies (Table).

This offset differed significantly from zero in two measurements. These findings suggest sensorineural hearing impairment at 4 to 8 kHz being present well before the presumed age at onset of hearing impairment in the lower frequencies as well as vestibular impairment. More appropriate analysis of these findings may be performed after having genotyped the youngest family members.

All available threshold data are shown in Figure 3, with separate trend lines for each of the two sets of low and high frequencies. It should be noted that formal regression analysis of the data shown was not permitted, because of the replications (that is, the presence of longitudinal individual measurements and

separate entries for the two ears in most cases). At 0.25 to 2 kHz, we assumed an average trend with onset at 40 years of age and progression by 3 dB/year (see mean values in the Table). At the 4 to 8 kHz frequencies, we assumed an average threshold trend from 50 dB at the age of 35 years to 120 dB at the age of 75 years (that is, average annual progression about 1.8 dB, see Table I).

Table 1 Relevant results of the longitudinal analyses (i.e. linear regression analysis for n > 2) of the binaural mean threshold in relation to age applicable to cases with suitable observation intervals.

Case	*age interval (y)*	*n*	*ATD (dB/y)*[a]	*Onset age (y)*[a]	*Offset (dB)*[b]
III-2	58-72	7	lo 3, hi 0	lo 46	R 7/58, L **85**/129
III-18	52-80	8	Lo 1		R 28/na, L 51/na
III-23	55-73	7	Lo 2	lo 18	R na, L na
III-24	65-70	3	Lo 4	lo 50	R na, L na
III-26	44-47	3	R lo 5, L lo 24	L lo 44	R 28/na, L na/23
	53-69	10	lo* 3, hi* 2	lo* 41	
III-29	66-71	4	Lo 3	lo 51	R na, L na
III-33	51-69	9	Lo 4	lo 45	R na/72, L na/89
III-35	46-67	18	lo 3, hi 1	lo 34, hi 0	(nonlinear)
III-37	49-66	7	lo 3, hi 2	lo 36, hi 19	R na, L na
III-43	56-69	9	lo 3, hi 1	lo 47	R **117**/49, L 20/na
Mean			*lo 3.0, hi 1.8*	*lo 41*	
IV-46	42-48	13	lo 6-16, hi 4-5	lo 36-41, hi 27-30	R na, L na

[a], pooled regression estimate; [b], 4 kHz/8 kHz ; significant difference from zero in bold; lo, low frequencies (0.25-2 kHz; *, 0.25-1 kHz); hi, high frequencies (4-8 kHz; *, 2-8 kHz); R, right ear; L, left ear; na, not applicable (i.e. negative value)

Vestibulo-ocular examination was performed on all affected family members (Figure 2), except in cases IV-37 (previous ear surgery) and III-18 (not fit to undergo the tests). All cases had normal ocular motor function. In generation III, all affected family members (aged over 60 years), except for one individual (case III-2) had an absent vestibulo-ocular reflex, but their cervico-ocular reflex was enhanced compared with normal subjects, which is typical of labyrinthine-defective patients[14]. In case III-35 the same findings had been obtained seven years earlier. In Case III-2 severe hyporeflexia of the vestibulo-ocular reflex was evident, but with a well-developed cervico-ocular reflex; this individual had relatively late onset of hearing impairment symptoms. Similar vestibular findings were obtained in generation IV in cases IV-48 and IV-54 (aged 45 and 40 years, respectively). These three cases showed a very short vestibulo-ocular reflex time constant (1-4 s). In generation IV, only case IV-46 (aged 47 years) showed bilateral

vestibular areflexia, whereas cases IV-39 (aged 46 years) and IV-50 (aged 43 years) exhibited unilateral caloric areflexia.

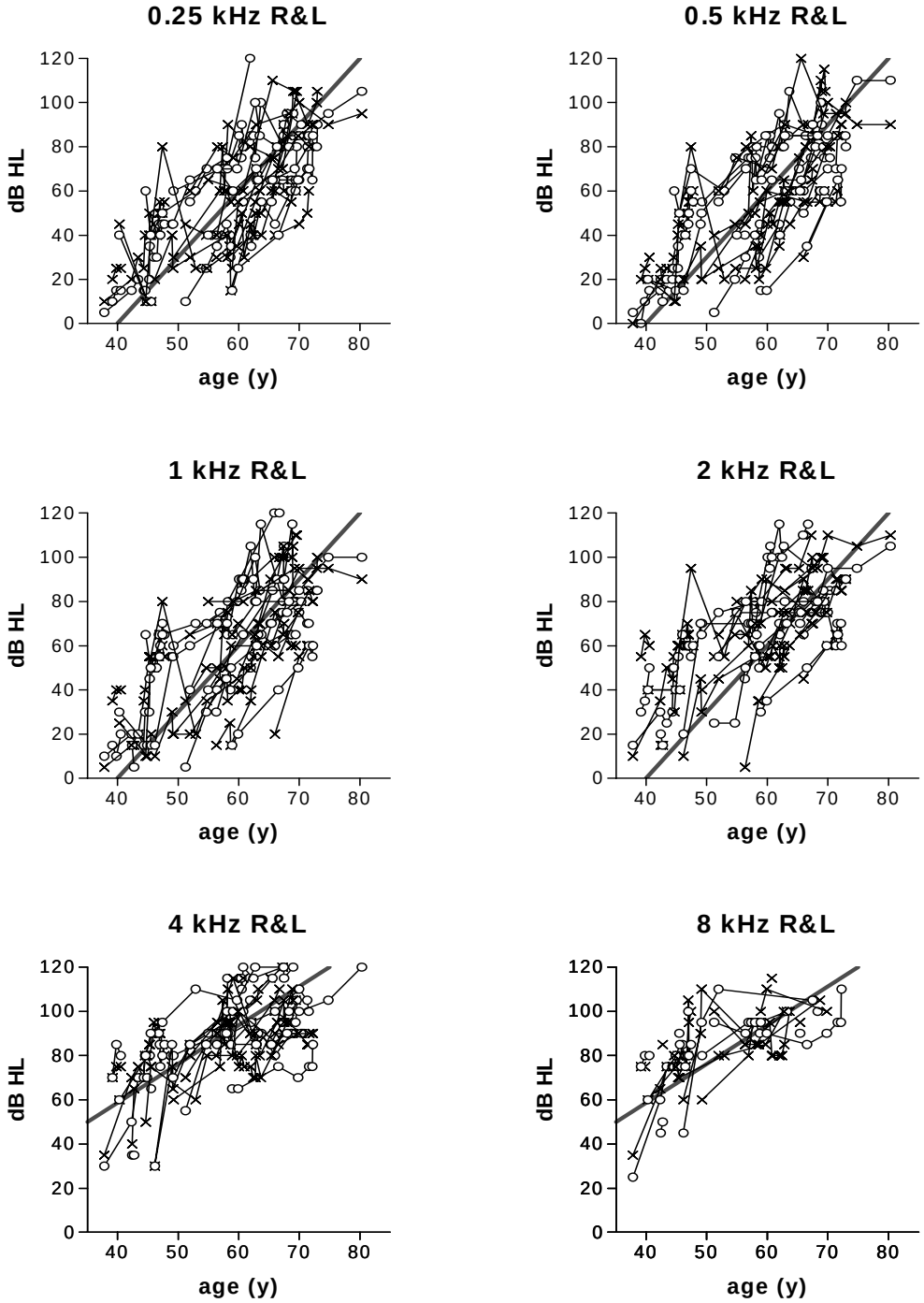

Figure 3 Plots of all the available threshold-on-age data for each frequency with “trend lines" (bold). For each ear (o, right; x, left) longitudinal data points are connected by thin lines. The trend line for each of the frequencies 0.25, 0,5, 1 and 2 kHz is based on the mean values (slope and onset age) in the Table. The trend line for each of the frequencies 4 and 8 kHz has a mean slope of 1.8 dB/year (Table) and was arbitrarily drawn at an offset level of 50 dB at age 35 years.

Discussion

This clinical study of a family (family W98-011[9]) with the DFNA9/*COCH* mutation revealed distinct characteristics of cochleovestibular impairment.

Considerable intersubject and interaural variability in the development of hearing threshold with advancing age were found (Figure 2 and Table). Most of the progression occurred at the low frequencies (3 dB/year, Figure 3). At the high frequencies, sensorineural hearing impairment may have been due to presbyacusis. In the Table annual increases in threshold levels of about 1 to 2 dB are shown for the high frequencies in cases where this could be evaluated. We simulated average presbyacusis by plotting P_{50} presbyacusis threshold values[12] for each frequency at several equidistant ages over the age interval of 50 to 70 years. We calculated virtual values for annual threshold deterioration (ATD) and related these values to median presbyacusis values by linear regression analysis for these hypothetical threshold data. The resulting values were between 0.7 dB/year (women) and 1.4 dB/year (men) at 4 kHz and between 1.3 and 1.8 dB/year at 8 kHz. These estimates are in the same range as most of the ATD values estimated for the high frequencies in the Table.

Vestibular areflexia was found from the age of 47 years (case IV-46) onwards, whereas at a younger age (40 to 46 years) the affected individuals showed either severe hyporeflexia or unilateral caloric areflexia. Those younger subjects are likely to develop vestibular areflexia as well.

The DFNA9 family reported on by Manolis et al.[15] displayed onset of low-frequency hearing impairment at about 16 to 28 years of age, which seems earlier than in the family in the present study. Khetarpal et al.[16] reported that in this kindred onset age was about 20 years of age, whereas in another family it would be about 40 years. From previous reports[15-17] we derived individual annual threshold progression values in the range of 3 to 8 dB, predominantly at the low frequencies. Some of our cases exhibited more rapid progression (up to about 24 dB/year), especially at the beginning of the observation period. Given this finding, the possibility cannot be excluded that in some of the previously described cases such progression was only transient and escaped detection.

Three distinct *COCH* mutations pertaining to three American families with nonsyndromic autosomal dominantly inherited hearing impairment, different from the mutation in the family of the present study, were described by Robertson et al.[18] The P51S mutation in the family in the present study was also found in three additional Dutch families (families W98-065, W98-066, and W98-094)[9]. All families originated from the southern region of the Netherlands. One of them has previously been described by Verhagen et al.[19] (family W98-094[9]). A Flemish family with the P51S mutation has recently been studied by Fransen et al. (personal communication), who also found this mutation in two other previously described Dutch families[20-22]. Only one American patient with DFNA9-linked sensorineural hearing impairment had proven hypoactive labyrinths at age 49 and inactive labyrinths at 53 years (case IV-3, kindred 2)[16].

Cardiovascular disease was a prominent additional finding in the family in the present study, as in a family described by Verhagen et al.[21] The latter family was recently shown to have the present DFNA9/*COCH* mutation (Fransen et al., personal communication).

Some histopathological descriptions of DFNA9 have been reported. Khetarpal et al.[16] and Robertson et al.[18] discovered an acidophiylic mucopolysaccharide-containing ground substance in the cochleas, maculas and cristas of DFNA9 patients, as well as severe degeneration of vestibular and cochlear sensory elements and dendrites. These depositions occurred at sites similar to those where *COCH* gene expression is seen in the chicken inner ear[18]. Such a deposition may cause functional impairment in a straightforward manner - in terms of "strangulating" nerve endings - as has been hypothesized by Khetarpal et al.[16]. It also seems possible, however, that this deposition does not cause structural or functional impairment, but rather is a result from it. Nevertheless, more conclusive pathophysiological explanations require further study.

Conclusions

The findings presented in this family study suggest a gradual development of cochleovestibular impairment caused by a 208C→T mutation in the *COCH* gene located in chromosome 14q12-13 (DFNA9). The gross characteristics of this trait

are progressive middle-age onset (at the age of approximately 40 years) low-frequency hearing impairment of about 3 dB/year and high-frequency threshold increases from approximately 50 dB hearing level at the age of 35 years to approximately 120 dB hearing level at the age of 75 years. At the time of onset of low-frequency hearing impairment, development of vestibular areflexia starts as well.

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Chapter 4

DFNA20/26

A Dutch family with hearing loss linked to the DFNA20/26 locus. Longitudinal analysis of hearing impairment

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Abstract

Objective: To perform linkage analysis and to outline hearing loss characteristics in a family exhibiting a non-syndromic, autosomal dominant type of progressive sensorineural hearing loss.

