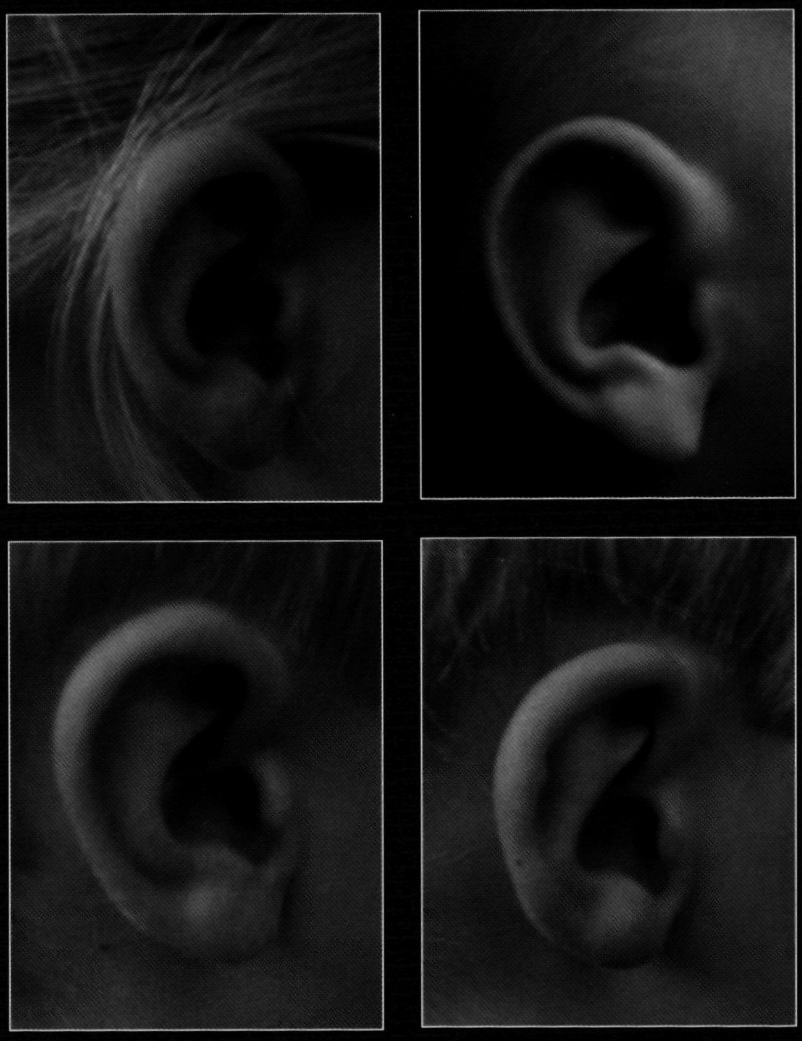
Genetic hearing impairment



Clinical and Genetic aspects in BOR-syndrome, Pendred syndrome and DFNB1

CHRISTEL STINCKENS

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General Introduction

INTRODUCTION

INTRODUCTION

In Western Europe, prevalence of genetic hearing loss is about 1 27 in 1000 births (with hearing level in the best ear > 25dB)[1] At least half of these cases are probably inherited[2 3] Incidence of autosomal recessive inheritance patterns is estimated on approximately 70-80%, autosomal dominant inheritance patterns on 20-30% and X-linked patterns on 1-2%[16 18] Mitochondrial deafness has also been described[4]

Historical notes

One of the pioneers in research of genetic deafness was the Irish otologist Sir William Wilde In 1853, thanks to an Irish census to which he was allowed to add a questionnaire, he was able to describe pedigrees of deaf families, which made him the first to describe the direct (dominant) way of inheritance[5]

In 1880, the Berlin otologist Arthur Hartmann first mentioned an indirect (recessive) way of inheritance He wondered why children who had consanguineous parents were predestined to have more frequently congenital deficits than others[6] His theory on direct or dominant and indirect or recessive inheritance was subscribed by Adam Politzer (otologist in Vienna) in his 2nd edition of the Lehrbuch of Ohrenheilkunde[7]

The first descriptions of syndromes with hearing loss date from the 19th century In 1858, the German ophthalmologist von Graefe was the first to describe the occurrence of retinitis pigmentosa and congenital deafness[8] Evidence for the influence of consanguinity came from studies in the Jewish population, which were followed by the English description by the Scottish ophthalmologist Charles Usher whose name became an eponym for the syndrome[9 11] Other examples of syndromes which were first described in the 19th century are the Branchio-Oto-Renal syndrome and Pendred syndrome, which form also part of this thesis

In 1896, the English physician Vaughan Pendred described a family with two deaf daughters who had goitres[12] This syndrome did not get much attention, until the metabolic disorder which is causing the syndrome and its epidemiological importance were described by Morgans and Trotter (1958)[13] and Fraser (1960, 1965)[14 15]

Although non-syndromic hearing loss accounts for the majority of hereditary hearing impairments, most attention has been paid to syndromic hearing disorders, as they can easily be differentiated based on their associated

characteristics. At this moment, more than 400 syndromes with hearing loss have been described[16].
With the introduction of the audiometer in the late nineteen thirties, it became possible to describe and differentiate the non-syndromic forms of hearing impairment and this resulted in the first publications on families with non-syndromic hearing loss in the nineteen fifties. The non-syndromic forms were classified by type of audiogram (low-, mid- and high-frequency hearing loss), severity of hearing impairment, age of onset and presence or absence of progression[16].

Since 1992, major progress has been made in molecular genetic diagnosis of hereditary sensorineural hearing loss. Thanks to the improvement of the methods to investigate DNA, phenotype was not longer the only criterion to distinguish different types of hereditary hearing loss. An entire new domain of investigation opened for non syndromic hearing loss, which before was not a very popular subject for investigators. This resulted in a rapid increase of knowledge about different types of non-syndromic hearing loss, which were designated DFNA (autosomal dominant), DFNB (autosomal recessive) and DFN (X-linked).
Nowadays, 51 different loci are known to be responsible for non-syndromic autosomal dominant hearing loss, and 39 for the non-syndromic autosomal recessive forms (table 1,2).
In several syndromic as well as non syndromic forms, the gene responsible for the hearing impairment has been cloned. In some cases, it has already become possible to use molecular tests for genetic diagnosis.

Branchio-Oto-Renal (BOR) syndrome
The association of branchial, otic and renal anomalies appeared in literature possibly in a case report written by Heusinger[17]. In 1878, Sir James Paget described the syndrome in almost its full extent after discovering it in two generations of one family[18]. In 1955, the combination of preauricular and cervical sinuses and fistulas with hearing impairment was for the first time considered as a separate syndrome by Fourman and Fourman[19].
It was not until Melnick and Fraser described kindreds segregating autosomal dominant hearing loss, bilateral preauricular pits, bilateral cervical fistulae,
auricular deformity and bilateral renal dysplasia that branchio-oto-renal (BOR) dysplasia was recognized as a syndrome[20 21].
The estimated prevalence of BOR syndrome is 1:40,000 and it affects about 2% of profoundly deaf children[22].

Table 1: Autosomal dominant loci

Locus	Chromosomal localisation	Gene	Most important reference
DFNA1	5q31	HDIA1	Lynch 1997[74]
DFNA2	1p34	GJB3/KCNQ4	Xia 1999[75], Kubish 1999[76]
DFNA3	13q12	GJB2/GJB6	Denoyelle1998[77], Grifa 1999[78]
DFNA4	19q13		Chen 1995[79]
DFNA5	7p15	DFNA5	Van Laer 1998[80]
DFNA6/14/38	4p16 3	WFS1	Bespalova 2001[81], Young 2001[82]
DFNA7	1q21-q23		Fagerheim 1996[83]
DFNA8/12	11q22-24	TECTA	Verhoeven 1998[84]
DFNA9	14q12-q13	COCH	Robertson 1998[85]
DFNA10	6q22-q23	EYA4	Wayne 2001[86]
DFNA11	11q12 3-q21	MYO7A	Liu 1997[87]
DFNA13	6p21	COL11A2	Mc Guirt 1999[88]
DFNA15	5q31	POU4F3	Vahava 1998[89]
DFNA16	2q24		Fukushima 1999[90]
DFNA17	22q	MYH9	Lalwani 2000[91]
DFNA18	3q22		Bonsch 2001[92]
DFNA19	10 pericentr		Green 1998[93]
DFNA20/26	17q25	ACTG1	Morell 2000[94] van Wijk et al 2003[174] Zhu et al 2003[170]
DFNA21	6p21		Kunst 2000[95]
DFNA22	6q13	MYO6	Melchionda 2001[96]
DFNA23	14q21-q22		Salam 2000[97]
DFNA24	4q		Hafner 2000[98]
DFNA25	12q21-24		Greene 1999[99]
DFNA20-26	17q25	ACTG1	Yang 2000[100] van Wijk et al 2003[174] Zhu et al 2003[170]
DFNA27	4q12		Fridell 1999[101]
DFNA28	8q22	TFCP2L3	Peters 2002[102]
DFNA29		reserved	
DFNA30	15q25-26		Mangino 2001[103]
DFNA31		reserved	
DFNA32	11p15		Li 2000[104]
DFNA33		reserved	
DFNA34	1q44		Kurima 2000[105]
DFNA35		reserved	
DFNA36	9q13-q21	TMC1	Kurima 2002[106]
DFNA37	1p21		Talebizadeh 2000[107]
DFNA38	-	-	-
DFNA39	4q21 3	DSPP	Xiao 2001[108]
DFNA40	16p12	reserved	
DFNA41	12q24-qter		Blanton 2002[109]
DFNA42			
DFNA43	2p12		Flex 2003[110]
DFNA44	3q28-29		Modamio-Hoybjor 2003[111]
DFNA45		reserved	
DFNA46		reserved	
DFNA47	9p21-22		D'Adamo 2003[112]
DFNA48	12q13-14		D'Adamo 2003[113]
DFNA49		reserved	
DFNA50		reserved	
DFNA51	9q21		

Table 2: Autosomal recessive loci

Locus	Chromosomal localisation	Gene	Most important reference
DFNB1	13q12	GJB2	Kelsell 1997[114]
DFNB2	11q13.5	MYO7A	Liu 1997[115], Weil 1997[116]
DFNB3	17p11.2	MYO15	Wang 1998[117]
DFNB4	7q31	SLC26A4	Li 1998[118]
DFNB5	14q12		Fukushima 1995[119]
DFNB6	3p14-p21	TMIE	Naz 2002[120]
DFNB7	9q13-q21	TMC1	Kurima 2002[121]
DFNB8	21q22	TMPRSS3	Scott 2001[122]
DFNB9	2p22-p23	OTOF	Yasunaga 1999[123]
DFNB10	21q22.3	TMPRSS3	Scott 2001[124]
DFNB11	9q13-q21	TMC1	Kurima 2002[125]
DFNB12	10q21-q22	CDH23	Bork 2001[126]
DFNB13	7q34-36		Mustapha 1998[127]
DFNB14	7q31		Mustapha 1998[128]
DFNB15	3q21-q25; 19p13		Chen 1997[129]
DFNB16	15q21-q22	STRC	Verpy 2001[130]
DFNB17	7q31		Greinwald 1998[131]
DFNB18	11p14-15.1	USH1C	Ahmed 2002[132]
DFNB19	18p11		Green 1998[133]
DFNB20	11q25-qter		Moynihan 1999[134]
DFNB21	11q	TECTA	Mustapha 1999[135]
DFNB22	16p12.2	OTOA	Zwaenepoel 2002[136]
DFNB23	10p11.2-q21	PCDH15	Ahmed et al 2003[171]
DFNB24	11q23		Smith, unpublished
DFNB25	4p15 3-q12		Smith, unpublished
DFNB26	4q31		Riazuddin 2000[137]
DFNB27	2q23-q31		Pulleyn 2000[138]
DFNB28	22q13		Walsh 2000[139]
DFNB29	21q22	CLDN14	Wilcox 2001[140]
DFNB30	10p12 1	MYO3A	Walsh 2002[141]
DFNB31	9q32-q34	WHRN	Mustapha 2002[142], Mburu 2003[143]
DFNB32	1p13.3-22.1		Masmoudi 2003[144]
DFNB33	9q34 3		Medlej-Hasim 2002[145]
DFNB34		reserved	
DFNB35	14q24.1-24.3	Unknown	Ansar 2003[146]
DFNB36		reserved	
DFNB37	6q13	MYO6	Ahmed 2003[147]
DFNB38	6q26-q27	Unknown	Ansar et al.2003[172]
DFNB39	7q11.22-q21.12	Unknown	Wajid et al 2003[173]

Phenotype

BOR syndrome has a wide spectrum of variable clinical manifestations in ear, branchial arch and kidney. Main features are slight malformations of the auricles, the ear canal, the middle and/or inner ear, preauricular sinuses, hearing impairment, cervical sinuses or fistulas of the second branchial arch and renal dysplasia. Less common features are facial or palatal anomalies, lacrimal duct stenosis and external auditory canal stenosis[22,23].

Hearing loss can be conductive, sensorineural or mixed and is mostly stable, although progressive hearing loss has been described[19 21 24 27] Middle ear anomalies can include malformation, malposition, dislocation or fixation of the ossicles, and reduction in size or malformation of the middle ear cavity Inner ear malformations include cochlear hypoplasia, enlargement of the cochlear and vestibular aqueducts, and hypoplasia of the lateral semicircular canal [27 29] Renal anomalies range from hypoplasia to aplasia, either unilaterally or bilaterally Anomalies of the collecting system affect the ureter, calyx and renal pelvis[30 31]
Multiple family studies have shown that incomplete penetrance and variable expressivity - particularly the severity of hearing loss and the renal abnormalities- are common, with phenotypic variation between families and also within families This is the reason why several investigators made the distinction between branchio-oto (BO) and branchio-oto-renal (BOR) syndrome[32] This also led to the assumption that more than one autosomal dominant mutation was responsible for the different clinical presentation forms[33 34], which was later questioned by other authors[23 30 35]

Genetics

In 1997, Abdelhak et al identified mutations in a novel gene called EYA 1 in seven patients with BOR-syndrome This gene, which is localized to chromosome band 8q13 3, is a human homologue of the Drosophila eyes absent gene (eya) and was therefore called EYA 1 The gene is composed of 17 exons that span 156kb of genomic DNA and encode a 559-amino acid protein The eya homologous region (eyaHR) from exons 9-16 of EYA1 is highly conserved within the EYA gene family and is the site of the majority of BOR mutations Mutations in EYA1 can also cause branchio-otic syndrome (BO)[36 39] About 70% of families whose members had BOR syndrome investigated by Kumar et al did not show mutations in the EYA1 gene This suggests either that most of the mutations lie in the untranslated region or that several genes are involved in the brachiogenic disorder In 1998, Kumar et al investigated a large Dutch family with branchio-otic type syndrome, with preauricular sinus or cysts, commissural lip pits, an external ear anomaly and hearing impairment Linkage analysis excluded linkage to the 8q13 region, and in 2000, conclusive evidence of linkage with markers on chromosome 1q31 was obtained, establishing a genetic heterogeneity associated with BOR syndrome[40 41]
In 2004 Six 1 on 14q23 1 was identified as a gene causing BOR and BO syndromes[175]