Design: Genetic analysis was performed using microsatellite markers. Audiometric data were collected and analyzed longitudinally. Sigmoidal dose-response curves enabled us to perform nonlinear regression analysis per frequency and on phoneme recognition scores. Speech recognition scores were compared with those of DFNA2, DFNA5, DFNA9 and presbyacusis subjects.

Subjects: Affected family members of a Dutch family (W99-060).

Results: We revealed linkage of hearing loss to the DFNA20/26 locus (maximum lod score 3.1 at $\theta = 0.04$) and reduced the critical region from 12 cM to 9.5 cM. Patients younger than 15 years already showed gently downsloping audiograms. At the ages of 15-20 and 25-40 years, hearing loss was profound at 8 and 1-4 kHz. The 0.25-0.5 kHz thresholds showed more gradual progression by about 1.5-2 dB/year. From about 40 years onwards hearing was residual. Hearing impairment took a more severe course than in a known DFNA20 family. Score recognition in DFNA20/26 was better than in DFNA9 at any pure tone average (1-4 kHz) threshold. Compared with subjects having DFNA2 and DFNA5, speech recognition in DFNA20/26 scored better at threshold levels below 85 dB HL, but worse at levels above 90 dB. Compared with presbyacusis subjects, those with DFNA20/26 scored better in speech recognition at levels below 100 dB and worse at levels above 100 dB.

Conclusions: Autosomal dominant hearing loss is linked to the DFNA20/26 locus in this Dutch family. The critical region is reduced from 12 to 9.5 cM. Phenotypically, patients are more severely affected compared to patients of the original DFNA20 family.

Introduction

Genetic hearing loss is one of the most frequent forms of sensorineural deficits handicapping people of all ages all over the world. Ten percent of the population older than 65 years and 50% older than 80 years are affected[1]. About one child in a thousand is born with prelingual hearing loss and in at least half of these cases the cause is inherited[2,3]. According to Morton[1] approximately 77% of the non-syndromic inherited forms of moderate to profound hearing loss in early childhood shows an autosomal recessive pattern of inheritance (DFNB), in contrast to 22% with an autosomal dominant (DFNA) type. The percentage of X-linked hearing loss (DFN) is 1%, whereas hearing loss with a mitochondrial pattern of inheritance occurs sporadically[1,4]. It seems that most of the hereditary types of postlingual hearing loss are due to either autosomal dominant or mitochondrial mutations[5]. In recent years, mapping of deafness loci has become a common research effort. So far, 41 autosomal dominant, 33 autosomal recessive and 6 X-linked loci associated with non-syndromic hearing impairment have been mapped and 29 different genes have been identified[6].

Recessive forms of hearing loss generally involve all frequencies, are mostly congenital or prelingual, and range in severity from severe to profound[7]. For dominantly inherited hearing loss there is more variation and clearly different types can be distinguished on the basis of the frequencies involved, severity, age of onset and speech recognition scores[7-10]. Despite intra- and interfamilial variation in hearing loss caused by specific loci/genes, it is possible to differentiate between a number of these loci based on their clinical characteristics[7,9,11,12]. Phenotypic and genotypic characterization of families is important for insight into intra- and inter-locus variation in hearing loss.

Herein, we describe a Dutch family (W99-060) with progressive sensorineural autosomal dominant hearing impairment linked to the DFNA20/26 locus. Statistical analysis was performed on pure tone audiometry data and on speech recognition scores. The results were compared to those previously reported for 4 affected family members of a known DFNA20 family[13]; speech recognition scores were compared with those found in subjects with DFNA2[8], DFNA5[9], DFNA9[8] and presbyacusis[14].

Patients and methods

In 1999, we began our investigations of hearing loss in a large Dutch family (W99-060) spanning six generations (Figure 1) with the approval of the institutional review board. Fourteen family members had a history of progressive hearing impairment that first manifested in adolescence. After having obtained written and informed consent, we obtained pure-tone and speech audiograms from 22 individuals using standard procedures and, in some cases, a portable audiometer. Previously obtained audiograms were retrieved for 13 individuals. Blood samples were collected from 11 presumably affected and 22 unaffected persons for linkage analysis. Special attention was paid to the presence of syndromic features possibly accompanying hearing loss.

Linkage analysis

Genomic DNA was extracted from peripheral blood lymphocytes according to established procedures[15]. Analysis of polymorphic markers involved amplification by polymerase chain reaction (PCR). Each reaction contained 100 ng genomic DNA and 30 ng of each primer, in 15 µl Supertaq buffer (50 mM KCl, 1.5 mM $MgCl_2$, 10 mMTris-HCl pH 9.0, 0.1% Triton X-100, 0.01% (w/v) gelatin) in the presence of ^{32}P-dCTP with 0.06 U Supertaq (HT Biotechnology LTD, Cambridge, England). Amplification was achieved by 35 cycles of 1 min 94°C, 2 min 55°C and 3 min 72°C with microsatellite markers. The radiolabeled PCR products were mixed with 15 µl sample buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue) heated to 95°C for 3 minutes and 4 µl of this mixture were separated on a 6.6% denaturing polyacrylamide gel. Subsequently, the gel was dried and exposed overnight to Kodak X-OMAT film to visualize the separated allelic bands. Two-point lod scores were calculated using the MLINK subroutine of the computer program LINKAGE (version 5.1)[10] on the basis of autosomal dominant inheritance. For the calculation, the relative prevalence of the disease allele was assumed to be 0.0001 and penetrance 95%. A relative prevalence of 0.001 was assumed for phenocopies. The cutoff age for unaffected family members was 20 years.

Audiometric analysis

Audiometric configuration and threshold asymmetry were evaluated according to the criteria and classification established by the European Work Group on Genetics of Hearing Impairment[16]. Serial audiometry was available in 8 patients

and suitable for longitudinal analysis in 5 of them (VI-4, IV-19, V-6, V-15, V-21). Nonlinear longitudinal regression analysis (air-conduction threshold on age) was performed using a commercial program (Prism 3.02, GraphPad, San Diego, CA, USA). The bone conduction threshold was measured to exclude conductive hearing loss. One-way analysis of variance (ANOVA) was used to detect significant differences between any (sub)group of patients. Pooling was only performed where it was permitted according to the results of such tests. All these data enabled us to construct age related typical audiograms (ARTA). Speech recognition scores were measured using (phonetically balanced) standard consonant-vocal-consonant syllables (Dutch CVC word lists). The phoneme score was analyzed in relation to age and pure tone average of the thresholds at 1, 2 and 4 kHz ($PTA_{1\text{-}4\ kHz}$). Nonlinear regression analysis was performed using a sigmoid response curve with variable slope. Details can be found in a previous report[8].

Analysis of variance and the t test (with Welch's correction if Bartlett's test demonstrated significantly unequal variances) were used to compare the results with those previously obtained in: (1) a group of patients with presbyacusis[14]; (2) a group of DFNA9 patients[8]; as well as (3) a group of DFNA5 subjects[9].

Vestibulo-ocular examination and imaging techniques

Seven family members (IV-7, IV-11, IV-22, V-5, V-6, V-15 and VI-4) underwent vestibular and ocular motor tests. These included evaluation of vestibulo-ocular responses, using electronystagmography with computer analysis and saccadic, smooth pursuit and optokinetic nystagmus responses. Vestibular stimulation comprised rotatory and caloric tests. Details and normal values have been previously described[17]. A CT scan of the petrosal bones of case IV-22 was performed (Siemens Somatom Plus 4, Siemens, Forchheim, Germany).

Results

The hearing loss trait in the family (Figure 1) exhibits an autosomal dominant pattern of inheritance. The case histories and physical examinations excluded syndromic involvement. Most of the patients dated their first symptoms of hearing loss to the first 2 decades of life. Given the normal speech and language

development and the substantial progression of hearing loss, especially in the 2nd decade, the hearing loss is expected to be mainly postlingual in origin.

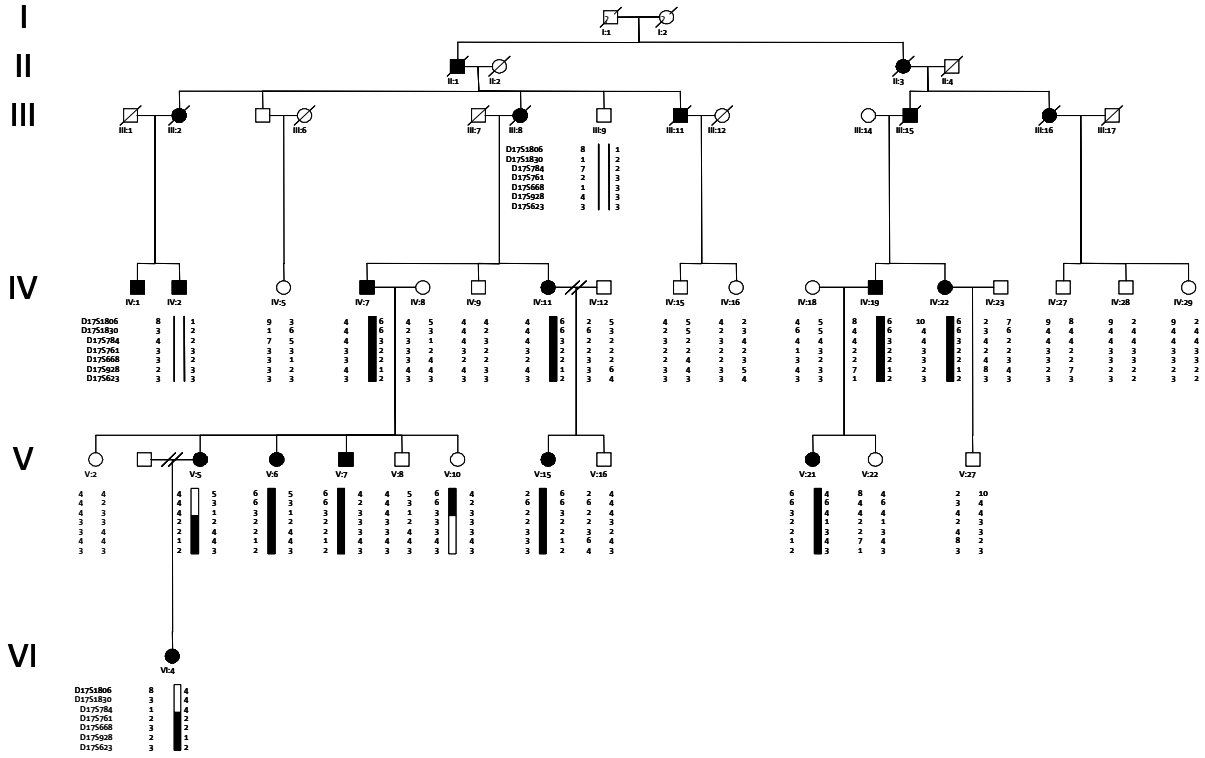

Figure 1 Pedigree of family W99-060 and genotypic data for 17q25 markers, listed in centromere-to-telomere order. The most likely haplotypes are shown. A black bar indicates the haplotype that is associated with the affected status. Solid lines indicate an unknown phase. The order of the markers D17S1806, D17S784, D17S668 and D17S928 was according to the Marshfield genetic map (http://research.marshfieldclinic.org/genetics) and the Decode high-resolution recombination map[20]. The additional markers were positioned as given in the most recent freeze (April 2003) of the Human Genome Working Draft (http://genome.ucsc.edu) and the Celera database. □, man; ○, woman; ■/●, affected persons; /, deceased; //, separated; ?, affected by history

Linkage analysis results

Because of the apparent similarity of the present type of hearing loss to DFNA5, this locus was tested first with polymorphic markers (e.g. D7S673, D7S2444 and D7S2493). The locus was excluded by lod scores lower than −2 (data not shown). Subsequently, a genome scan was initiated and after exclusion of about one third of the genome, linkage was detected with marker D17S928 (17q25) with a maximum two-point lod score of 3.1 at θ= 0.04. This marker flanks the DFNA20 interval at the telomeric side[18]. Additional markers derived from this region were tested and two-point lod scores were calculated (Table 1).

Table 1 Two-point lod scores between the hearing loss and polymorphic markers of the DFNA20/26 region

Theta / Marker	0.00	0.01	0.05	0.1	0.2	0.3	0.4	Z_{max} (θ)
D17S1806	-12.03	-6.06	-4.65	-3.86	-2.04	-1.04	-0.42	
D17S1830	-4.08	0.54	1.71	1.95	1.65	0.97	0.29	
D17S784	-9.34	-3.62	-1.64	-0.91	-0.48	-0.39	-0.25	
D17S761	-1.61	0.38	0.89	0.94	0.70	0.38	0.09	
D17S668	2.20	2.06	1.89	1.65	1.12	0.54	0.04	
D17S928	1.89	2.87	3.07	2.78	1.84	0.79	-0.03	3.10 (0.04)
D17S623	0.79	2.07	2.36	2.18	1.52	0.74	0.00	

Abbreviations: θ, recombination fraction; Z_{max}, maximum logarithm of odds score

The most likely haplotypes were constructed to determine the borders of the critical region (Figure 1). This revealed that individual IV-2 shares only the allele for marker D17S668 as seen with the affected haplotype. A genotyping error was excluded by analyzing DNA from two independent samples. Since both parents had died we were unable to determine whether or not allele 2 is derived from the affected mother. Therefore, we decided to determine the critical region primarily on the basis of the remaining part of the pedigree. On the centromeric side, the critical region is flanked by D17S784 as can be deduced from a recombination event seen in the affected individual V-5. Individual V-10 (33 years old at examination) also displays a recombination event suggesting that marker D17S784 is the proximal flanking marker. However, for this individual non-penetrance cannot be excluded. The given location of the marker D17S1830 relative to D17S784 is based on physical maps (see Figure 1 and 6) and might therefore be less reliable than marker orders based on genetic maps or radiation hybrid maps. In case markers D17S1830 are located distally from D17S784 the former marker would be the proximal flanking marker. There is no recombination seen for the most telomeric marker D17S623 and thus the linkage interval for this family is in between D17S784 and 17qter. Marker D17S623 is the only marker shown for which the position in the physical map is not compatible with that in the Marshfield genetic map in which it is at the same position as D17S1830 and D17S784 (http://research.marshfieldclinic.org/genetics). Recombination events seen in the individuals V-5 and V-10 indicate that D17S623 is located at the telomeric side of D17S784. The definition of the critical region is not dependent on the position of D17S623.

Regarding the allele of marker D17S668 in individual IV-2 as being derived from the haplotype that carries the mutation, the critical region based on the given

marker order would be delimited to the interval between D17S761 and D17S928. However, because of the extent of genetic heterogeneity and environmental causes of hearing loss, the results for individual IV-2 must be regarded with caution. Unfortunately patient IV-1 refused to participate in this study. Assuming that one gene is involved in the hearing loss in the four families known to be linked to 17q25[5,18,19], our data reduce the critical region from 12 cM[18], between D17S1806 and D17S668, to 9.5 cM between D17S784 and D17S668. In the recently published high resolution recombination map of Decode[20] this distance measures only 6.1 cM.

Audiometric analysis

The available audiograms of 10 affected cases are shown in Figure 2 (page 154). The patients showed gently downsloping audiograms already at age < 15 years. By the ages of 15-20 and 25-40 years, hearing loss had become severe to profound at 8 and 1-4 kHz respectively. The thresholds at 0.25-0.5 kHz showed more gradual progression at an average increase of about 1.5-2 dB/year. There was residual hearing, i.e. mainly at the lower frequencies, at ages from about 40 years onwards. Figure 3 shows the age-related typical audiograms (ARTA) of the present and, for the sake of comparison, of a known DFNA20 family.

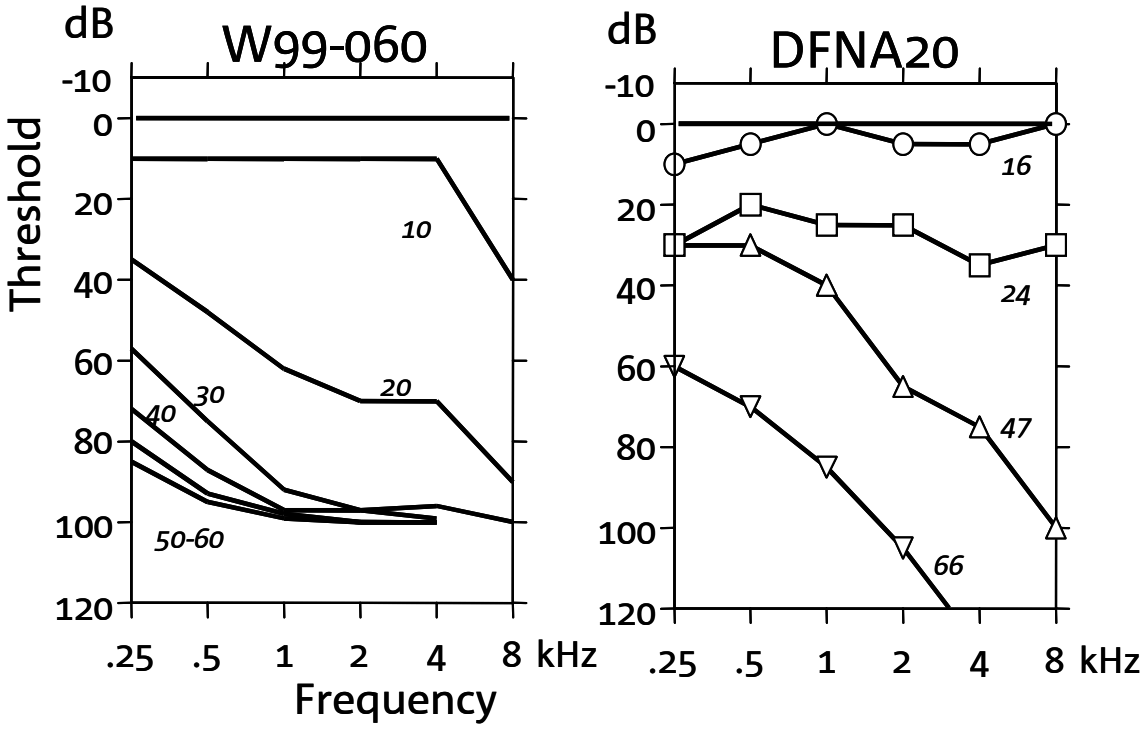

Figure 3 Age Related Typical Audiograms (ARTA) of family W99-060 and of a known American DFNA20 family[13]. Italics indicate age in years

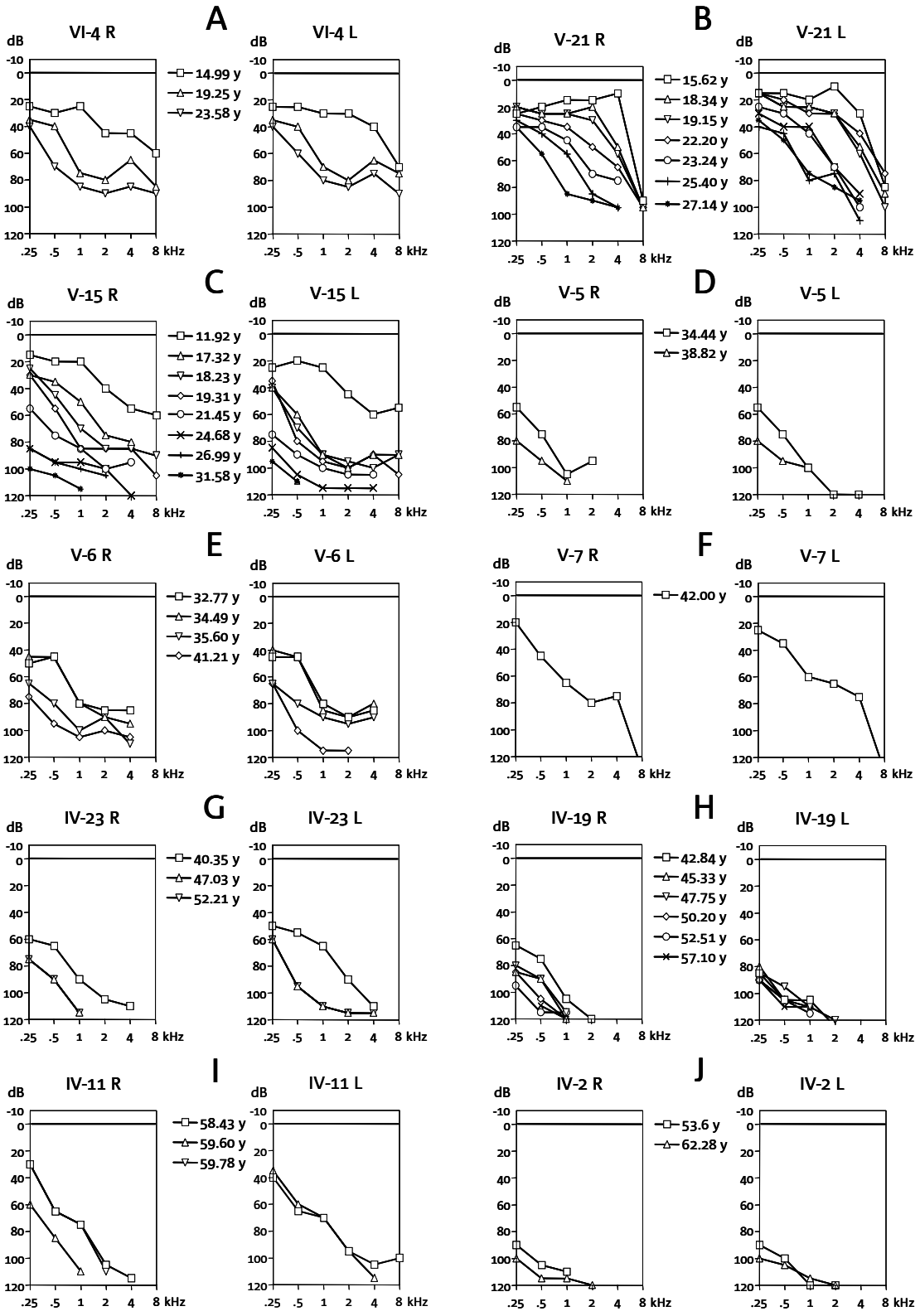

Figure 2 Serial audiograms of 10 family members (A-J) shown for the right (R) and left (L) ear, separately (air conduction threshold in dB HL). Note that the panels are ordered (top left to bottom right) by age (y = year) at the last visit. Some of the serial audiograms have been omitted for clarity

The plots in Figure 4 combine the longitudinal analyses in the suitable cases with the (cross-sectional) data in the other cases.