Pendred syndrome

The clinical phenotype known as Pendred Syndrome (PS) was first observed in 1896 by a British physician, Vaughan Pendred He described an Irish family in which two of ten offspring were congenitally deaf and had goitres that could not be attributed to environmental factors (endemic goitre)[12] In 1956 it became clear that the inheritance pattern of this syndrome was autosomal recessive, and in 1958, it was reported that the thyroid enlargement was caused by a defect in organification of iodide, resulting in impairment of thyroxin synthesis[42 43]

In 1967, Jensen reported a Mondini–type cochlea in a patient with Pendred syndrome, and in 1978 Valvassori and Clemis for the first time described an enlarged vestibular aqueduct[44 45] The presence of an enlarged vestibular aqueduct in almost all, and Mondini type cochlea in some patients with Pendred syndrome was later confirmed by several authors[47 49]

The prevalence of PS is estimated to be 1-8% of congenital deafness

Phenotype

The classical features of Pendred syndrome include sensorineural hearing loss and goitre

Originally, hearing loss was mainly considered as congenital sensorineural hearing loss It is generally profound and the audiogram has a steeply down sloping configuration Since 1980, several authors mentioned progression and/or fluctuation of hearing loss in Pendred syndrome documented by means of audiograms[46 47 50 53] Progression is particularly rapid in early childhood Episodic vertigo with decreased peripheral vestibular function has been described in different cases[46 51 52 54] In almost all individuals with Pendred syndrome, an enlarged vestibular aqueduct is found on CT-scan[47 49]

Thyroid enlargement is not always present, and usually develops during puberty Affected individuals generally remain euthyroid despite the goitre The thyroid defect is associated with abnormal iodide processing, that often can be diagnosed using the perchlorate discharge test, which is based on the release of radioactivity following administration of radioactive iodide[13 15] Recently it has become clear that the perchlorate discharge test is not as reliable as previous thought for diagnosing Pendred syndrome Masmoudi et al studied two families with Pendred syndrome all had the same mutation, but only 11 had palpable goitre and in all 8 tested individuals the perchlorate discharge test was negative[55]

Genetics

In 1996 Pendred syndrome was mapped to a 9-cM region on the long arm of chromosome 7 (7q31)[56 57] In 1997, Everett et al cloned the causative gene and initially named it PDS, later PDS has been renamed [SLC26A4][58] The protein encoded by the [SLC26A4] gene was predicted to be a 780-amino-acid protein and was named pendrin As initially was thought that pendrin was a putative sulphate transporter, in 1999 Scott et al demonstrated that [SLC26A4] encodes a chloride-iodide transport protein[59]

A form of non-syndromic deafness, DFNB4, localizes to the same genomic region and is allelic to Pendred syndrome[60 61] Patients with DFNB4 have sensorineural hearing loss and an enlarged vestibular aqueduct, but do not have any thyroid anomalies Hearing loss is comparable to that in Pendred syndrome a down sloping audiogram with progression and/or fluctuation (often episodes of sudden hearing loss with afterwards (partial) recuperation) in hearing loss[62 64] Episodic vertigo often is present, most of the time in combination with sudden hearing loss[54 65 66] Goitre or thyroid dysfunction are absent

Functional studies by Scott et al suggest that the observed phenotype correlates with the degree of residual function of the encoded protein, pendrin Alleles with mutations in [SLC26A4] associated with Pendred syndrome had complete loss of pendrin-induced chloride and iodide transport, those with mutations in [SLC26A4] associated with DFNB4 still had the possibility to transport both iodide and chloride, but at a much lower level than wild-type pendrin Thus, mutations that result in no residual transport function appear to be associated with the Pendred syndrome phenotype, minimal transport ability would prevent thyroid dysfunction but not the sensorineural hearing loss and temporal bone anomalies that characterize DFNB4[67] This suggests that DFNB4 could be a milder form of Pendred syndrome To date, 87 mutations in [SLC26A4] have been found in a total of 167 families

In the ear, pendrin is predominantly expressed in the endolymphatic duct and sac, and to a lesser extent in the nonsensory regions of the utricle, saccule and cochlea[68 69] This suggests that anion transport depending on normal pendrin plays an important role in maintaining the endolymphatic homeostasis, which is essential to a normal inner ear function [SLC26A4] is also expressed in the thyroid, kidney and placenta Pendrin was found to be expressed in the apical membrane of thyrocytes, the intercalated cells of cortical collecting ducts in the kidney, as well as in the brush border membrane of cytotrophoblasts[70 71] Studies with a Pds-knockout mouse showed complete absence of hearing, combined with a variable vestibular phenotype, going from absence of vestibular signs to severe

vestibular signs All mice showed a dilated endolymphatic duct and sac starting from embryonic day 15, followed by dilation of the cochlea and saccule, and in some cases also the semicircular canals At postnatal day 7, normally developed sensory hair cells of the cochlea and vestibular macula were present, but by postnatal day 15, there was clear evidence of degeneration of these structures, which progressively worsened through postnatal day 45 This seems to occur at the same time as the establishment of a mature endolymphatic fluid composition and the development of the endocochlear potential, suggesting a progressive deterioration and swelling of a near-mature endolymphatic compartment after the development of an anatomically normal inner ear and not –as previously thought- a simple arrest of development This mechanism is reminiscent of the endolymphatic hydrops in Menieres disease The investigators did not show any biochemical or histological evidence of thyroid disease[72] A widened vestibular aqueduct has become a maior feature of Pendred syndrome, indicative to go for a mutation screening of [SLC26A4]

Diagnosing Pendred syndrome

As mentioned above, clinical presentation and perchlorate test are unreliable features to diagnose Pendred syndrome Mutation screening of [SLC26A4] has become the most reasonable diagnostic test in individuals with sensorineural hearing loss and cochlear malformations

DFNB1

Non-syndromic deafness

Prelingual non-syndromic deafness is the most frequent hereditary sensory defect In more than 80% of the cases, the mode of transmission is autosomal recessive Before genetic testing was available, non-syndromic forms of hearing loss could only be distinguished based on the mode of inheritance, the age of onset of hearing loss and on the shape of the pure tone audiogram Forms of progressive hearing loss were divided into low frequency, mid-frequency and high frequency hearing loss[16] With the development of gene-linkage studies, it became clear that much more different genotypes for non-syndromal hearing loss could be recognized To date, 51 loci have been identified for the autosomal dominant forms (DFNA) and 39 loci for the autosomal recessive forms (DFNB) (table 1 and 2)[73]

DFNB1

In 1994, Guilford et al were able to localize a gene causing recessive and profound sensorineural hearing loss to the pericentromeric region of chromosome 13q, in two families from Northern Tunisia They called this form of deafness NSRD1, which was later renamed DFNB1[148] In 1996, Brown et al demonstrated that this locus was also responsible for hearing impairment in a large Pakistani family, and that the DFNB1 locus is proximal to the marker D13S175[149] In 1997, Kelsell et al were the first to find connexin 26 mutations in three autosomal recessive non-syndromic sensorineural deafness pedigrees who were genetically linked to chromosome 13q11-12 (DFNB1) where the connexin 26 gene is localized[150]

Following these results, to date 75 autosomal recessive mutations in the GJB2 gene, which is coding for connexin 26, have been described, and a deletion of G in position 30 to 35 (mutation 35delG) seems to be the most frequent mutation in GJB2 Detailed information on these mutations can be acquired on the connexin home page http www crg es deafness[151] About 50% of all autosomal recessive non-syndromal deafness are due to connexin 26 mutations, and the 35delG mutations accounts for the majority (>70%) of the connexin 26 mutant alleles[152 153] Connexin genes code for the subunits of gap junction proteins, which form intercellular channels in plasma membranes for transport of fluid and small molecules These gap junctions are essential to recycle potassium ions needed for initiation of action potentials in hair cells[154] Cohen-Salmon et al were able to demonstrate that mice with targeted ablation of Connexin 26 had normally developing inner ears, but with cell death appearing on postnatal day 14, i e soon after the onset of hearing They concluded that the Cx26 containing epithelial gap junction network is essential for cochlear function and cell survival[155]

Hearing loss in patients with connexin 26 mutations has a prelingual onset, and it may be mild, moderate, severe or profound Both ears present a similar degree of hearing loss and the deafness either preferentially affects high frequencies or affects all frequencies to the same extent Intrafamilial variations of the severity of the hearing loss are common Hearing loss is generally not or only mildly progressive It is not clear yet if there is a correlation between particular connexin 26 mutations and severity of hearing loss, as some authors didn t and others did find evidence for such a correlation[156 159] In most subjects no radiological anomalies of the inner ear are present, although Kenna et al reported on temporal bone anomalies in 4 individuals[160 163] In several hearing impaired individuals, only one mutated gene is found, which questions pathogenicity [162 164]

In 1999, Kelley et al described connexin 30 (GJB6), which is co-localized to connexin 26 and whose structure is similar to this of connexin 26, as a candidate gene for non-syndromic hearing loss[165] Because of the fact that a large fraction of patients with GJB2 mutations only have one mutant allele, del Castillo et al looked for mutations other than those involving GJB2 in patients with only one mutant (GJB2) allele and with evidence of linkage to DFNB1 They were able to identify a 342-kb deletion in the gene encoding connexin 30 (GJB6), a protein that is reported to be expressed with connexin 26 in the inner ear They concluded that this deletion in GJB6 is the second most frequent mutation causing prelingual deafness in the Spanish population and that mutations in the locus DFNB1 can result in a monogenic or a digenic pattern of inheritance of prelingual deafness They postulated that DFNB1 is a complex locus containing two genes (GJB2 and GJB6) and that the loss of any two of the four alleles from these genes results in hearing impairment[166] These findings were subscribed by several other investigators[167 168]
Teubner et al demonstrated normally developing inner ear structures in Cx30 deficient mice, but they observed a loss of cells in the sensory epithelium of their cochlea up to postnatal day 18, which was slowly progressive They postulated that in the absence of Cx30, the deficiency of the gap junctions between supporting cells may lead to a local extracellular accumulation of K^+ ions around the hair cells, which in turn might trigger cell apoptosis because of chronic depolarisations or by other mechanisms They also found that the endocochlear potential was virtually undetectable and that the endolymphatic K^+ concentration was significantly decreased compared to this in wild-type mice[169]

The goal of this Ph D study was to study clinical and genetic aspects of genetic hearing impairment To get access to the population with a genetic hearing impairment the support of the Institute Spermalie (dr L Standaert) in Brughes has been sought So the start had been made to perform the studies in BOR syndrome and in DFNB1 For the CT and MRI studies in the Flemish BOR syndrome family the support of dr Jan Casselman (Brughes) has been sought

The outcome of this Belgian MRI study in BOR syndrome helped to start up a similar but larger MRI study in BOR syndrome in the Nijmegen University Hospital In the BOR study it will be emphasized to study clinical presentation especially regarding the degree of hearing impairment related to the inner ear structures The study in the families with in total 15 persons with a profound non syndromic childhood deafness (Chapter IV) has started to trace the gene(s) and mutation(s)

involved. As part of the phenotype genotype correlation study in those persons, this opportunity will be used to report on the severity and progression of the hearing impairment.

In chapter III the focus will be on Pendred syndrome. The gene involved in Pendred syndrome proved to be the same as in the Enlarged Vestibular Aqueduct syndrome, well known for its fluctuating hearing loss and attacks of vertigo correlated with the presence of an enlarged vestibular aqueduct. This brought up the question if hearing impairment in the classical Pendred syndrome could also have those features that might have been overlooked in the past. The literature available on this topic was very scarce. The Nijmegen series of Pendred syndrome was restudied to trace three patients with a fluctuant hearing loss associated with Meniere like vertigo. Later on a new Belgian family became available providing again an opportunity to describe in detail the clinical presentation based on a thorough clinical and genetic evaluation.

By doing so this Ph.D. study likes to provide new knowledge to the field of genetic hearing impairment especially focussed on BOR syndrome, Pendred syndrome and non syndromic profound childhood deafness especially in Flandres and The Netherlands.

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1.2

Non-syndromic dominant sensorineural hearing loss: from a few phenotypes to many genotypes

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ABSTRACT

Sensorineural hearing loss affects approximately 1 in 2 persons at about 80 years of age and 1 in 750 in childhood The best known forms of hearing loss with an autosomal dominant pattern of inheritance are the syndromic-mediated ones At present, the non-syndromic autosomal dominant inherited forms can only be distinguished by the shape of the tone-audiogram Based on gene linkage studies twelve different genotypes for autosomal dominant hereditary non-syndromic forms of sensorineural hearing loss have been recognized in a period of almost 2 years In view of the great diversity of types that have been recognized in such a short period, it can be expected that over the next 10 years, several dozens genetically-mediated forms of autosomal dominant inherited sensorineural hearing loss will be detected Similar developments are taking place in the non-syndromic autosomal recessive hereditary forms of sensorineural hearing loss and deafness The above indicates clearly that before too long, new genetic investigation techniques will enable us to distinguish between forms of sensorineural hearing loss that could not be distinguished in the past

INTRODUCTION

Sensorineural hearing loss affects approximately 1 in 2 persons at about 80 years of age and 1 in 750 in childhood

Inaccessibility of the inner ear to discriminant diagnostic procedures and the great diversity of possible causes of deafness, are reasons why it can be difficult to establish the causes in individual cases

The best known forms of hearing loss with an autosomal dominant pattern of inheritance are the syndromic-mediated ones, such as osteogenesis imperfecta type 1 and otosclerosis At present, the non-syndromic autosomal dominant inherited forms can only be distinguished by the shape of the tone audiogram[1]

Progressive sensorineural hearing loss is divided into low frequency, mid-frequency or high frequency hearing loss depending on whether the hearing loss is most pronounced in the low tone, middle tone or high tone range Apparently, the possibility that flat progressive perceptive hearing loss can also have an autosomal dominant pattern of inheritance, has been overlooked

Depending on the age at onset, various separate types can be distinguished within this classification Table 1 lists the various forms of sensorineural hearing loss that can presently be distinguished on clinical grounds[1] Until recently it was unclear whether the clinical classification of these types of non-syndromic autosomal

dominant inherited hearing loss would still hold its ground with the emergence of new genetic knowledge

Table 1 Autosomal dominant inherited types of hearing impairment that can be distinguished on clinical grounds

1	Congenital severe sensorineural hearing loss
2	Congenital low-frequency sensorineural hearing loss
3	Progressive low-frequency sensorineural hearing loss with childhood onset
4	Mid-frequency sensorineural hearing loss
5	Progressive high-frequency sensorineural hearing loss
6	Progressive mixed hearing loss
7	Unilateral sensorineural hearing loss
8	Progressive vestibulo-cochlear dysfunction and sensorineural hearing loss

1 1 *X-dominant inherited hearing loss*

When speaking in terms of dominant hereditary syndromes, it is necessary to realise that an X-dominant pattern of inheritance is also possible[2] A well-known example of the latter is Alport's syndrome, characterized by progressive perceptive hearing loss, haematuria, eye abnormalities and progressive deterioration of renal function, which until a few decennia ago, was considered to have an autosomal dominant pattern of inheritance Occasionally, an autosomal recessive form of this syndrome is encountered It is unclear whether there might also be an autosomal dominant inherited form Besides the specific pattern of inheritance, the fact that men are more severely affected than women should have drawn attention to the possibility of an X-dominant mode of inheritance at a much earlier date A non-syndromic X-dominant inherited type of sensorineural hearing loss has still not been recognized

1 2 *Non-Mendelian mitochondrial inherited deafness*

Recently, there has been a great deal of interest in the mitochondrial mode of inheritance Mitochondria are small organelles that are responsible for the cell's energy production They have their own circular DNA that codes for the 13 polypeptides necessary for oxidative phosphorylation Mitochondria are passed on to the offspring exclusively by the mother via the cytoplasm in the ovum

Table 2: Mitochondrial DNA mutations with hearing impairment as main symptom or additional symptom

Author	Clinical symptoms	Hearing impairment	Mt-DNA mutation
Mt-DNA mutations specifically associated with hearing impairment as the main presenting symptom			
Hu D, Prezant TR[7,8]	High susceptibility to antibiotic ototoxicity aminoglycoside (streptomycine) related deafness	Bilateral profound deafness	12SrRNA A1555G
Jaber L, Prezant TR[9,8]	Progressive hearing loss starting in childhood	Profound bilateral hearing loss without any additional symptoms	12SrRNA A1555G
Vernham GA, Reid FM, Fischel-Ghodsian N[10,11,12]	Progressive hearing loss	Variable hearing loss, predominantly high frequencies Onset between 3-18 years	tRNA-ser (UCN) T7445C
Tiranti V[13]	Bilateral hearing loss, ataxia and myoclonus	Moderate to severe hearing loss	tRNA-ser(UCN) 7472insC
Mt-DNA mutation in which hearing impairment is an additional symptom (mitochondrial encephalopathy)			
Pavlakis SG[14]	MELAS Mitochondrial encephalopathy, episodic nausea and vomiting, migrain-like headaches, stroke-like episodes, seizures, gray matter spongiosis	Hearing loss in about 30% of the cases	Heteroplasmic point mutation in tRNA gene, 3243
Goto Y[15]	Mitochondrial encephalopathy, lactic acidosis, stroke-like episodes, diabetes and deafness	Hearing loss	Heteroplasmic point mutation in tRNA gene, 3271
Shofner JM, Berkovic SF, Hammans S, Silvestri G, Zebiani M[16,21]	Myoclonic epilepsy and red ragged fibres, ataxia, dementia and optic atrophy (MERFF)	Hearing Loss	Heteroplasmic point mutation in tRNA gene, 8344,8356
Zeviani M, Poulton J[22,23]	Kearns Sayre syndrome, PEO and Pearson syndrome, Ptosis, retinopathy, heart block, Cerebellar syndrome, endocrinopathies, elevated CSF protein, white matter spongiosis	Hearing loss	Large heteroplasmic duplications and deletions in mt-DNA

MERRF, myoclonic epilepsy and ragged red fibres

Every cell contaınes hundreds of mıtochondrıa and every mıtochondrıon contaıns varıous copıes of mıtochondrıal DNA (Mt-DNA) Mıtochondrıal DNA ıs more sensıtıve to mutatıon than nuclear DNA Therefore, some Mt-DNA dıseases can arıse as a result of spontaneous mutatıon, ın addıtıon to the maternally transmıtted Mt-DNA dıseases A late onset ıs characterıstıc of these syndromes Hearıng ımpaırment often forms an addıtıonal problem, but deafness can also be the only expressıon of a syndrome[3 4] Table 2 gıves an overvıew of the currently known Mt-DNA medıated forms of hearıng loss

1 3 *Gene lınkage for non-syndromıc autosomal domınant ınherıted hearıng loss*

In a famıly wıth ten to twenty persons affected by an autosomal domınant hereditary anomaly, gene-lınkage studıes can be performed to determıne whıch part of a chromosome ıs responsıble for the genetıcally-medıated anomaly The posıtıon on the chromosome can be ısolated wıth ıncreasıng accuracy, because an ıncreasıng number of markers are becomıng avaılable for whıch the localısatıon on the chromosome ıs known wıth great accuracy Moreover, ıt ıs possıble to perform gene-lınkage studıes on multıple famılıes[5]

On the basıs of these ınvestıgatıon technıques, eleven dıfferent genotypes for autosomal domınant hereditary non-syndromıc forms of sensorıneural hearıng loss have been recognızed ın a perıod of almost 2 years (table 3)

Table 3 Non-syndromıc autosomal domınant ınherıted types of sensorıneural ımpaırment and theır gene lınkage

DFN	Lınkage	McKusıck catalogs	Gene cloned	Year	Author
Low frequency hearıng loss (DFN A1)	5q[31]	124 900	--	1992	(24)
A2	1p[32]	124 800	--	1994	(25)
A3	13q[12]	124 800	--	1994	(26)
A4	19p[13]	124 800	--	1995	(27)
A5	7p[15]	124 800	--	1995	(28)
A6	4p[16 3]	124 800	--	1995	(29)
A7	1q[21 23]	124 800	--	1995	(30)
A8	11q	124 800	--	1995	(31)
A9	14q[12 13]	124 800	--	1996	(32)
A10	6q[22 23]	124 800	--	1996	(33)
A11	11q[13 5]	124 800	--	1996	(43)
A12	11q[22 24]	--	-	--	(47)

In vıew of the great dıversıty of types that have been recognızed ın such a short perıod, ıt can be expected that over the next 10 years, several dozen genetıcally-

mediated forms of autosomal dominant inherited sensorineural hearing loss will be detected Owing to the fact that large families with autosomal dominant forms of hearing loss are relatively common in Western industrialized society, there should be very few obstacles in these developments

It can be expected along similar lines that it will even become possible to recognize genetically-mediated anomalies on a gene level[5 6] It will then become apparent which substances play a role in the physiology and pathophysiology of the mechanism of hearing It was recently discovered, for example, that myosin VIIA plays a role in the Usher syndrome type IB In addition, the clinical syndrome, i e the variation in severity and progression of hearing loss, can be accurately documented in families in whom earlier successful gene-linkage studies had been completed Before long, it can be expected that textbooks about hereditary non-syndromic forms of sensorineural hearing loss will have to be rewritten It is therefore necessary to document the level of hearing loss in relation to age and the variation in severity of the hearing loss within each family, so that we can recognize the specific clinical symptoms that represent each separate genetic type In the wake of this, our knowledge about the working mechanism of the inner ear will make great leaps forward

Similar developments are taking place in the non-syndromic autosomal recessive hereditary forms of sensorineural hearing loss and deafness (table 4)

Table 4 Non-syndromic autosomal recessive inherited types of sensorineural hearing impairment and their gene linkage

DFN	Linkage	McKusick catalogs	Gene cloned	Year	Author
B1	$13q^{12}$	220 700 and 220 800	--	1994	(34)
B2	$11q^{13\,5}$	220 700 and 220 800	--	1994	(35)
B3	$17p^{11\,2}q^{12}$	220 700 and 220 800	--	1995	(36)
B4	$7q^{31}$	220 700 and 220 800	--	1995	(37)
B5	$14q^{12}$	220 700 and 220 800	--	1995	(38)
B6	$3p^{14\,21}$	220 700 and 220 800	--	1995	(39)
B7	$9q^{13\,21}$	220 700 and 220 800	--	1995	(40)
B8	$21q^{22}$	220 700 and 220 800	--	1996	(41)
B9	$2p^{22\,23}$	220 700 and 220 800	--	1995	(42)
B10	$21q^{22\,3}$	220 700 and 220 800	--	1996	(44)
B11	$9q^{13\,21}$	--	--	--	(45)
B12	$10q^{21\,22}$	220 700 and 220 800	--	1996	(46)
B13	--	--	--		reserved
B14	--	--	--		reserved
B15	--	--	-		reserved

New techniques will soon make it possible to establish the cause of deafness in young children and the tests can also be used in the form of early diagnostics to

make causative genetic diagnoses Owing to the fact that large isolates with hereditary autosomal recessive hearing loss are fairly scarce in Western industrialized society compared to other parts of the world, the detection of these gene links and the identification of the genes involved will be more difficult and time-consuming in Western industrialized society The above indicates clearly that before too long, new genetic investigation techniques will enable us to distinguish between forms of sensorineural hearing loss that could not be distinguished in the past

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1.3

NIET-SYNDROMAAL GEHOORVERLIES. VAN ENKELE FENOTYPEN NAAR VELE GENOTYPEN

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SAMENVATTING

Meer dan 60 procent van de gevallen van ernstige, vroeg beginnende slechthorendheid is genetisch bepaald, meestal op basis van autosomaal recessieve genmutaties Aangezien het zowel voor de NKO-arts als voor de algemene arts belangrijk is een erfelijke vorm van gehoorverlies te onderkennen, wordt een overzicht van de erfelijke vormen van niet-syndromaal gehoorverlies, met name de autosomaal dominante, de autosomaal recessieve, de X-gebonden en de mitochondriele, gegeven
De genetica heeft in de voorbije jaren een enorme vooruitgang gemaakt in het koppelen van genen en het opsporen van mitochondriele gendefecten die niet-syndromaal gehoorverlies veroorzaken Zo werden reeds 13 genen gelokaliseerd die elk verantwoordelijk zijn voor een vorm van autosomaal dominant niet-syndromaal progressief gehoorverlies en 15 die een autosomaal recessief niet-syndromaal gehoorverlies veroorzaken
Verwacht wordt dat men in de komende jaren nog meer genen zal lokaliseren en de genen, dus ook hun produkten, zal kunnen herkennen Op die manier zal enerzijds de kennis over de werking van het binnenoor en anderzijds de mogelijkheid genetisch advies te geven een grote vooruitgang maken

INLEIDING

Binnenoorslechthorendheid treft bij benadering 1 persoon op 2 vanaf de leeftijd van tachtig jaar en ongeveer 1 op 750 kinderen Doofheid en slechthorendheid betekenen in onze sterk communicatieve maatschappij een grote belemmering om goed te kunnen functioneren Daarom is het belangrijk om tijdig een goede diagnose te stellen en de patient op optimale wijze te begeleiden, zowel via aangepast onderwijs als bij de orientatie van de beroepskeuze
Meer dan zestig procent van de gevallen van ernstige vroeg beginnende slechthorendheid wordt veroorzaakt door genetische factoren, meestal autosomaal recessieve mutaties van eén enkel gen Autosomaal dominant overervende binnenoorslechthorendheid komt daarentegen vooral op volwassen en oudere leeftijd voor
Naast het vaststellen van de slechthorendheid is het tevens de taak van de arts de erfelijke factor te onderkennen, om op die manier optimale genetische informatie te kunnen geven Hiervoor is het belangrijk een goed inzicht te hebben in en kennis van de erfelijke vormen van gehoorverlies Daar het onderwerp "erfelijke

doofheid" zeer uitgebreid is, beperkt deze bijdrage zich tot een bespreking van de niet-syndromale vormen van erfelijk gehoorverlies

AUTOSOMAAL DOMINANT EN RECESSIEF OVERERVEND NIET-SYNDROMAAL GEHOORVERLIES

Tot voor kort kon men de verschillende vormen van autosomaal dominant overervende slechthorendheid enkel van elkaar onderscheiden op basis van het toonaudiogram[22] Afhankelijk van het feit of het gehoorverlies het meest uitgesproken was in de lage tonen, het middengebied of de hoge tonen, was er sprake van een lage tonen, een komvormig of een hoge tonen progressieve slechthorendheid Kennelijk is de mogelijkheid dat ook een vlak progressief perceptieverlies autosomaal dominant overerfbaar kan zijn, over het hoofd gezien Op grond van het tijdstip van intreden werden bij deze indeling nog afzonderlijke types onderscheiden In tabel 1 worden deze op klinische gronden onderscheiden vormen van binnenoorslechthorendheid opgesomd[22] De mate van progressie van slechthorendheid en de relaties tussen de ernst van het gehoorverlies en de leeftijd is over decennia gedetailleerd bestudeerd bij een grote Nederlandse familie met een progressieve binnenoorslechthorendheid[28 30 63 64] Het was tot voor kort onzeker of deze in tabel 1 voorgestelde indeling, die louter op klinische gegevens gesteund is, stand zou houden zodra nieuwe genetische kennis ter beschikking zou komen

Autosomaal recessief overervende niet-syndromale slechthorendheid begint meestal op zeer jonge leeftijd en is meestal ernstiger dan de autosomaal dominante vorm

Tabel 1 Klinisch onderscheiden autosomaal dominant overervende vormen van gehoorverlies (cf Gorlin et al 1995)[22]

1	Autosomaal dominant congenitaal ernstig gehoorverlies
2	Autosomaal dominant congenitaal sensorineural gehoorverlies voor de lage frequenties
3	Autosomaal dominant progressief gehoorverlies in de lage frequenties
4	Autosomaal dominant progressief gehoorverlies in de middenfrequenties
5	Autosomaal dominant progressief gehoorverlies in de hoge frequenties
6	Autosomaal dominant progressief gemengd gehoorverlies
7	Autosomaal dominant unilateraal sensorineural gehoorverlies
8	Autosomaal dominant progressieve vestibulo-cochleaire dysfunctie met sensorineuraal gehoorverlies

Het is in de meeste gevallen moeilijk na te gaan of de slechthorendheid progressief is, daar het gehoor meestal reeds zeer slecht is op de leeftijd waarop een betrouwbaar audiogram kan worden afgenomen Daarom werd in het verleden ook geen onderscheid gemaakt tussen verschillende fenotypen, zoals wel gebeurde bij de autosomaal dominante vormen

X-GEBONDEN NIET-SYNDROMAAL OVERERVEND GEHOORVERLIES

X-gebonden doofheid is een zeldzame vorm van erfelijke doofheid, die instaat voor ongeveer 1,7% van alle erfelijke vormen van slechthorendheid Van de X-dominante slechthorendheid zijn enkel syndromale vormen, zoals het syndroom van Alport, gekend In de groep van de X-recessieve doofheid komen wel niet-syndromale vormen voor Deze laatste werden in het verleden op basis van het audiogram ingedeeld in vier groepen een congenitaal sensorineuraal type, een vroeg beginnend sensorineuraal type, een gemengd gehoorverlies met fixatie van de voetplaat van de stapes die de oorzaak is van de perilymfatische 'gusher" bij heelkunde, en een progressief sensorineuraal type[46] Ook hier was onzeker of deze fenotypische indeling door het genetisch onderzoek bevestigd zou worden

MITOCHONDRIEEL NIET-SYNDROMAAL OVERERVEND GEHOORVERLIES

Recent wordt ook veel aandacht besteed aan de mitochondriele overervingswijze Mitochondrien, die hun eigen DNA bevatten, worden uitsluitend door de moeder doorgegeven aan het nageslacht via het cytoplasma van de eicel Door het dominante karakter van de overerving kan men in eerste instantie de indruk hebben dat het hier gaat om een autosomaal dominant beeld Dat de slechthorendheid alleen door de moeder wordt doorgegeven, waarbij in principe elk kind van een slechthorende moeder de afwijking heeft, kan hier dan helpen om de juiste diagnose te stellen Gehoorproblemen kunnen de enige uiting zijn van het ziektebeeld, maar meestal is de slechthorendheid een additioneel probleem De door aminoglycoside-ototoxiciteit geinduceerde doofheid, zoals bij streptomycine-inname aan een normale dosis, is een bekend voorbeeld van mitochondrieel bepaalde doofheid zonder andere afwijkingen[27 44] Hoewel buiten het bestek van dit artikel, lijkt het zinvol de combinatie van doofheid met diabetes mellitus, die optreedt op latere leeftijd en onder andere werd beschreven in een Nederlandse familie[58], te vermelden