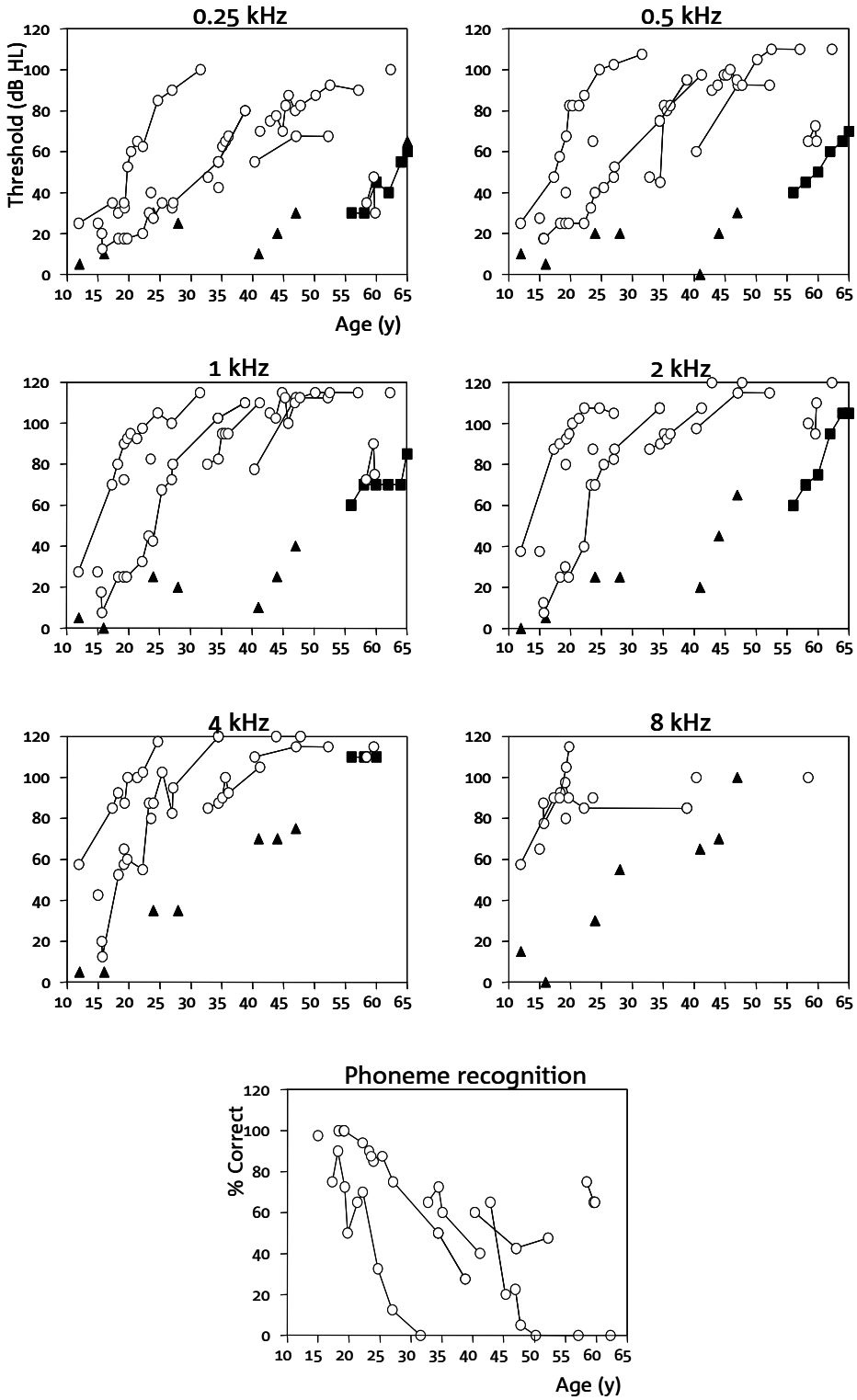

Figure 4 Plots combining individual, longitudinal data (with connection lines) and separate, cross-sectional data for binaural mean air conduction threshold and phoneme recognition score (open circles) against age (bottom panel). Black triangles and squares indicate cross-sectional and longitudinal data from the American family described by Elfenbein et al.[13]

Cross-sectional data reported by Elfenbein et al.[13] are included for the purpose of comparison. The bottom panel shows the phoneme recognition score in relation to age. The threshold data showed much higher progression at the high frequencies than at the lower frequencies. The maximum rate of progression culminated within the age range of 10 to 35 year and varied in individual cases between about 3 and 8 dB/year. Onset ages were in the range of 5 to 25 years, showing an apparent decrease at increasing frequencies in some cases. At age 15 to 35 years, 50% of the final degree of deterioration had developed, and by age 30 to 50 years 90% of the final degree of deterioration had developed.

Appreciable deterioration of speech recognition (score < 90%) began between ages 15 and 40 years and showed large intersubject variations (Figure 4, bottom panel). At 20-45 years of age, recognition scores deteriorated maximally (range 5 to 20 %/year). With few exceptions, speech recognition became problematic (maximum phoneme score < 50%) from an age of about 25-45 years onwards. Between the ages of 30 and 60 years, speech recognition was almost completely lost, except in 1 patient. In relation to the corresponding threshold level (i.e., $PTA_{1\text{-}4\ kHz}$), the phoneme recognition score was relatively good compared to that previously obtained at our clinic in patients with presbyacusis[14], DFNA2[8], DFNA5[9] and DFNA9[8] patients. The slope at which the phoneme recognition score decayed with increasing PTA level appeared to be steeper than in the aforementioned different groups of patients[8,9,14] (Figure 5). The recognition score in DFNA20/26 was better than in DFNA9 at any PTA. Compared with DFNA2 and DFNA5 subjects, DFNA20/26 subjects scored better in speech recognition at PTAs lower than 85 dB of hearing loss, but worse at PTAs higher than 90 dB. Compared with presbyacusis subjects, those with DFNA20/26 scored better in speech recognition at PTAs lower than 100 dB and worse at PTAs higher than 100 dB.

Vestibulo-ocular examination and imaging results

While caloric testing revealed no abnormalities, patient IV-7 exhibited vestibular hyporeflexia and asymmetrical responses to rotatory tests. Severe vestibular hyporeflexia and an enhanced cervico-ocular reflex were noted in family member IV-22. Vestibular testing in six other participants (IV-11, IV-22, V-5, V-6, V-15 and VI-4) revealed no abnormalities. The middle and inner ear structures of family member IV-22 had a normal appearance on CT scans.

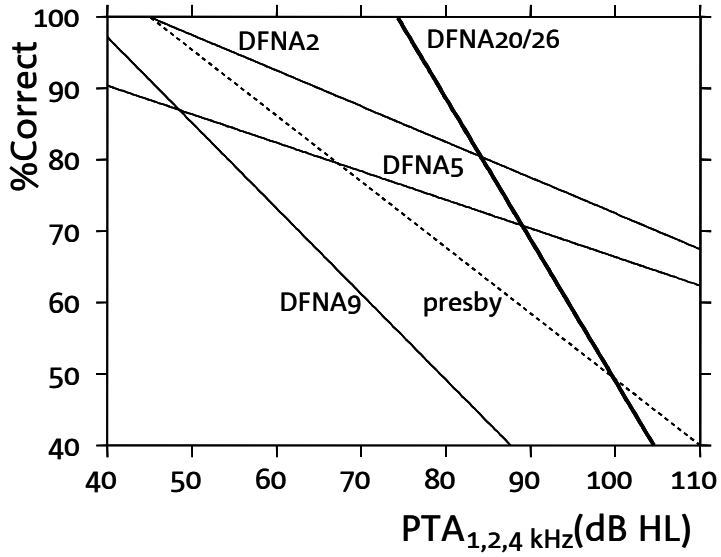

Figure 5 Plot showing relationship between phoneme recognition score (% Correct) and binaural mean $PTA_{1\text{-}4\ kHz}$ (dB HL) schematically by lines drawn for DFNA2, DFNA5, DFNA9 and the Dutch DFNA20/26 family. Presbyacusis (presby) is represented by a dotted line.

Discussion

The Dutch family in the present study shows postlingual non-syndromic progressive sensorineural hearing loss with probably no or very limited vestibular involvement. This is the fourth DFNA-family found to have hearing loss linked to chromosome 17q25. The critical region, originally described by Morell et al.[18], is located between the markers D17S1806 and D17S668, and occupies an interval of about 12 cM (Figure 6). Yang and Smith[19] presented two unrelated American families with progressive autosomal dominant hearing loss with linkage to a region overlapping the DFNA20 interval[6]. The locus for these two families was designated DFNA26[6]. Flanking markers for the DFNA26 locus have not been reported so far. Extensive clinical comparison with these DFNA26 families is prohibited by the present lack of reported audiometric data.

The originally reported type of hearing impairment associated with DFNA20 showed progressive sensorineural hearing impairment with a relatively late onset (age 20) that predominantly affected the high frequencies. The pattern of hearing loss was suggested to resemble presbyacusis with an onset that is 30 years earlier than normal[18]. Recently, audiometric data were reported for 4 affected family members by Elfenbein et al.[13], who described downsloping sensorineural hearing loss, first evident at 6 kHz and later followed by 8 kHz. This pattern could be

demonstrated in some cases in their early teens but was clearly evident only by age 24 to 29 years. By the end of the third and fifth decades, clear differences were found at some frequencies between affected and unaffected persons. With increasing age, hearing loss increased at all frequencies, ultimately leading to a "corner audiogram" configuration. The hearing loss of the presented Dutch DFNA20/26 family shows some similarities with the American family reported by Elfenbein et al. However, the audiometric data also revealed apparent differences. We demonstrated that hearing loss was profound by the ages of 15-20 and 25-40 years at 8 and 1-4 kHz, respectively. Loss at the lower frequencies, i.e. 0.25-0.5 kHz, showed more gradual progression with an average increase of ~1.5-2 dB/year. Affected individuals have only residual hearing from an age of about 40 years onwards. Thus, hearing impairment in the Dutch DFNA20/26 family has a more severe appearance than that in the American family (Figure 3). Higher threshold levels were attained at an earlier age at any given frequency. Obviously, comparing purely longitudinal data of both families would be more appropriate, however, Figure 3 may give some indication of the difference in severity.
The DFNA20/26 patients in the present family showed better maximum speech recognition scores in relation to the level of pure tone hearing impairment at levels below 80-90 dB HL than was found in DFNA2, DFNA5, DFNA9 and presbyacusis patients. However, owing to a steeper slope of the trend line pertaining to DFNA20/26, these patients showed lower scores at levels above 90 dB than the DFNA2 and DFNA5 patients and scores similar to presbyacusis at about 100 dB (Figure 5). Elfenbein et al.[13] mentioned the proband's poor speech recognition scores without details and included data on acoustic emissions, but no detailed data on speech recognition scores were given[13]. We did not evaluate otoacoustic emissions.

A survey of the critical region for candidate genes for DFNA20/26 suggested the *P4HB* gene, encoding the beta subunit of prolyl 4-hydroxylase, as the most promising candidate gene. The P4HB protein catalyses the formation of 4-hydroxyproline in collagens and thereby is important for the structure and function of collagen. In addition, the protein functions as protein disulphide isomerase and as a cellular thyroid hormone binding protein. However, a disease-causing mutation in this gene could not be demonstrated in the DFNA20[18] family and in the present family.

The difference in phenotype between the previously described American DFNA20 family[18], based on data of only four family members[13], and the present family does not exclude the involvement of the same causative gene. Different types of mutations might lead to different phenotypes. However, it can also be hypothesized that different genes are causing different traits linked to the DFNA20/26 interval. An example is the recent localization of a gene for Usher syndrome type 1G (*USH1G*) to 17q24-q25[21] overlapping with the DFNA20 interval as it was described by Morell et al.[18]. Since the critical region for USH1G is flanked by D17S1830 at the telomeric side, this locus does not overlap with the critical region determined for the Dutch family (Figure 1, Figure 6).

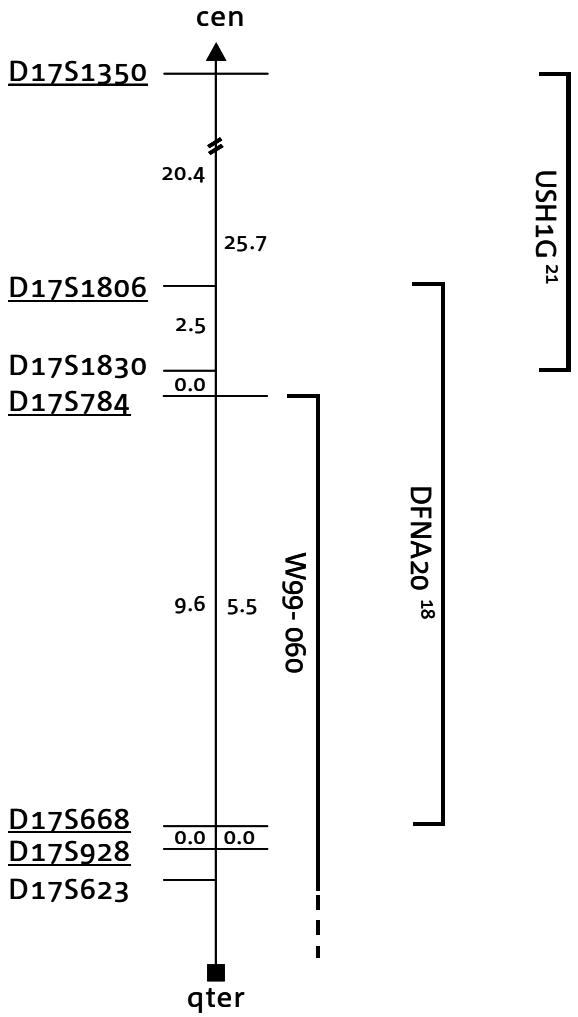

Figure 6 Representation of the critical regions of family W99-060 of the present study, a known DFNA20 family[18] and the USH1G locus[21]. Order and distances in centimorgans of the underlined markers are according to the Marshfield genetic map (available at http://research.marshfieldclinic.org/genetics) (left) and/or the Decode genetic map[20] (right). Additional markers are located according to their positions in the Human Genome Working Draft (April 2003 freeze; available at:http://genome.ucsc.edu) and the Celera database.

Therefore, we conclude that the distal part of chromosome 17q harbours at least two causative genes for hearing loss. The mouse mutation jackson-shaker (*js*) associated with deafness and vestibular impairment is located in the region of mouse chromosome 11 homologous to human chromosome 17q25[22]. Given the vestibular impairment it seems more likely that the mutated gene in the *js* mouse is the orthologue of the USH1G gene than that of the gene for nonsyndromic hearing loss in the present family. Already three genes have been found to be involved in both Usher syndrome and in non-syndromic hearing loss[7,12,16,23-29]. Therefore, the identification of the disease-causing mutations is needed to elucidate whether DFNA20 and DFNA26 are caused by mutations in the same gene. This will also show whether the USH1G gene is involved.

The present research was successful in mapping the causative gene for hearing loss in a Dutch family to the DFNA20/26 interval and in refining its critical region. The present report is the second to provide detailed tone and speech audiometric data for this locus. The publication of additional data available from other DFNA20/26 families is needed to improve phenotypic comparison. Clinical features of the two available families show some audiometric similarity. However, members of the Dutch family appear to be more severely affected already at an earlier age. As yet, no gene or disease-causing mutations have been identified for DFNA20/26. It has been previously suggested that DFNA20 might represent a suitable model of presbyacusis[13].The present data however, do not demonstrate any striking similarity between the phenotype of DFNA20/26 and presbyacusis.

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Chapter 5

DFNB1

Hearing loss and connexin 26

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Abstract

Deafness is genetically heterogeneous. Approximately half of the autosomal recessive forms of deafness are caused by mutations in *GJB2*, the gene coding for connexin 26. This protein is believed to play an essential role in the transport of K^+ ions in the endolymph of the inner ear after sound stimulation. Mutation analysis of the *connexin 26* gene (*GJB2)* is being performed fairly widely in the western world. This offers a good starting point for the further investigation of autosomal recessive and sporadic forms of hearing impairment.

Introduction

Hearing impairment is a sensory disability that affects millions of people all over the world. Though not life-threatening, it can become a major burden in social and professional life. In the industrialized world, deafness of infective and/or environmental origin has become less frequent, with a consequent rise in the proportion of hereditary hearing impairment. Deafness occurs in 1:1000 neonates[1] and the cause is hereditary in about half. This type of hearing impairment is sometimes referred to as prelingual, as it affects the child before the age of speech development. A distinction can be made between syndromic deafness, in which the deafness is accompanied by other specific abnormalities, and non-syndromic deafness (about 75%), in which there are no additional abnormalities. Approximately three-quarters of the non-syndromic forms are caused by a recessive disorder[1-4]. Table 1 gives an overview of some epidemiological features.

Table 1 Epidemiological features of prelingual hearing loss

	Prelingual hearing loss
Incidence	1/1000
Percentage of genetically caused cases of hearing loss	50 %
Syndromic vs non-syndromic causes	25 vs 75 %
Autosomal dominant	20%
Autosomal recessive	74%
X-linked	5%
Mitochondrial	1%

Between 1997 and today, many non-syndromic hereditary forms of deafness have been localized on the human genome by genetic linkage techniques. Depending on the pattern of inheritance of the deafness, these loci are designated DFNA (autosomal dominant), DFNB (autosomal recessive) or DFN (X-linked). They are numbered in chronological order of discovery. For the majority of these loci the underlying disease-causing genes have not been identified so far. On the Hereditary Hearing Loss Homepage[5] all these currently known forms of hereditary deafness are summarized. Tables 2–5, derived from this homepage, illustrate the achievements in this field of research.

Table 2 Loci and genes associated with autosomal dominant, non-syndromic hearing impairment, with the year of publication (*, unpublished observations)

Locus	*Localisation*	*Reference*	*Year*	*Associated Gene*	*Reference*	*Year*
DFNA1	5q31	León *et al.*	1992	*HDIA1*	Lynch *et al.*	1997
DFNA2	1p34	Coucke *et al.*	1994	*GJB3 (CX31)*	Xia *et al.*	1998
				KCNQ4	Kubisch *et al.*	1999
DFNA3	13q12	Chaib *et al.*	1994	*GJB2 (CX26)*	Denoyelle *et al.*	1998
				GJB6 (CX30)	Grifa *et al.*	1999
DFNA4	19q13	Chen *et al.*	1995			
DFNA5	7p15	Van Camp *et al.*	1995	*DFNA5*	Van Laer *et al.*	1998
DFNA6	4p16.3	Lesperance *et al.*	1996	*WFS1*	Bespalova *et al.**	2001
DFNA7	1q21-23	Fagerheim *et al.*	1996			
DFNA8/12	11q22-24	Kirschhofer *et al.*	1996	*TECTA*	Verhoeven *et al.*	1998
DFNA9	14q12-13	Manolis *et al.*	1996	*COCH*	Robertson *et al.*	1998
DFNA10	6q22-23	Ò'Neill *et al.*	1996	*EYA4*	Wayne *et al.*	2001
DFNA11	11q12.3-21	Tamagawa *et al.*	1996	*MYO7A*	Liu *et al.*	1997
DFNA12	11q22-24	Verhoeven *et al.*	1997	*TECTA*	Verhoeven *et al.*	1998
DFNA13	6p21	Brown *et al.*	1997	*COL11A2*	McGuirt *et al.*	1999
DFNA14	4p16	Van Camp *et al.*	1999	*WFS1*	Bespalova *et al.**	2001
DFNA15	5q31	Vahava *et al.*	1998	*POU4F3*	Vahava *et al.*	1998
DFNA16	2q24	Fukushima *et al.*	1999			
DFNA17	22q	Lalwani *et al.*	1999	*MYH9*	Lalwani *et al.*	2000
DFNA18	3q22	Boensch *et al.*	1998			
DFNA19	10	Green *et al.*	1998			
DFNA20	17q25	Morell *et al.*	2000			
DFNA21						
DFNA22	6q13	Melchionda *et al.*	2001	*MYO6*	Melchionda *et al.*	2001
DFNA23	14q21-22	Salam *et al.*	2000			
DFNA24	4q	Häfner *et al.*	1999			
DFNA25	12q21-24	Greene *et al.*	1999			
DFNA26	17q25	Yang *et al.*	2000			
DFNA27	4q12	Fridell *et al.*	1999			
DFNA28	8q22	Anderson *et al.*	1999			
DFNA29		reserved			reserved	
DFNA30	15q26	Mangino *et al.*	1999			
DFNA31		withdrawn			withdrawn	
DFNA32	11p15	Li *et al.*	2000			
DFNA33		reserved			reserved	
DFNA34	1q44	Kurima *et al.*	2000			
DFNA35		reserved			reserved	
DFNA36	9q13-21	Kurima *et al.*	2000			
DFNA37	1p21	Talebizadeh *et al.*	2000			
DFNA38	4p16.3	Young *et al*	2001	*WFS1*	Young *et al.**	2001
DFNA39	4q21.3	Xiao *et al.*	2001	*DSPP*	Xiao *et al.*	2001
DFNA40		reserved			reserved	

Table 3 Loci and genes associated with autosomal recessive, non-syndromic hearing impairment, with the year of publication

Locus	*Localisation*	*Reference*	*Year*	*Associated Gene*	*Reference*	*Year*
DFNB1	13q12	Guilford *et al.*	1994	*GJB2 (Cx26)*	Kelsell *et al.*	1997
DFNB2	11q13.5	Guilford *et al.*	1994	*MYO7A*	Liu *et al.*	1997
					Weil *et al.*	1997
DFNB3	17p11.2	Friedman *et al.*	1995	*MYO15*	Wang *et al.*	1998
DFNB4	7q31	Baldwin *et al.*	1995	*SLC26A4*	Li *et al.*	1998
DFNB5	14q12				Fukushima *et al.*	1995
DFNB6	3p14-p21				Fukushima *et al.*	1995
DFNB7	9q13-q21				Jain *et al.*	1995
DFNB8	21q22	Veske *et al.*	1996	*TMPRSS3*	Scott *et al.*	2001
DFNB9	2p22-23	Chaib *et al.*	1996	*OTOF*	Yasunaga *et al.*	1999
DFNB10	21q22.3	Bonné-Tamir *et al.*	1996	*TMPRSS3*	Scott *et al.*	2001
DFNB11	9q13-q21	Scott *et al.*	1997			
DFNB12	10q21-q22	Chaib *et al.*	1996	*CDH23*	Bork *et al.*	2001
DFNB13	7q34-36	Mustapha *et al.*	1998			
DFNB14	7q31	Mustapha *et al.*	1998			
DFNB15	3q21-q25*	Chen *et al.*	1997			
	19p13*					
DFNB16	15q21-q22	Campbell *et al.*	1997			
DFNB17	7q31	Greinwald *et al.*	1998			
DFNB18	11p14-15.1	Jain *et al.*	1998			
DFNB19	18p11	Green *et al.*	1998			
DFNB20	11q25-qter	Moynihan *et al.*	1999			
DFNB21	11q	Mustapha *et al.*	1999	*TECTA*	Mustapha *et al.*	1999
DFNB22					reserved	
DFNB23	10p11.2-q21				reserved	
DFNB24	11q23				reserved	
DFNB25	4p15.3-q12				reserved	
DFNB26	4q31	Riazuddin *et al.*	2000			
DFNB27	2q23-q31	Pulleyn *et al.*	2000			
DFNB28	22q13	Walsh *et al.*	2000			
DFNB29	21q22	Wilcox *et al.*	2001	*CLDN14*	Wilcox *et al.*	2001
DFNB30	10p				reserved	

*, the two loci revealed similar LOD scores

Table 4 Loci and genes associated with X-linked, non-syndromic hearing impairment, with the year of publication

Locus	*Localisation*	*Reference*	*Year*	*Associated Gene*	*Reference*	*Year*
DFN1*	Xq22	Tranebjaerg *et al.*	1995	*DDP*	Jin *et al.*	1996
DFN2	Xq22	Tyson *et al.*	1996			
DFN3	Xq21.1	De Kok *et al.*	1995	*POU3F4*	De Kok *et al.*	1995
DFN4	Xp21.2	Lalwani *et al.*	1994			
DFN5					withdrawn	
DFN6	Xp22				del Castillo *et al.*	1996
DFN7					withdrawn	
DFN8					reserved	

*, later recognised as syndromic

Table 5 Mitochondrial mutations associated with non-syndromic hearing impairment, with the year of publication

Gene	*Mutation*	*Reference*	*Year*
12S rRNA	1555A→G	Prezant *et al.*	1993
		Usami *et al.*	1997
		Estivill *et al.*	1998
tRNASer (UCN)	1445A→G	*Reid *et al.*, *Fischel-Ghodsian *et al.*	1994 1995
		*Sevior *et al.*	1998
	7472insC	*Tiranti *et al.*	1995
		*Jaksch *et al.*	1998
		*Schuelke *et al.*	1998
		Verhoeven *et al.*	1999
	7510T→C	Hutchin *et al.*	1999
	7511T→C	Friedman *et al.*	1999
		Sue *et al.*	1999

*, additional symptoms were present in some patients

Certain research groups, having found preliminary evidence of a new locus, have claimed ('reserved') loci in advance. 'Withdrawn' indicates those which turned out not to be correct. Most of these genetic types of hearing impairment are quite rare, with the exception of DFNB1. This paper addresses DFNB1, which is caused by mutations in the connexin 26 gene.

Hearing impairment

Although the connexin 26 gene *GJB2*, is also involved in an autosomal dominant form of deafness (DFNA3), most mutations in this gene cause recessive hereditary bilateral deafness/hearing impairment, so-called DFNB1. This form of sensorineural non-syndromic hearing loss is prelingual and its severity varies from mild to profound, depending to some extent on the type of mutation[6,7]. Hearing loss in the high-tone range has recently been described as a characteristic feature, but all frequencies are affected[8]. In two thirds of cases, the hearing loss is non-progressive and there usually no vestibular and/or labyrinthine abnormalities.

Genetics

DFNB1 was the first locus incriminated in autosomal recessive deafness; in 1997, *GJB2* was found to be responsible[9]. *GJB2* is a small gene situated on chromosome 13q11; it has a length of about 5.5 kilobases. There are two exons, of which only one contains the coding sequence. The mRNA is 2.4 kilobases long and translates into a

protein with 226 aminoacids. This protein belongs to the connexin family, which currently has more than a dozen members[10].