Tabel 2: Mitochondrieel bepaalde vormen van gehoorverlies

a Mt DNA mutaties specifiek geassocieerd met gehoorverlies als hoofdsymptoom

Auteur	Klinisch beeld	Gehoorverlies	Mt DNA mutatie
Hu et al[27] Prezant et al 1993[44]	Hoge gevoeligheid voor antibiotische ototoxiciteit amino glycoside (streptomycine) gerelateerde doofheid	Bilaterale ernstige doofheid	12SrRNA A1555G
Jaber et al 1992[31] Prezant et al 1993[44]	Progressief gehoorverlies beginnend op kinderleeftijd	Ernstig bilateraal gehoorverlies zonder bijkomende symptomen	12SrRNA A1555G
Vernham et al 1994[59] Reid en Vernham 1994[47]	Progressief gehoorverlies	Variabel gehoorverlies vooral in de hoge frequenties	tRNA-ser(UCN) T7445C
Fischel Ghodsian et al 1995[18]	Aanvang tussen 3 en 18 jaar		tRNA ser(UCN) T7445C

b Mt DNA-mutaties met gehoorverlies als bijkomend symptoom

Auteur	Klinisch beeld	Gehoorverlies	Mt DNA-mutatie
Pavlakis et al 1984[42]	MELAS Mitochondriele encefalopathie, episodische nausea en braken, migraineachtige hoofdpijn episodische beroertes, stuipen spongiosis van de grijze stof	Gehoorverlies in ongeveer 30% van de gevallen	Heteroplasmische puntmutatie in tRNA-gen A3243G
Van den Ouweland et al 1992[58] Atawa et al 1992[3] Katagiri et al 1994[33] Odawara et al 1995[39] Alcolado et al 1995[2] Vialettes et al 1995[62] Aitman TJ 1995[1] Oshima et al 1996[41] Yamasoba et al 1996[65]	Niet insuline dependente diabetes mellitus	Gehoorverlies	Puntmutatie in tRNA-gen 3243
Goto et al 1991[23]	Mitochondriele encefalopathie lataatacidose, episodische beroertes, diabetes en doofheid	Gehoorverlies	Heteroplasmische puntmutatie in tRNA gen 3271
Shoffner et al 1990[49] Berkovic et al 1991[5 6] Hammans 1994[26] Silvestri et al 1992[50] Zeviani et al 1993[67]	Myoklonische epilepsie en red ragged fibers ataxie dementie en opticus atrofie (MERRF)	Gehoorverlies	Heteroplasmische puntmutatie in tRNA genen A8344G en A8356G
Zeviani et al 1989[66]	Kearns-Sayre syndroom PEO en Pearson syndroom	Gehoorverlies	Grote heteroplasmische duplicaties en deleties in mt DNA
Poulton et al 1989[43]	Ptosis retinopathie hartblok cerebellair syndroom endocrinopathieen, verhoogd CSV-proteine met spongiose van de witte stof	Gehoorverlies	
Tiranti et al 1952[52] Ensink et al 1996[17]	Bilateraal gehoorverlies, ataxie en myoclonus	Matig tot ernstig gehoorverlies	tRNA-ser(UCN)7472insC

In tabelvorm wordt een overzicht gegeven van mitochondrieel overervende vormen van gehoorverlies, waarbij een onderscheid gemaakt wordt tussen gehoorverlies als hoofdkenmerk en gehoorverlies als geassocieerd kenmerk (tabel 2). Deze laatste groep kan beschouwd worden als een vorm van syndromaal gehoorverlies, maar wordt toch vermeld om een duidelijker overzicht te geven van deze groep genetische afwijkingen.

KOPPELINGSONDERZOEK BIJ NIET-SYNDROMAAL ERFELIJK GEHOORVERLIES

De voorbije jaren heeft de genetica een enorme vooruitgang gemaakt op het gebied van niet-syndromale erfelijke slechthorendheid.
Met behulp van klinisch genetisch onderzoek van grote families met één bepaalde vorm van gehoorverlies kan men aan de hand van genkoppelingsonderzoek de meest waarschijnlijke plaats van het gen verantwoordelijk voor het gehoorverlies bepalen. Met deze methode zijn de genen voor enige tientallen vormen van syndromale doofheid geïdentificeerd[12,13].
Ook op het gebied van de niet-syndromale doofheid zijn in een periode van twee jaar reeds dertien verschillende genotypen herkend voor autosomaal dominant niet-syndromaal bepaalde vormen van gehoorverlies. Deze verschillende vormen worden aangeduid met de notatie DFNA, gevolgd door een cijfer (tabel 3).

Tabel 3: Genkoppelingsresultaten bij niet-syndromaal autosomaal dominant overervend gehoorverlies.

	Koppeling	Gen	Jaar	Auteur
DFNA1	5q31	onbekend	1992	Leon et al.1992[36]
DFNA2	1p34	onbekend	1994	Coucke et al. 1994[14]
DFNA3	13q12	onbekend	1994	Chaïb et al. 1994[8]
DFNA4	19p13	onbekend	1995	Chen et al. 1995[11]
DFNA5	7p15	onbekend	1995	Van Camp et al. 1995[57]
DFNA6	4p16.3	onbekend	1995	Lesperance et al. 1995[37]
DFNA7	1q21-23	onbekend	1995	Tranebjaerg et al. 1995[54]
DFNA8	11q	onbekend	1996	Kirschhofer et al. 1996[34]
DFNA9	14q12-13	onbekend	1996	Manolis et al. 1996[38]
DFNA10	6q22-23	onbekend	1996	O'Neill et al. 1996[40]
DFNA11	11q13.5	onbekend	1996	Tamagawa et al. 1996[51]
DFNA12	11q22-24	onbekend	1996	Verhoeven et al 1996[60]
DFNA13	6p21	onbekend	1997	University of Iowa

Voor de autosomaal recessieve niet-syndromale slechthorendheid heeft men vijftien genen gelokaliseerd, die naar analogie met de dominante vormen worden aangeduid met de notatie DFNB, gevolgd door een cijfer (tabel 4)
Ten slotte werden ook bij de X-recessieve vormen van niet-syndromaal gehoorverlies reeds acht verschillende genotypen onderkend Deze worden aangeduid met de notatie DFN, gevolg door een cijfer (tabel 5) Enkel van DFNB2 en DFN3 is het verantwoordelijke gen reeds geïdentificeerd

Tabel 4 Genkoppelingsresultaten bij niet-syndromaal autosomaal recessief overervend gehoorverlies

	Koppeling	Gen	Jaar	Auteur
DFNB1	13q12	onbekend	1994	Guilford et al 1994a[24]
DFNB2	11q13 5	onbekend	1994	Guilford et al 1994b[25]
DFNB3	17p11 2-q12	onbekend	1995	Friedmann et al 1995[19]
DFNB4	7q31	onbekend	1995	Baldwin et al 1995[4]
DFNB5	14q12	onbekend	1995	Fukushima et al 1995a[20]
DFNB6	3p14-p21	onbekend	1995	Fukushima et al 1995b[21]
DFNB7	9q13-q21	onbekend	1995	Jain et al 1995[32]
DFNB8	21q22	onbekend	1996	Veske et al 1996[61]
DFNB9	2p22-p23	onbekend	1996	Chaib et al 1996a[9]
DFNB10	21q22 3	onbekend	1996	Bonne-Tamir et al 1996[7]
DFNB11	9q13-q21	onbekend	1996	Scott et al 1996[48]
DFNB12	10q21-22	onbekend	1996	Chaib et al 1996b[10]
DFNB13	gereserveerd			
DFNB14	gereserveerd			
DFNB15	3q21-q25 of 19p13	onbekend	1997	University of Iowa

Tabel 5 Genkoppelingsresultaten bij niet-syndromaal X-gebonden recessief gehoorverlies

	Koppeling	Gen	Jaar	Auteur
DFN1	Xq22	onbekend	1995	Tranebjaerg et al 1995[55*]
DFN2	Xq22	onbekend	1996	Tyson et al [56]
DFN3	Xq21 1	POU3F4	1995	De Kok et al 1995[15]
DFN4	Xq21 2	onbekend	1994	Lalwani et al 1994[35]
DFN5	gereserveerd			
DFN6	Xp22	onbekend	1996	del Castillo et al 199616
DFN7	gereserveerd			
DFN8	gereserveerd	onbekend		

*Deze vorm van gehoorverlies werd oorspronkelijk als niet-syndromaal beschouwd, maar bij nader familieonderzoek bleek het hier te gaan om een syndromale vorm[53]

Rekening houdend met deze grote verscheidenheid die op zo korte tijd aan het licht kwam, mag men verwachten dat in de komende jaren nog meer enkel genetisch te onderscheiden vormen van niet-syndromaal erfelijk gehoorverlies zullen worden ontdekt Omdat grote families met autosomaal dominante vormen van slechthorendheid relatief gemakkelijk beschikbaar zijn in de westerse geïndustrialiseerde samenleving, zal dit ook geen remming betekenen voor die ontwikkelingen Voor koppelingsonderzoek voor een recessief overervend gehoorverlies is het nodig grotere families met meerdere afzonderlijke consanguine huwelijken met slechthorende of dove kinderen te onderzoeken Die vindt men vooral in religieuze of geografisch bepaalde isolaten, waar consanguiniteit frequenter is Vooral in islamitische gemeenschappen is er sprake van een zeer hoge consanguiniteitsratio Succesvol koppelingsonderzoek voor autosomaal recessief overervend gehoorverlies heeft tot nu toe alleen plaatsgehad onder deze islamitische bevolkingsgroepen buiten Europa en is nadien bevestigd in Europa bij geïmmigreerde islamitische bevolkingsgroepen In Europa bekende geografische isolaten met daarin veelvuldig voorkomend autosomaal recessief overervende doofheid en slechthorendheid zijn tot nu toe onvoldoende onderzocht Voor deze erfgang zullen meer genafwijkingen herkend worden[12 13] Op die manier kan men meer kennis verwerven over de genproducten die in de fysiologie en de pathofysiologie van het gehoororgaan een rol blijken te spelen, waardoor de kennis over het werkingsmechanisme van het binnenoor grote stappen voorwaarts zal maken

Anderzijds zal het klinische ziektebeeld, hier de variatie in ernst en progressie van het gehoorverlies, nauwkeurig bepaald kunnen worden in deze families bij wie eerder succesvol een genkoppelingsstudie werd afgerond Voor de vorm DFNA5, gelokaliseerd op 7p15 zijn eerder over decennia verspreid bij een zeer grote Nederlandse familie studies over de ernst van het gehoorverlies in verhouding tot de leeftijd en zo over de mate van progressie van het gehoorverlies verricht[28 29 30 57 63 64] Bij Indonesische, Vlaamse, Amerikaanse en Nederlandse families met DFNA2 zijn soortgelijke klinische studies naar de ernst en het verloop van het gehoorverlies uitgevoerd Afzonderlijke publicaties hierover zijn in druk

Voor de andere DFNA-vormen is binnen deze families nog maar weinig bekend over het klinisch beloop van het gehoorverlies Omwille van de voorlichting zullen dergelijke studies ook nodig zijn voor de andere erfelijke vormen van autosomaal dominant overervend gehoorverlies

De vraag die zich nu stelt, is of de ernst en de progressie van het gehoorverlies in families met eenzelfde genotype zullen overeenkomen en of er opmerkelijke

onderlinge verschillen zullen blijken te bestaan. Zo zal dan ook duidelijk worden of de vele genotypen op grond van hun fenotype onderscheiden kunnen worden. Daarvoor zijn wel nauwkeurige klinische beschrijvingen vereist van de ernst van het gehoorverlies in verhouding tot de leeftijd, van de vorm van het audiogram en van de variatie in expressie van het gehoorverlies binnen één familie en tussen verschillende families onderling. Zo zal het klinisch beeld passend bij elk afzonderlijk genetisch type herkend kunnen worden. Deze informatie kan van betekenis zijn voor jonge aangedane familieleden, ondermeer met betrekking tot scholing en beroepskeuze. Een andere toepassing geldt bij personen met een autosomaal dominant overervend gehoorverlies, bij wie men op grond van een te voorspellen progressie van het gehoorverlies een betere invaliditeitsschatting zal kunnen maken.

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Chapter II

The Branchio-Oto-Renal syndrome

2.1

THE BRANCHIO-OTO-RENAL SYNDROME

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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 or 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 or 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 summarized 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%)

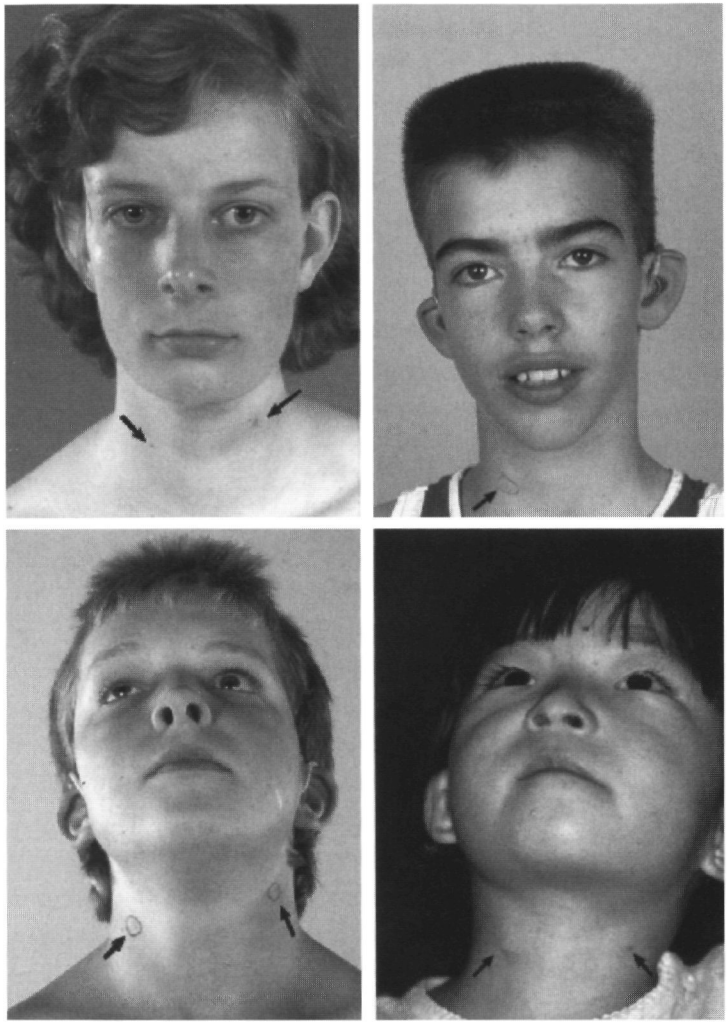

Figure 1: Pictures of typical clinical features in different BOR patients

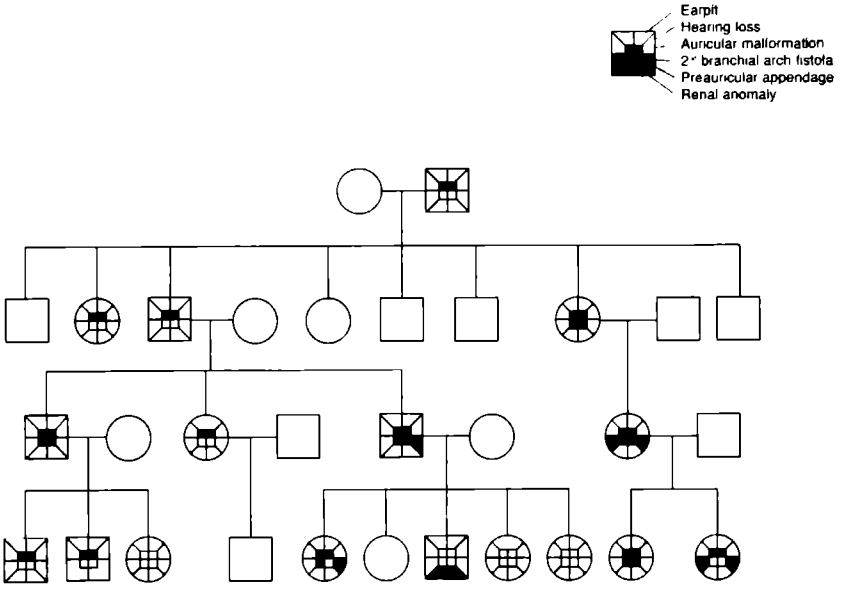

Figure 2: Example of a pedigree of a BOR family (with courtesy of Stinckens et al[6])

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 characterized by great variability, ranging from asymptomatic minor deformities to severe dysplastic kidneys or even kidney agenesis[3-5,10,11]. The expression of important 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].

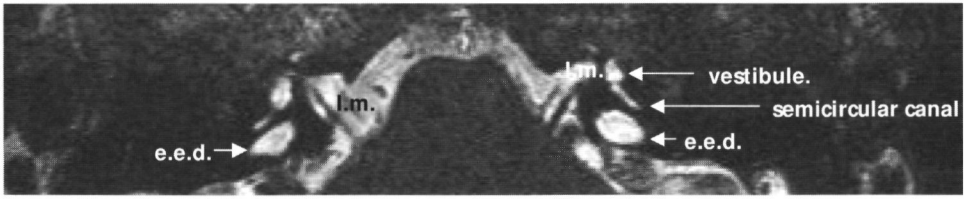

Figure 3: High resolution (CISS) heavily T2 weighted MR image of the temporal bone at the level of the internal meatus (l.m.). Typical example of the enlarged endolymphatic duct (e.e.d.) on both sides.

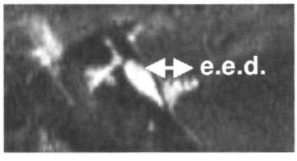

Figure 4: Multiplanar reformatted image (MPR) of the same patient (Figure 3). Semisagittal plane through the endolymphatic duct (e.e.d.) on the left side showing the course of this duct in the longitudinal direction.

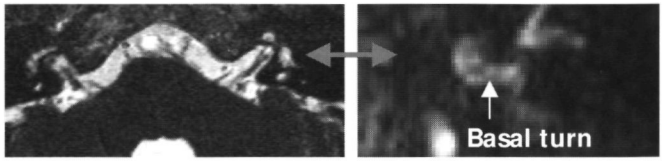

Figure 5: Another typical sign is the hypoplastic cochlea as shown here by an MPR image of an affected cochlea. Image in the axial plane at the level of the internal meatus and apex of the cochlea. Semicoronal section through the turns of the cochlea shows only 1 complete turn and no middle and apical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

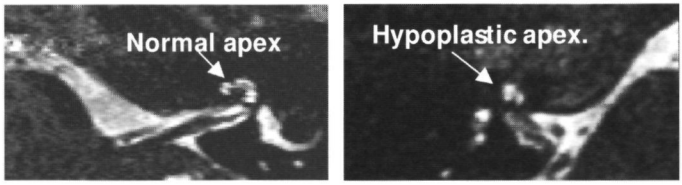

Figure 6: For comparison a normally developed cochlea (left) and an example of an affected cochlea (right) showing absent apical turns in the axial plane.

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).

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

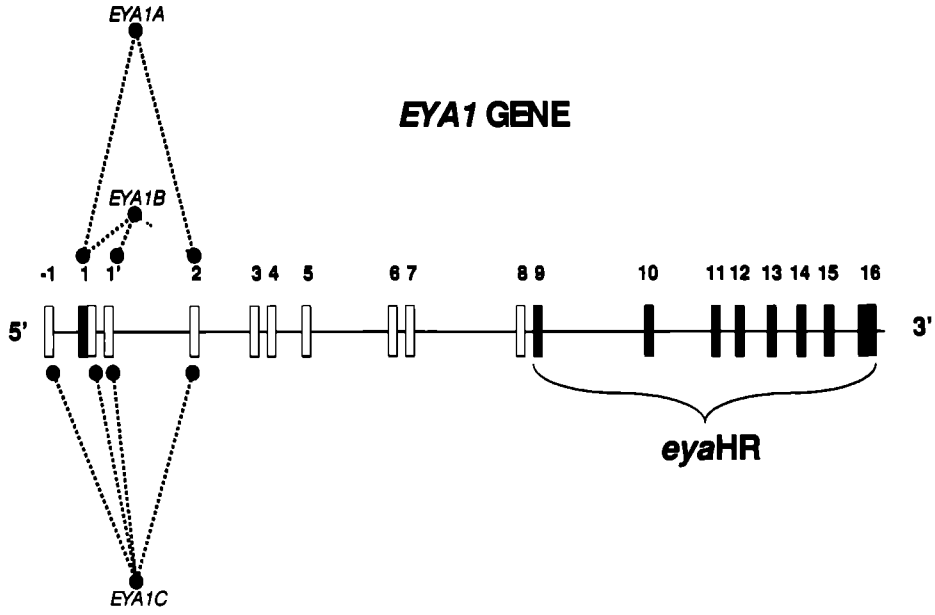

Figure 7 Schematic representation of the *EYA1* gene structure (unscaled) All boxes are coding exons except for the black-filled boxes The grey-filled boxes indicate the *eya*-homologous region The dotted lines indicate how the different isoforms (*EYA1A, EYA1B and EYA1C*) are built up

GENETICS

The *EYA1* gene (OMIM #601653) has been found to underlie the BOR syndrome[19] This is the human homologue of the drosophila 'eyes absent' gene

one (*eya1*) and is localized 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

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 recognized 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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2.2

THE PRESENCE OF A WIDENED VESTIBULAR AQUEDUCT AND PROGRESSIVE SENSORINEURAL HEARING LOSS IN THE BOR SYNDROME. A FAMILY STUDY

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ABSTRACT

Objective A new large family with the BOR syndrome is reported with special reference to the presence of a widened vestibular aqueduct and a progressive sensorineural component in the mixed hearing loss A review of the BOR literature of 184 patients is given

Setting University Hospitals

Results A BOR family with 17 affected members was studied Fourteen of those were still alive and 12 of those cooperated in this clinical study Detailed radiological studies showed in three out of 12 affected family members a widened vestibular aqueduct and progressive sensorineural hearing loss This raises the question whether there is a true correlation or whether those are coincidental

Conclusions In our family with the Branchio-Oto-Renal syndrome, a widened vestibular aqueduct and progressive hearing loss is found in a few affected family members Imaging of the temporal bones and long-term audiometric follow-up could help to reveal whether the widened vestibular aqueduct is the cause for the progressive hearing loss

INTRODUCTION

The Branchio-Oto-Renal (BOR) syndrome (McK No 601653)[1] has an autosomal dominant pattern of inheritance Main features are slight malformation of the auricles, preauricular sinuses, hearing loss, branchiogenic cervical fistulas of the second branchical arch and renal dysplasia Penetrance of the disease is almost totally complete, but expression of the symptoms varies

Particularly the severity of the hearing loss and renal abnormalities varies[1 8] Hearing loss can be of the conductive, mixed or sensorineural type

Hypoplasia of the cochlea has been demonstrated histologically and radiologically[4 9 52] A widened vestibular aqueduct has been described after histological examination[9] and after radiological examination[8 10] A review of the literature showed progression of the sensorineural component of the hearing loss in a few cases[3 11 14] Reconstructive surgery for the congenital conductive component is possible, but it is generally difficult to achieve satisfactory results[5 15 16] Prevalence of the BOR syndrome is estimated to be 1 40000 The BOR gene is *EYA1*, which lies on 8q13 3[17 19] The BOR syndrome is genetically and clinically different from a very similar branchiogenic syndrome This syndrome is not linked to the EYA1-locus[20 21]

To see whether the sensorineural component of the hearing loss in the BOR syndrome was progressive and whether this was associated with a widened vestibular aqueduct, we investigated a new family with this syndrome; there were 17 affected family members Special attention was paid to long-term audiometric follow-up and to the results of CT scanning and MRI of the temporal bones, especially the presence of an enlarged vestibular aqueduct.

PATIENTS AND METHODS

As part of an etiological evaluation of the causes of deafness in patients at the Royal Institute Spermalie for the deaf and hard of hearing in Brughes, Belgium, a family was encountered with the BOR syndrome. A pedigree (figure 1) of the family was drawn and the different branches of the family were contacted. Permission was obtained to perform a clinical-genetic study, including clinical evaluation of the typical BOR features, audiometry, CT scanning or MRI of the temporal bones. To evaluate the size of the vestibular aqueduct radiologically, we used the criterion that the width of the vestibular aqueduct must be smaller than the diameter of the posterior semicircular canal in the axial plane to be considered as normal. Previous audiograms were traced. Blood samples were taken from eight affected family members (figure 1) and some others for gene linkage and mutation analysis[19]. Linkage analysis was performed on a personal computer using the LINKAGE computer package (versions 5.1).

Two-point linkage analysis was performed using the FASTLINK version 3.0 (Cottingham et al., 1993) and LOD scores were calculated using a model of autosomal dominant mode of inheritance. The marker allele frequency was assumed to be uniformly distributed. Mutation analysis of the *EYA1* gene was not yet successful. To evaluate individual progression in hearing impairment, we followed the methode described by Cremers et al.[48]. We used the commercial programme Prism, version 2.0 (GraphPad, San Diego, USA) for non-linear fitting. In the regression equation, the hyperbolic quation $T = T_{max}/t_{0.5} + t)$ was used, where T is the threshold (db HL), t_{max} is the asymptotic saturation threshold, t is age (in years) and $t_{0.5}$ is 'half-value' age. Results of previous renal function tests were reviewed. Family members who had been tested before as well as those who had not did not agree to undergo additional renal tests

The literature on the BOR syndrome was reviewed thoroughly regarding the stability and the severity of the hearing impairment[2-4 7 10 15 16 22 46 56].

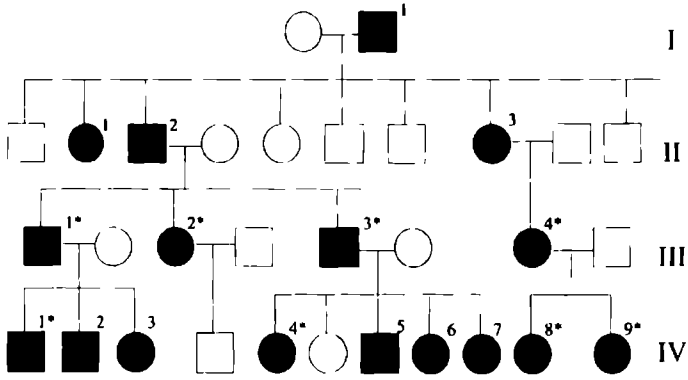

Figure 1: Pedigree of the family; □ male, ○ female; ●■ affected members; * included in the study

RESULTS

A four generation pedigree is shown in figure 1. Fourteen out of the 17 affected members are still alive. Case IV-5 died 8 h after birth as result of renal failure. The syndromal features of these 13 affected persons are presented in table 1. The frequency of the main features in this family is in agreement with the data obtained from a personal review of 184 BOR patients from the literature (table 2).

Preliminary gene linkage results showed a positive lod score of 1.07 for marker D8S286. This is suggestive for linkage to 8q.

The hearing losses of the 12 affected family members are presented in more detail in fgure 2.

The binaural median air conduction threshold (0.5, 1 and 2 kHz, most recent measurements) was 100 dB and the range was 75-120 dB. A review of the literature concerning the degree of hearing impairment in 82 BOR patients was performed. We retrieved the air conduction thresholds (dB HL), bone conduction thresholds and air bone gaps.

It appeared that the mean (i.e. averaging 0.5, 1 and 2 kHz in each ear, separately) air and bone conduction thresholds as well as the air bone gap, showed normal distributions, i.e. no significant deviation from a normal distribution in appropriate tests (Prism 2.0). Owing to the fact that many air conduction thresholds were off the scale, we selected the median threshold as the key parameter. Median values were 50 dB for air conduction, 30 dB for bone conduction and 20 dB for the air bone gap. The ranges were 20 dB to off the scale for the air conduction thresholds, 0-65 dB for thee bone conduction thresholds and 0-55 dB for the air bone gaps.

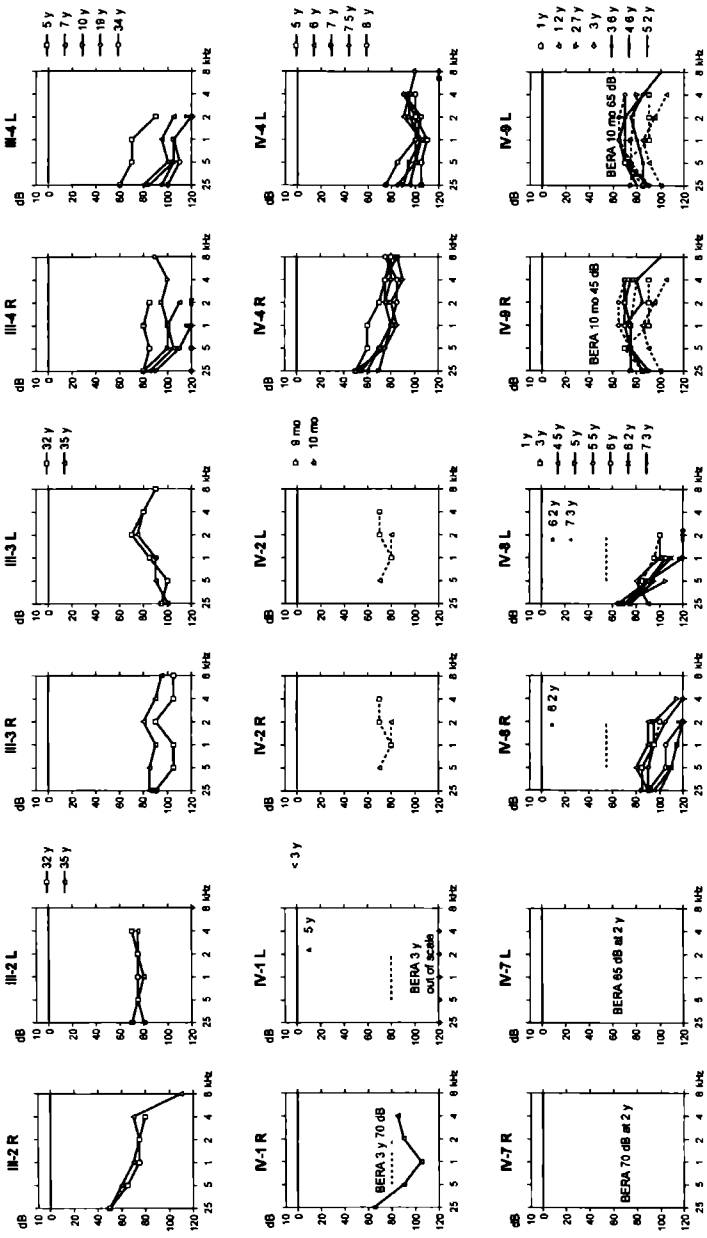

Figure 2. Serial audiograms (R, right ear, L, left ear; air conduction threshold in dB HL) of nine affected members of this family. Age in years (y) or months (mo); see the keys to each panel. Dashed lines related to free-field measurements. Filled symbols mark off the scale thresholds; BERA, brainstem evoked response audiometry.