The protein

Connexins are membrane proteins with four transmembrane domains. Six chains of these proteins form a complex (a hexamer), called connexon. Two hexamers in the membranes of adjacent cells form a cell-to-cell channel, a so-called gap junction, which allows the transport of small molecules and ions between cells. A hexamer can contain various types of connexin, and various types of hexamer can form cell-to-cell channels. The channel constituents determine which molecules or ions can pass through[11].

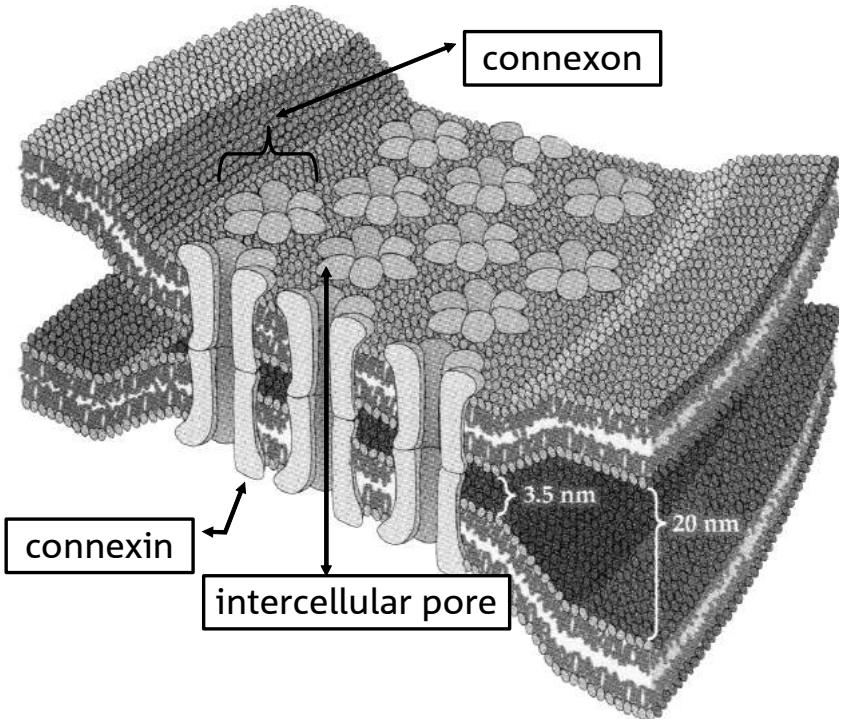

Figure 1 Schematic representation of a gap junction. Six connexins form a connexon. Two connexons of neighbouring cells form pores which allow intercellular transport of small molecules. (Adapted from Furshpan and Potter, 1959[19])

The cell

Recently, the hypothesis was put forward that CX26 protein is essential for maintaining the high K^+ concentration in the endolymph of the inner ear. Sound stimulation of the ossicular chain causes vibrations in the endolymph. K^+ ions enter the hair cells under the influence of these vibrations and the vibration signal is ultimately converted into a neural signal. The system is regenerated by the release of K^+ from the hair cells into the supporting cells. The K^+ ions are then

passed from cell to cell via gap junctions and are eventually released into the endolymph. Except for sensorineural cells, the connexin 26 protein is present in gap junctions connecting all cell types in the cochlea, including the spiral limbus, the supporting cells, the spiral ligament and the basal and intermediate cells of the stria vascularis. It is therefore very likely that connexin 26 is involved in K^{+} recycling in the cochlea[11].

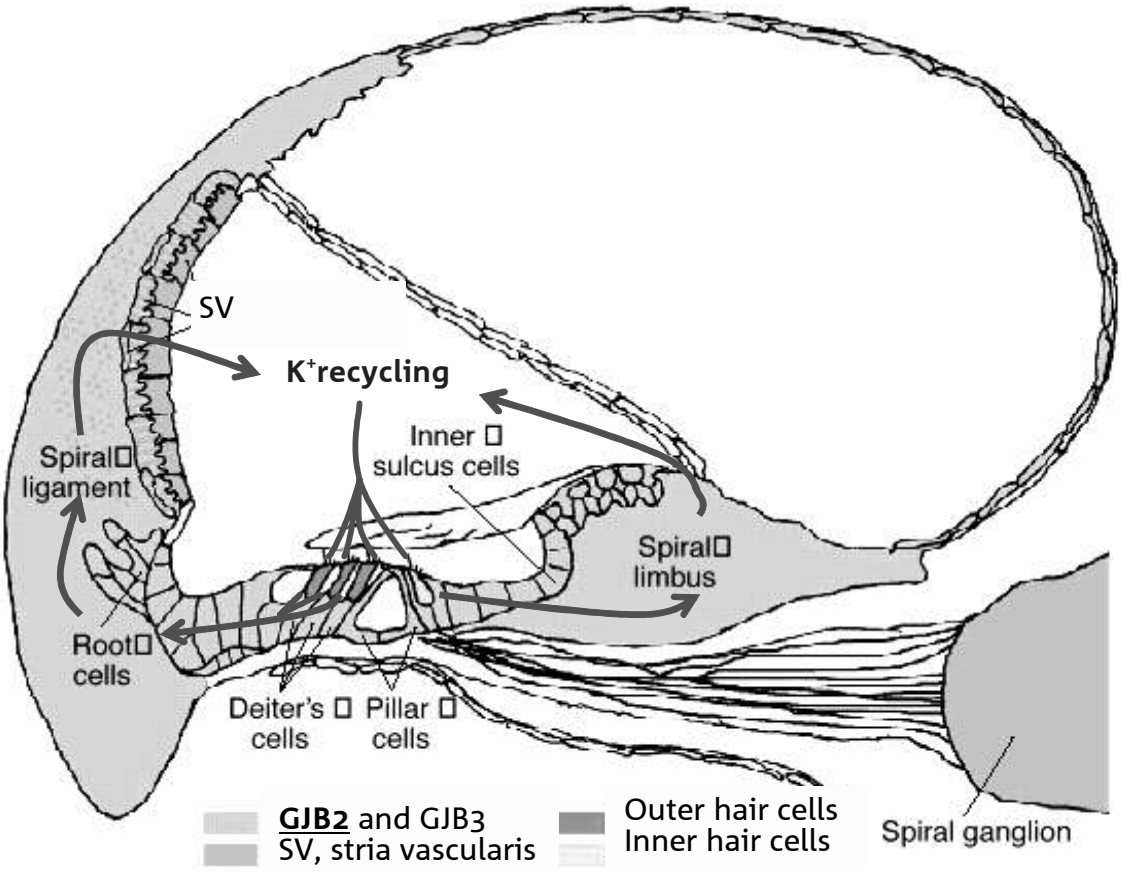

Figure 2 Schematic section through the human cochlea with different colours (given in the key) showing the K^{+} recycling pathway and the expression of connexin 26 (*GJB2)*.(Adapted from Steel and Bussoli, 1999[23])

Epidemiology

Mutations in *CX26* are the most common cause of autosomal recessive deafness throughout the world. This gene is believed relevant to half of all cases of hereditary deafness. *CX26* shows diverse mutations, but one mutation occurs very frequently in Europe: the 35delG mutation. Average carrier frequency in Europe is 1:51 (north/middle Europe 1:79, south Europe 1:35)[12] (Table 6). In the Mediterranean countries the carrier frequency exceeds even that of the ΔF508 mutation in the *CFTR* gene which causes cystic fibrosis. Carrier frequencies in North America and Australia are comparable to those in north/middle Europe. In oriental populations and the

Ashkenazi Jews, other mutations in the same gene play a more important role (234delC[13] and 176delT[14], respectively). The high frequency of connexin-26-related hearing impairment in certain populations may be the result of the tradition of marriages between hearing-impaired persons[15]. The 35delG mutation gives rise to a severely shortened, non-functional protein[16]. More than sixty other, far less frequent, mutations have been described in *CX26*[17]. Uncertainty about the pathogenicity of some of the mutations complicates interpretation of mutation analysis[18].

Table 6 Carrier frequency of mutation 35delG in the *GJB2* gene in 17 European countries. Adapted from Gasparini et al, 2000[12]

Country	*Carrier frequency*
Northern and Central Europe	
Norway	1/47.5
Denmark	1/190
Estonia	1/22.5
United Kingdom	0/119
Germany	1/50
Belgium	1/190
Holland	1/44.5
France (Brittany)	1/96
France	1/200
Czech Republic	1/48.7
Slovenia	1/182
Bulgaria	1/157
Total	1/79.3
Southern Europe	
Portugal	1/45
Spain	1/40
Italy	1/32
Italy (sardinia)	1/29.5
Malta	1/36
Greece	1/33
Turkey	1/37.5
Total	1/35.2
Total average of Europe	1/51.1

Denoyelle *et al.*[7] found mutations in the *CX26* gene in 49% of the families from France, Great Britain and New Zealand who had severe to profound prelingual hearing loss. *CX26* mutations were present in 51% of the group with, versus 31% in the group without, a clear familial history of hearing impairment; 86% of the *CX26* mutations were 35delG mutations. Mueller *et al.*[19] studied a group of 284 English patients with early childhood hearing impairment or deafness, with and without hereditary causes. They found *CX26* mutations in 27.8% of the familial cases and in 7.9% of the sporadic cases; 70% of the *CX26* mutations were 35delG mutations. This difference can be explained by the fact that families with different ethnic backgrounds were included in the study. The prevalence of non-familial, sporadic

hearing impairment based on *CX26* mutations in an English-Belgian population of 68 children was 10%[20].

Diagnosis

An increasing number of medical centres can perform mutation analysis to determine involvement of the *CX26* gene in congenital hearing impairment. This method has been available for several years at the department of medical genetics in Nijmegen. We retrospectively analysed the outcome of ninety-one *CX26* mutation analysis requests covering a fixed period of time. Nineteen unrelated cases were shown to have two mutations in the gene. Twelve of them turned out to be homozygous, whereas four others were heterozygous for the 35delG mutation. Overall, the 35delG mutation was involved in 84% of these cases, thirteen cases originated from multi-affected families, whereas three others were sporadic cases. Information on the remaining three families could not be retrieved. Table 7 gives an overview of the *CX26* mutations found in Nijmegen.

Mutation analysis applies not only to children with a clear family history, but also to children whose parents have normal hearing (sporadic cases). Moreover, if a mutation in *CX26* is present, genetic counselling can be offered to provide information on the aetiology answers and on the likelihood of recurrence in future offspring. When a mutation analysis is positive there will usually be no need for further investigations such as imaging and ophthalmological tests, because other causes of congenital deafness no longer have to be excluded. In these cases, attention can immediately be focused on optimizing the child's hearing. Histopathological examination of the cochlea in a patient with confirmed *CX26* mutation has revealed an intact acoustic nerve[21]. This means that these patients are suitable candidates for cochlear implantation, provided that their hearing loss is sufficiently profound. Early diagnosis leads to early treatment, which gives the best results with cochlear implantation.

Table 7 Overview of the CX26 mutations found in Nijmegen

Patient	*First mutation→*	*Implication for protein structure*	*2nd mutation→*	*Implication for protein structure*
1	35delG	Shortened	35delG	shortened
2	35delG	Shortened	109G>A	V37I
3	35delG	Shortened	35delG	shortened
4	35delG	Shortened	313del14	shortened
5	35delG	Shortened	35delG	shortened
6*(f)	71G>A	W24X	407insA	shortened
7*(m)	71G>A	W24X	427C>T	R143W
8*(d)	71G>A	W24X	407insA	shortened
9	35delG	Shortened	35delG	shortened
10	35delG	Shortened	35delG	shortened
11	35delG	Shortened	35delG	shortened
12	35delG	Shortened	35delG	shortened
13	35delG	Shortened	35delG	shortened
14	35delG	Shortened	35delG	shortened
15	35delG	Shortened	449delT	shortened
16	35delG	Shortened	35delG	shortened
17	101T>C	M34T	427C>T	R143W
18	35delG	Shortened	IVS1+1G>A	unknown
19	35delG	Shortened	35delG	shortened
20	109G>A	V37I	109G>A;	V37I
21	35delG	Shortened	35delG	shortened

*, belong to the same family; (f), father; (m), mother; (d), daughter

Conclusion

Unlike many other genes *CX26* is small, so that screening for mutations is fast and relatively simple. Besides, the overall high involvement of *CX26* mutations in autosomal recessive non-syndromic forms of deafness, and even in sporadic cases, makes mutation analysis distinctly worthwhile. *CX26* mutation analysis has therefore secured a place as a useful tool in clinical practice. So far, many different mutations in the *CX26* gene causing DFNB1 have been identified[17]. The uncertainty about the pathogenicity of the mutation demands close collaboration with geneticists who are familiar with deafness[18]. Nevertheless, *CX26* mutation analysis provides a good starting-point in the molecular diagnosis of patients with non-syndromic congenital deafness.

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Chapter 6

Summary and discussion

Samenvatting en discussie

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Summary

Genetic hearing impairment became a topic of research in Nijmegen ORL department in the early seventies, about three decades ago. The application of gene linkage studies for genetic hearing impairment started in the late eighties, focusing mainly on genetic syndromes. In the early nineties these techniques started to be used for non-syndromic types of genetic hearing impairment. A co-operation has been established between the Nijmegen University ORL department and the otogenetic laboratory of Boys Town, Omaha, Nebraska, USA (Prof. dr. W.J. Kimberling and Prof. S. Kumar) for BOR and Usher syndrome. With the Antwerp Department of Human Genetics the cooperation mainly regarded genotyping of DFNA families and otosclerosis. The establishment of the Nijmegen otogenetic laboratory (dr. H. Kremer) in collaboration with the Nijmegen Department of Human Genetics (Prof. dr. H. Brunner, dr. F. Cremers), has provided new and forceful opportunities for research on the genetics of hearing impairment.

This PhD thesis project took advantage of the previous efforts in the field of genetic hearing impairment. The BOR families had part of their mutations established early on in Boys Town. The DFNA9 and DFNA20/26 linkage studies were performed successfully at the Nijmegen Research laboratory and so this laboratory succeeded to identify the genes and their mutations involved in DFNA9 and DFNA20/26. The co-operation with the Department of Human Genetics (dr L Hoefsloot) was successful in providing important diagnostic mutation analysis data for DFNB1 (*GJB2*). Outcomes of these research projects have been brought together in chapters 2, 3, 4 and 5.

This thesis includes pheno- and genotypic descriptions of several types of hereditary hearing loss. Successively scientific reports on autosomal dominant syndromic (BOR syndrome, *Chapter 2*), autosomal dominant non-syndromic (DFNA9 and DFNA20/26, *Chapters 3-4*) and autosomal recessive non-syndromic (DFNB1, *Chapter 5*) forms of hereditary hearing loss are presented in four separate chapters. *Chapter 1* presents a general introduction to the field of genetic hearing impairment.

Chapter 2 discusses BOR syndrome and provides a review on this clinical entity in *Chapter 2.1*. The first report (*Chapter 2.2*) is a clinical report on a father and his

son from a small BOR family focusing on the presentation of a long-term audiometric follow-up related to its radiological appearance. This provided us with the first notion of the presence of concomitant progressive fluctuating hearing loss and an enlarged endolymphatic duct and sac (LEDS) in the youngest of the studied patients. This encouraged us to include five other Dutch BOR families in a magnetic resonance imaging (MRI) study (*Chapter 2.3*). These findings, together with long-term audiometric data and mutation analysis results, were studied and analysed (*Chapter 2.4*). Although we observed many different forms of middle-ear and inner-ear anomalies, none of them could be pinpointed as pathognomical for BOR syndrome (*Chapter 2.3*). We were able to substantiate progression and even fluctuation of hearing thresholds in some of the studied cases. Patients with LED(S) and/or an age of 25 years or under seemed to be prone to be more severely affected in terms of hearing loss thresholds and suffered from progressive fluctuating hearing loss (*Chapter 2.4*). Each family in this study showed linkage data compatible with *EYA1* involvement. So far mutation analysis has disclosed three different mutations in three of the six families. The limited amount of genetic data and a high degree of big inter- and intra-familial variability prohibited finding a clear geno-phenotype correlation. The recently published observation that genomic rearrangements of the *EYA1* gene are responsible for a high proportion of formerly undiagnosed BOR patients is of great interest. This sheds a new light on the available genetic data of undiagnosed BOR families and suggests an adaptation of the present method of *EYA1* mutation analysis. Expanding this diagnostic procedure might provide us with more genetic and clinical data on BOR syndrome. Hopefully, this will help to understand the issues raised during our work, as well as on the pathogenicity of this syndrome in general.

Chapter 3 reports on DFNA9/*COCH* and begins with a review on this subject (*Chapter 3.1*). In *Chapter 3.2* we describe the genetic and phenotypic analysis of a Dutch family carrying a G88E/*COCH* mutation. Hearing loss was found to be progressive, first affecting the high frequencies and, at a later stage, the mid and low frequencies. The age of onset was between the 5th and 6th decade and hearing loss thresholds deteriorated at approximately 3.6-4.6 dB/year. Almost all patients with obvious hearing impairment showed lack of vestibular function. We did not detect any substantial phenotypic differences between the original and

the present Dutch family. *Chapter 3.3* describes a large Dutch family affected by cochleovestibular impairment due to the P51S/*COCH* mutation. This mutation is a founder mutation in Belgium and the Netherlands and has frequently been encountered amonst patients visiting the Nijmegen ORL department. Analysis showed predominant high frequency hearing impairment with an onset in the fourth to fifth decade of life, progressing by 3dB/year. Vestibular impairment up to and including areflexia developed concomitantly. Since then various other P51S/*COCH* family studies comprised audiometrical, genetic and vestibular test analyses and revealed a recognisable phenotype. DFNA9 is histopathologically characterised by extracellular glycosaminoglycan mucopolysaccharide depositions in the cochlea. This observation was first made in the temporal bone of a deceased patient affected by a G88/*COCH* mutation. Recent observations have shown that the mutated protein cochlin is normally processed and secreted in inner ear cells. The major effect probably occurs beyond the point of excretion in the extracellular matrix of the inner ear, and might be the result of interference with protein-protein interactions or mechanical problems due to cochlin aggregation. At present a post-mortem temporal bone study is being performed in the P51S/*COCH* family. It will be interesting to compare these results to the above-mentioned findings.

In *Chapter 4*, we present a Dutch family with an autosomal dominant type of hearing loss linked to the DFNA20/26 locus at chromosome 17q25. We reduced the critical region from 12 cM to 9.5 cM. Audiometric longitudinal data, including speech recognition scores, and vestibular data were collected and analysed. At age < 15 years hearing loss thresholds were gently downsloping and hearing loss became profound at the ages of 15-20 years (8 kHz) and 25-40 years (1-4 kHz). The 0.25-0.5 kHz thresholds progressed more gradually at ~1.5-2 dB/year. From ~40 years onwards hearing was only residual. Although speech recognition begun to be problematic between 25 and 45 years of age, maximum phoneme recognition scores were higher than those found in DFNA2, DFNA5 or DFNA9. Compared to the original North-American DFNA20 family, the present DFNA20/26 family is more severely affected. The DFNA20/26 locus harbours the gamma 1 actin gene (*ACTG1*). Recently, colleagues of the Nijmegen Otogenetic Laboratory identified a Thr278Ile mutation in this gene, which suggests its involvement in DFNA20/26. The consequences of such a mutation on the protein itself, on protein-protein

interactions and on cochlear stereocilia function can be considered new goals for future research.

Chapter 5 deals with DFNB1/*GJB2*. Mutations in this gene, encoding the protein connexin 26, play a major role in autosomal recessive and sporadic cases of hearing loss. *GJB2* mutation analysis has become a successful diagnostic tool, which can be easily implemented in a diagnostic DNA laboratory. This chapter reviews this subject and summarises the first results of this diagnostic procedure in Nijmegen. Nineteen unrelated cases were shown to have two mutations in the gene. Twelve of them turned out to be homozygous, whereas four others were heterozygous for the 35delG mutation. Overall, the 35delG mutation was involved in 84% of these cases. These findings are in line with results from our neighbouring countries. Additional studies should be conducted to investigate whether the type of mutation is correlated to the severity and the type of hearing impairment.

Discussion

In summary, clinical and genetic data on one syndromic and three non-syndromic types of hereditary hearing are outlined and discussed in this thesis. The complexity of the auditory system and the high number of identified loci prove that many other genes are involved, many of which have not yet been identified. Since the first description of a locus involved in hereditary hearing loss in 1992, the number of loci for this impairment seems to grow exponentially. Due to the laborious nature of identifying disease-causing genes and the fact that the human genomic sequence and its annotation are still incomplete, the discovery of deafness genes progresses at a slower rate. Performing studies leading to accurate standardised clinical descriptions, e.g. the analysis of audiometric and vestibular data, is essential for the proper distinction of different phenotypes. Although such a distinction may seem trivial, the smallest difference or the slightest resemblance in clinical characteristics may be directive for candidate gene selection. Furthermore, the function of genes involved in hearing will be better understood, if all effects of a mutated gene are thoroughly known. Taken together this will hopefully lead to developments of new molecular diagnostic tools, perhaps even contribute to new therapeutic options and, last but not least, will optimise patient care in general ENT practise as well as in genetic counselling.

Samenvatting

Ongeveer dertig jaar geleden, in het begin van de jaren zeventig van de vorige eeuw, werd erfelijke slechthorendheid een wetenschappelijk aandachtspunt van de Nijmeegse KNO afdeling. Eind jaren tachtig startten de eerste genkoppelingsstudies naar erfelijke slechthorendheid, die vooral gericht waren op syndromale afwijkingen gepaard gaande met slechthorendheid. Dezelfde techniek werd gebruikt voor erfelijke niet-syndromale vormen van slechthorendheid in het begin van de jaren negentig. Een samenwerkingsverband voor onderzoek naar het BOR- en het Usher-syndroom werd opgericht met deelname van de Nijmeegse KNO afdeling en het otogenetische laboratorium van Boys Town in Omaha (Nebraska, USA) onder leiding van Prof. dr. W.J. Kimberling en Prof. S. Kumar. De gezamelijke inspanningen van de afdeling Humane Genetica uit Antwerpen (dr. G. Van Camp) en de Nijmeegse KNO afdeling zijn meer gericht op genotypering van autosomaal dominante vormen van slechthorendheid en otosclerose. Het steeds intensievere contact met de afdeling Humane Genetica van het UMC St Radboud (Prof. dr. H. Brunner, dr. F. Cremers) heeft geleid tot de oprichting van het Nijmeegs otogenetisch laboratorium (dr. H. Kremer). Dit laboratorium is een zeer bruikbare toevoeging en biedt nieuwe én krachtigere mogelijkheden voor onderzoek naar erfelijke slechthorendheid.

Dit promotie-onderzoek heeft veel profijt gehad van eerder verrichte onderzoeksinspanningen op het gebied van erfelijke slechthorendheid. Zo werd in een eerder stadium de mutatie-analyse in de verschillende families met het BOR syndroom uitgevoerd. Het koppelingsonderzoek in de DFNA9 en DFNA20/26 families werd met succes afgerond in het Nijmeegse otogenetisch laboratorium en heeft geleid tot de identificatie van de ziekte veroorzakende mutaties in de desbetreffende genen. De Nijmeegse afdeling DNA diagnostiek (dr. L Hoefsloot) heeft de gegevens met betrekking tot de uitslagen van DFNB1/connexine 26 (*GJB2*) mutatie-analyse ter beschikking gesteld. Het resultaat van al deze onderzoeksprojecten komen in de hoofdstukken 2, 3, 4 en 5 aan de orde.

In dit proefschrift worden diverse vormen van erfelijke slechthorendheid feno- en genotypisch beschreven. In vier afzonderlijke hoofdstukken worden achtereenvolgens enkele wetenschappelijke aspecten en resultaten besproken met betrekking tot een syndromale (BOR syndroom, *Hoofdstuk 2*), autosomaal

dominante (DFNA9 and DFNA20/26, *Hoofdstuk 3 & 4*) en een autosomaal recessieve (DFNB1, *Hoofdstuk 5*) vorm van erfelijke slechthorendheid. *Hoofdstuk 1* is een algemene inleiding over deze aandoening.