Table 1 Clinical features of the BOR syndrome in a new family

Case	Malformed auricles	Preauricular sinus	Second branchial arch fistula	Hearing impairment	Renal anomaly
III-1	-	+	+	+	?
III-2	-	+	+	+	?
III-3	-	+	+	+	-
III-4	-	+	+	+	-
IV-1	-	+	-	+	-
IV-2	-	+	-	+	?
IV-3	-	+	+	+	-?
IV-4	-	+	+	+	-
IV-5	?	?	?	?	+
IV-6	-	+	-	+	-
IV-7	-	+	-	+	?
IV-8	-	+	+	+	?
IV-9	-	+	+	+	?

Table 2 Frequency of the main features in the BOR syndrome based on a review of 184 cases from the literature

	Reported presence or 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%)

The average air conduction threshold in the present family was clearly much poorer than that derived from earlier reported cases It can not be excluded that the average hearing threshold in the present family will increase as the subjects grow older, presumably, most of our patients were younger than the earlier reported ones

Follow-up audiometry in patients III-4 and IV-8 showed progressive hearing loss (figure 2 and 3) In case IV-8 progressive hearing loss was noted between 3 and 7 years of age Free field audiometry at 12 months suggested only 50-60 dB hearing loss In case III-4, progressive hearing loss was noted between 5 and 34 years of age. In case IV-1 brain stem audiometry performed at age 3 years showed a hearing level of 70 dB in the right ear and no response from the left ear Pure tone audiometry at age 5 years confirmed the lack of response in the left ear and 95 dB hearing level in the right ear These findings are suggestive of progressive hearing

loss We were able to trace the previous audiograms of the other subjects Their follow-up varied from 1 to 6 years (III-2, III-3 IV-1, IV-2, IV-4, IV-9) These data reflected stability in their hearing impairment Several affected members of this family (nos III-4, IV-8, IV-9, III-3, IV-4) mentioned feeling unsteady in a dark environment They were also relatively late to start walking at 22 months in the most delayed of them This is suggestive for vestibular areflexia Two family members (III-3, IV-4) agreed to have this confirmed by vestibular testing with a rotating chair and electronystagmography

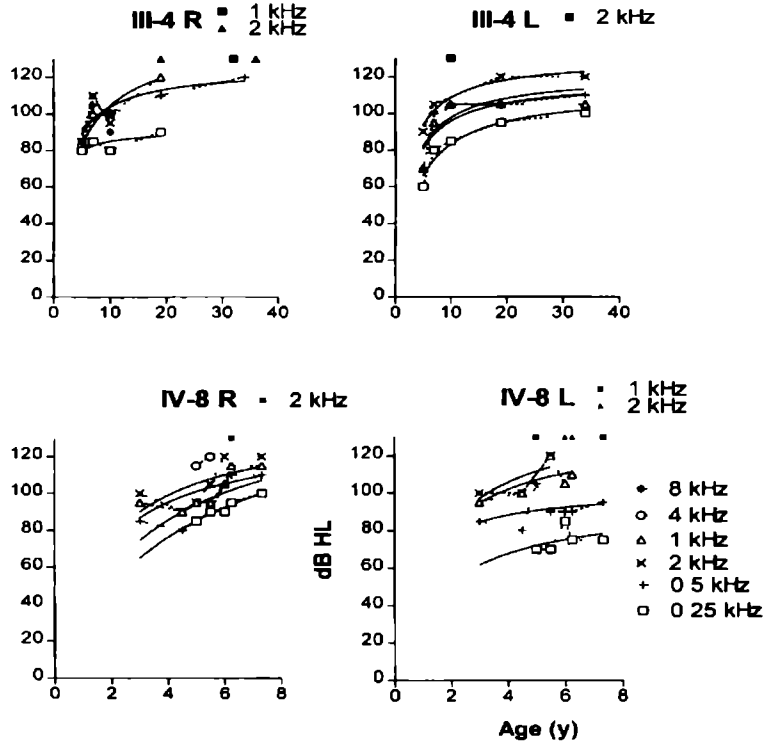

Figure 3 Threshold against age plots for two apparently progressive cases (III-4 and IV-8 in the pedigree of figure 1) Longitudinal measurements of air conduction threshold (dB HL) are interconnected by dashed lines The continuous lines pertain to fitted hyperbolic curves (see Section 2) The fitted T_{max} values were in the range of 90-150 dB, increasing with the frequency, the fitted $t_{0.5}$ values were in the range of 1-4 year, independent of frequency In case IV-8 at 0 25 Hz an outlying value of 90 dB (R,L) was excluded from the analysis to avoid erratic fitting of the hyperbolic function Filled symbols relate to out-of-scale measurements (in-panel symbol keys)

There was no spontaneous nystagmus. Caloric testing showed no response in patient III and some response (hyporeflexia) bilaterally in patient IV-4. In patient IV-7 epilepsy was reported at the age of 2 months. At the age of 4 months, hydrocephalus was diagnosed. Antiepileptic medication could be stopped at the age of 2 years.

Renal tests were studied in six cases (III-4, IV-1, III-3, IV-4, IV-6, IV-7). In case IV-5, autopsy was performed on the day of birth. Bilateral hydronephrosis and cystic kidneys were found. Intravenous Pyelography (IVP) had been performed in two out of the six cases (III-4, IV-1). Ultrasound examinations of the kidneys had been performed in five out of the six cases (III-4, III-3, IV-4, IV-6, IV-7). In all these cases, IVP and ultrasound of the kidneys were reported to be normal.

The results of CT scanning and MRI of the temporal bones in eight patients (III-1, III-2, III-3, III-4, IV-1, IV-4, IV-8, IV-9) out of the 12 affected family members are presented in table 3.

Table 3: CT and MRI findings of the temporal bones in eight BOR patients.

Patient	Hypoplasia cochlea	Widened vestibular aqueduct
III-1	+	-
III-2	+	-
III-3	+	-
III-4	+	+
IV-1	+	+
IV-4	+	-
IV-8	+	+
IV-9	+	-

Excluded were cases IV-2, IV-3, IV-6, IV-7 mainly because of their age and because it was inconvenient for them to be investigated. A widened vestibular aqueduct and an enlarged endolymphatic duct and sac (figures 4) were seen in three cases (III-4, IV-1, IV-8).

Case III-4 and IV-1 were only examined by CT-scanning. Case III-4 presented a bilateral and Case IV-1 a unilateral (right) enlarged vestibular aqueduct.

Case IV-8, who was examined by CT and MRI scanning, presented on CT a clearly enlarged right vestibular aqueduct. The width of the left vestibular aqueduct was the same as this of the posterior semicircular canal. On MRI on the other hand, an enlarged endolymphatic duct and sac were diagnosed, which was not convincing on CT. It is remarkable that the audiometric data of these three cases showed a progressive hearing loss, since this finding is very unusual in the literature.

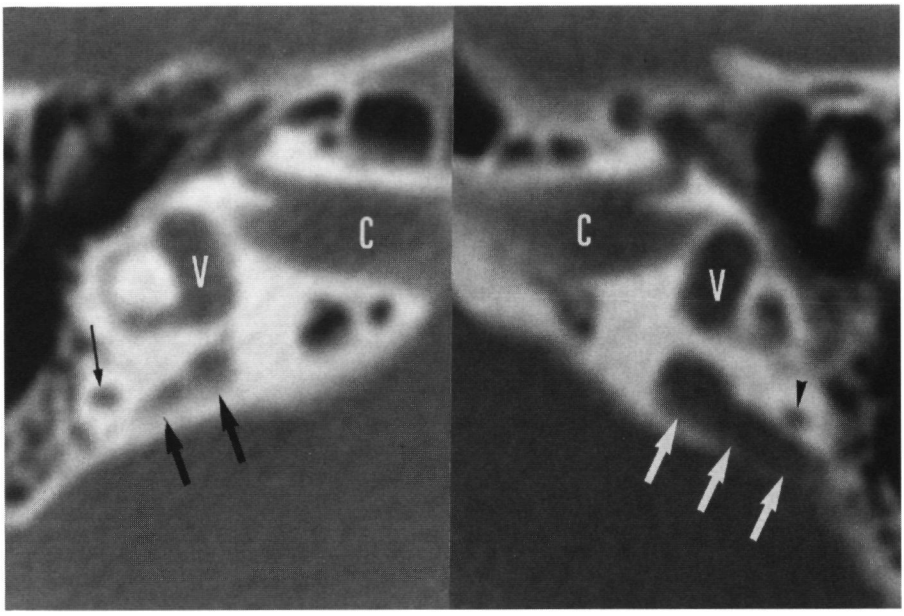

Figure 4: Patient III-4, bilateral large vestibular aqueduct. Axial CT image made at the level of the right and left vestibule. The right (large black arrows) and especially the left (large white arrows) vestibular aqueduct is enlarged and is much wider than the right (long black arrow) and left (black arrowhead) posterior semicircular canal. Normally the width of the vestibular aqueduct is smaller than the diameter of the posterior semicircular canal in the axial plane. Vestibule (V), internal auditory canal C.

DISCUSSION

A new BOR-family was studied in detail showing for this syndrome the unusual finding of a widened vestibular aqueduct and a progressive hearing impairment. It is still unknown how frequent those features are in this BOR-syndrome and whether they are related or not.

Alexander[57] was the first in 1904 to give a histological report on the presence of a widened vestibular aqueduct combined with hypoplasia of the cochlea in a man with congenital deafness.

In 1976, Fitch et al.[9] also reported the presence of a widened vestibular aqueduct in a patient with the BOR syndrome, based on histological findings. Dagillas et al.[10], Chen et al.[8] confirmed this by radiological studies. In the BOR literature, there are some references[3,11-14] about progressive hearing loss. Fourman and Fourman[13] mentioned that the deafness varied from mild to severe. In some cases

it has been recognized in childhood, while other cases were quite certain that they had been able to hear perfectly until they were about 20 years old, when their hearing had begun to deteriorate Brusis[12] reported progressive hearing loss in an affected mother who had been hard of hearing during childhood and had become deaf later in life bilateral hearing loss of 90 dB Her 31-year-old affected son was reported to have experienced recently a progression of his hearing impairment (Fraser et al [3], Shenoi[11] and Bourguet et al [14]) This study on a new BOR family was the first to find an association between a progressive sensorineural component in the hearing loss and a widened vestibular aqueduct and/or an enlarged vestibular sac Larger numbers of well studied BOR families are required to provide the evidence needed, whether those findings are accidental or not
Recently, the combination of progressive sensorineural hearing impairment and an enlarged vestibular aqueduct was found to be part of the Pendred syndrome[47 49]
Mutation analysis of the *PDS* gene has been started to see whether isolated cases with the enlarged vestibular aqueduct syndrome are non-diagnosed cases of Pendred's syndrome A widened vestibular aqueduct has also been reported in the deafness oligodontia syndrome[50]

In 1997, a report was published on three families with two affected sibs each They had fluctuating progressive sensorineural hearing loss, an enlarged vestibular aqueduct and a genetic background of a widened vestibular aqueduct[51] There are also other reports showing a genetic background for an enlarged vestibular aqueduct in siblings with sensorineural hearing loss[53 54] The Pendred gene (*PDS* gene) was also found to be affected in the non-syndromic autosomal recessive type of childhood deafness (DFNB4)[55] The description of DFNB4 was based on a large consanguineous family from South West India 10 individuals (aged between 5 and 38 years) had congenital, profound, non-sydromic deafness[55] Stigmata of syndromic deafness, such as a palpable thyroid, were excluded However, the perchlorate discharge test was not performed, so the diagnosis of Pendred's syndrome could have been missed In the near future, mutation analysis of the *PDS* gene will show how frequently Pendred's syndrome is the diagnosis in isolated cases of the enlarged vestibular aqueduct syndrome
The BOR syndrome might form a second clinical-genetic model on which to study the effect of an enlarged vestibular aqueduct Long-term audiometric follow-up data, CT scanning and MRI of the petrous bones are needed from thoroughly documented BOR families, to evaluate relationship between an enlarged vestibular aqueduct and progressive sensorineural hearing loss in BOR syndrome

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2.3

TEMPORAL BONE ANOMALIES IN THE BRANCHIO-OTO-RENAL SYNDROME: DETAILED COMPUTED TOMOGRAPHIC AND MAGNETIC RESONANCE IMAGING FINDINGS

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ABSTRACT

Objective:To inventory computed tomographic and magnetic resonance imaging findings in the Branchio-Oto-Renal (BOR) syndrome.

Study design: A prospective computed tomographic and magnetic resonance study on a family with the BOR syndrome.

Setting: Department of Medical Imaging and magnetic resonance imaging at St. Jan Brugge, Brugge, Belgium

Patients: Eight affected members of a Belgian BOR family. Younger affected family members were excluded because of their age.

Results: Computed tomography showed inner ear malformations in all eight affected patients. Magnetic resonance imaging was performed on five patients and showed inner ear malformations. To define hypoplasia or congenital enlargement of the inner ear structures, measurements obtained from a control group of normal subjects were used for comparison. Almost symmetrical cochlear abnormalities were observed on the three-dimensional Fourier transformation-constructive interference in steady state images of the five patients who underwent magnetic resonance imaging; four had dysplasia of the cochlea, and one had hypoplasia. The vestibule was slightly enlarged in one patient; computed tomography and magnetic resonance imaging showed semicircular canal malformations. Magnetic resonance imaging clearly showed bilateral enlarged endolymphatic sacs and ducts, whereas computed tomography showed only unilateral widening of the vestibular aqueduct and borderline widening of the vestibular aqueduct. Magnetic resonance imaging showed bilateral hypoplasia of the cochlear branch of the eighth nerve in one patient.

Conclusion: Hypoplasia and dysplasia of the cochlea were consistent findings and only magnetic resonance imaging was able to evaluate the intracochlear changes in detail and corrected computed tomography in most patients. Moreover magnetic resonance imaging also detected bilateral hypoplasia of the cochlear branch of the eighth nerve in one patient. A widened vestibular aqueduct and a widened vestibular sac were frequent but not obligatory features of the BOR syndrome. Other malformations of the middle ear included a reduced middle ear cavity and malformations of the ossicular chain.