Hoofdstuk 2 is gewijd aan het BOR syndroom en bevat een overzichtsartikel over deze aandoening (*Hoofdstuk 2.1*). *Hoofdstuk 2.2* is een klinische beschrijving van een vader en zijn zoon met het BOR syndroom, met speciale aandacht voor de audiometrische follow-up in relatie tot het beeldvormend onderzoek. Hierbij vertoonde de jongste patiënt de opvallende combinatie van progressieve fluctuerende slechthorendheid met een verwijding van de ductus en sacculus endolymphaticus (LEDS). Dit is aanleiding geweest om vijf andere families met het BOR syndroom radiologisch te onderzoeken (MRI) (*Hoofdstuk 2.3*). De resultaten van dit onderzoek zijn samen met gedetailleerde audiometrische en genetische gegevens geanalyseerd. Hoewel er op MRI diverse afwijkingen van zowel het midden- als het binnenoor zichtbaar waren, kon geen van deze afwijkingen beschouwd worden als typisch voor het BOR syndroom (*Hoofdstuk 2.3*). Het was mogelijk om in sommige van de bestudeerde patiënten achteruitgang en/of fluctuatie van het gehoor aan te tonen. BOR patiënten, bij wie een verwijding van de ductus en sacculus endolymphaticus (LED(S)) aantoonbaar is en die 25 jaar of jonger zijn, lijken gepredisponeerd te zijn om slechter te horen én vertonen progressief fluctuerende slechthorendheid (*Hoofdstuk 2.4*). Hoewel iedere participerende familie koppeling vertoonde met het *EYA1* locus, konden er maar drie mutaties in drie van de zes families aangetoond worden. Door de beperkte beschikbaarheid van genetische gegevens en de onderlinge klinische verschillen binnen en tussen de families was het niet mogelijk een betrouwbare fenotype-genotype correlatie te bepalen. Een recente wetenschappelijke publicatie heeft aangetoond dat herschikking binnen het *EYA1* gen verantwoordelijk zijn voor het grote gedeelte van de BOR patiënten, bij wie voorheen geen mutatie aangetoond kon worden. Dit werpt een nieuw licht op de beschikbare genetische data van dergelijke BOR families en vereist in feite een aanpassing van de huidige methode van *EYA1* mutatie-analyse. Aanpassing van deze methode zal mogelijkerwijs leiden tot het beschikbaar komen van meer genetische en klinische data over deze erfelijke aandoening. Wellicht draagt dit bij aan de beantwoording van de door ons geopperde vragen en zal het leiden tot meer inzicht in de pathogenese van deze aandoening.

Hoofdstuk 3 behandelt DFNA9/*COCH* en begint met een overzichtsartikel over dit onderwerp (*Hoofdstuk 3.1*). In *Hoofdstuk 3.2* wordt een Nederlandse familie beschreven die de G88E/*COCH* mutatie herbergt met de hierbij behorende genetische en klinische analyse. Het gehoorverlies heeft een progressief karakter, wat in eerste instantie de hoge en in een later stadium de midden en lage frequenties aantast. Het gehoorverlies begint tussen de 5e en 6e decade en verslechtert jaarlijks met ongeveer 3.6-4.6 dB. Bij vrijwel alle patiënten met een duidelijke beperking van het gehoor werd een verminderde evenwichtsfunctie geconstateerd. Een aantal mutatie-dragers waren nog te jong voor volledige expressie en vertoonden daarom geen klinische afwijkingen. Er waren nauwelijks klinische verschillen met de oorspronkelijke beschrijving van deze mutatie aantoonbaar. *Hoofdstuk 3.3* beschrijft een grote Nederlandse familie met patiënten bij wie zowel het gehoor als het evenwicht aangetast is op basis van de P51S/*COCH* mutatie. Deze mutatie is een founder-mutatie in Nederland en België en wordt regelmatig aangetroffen bij patiënten van de Nijmeegse KNO-kliniek. Audiometrische analyse laat zien dat het gehoorverlies tussen het 40e en het 50e levensjaar begint en zich grotendeels beperkt tot de hoge frequenties. Terwijl het gehoor jaarlijks met ongeveer 3 dB verslechtert, vermindert tegelijkertijd de functie van het evenwichtsorgaan. Dit leidt zelfs tot totale uitval van dit orgaan. Sinds deze beschrijving zijn verscheidene andere families met de P51S/*COCH* mutatie audiometrisch, genetisch en vestibulair onderzocht geanalyseerd, hetgeen geleid heeft tot de omschrijving van een karakteristiek fenotype. DFNA9 wordt histopathologisch gekenmerkt door extracellulaire glycosamineglycaan (mucopolysaccharide) deposities in de cochlea. Deze observatie werd voor het eerst gedaan in het os temporale van een overleden patiënt met de G88/*COCH* mutatie. Recent is bekend geworden dat het gemuteerde cochline eiwit zonder problemen wordt verwerkt en uitgescheiden door cellen van de cochlea. Het uiteindelijke effect van dergelijke deposities ontstaat waarschijnlijk pas na de secretie van cochline in de extracellulaire matrix. Wellicht dat mutaties leiden tot gestoorde eiwit-eiwit interacties óf dat aggregatie van dit product uiteindelijk zuiver mechanische problemen veroorzaakt. Op dit moment wordt er gewerkt aan een post-mortem histopathologisch onderzoek van het os petrosum van één van de P51S patiënten. Een vergelijking van de bevindingen met die van aanverwante binnenoorstudies zal wetenschappelijk interessant zijn.

In *Hoofdstuk 4* wordt een Nederlandse familie besproken met een autosomaal dominant overervende slechthorendheid die gekoppeld is aan het DFNA20/26 locus op chromosoom 17q25. De kritische regio werd gereduceerd van 12 cM tot 9.5 cM. Toon- en spraak-audiometrische gegevens, evenals vestibulaire gegevens zijn verzameld en geanalyseerd. Tot de leeftijd van 15 jaar werd een licht aflopend hoogfrequent gehoorverlies gezien, dat bij 8 kHz ernstig verslechterde tussen het 15e en het 20e jaar. Tussen het 25e en het 40e levensjaar verslechterde de kwaliteit van het gehoor bij 1-4 kHz. De lage tonen (0.25-0.5 kHz) verslechterden jaarlijks wat gematigder met een snelheid van ±1.5-2 dB. Vanaf het 40e levensjaar was het gehoor nauwelijks nog functioneel. Hoewel spraakherkenning problematisch begon te worden tussen het 25e en het 45e levensjaar, was de maximaal haalbare spraakherkenningscore beter dan bij DFNA2, DFNA5 en DFNA9. De huidige DFNA20/26 familie vertoont een ernstiger ziektebeeld dan de oorspronkelijk Noord-Amerikaanse familie. Het DFNA20/26 locus bevat het gamma-1 actine gen (*ACTG1*). Vrij recent hebben collega's van het Nijmeegs Otogenetisch Laboratorium een Thr278Ile mutatie gevonden in dit gen, wat betrokkenheid bij DFNA20/26 suggereert. De gevolgen van een gemuteerd *ACTG1* gen op het eiwit zelf, op eiwit-eiwit interacties onderling, evenals op de functie van cochleaire haarcellen zijn interessante vraagstukken voor toekomstig onderzoek.

Hoofdstuk 5 is gewijd aan DFNB1/*GJB2*. *GJB2* codeert het connexine 26 eiwit. *GJB2* mutaties spelen een belangrijke rol in autosomaal recessieve en sporadische vormen van slechthorendheid. Mutatie-analyse van het *GJB2* gen is relatief eenvoudig te verwezenlijken binnen een DNA diagnostische setting en is gebleken een succesvolle diagnostische procedure te zijn. Dit artikel geeft een overzicht over dit onderwerp en toont de eerste resultaten van deze diagnostische procedure in Nijmegen. De uitslag van eenennegentig mutatie-analyses bij personen met een autosomaal recessieve of sporadische vorm van erfelijke slechthorendheid zijn retrospectief geanalyseerd. Negentien niet-verwante personen vertoonden een mutatie op beide allelen. Twaalf hiervan hadden een homozygote 35delG mutatie, vier anderen een heterozygote 35delG mutatie. De 35delG-mutatie is betrokken bij 84% van de gevallen, waarin een mutatie aangetoond kon worden. Deze bevindingen zijn in overeenstemming met die van de ons omringende landen. Verder onderzoek naar de correlatie tussen de

soort mutatie en de ernst van het gehoorverlies zouden interessant kunnen zijn voor toekomstig onderzoek.

Discussie

Samengevat wordt er in dit proefschrift aandacht besteed aan de klinische en genetische aspecten van één syndromale en drie niet-syndromale vormen van erfelijke slechthorendheid. De complexiteit van het auditieve systeem en de grote hoeveelheid geïdentificeerde doofheidsloci bewijzen dat er nog veel meer onontdekte genen betrokken zijn bij deze aandoening. Vanaf de eerste beschrijving van een locus betrokken bij erfelijke slechthorendheid in 1992 lijkt het aantal loci vrijwel exponentieel te zijn gegroeid. Door de grotere bewerkelijkheid van het identificeren van de betrokken genen en het feit dat de humane genoomsequentie nog steeds incompleet is, verloopt de identificatie van slechthorendheid veroorzakende genen in een vooralsnog lager tempo. Het verrichten van klinische studies die leiden tot een nauwkeurig gestandaardiseerde klinische beschrijving met behulp van bijvoorbeeld audiometrie en vestibulaire gegevens, is essentieel om verschillende fenotypes te onderscheiden. Een dergelijk onderscheid mag dan misschien bijzaak lijken, maar zelfs de kleinste klinische verschillen of overeenkomsten kunnen van invloed zijn op de selectie van een kandidaat-gen. Daarnaast zal de kennis met betrekking tot de functie van genen in het binnenoor alleen verbeteren als alle klinische effecten van een genmutatie tot in details bekend zijn. Uiteindelijk zal dergelijk onderzoek hopelijk leiden tot de ontwikkeling van nieuwe moleculaire diagnostische procedures en misschien zelfs bijdragen aan de ontwikkeling van therapeutische mogelijkheden. Zeker niet op de laatste plaats zal een optimale kennis op het gebied van erfelijke slechthorendheid ten goede komen aan de patiëntenzorg in de algemene KNO praktijk, in het bijzonder bij genetische counseling.

Dankwoord

Een open deur wellicht, maar dit proefschrift was natuurlijk nooit tot stand gekomen zonder de hulp en inzet van een heleboel mensen. Het zijn niet alleen mijn spreekwoordelijke bloed, zweet en tranen geweest die gevloeid hebben. Een dankwoord is dan ook op zijn plaats.

Professor Cremers. Na een kort gesprek tussen OK 1 van het Radboud en (de enige telefoon in) het St Francis Hospital in Katete, Zambia, werd eind 1998 met succes een AGIKO aanstelling aangevraagd. Zo kreeg, in navolging van mijn wetenschappelijke stage, uw begeleiding voor mij een vervolg. Uw visie, tomeloze inzet en enthousiasme zijn bewonderenswaardig en hebben gezorgd dat ook deze otogenetische promotie succesvol afgerond is. Uw onderzoekslijn loopt als de spreekwoordelijke trein en het ziet er naar uit dat de “Otogenetica-Express” voorlopig niet tot stilstand komt.

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Curriculum Vitae

Martijn Kemperman werd op 10 december 1972 geboren in Haaren. In 1991 behaalde hij het eindexamen aan het R.K. Gymnasium Beekvliet te Sint-Michielsgestel. In dit zelfde jaar werd hij nageplaatst voor de studie geneeskunde aan de Katholieke Universiteit Nijmegen. In de afrondende fase van deze opleiding werd bij de afdeling keel-, neus- en oorheelkunde van het UMC St Radboud een wetenschappelijke stage gelopen op het gebied van erfelijke slechthorendheid. De hierbij behorende scriptie werd met een facultaire prijs bekroond. Het arts-examen werd in 1998 behaald, waarna hij werd aangesteld als arts-onderzoeker aan de afdeling keel-, neus- en oorheelkunde van het Universitair Medisch Centrum St Radboud. Een aantal maanden later kreeg dit wetenschappelijk onderzoek een vervolg in de vorm van een aanstelling als assistent-geneeskundige in opleiding tot klinisch onderzoeker (AGIKO). Het gehele onderzoeksproject was een nauwe samenwerking tussen de afdeling keel-, neus- en oorheelkunde en de afdeling antropogenetica van het UMC St Radboud. Zijn opleiding tot keel-, neus- en oorarts startte als AGIKO eind 1998 en zal naar verwachting eind 2005 worden afgerond.

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