INTRODUCTION

The Branchio-Oto-Renal (BOR) syndrome has an autosomal dominant pattern of inheritance Its main features are slight malformation of the auricles, preauricular sinuses, hearing loss, branchiogenic cervical fistulas of the second branchial arch and renal dysplasia Penetrance of the disorder is almost complete, but expression of the features varies Hearing loss is present in 85-90% of patients but can be sensorineural, conductive or mixed Rarely, the hearing loss is reported to be progressive Long-term audiometric follow-up data are scarce[1] The BOR syndrome is caused by heterozygous mutation of the *EYA1* gene[2] Mutation of the *EYA1* gene has not yet been found in all families with BOR[3]

A few studies of the inner ear and temporal bones in the BOR syndrome are available Anomalies include cochlear hypoplasia, often with a reduced size of the cochlea despite two or two and a half turns, absence or hypoplasia of the semicircular canals, a widened vestibular aqueduct, bulbous internal auditory canals, a deep posterior fossa, and acutely angled promontories[4 10] We recently conducted a clinical genetic study of a Belgian family with BOR-syndrome and performed detailed computed tomography (CT) and magnetic resonance imaging (MRI) on the temporal bones of eight affected members

PATIENTS AND METHODS

MRI and CT studies were performed on the temporal bones of eight members (3 males, 5 females - aged 5 to 39 years) of a family with the BOR syndrome The syndromic features of 14 affected persons are indicated in the pedigree (Figure 1) Twelve of the seventeen affected individuals are still alive All the patients had an important bilateral sensorineural component in the hearing loss of varying severity, which was verified audiologically Serial audiograms in two young patients showed some progression of the hearing loss There were no clinical signs of renal impairment Ultrasound examination had excluded renal abnormalities in four cases One member of the family died eight hours after birth Autopsy revealed bilateral cystic renal dysplasia Eight patients underwent CT examination of the temporal bones Axial images were obtained using a helical CT technique The scan parameters were 120 kV, 200 mAs, pitch 1 (1 mm per second), slice thickness 1 mm Images were also reconstructed every 0 2 mm to enable more detailed evaluation Additional coronal images were obtained in 4 cases using a conventional CT technique with the following parameters 120 kV, 450 mA, 1 mm thick contiguous slices

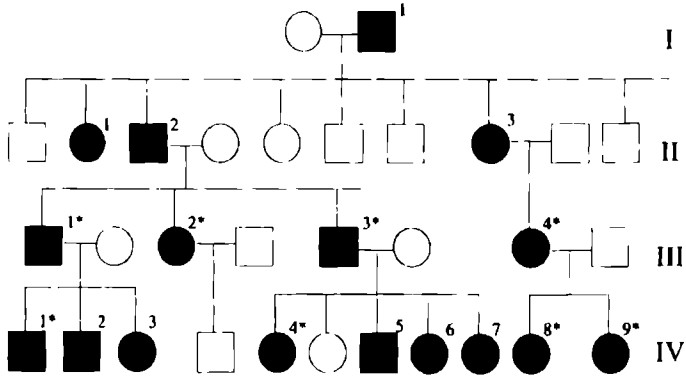


Figure 1: Pedigree of the family; □ male, ○ female; ●■ affected members; * included in the study

A field of view (FOV) of 14.5 cm was used on the coronal and axial images to visualize both temporal bones. High resolution images were obtained by processing the raw data retrospectively using software algorithms selected to emphasize bony detail. A small FOV of 9.6 cm and a matrix of 512 x 512 were used in this process to produce separate high-resolution displays of the right and left temporal bones.

Five of the patients also underwent MRI. Four of them were examined on a 1.5 Tesla system, using axial three-dimensional Fourier transformation – constructive interference in steady state (3DFT-CISS) images and axial T1-weighted spin-echo images (SE) before and after intravenous contrast administration (Gd-DTPA 0.1 mmoles/kg). The parameters of the 3 DFT-CISS sequences were: repetition time (TR) = 12 msec, echo time (TE) = 5.9 msec, excitations (NEX) = 2, flip angle = 70°, slice thickness = 0.7 mm, FOV = 140 x 160 mm, matrix = 316 x 512 (pixel size: 0.44 x 0.31 mm). The T-1 weighted spin echo sequence had the following parameters: TR = 490 msec, TE = 20 msec, NEX = 4, slice thickness 2 mm, contiguous slices. One patient was examined on a 1.0 Tesla system, using different parameters for the 3DFT-CISS sequence. This resulted in a lower spatial resolution (pixel size: 0.66 x 0.66 mm) but with a similar slice thickness of 0.7 mm.

In all the patients, the maximum diameter of the basal turn and second turn of the cochlea, the vestibule and the three semicircular canals were measured. Measurements of the cochlea and vestibule were performed on the CT (axial plane) and MR (3DFT-CISS) images, while the semicircular canals were measured on the CT images. Ten normal inner ears served as controls. The normal sizes were needed to distinguish between normal and hypoplastic cochlear turns, vestibules and semicircular canals in the patients with the BOR syndrome.

RESULTS

The CT and MRI findings in the middle and inner ears of eight members of a family with the BOR syndrome are summarized in Tables 1, 2 and 3.

In seven patients, CT revealed slight or severe malformations of the middle ear.

The size of the tympanic cavity and the external auditory canal was normal in all the patients.

In seven patients, the ossicular chain was affected. In 2 cases, the malleus and incus were fused and plump; in one of these cases, the head of the malleus was fixed to the anterior wall of the tympanic cavity bilaterally. In one patient the oval window was covered by a calcified plate.

A unilateral hypo-dysplastic stapes was recognized in four patients; this finding was associated with the absence or hypoplastic appearance of the pyramidal eminence; the stapedius muscle and tendon could not be seen on the affected side (Figure 2). In one patient, the footplate of the stapes was thickened unilaterally.

Computed tomography showed congenital inner ear malformations in all the patients. The diagnosis of hypoplasia or congenital enlargement of the inner ear structures was made by comparing the size of these structures to those measured in the 10 inner ears in the control group. The normal maximum diameters were: basal turn of the cochlea = 8-9 mm; second turn of the cochlea = 5-6 mm; vestibule = 6 x 3 mm, LSC = 6-7 mm, PSC = 7 mm, SSC = 7 mm.

Table 1: Imaging findings in the middle ear.

Subject	Side	Malleus-incus	Stapes	Pyramidal eminence	Oval window
III1	R	Normal	Hypodysplastic	Hypoplastic	Normal
	L	Normal	Normal	Normal	Normal
III2	R	Normal	Normal	Normal	Normal
	L	Normal	Hypodysplastic	Hypoplastic	Normal
III3	R	Normal	Normal	Normal	Normal
	L	Normal	Normal	Normal	Normal
III4	R	Normal	Hypodysplastic	Hypoplastic	Normal
	L	Normal	Normal	Normal	Normal
IV1	R	Fusion	Normal	Normal	Normal
	L	Fusion	Normal	Normal	Normal
IV4	R	Normal	Hypodysplastic	Hypoplastic	Normal
	L	Normal	Normal	Normal	Normal
IV8	R	Dysplasia,fusion	Dysplastic	Normal	Closed
	L	Dysplasia,fusion	Normal	Normal	Normal
IV9	R	Dysplasia,fusion,fixation	Normal	Normal	Closed
	L	Dysplasia,fusion,fixation	Normal	Normal	Normal

Table 2: Imaging findings in the internal ear.

Subject	Side	Cochlear hypodysplasia				V	LSC	PSC	SSC	VA/EDS
		BT	ST-AT	Md	Intrascalar separation					
III1	R	+	+	+	Complete	D	HD	N	N	N/N
	L	+	+	+	Complete	D	HD	N	N	N/N
III2	R	+/-	+	+	Incomplete	N	H	N	N	N/N
	L	+/-	+	+	Incomplete	N	H	N	N	N/N
III3	R	+/-	+/-	+/-	Complete	N	N	N	N	N/N
	L	+/-	+/-	+	Complete	N	N	N	N	N/N
III4	R	+/-	+	+	Not valuable	N	H	N	N	L/n.v.
	L	+/-	+	+	Not valuable	N	H	N	H	L/n.v.
IV1	R	+/-	+	+	Not valuable	N	HD	N	N	L/n v
	L	+/-	+	+	Not valuable	N	HD	N	N	N/n.v.
IV4	R	+/-	+	+	Doubtful	N	H	N	N	N/N
	L	+/-	+	+	Doubtful	N	H	N	N	N/N
IV8	R	+/-	+	+	Doubtful	N	PA	N	N	L/L
	L	+/-	+	+	Doubtful	N	HD	N	N	L/L
IV9	R	+/-	+	+	Not valuable	N	PA	PA	N	N/n.v
	L	+/-	+	+	Not valuable	N	PA	PA	N	N/n.v.

BT, basal turn; ST, second turn; AT, apical turn; Md, modiolus, V, vestibule; LSC, lateral semicircular canal; PSC, posterior semicircular canal, SSC, superior semicircular canal; VA, vestibular aqueduct; EDS, endolymphatic duct and sac; -, absent; +/-, present mild; +, present; n v , not valuable; N, normal, D, dysplastic, HD, hypodysplastic, PA partially absent; L, large

Table 3: Results of computed tomography and magnetic resonance imaging in the cochlea.

		CT cochlea			
		ST-AT separation	Scale separation		
			BT	ST	Modiolus
III1	R	Not visible	Doubtful	Absent	Sev. hypo
	L	Not visible	Present	Absent	Sev. hypo
III2	R	Not visible	Present	Absent	Sev. hypo
	L	Not visible	Present	Absent	Sev. hypo
III3	R	Not visible	Present	Present	Hypo
	L	Not visible	Present	Present	Sev. hypo
IV4	R	Not visible	Absent	Absent	Sev hypo
	L	Not visible	Doubtful	Absent	Sev. hypo
IV8	R	Not visible	Doubtful	Absent	Sev hypo
	L	Not visible	Absent	Absent	Sev hypo
		MR cochlea			
		ST-AT separation	Scale separation		
			BT	ST	Modiolus
III1	R	Visible	Present	Present	Sev. hypo
	L	Not visible	Present	Present	Sev. hypo
III2	R	Visible	Present	Incomplete	Hypo
	L	Visible	Present	Incomplete	Hypo
III3	R	Visible	Present	Present	Hypo
	L	Visible	Present	Present	Hypo
IV4	R	Visible	Present	Doubtful	Hypo
	L	Visible	Present	Doubtful	Hypo
IV8	R	Visible	Present	Doubtful	Hypo
	L	Visible	Doubtful	Doubtful	Hypo

(R, right side; L, left side, BT, basal turn; ST, second turn; AT, apical turn; sev.hypo, severely hypoplastic; hypo, hypoplastic)

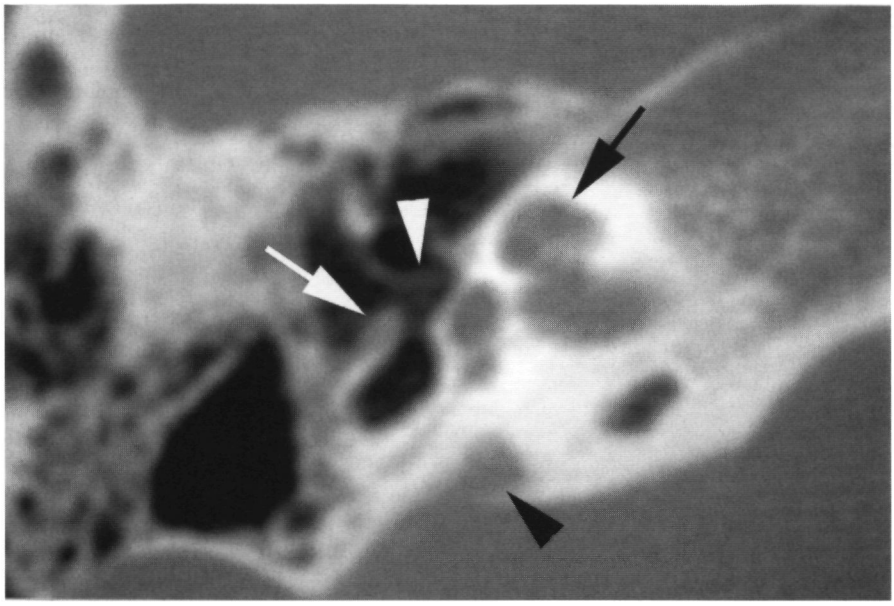
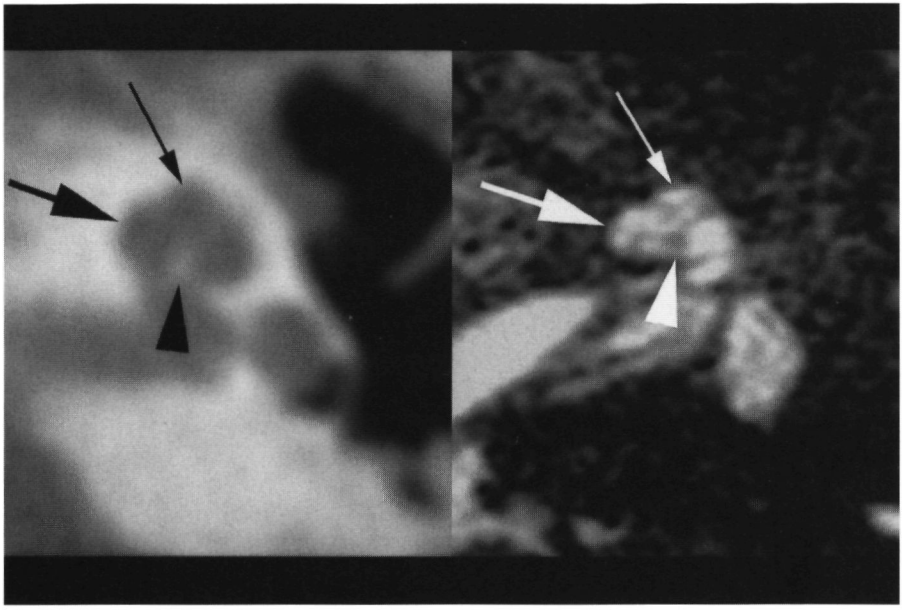


Figure 2: Axial computed tomography image at the level of the right pyramidal eminence obtained from patient III-4 showing hypoplasia/dysplasia of pyramidal eminence and stapes. The pyramidal eminence is hypoplastic and appears as a flat structure (white arrow). The muscle inside the pyramidal eminence and the capitulum of the stapes. The stapes is hypodysplastic, and only a small anterior crus of the stapes can be seen (white arrowhead). The apical and second turn of the cochlea can hardly be separated (black arrow) and the modiolus is hypoplastic. The large vestibular aqueduct is seen (black arrowhead).

A hypoplastic basal turn (7 mm) was found bilaterally in 2 patients and occurred unilaterally in 4 others. The shape of the basal turn was abnormal in all the patients. In two of them, the basal turn had a fairly pronounced bow shape and was narrowed. In all the patients, it was difficult to separate the second turn from the apical turn, while the modiolus appeared to be severely hypoplastic (Figure 3). The apical turn was hardly visible in 5 patients and could not be seen in 3 patients. The second turn was hypoplastic in 4 patients (4 mm) and had a normal size in the others (5 mm).

There was normal separation between the scala vestibuli and scala tympani in the basal turn of 4 cochleae (3 patients). It was unclear whether separation was normal in five other cochleae (Figure 4), whereas in the remaining seven cochleae, separation was absent. Separation between the scala vestibuli and scala tympani in the second turn of the cochlea was normal in 2 cochleae, indistinct in 5 and absent in 9 (Figure 5).



A B

Figure 3: Axial CT image (A) and three-dimensional Fourier transformation-constructive interference in steady state MRI image (B) at the level of the second and apical turn of the left cochlea obtained from patient III-3 showing separation between second and apical turns, invisible on CT scan and partially visible on MRI scan, as well as hypoplasia of the modiolus. The second turn (large arrow) and apical turn (small arrow) of the cochlea cannot be distinguished on CT scan. They are separated by an incomplete hypointense structure, however, representing the bony septum between the two turns of the cochlea (the bony spiral lamina and basilar membrane) on MRI. The modiolus is hypoplastic (arrowhead).

The vestibule was only slightly enlarged in one patient (7 x 4 mm) and was normal in all the others.

The lateral semicircular canal (LSC) was most frequently affected. It showed involvement and hypoplasia in 7 patients. Aplasia of the ampulla and adjacent LSC was found unilaterally in one patient and bilaterally in a second patient. In 4 other patients, the ampullae of the LSCs were narrowed (Figure 6). Dilatation of the posterior part of the LSC could also be seen in 2 of the latter patients.

In one patient, the LSC had a normal shape but was hypoplastic (diameter of only 5 mm). One patient showed bilateral partial absence of the PSC in combination with severely affected LSCs. Unilateral hypoplasia of the SSC was also present in another patient with LSC involvement.

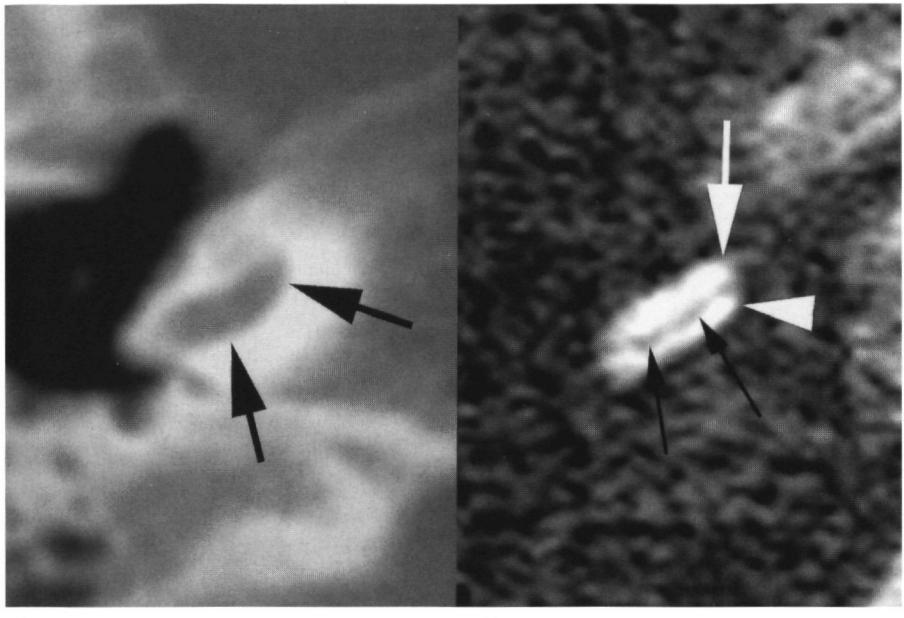


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Figure 4: Axial CT image (A) and axial three-dimensional Fourier transformation-constructive interference in steady–state MRI study (B) through the basal turn of the right coclea obtained from patient III-1 showing bow-shaped basal turn. The separation between scala tympani and vestibule appears dubious on CT scan but normal on MRI scan. The basal turn of the cochlea is bow-shaped on this CT image (large black arrows), a rather typical shape of this structure in patients with BOR syndrome. Inside this basal turn there is no bony separation between the scalae. However, on MRI, the bony spiral lamina and/or basilar membrane can be seen as a hypointense linear structure (small black arrows) between the scala tympani (white arrowhead) and scala vestibule/media (white arrow).

Three patients had an enlarged vestibular aqueduct: which was bilateral in one and unilateral in two. In one of the unilateral cases, the contra-lateral vestibular aqueduct had a borderline-widened size (Figure 7). The internal auditory canal was normal in all the patients. Inner ear malformations were seen in all five patients examined with MRI. Measurements of the inner ear structures in the control group were used to define hypoplasia or congenital enlargement of these structures in the patients with the BOR syndrome. The normal maximum diameters on MRI were as follows: basal turn of the cochlea = 8 mm, second turn of the cochlea = 5-6 mm, vestibule = 5-6 x 3 mm. Almost symmetrical cochlear abnormalities could be seen on the 3 DFT-CISS images of the 5 patients with BOR the syndrome. The basal turn was hypoplastic (7 mm) in only one of them.

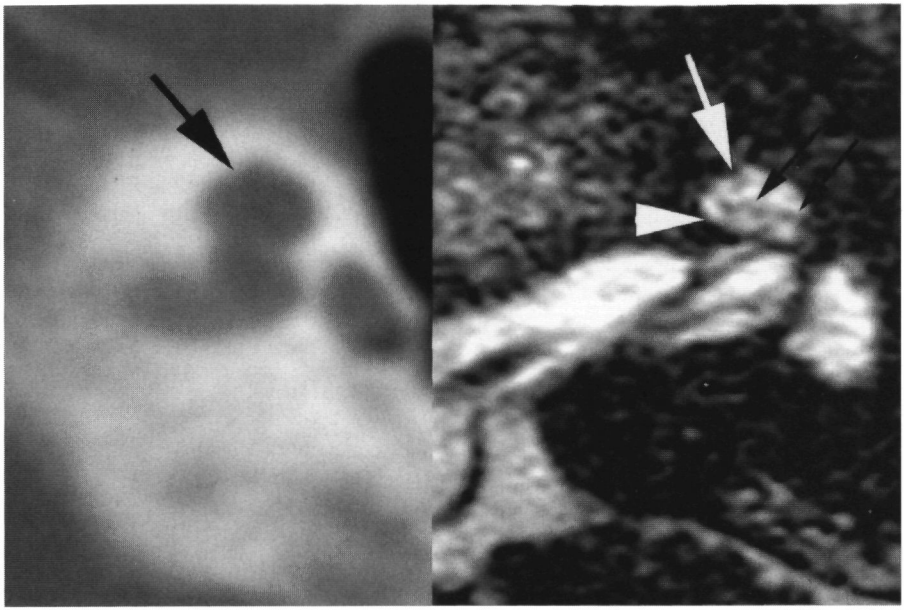

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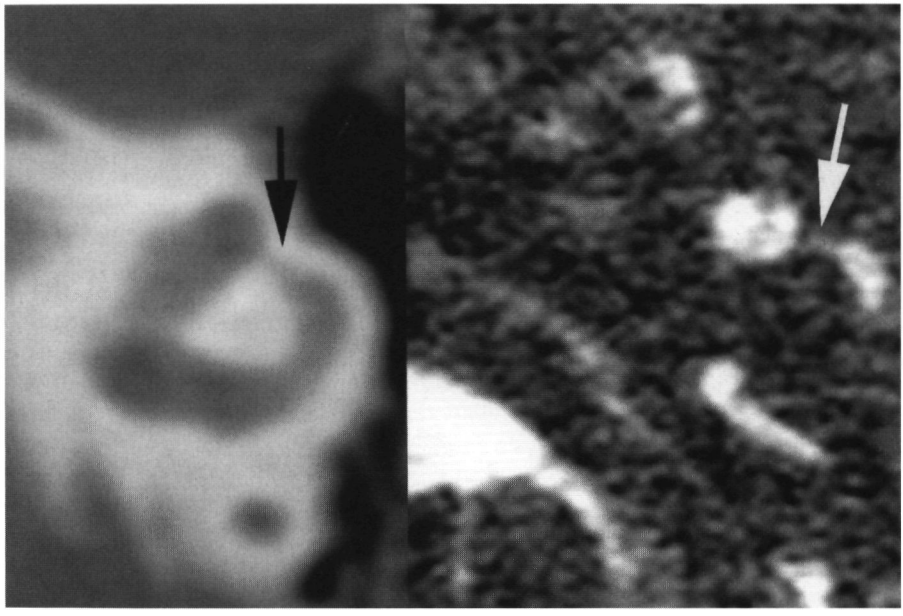
Figure 5: Axial CT image (A) and axial three-dimensional Fourier transformation-constructive interference in steady-state MRI study (B) through the second turn of the left cochlea obtained from patient III-1 showing separation between scala tympani and vestibule of the second turn absent on CT scan but normal on MRI scan. On CT scan, there is no separation between the scalae of the second turn of the cochlea (large black arrow). On MR, however, a hypointense line (small black arrow), representing the bony spiral lamina and/or basilar membrane between the scala tympani (white arrowhead) and the scala vestibuli/media (white arrow) can be seen. This hypointense line is incomplete. The apical turn cannot be depicted in its normal position lateral and adjacent to the second turn.

However, dysplasia was seen in all five patients. The second turn of the cochlea was hypoplastic in three patients; the apical turn was severely hypoplastic in four patients and was absent unilaterally in one patient (Figure 5b). In all patients, the second turn could be separated from the apical turn (when present) (Figure 3b). The modiolus always showed slight or severe hypoplasia (Figure 3b,7e,7f). Normal separation between the scala tympani and scala vestibuli was observed in the basal turn of the cochlea in four patients (Figure 4b). In the fifth patient, separation was dubious on one side. Separation in the second turn was incomplete in 2 patients (Figure 5b), doubtful in one patient and normal in the other two. The vestibule was slightly enlarged in one patient (7 x 4 mm), which was in accordance with the CT finding. MRI demonstrated similar semicircular canal malformations to those seen on CT (Figure 6). Only the patient with a large vestibular aqueduct (LVA) unilaterally on CT and contralateral borderline widened

vestibular aqueduct (LVA) was examined with MRI. In this patient MRI demonstrated clearly enlarged bilateral endolymphatic sacs and ducts (Figure 7 c-f). In 4 of the patients a normal superior vestibular, inferior vestibular and cochlear branch of the vestibulocochlear nerve and a normal facial nerve could be distinguished from one another inside the internal auditory canal and cerebello-pontine angle on the 3DFT-CISS images. In one patient the cochlear branch of the vestibulocochlear nerve was hypoplastic on both sides (Figure 8). No spontaneous intralabyrinthine hyperintensities or intralabyrinthine enhancements were visible on the non-enhanced and gadolinium-enhanced T1 weighted spin-echo images.



A **B**

Figure 6: Axial CT image (A) and axial three-dimensional Fourier transformation-constructive interference in steady-state MRI scan (B) through the left lateral semicircular canal obtained from patient IV-1 showing narrowing of the ampulla of the lateral semicircular canal. The ampulla of the lateral semicircular canal, normally the widest part of the canal, is clearly narrowed on both CT scan (black arrow) and MRI study (white arrow). This malformation was found in several members of this family with BOR syndrome.

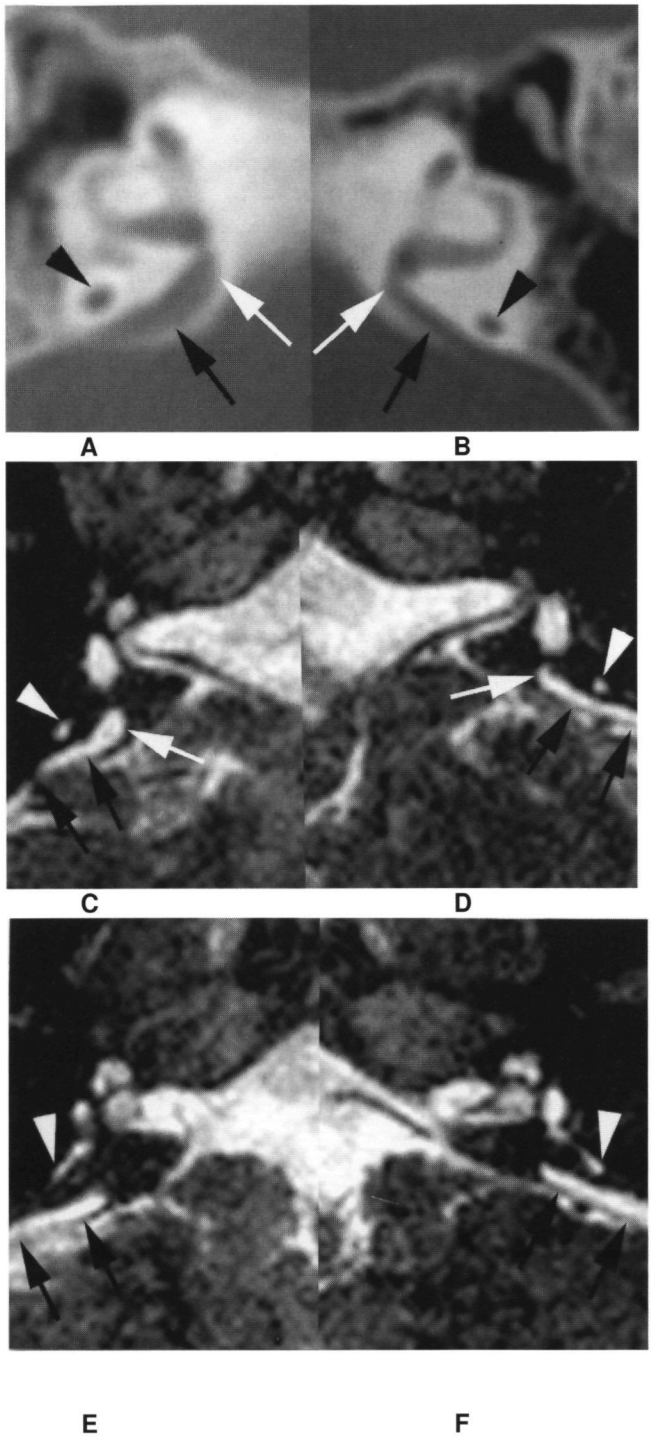


Figure 7: Images obtained from patient VI-8 showing unilateral large vestibular aqueduct on CT scan, bilateral enlarged endolymphatic duct/sac on MRI study. Axial CT images (A,B) and axial three–dimensional Fourier transformation-constructive interference in steady-state MRI scan (C,D) at the level of the ascending part and inferior part (E,F) of the posterior semicircular canal (PSC) of the right (A,C,E) and left (B,D,F) inner ear. **A.B.** The right vestibular aqueduct is enlarged (large arrows), and its diameter is clearly larger than that of the PSC (black arrowhead). The diameter of the left vestibular aqueduct (large arrows) is similar to that of the PSC and is therefore not enlarged but borderline. Notice the fusion of the head of the malleus with the corpus of the incus on both sides. Bilateral narrowing of the ampulla of the lateral semicircular canal can be seen. **C,D.** The right endolymphatic duct (white arrow) and sac (black arrows) have a longer diameter than the posterior semicircular duct (white arrowhead). The diameter of the left endolymphatic duct (white arrow) and sac (black arrows) are equal to or smaller than the diameter of the posterior semicircular duct (white arrowhead) and is therefore borderline/normal. **E,F** The right endolymphatic sac (black arrows) is larger in diameter than the right posterior semicircular duct (white arrowhead). At this level, it also becomes obvious that the left endolymphatic sac (black arrows) is larger in diameter than the left posterior semicircular duct (white arrowhead). The endolympohatic sac is easier to recognize on the MRI scan because it can be distinguished from the cerebrospinal fluid in the posterior fossa by the dura mater, which separates both fluid-containing structures. Notice the hypoplasia of the modiolus in the left and right cochlea.

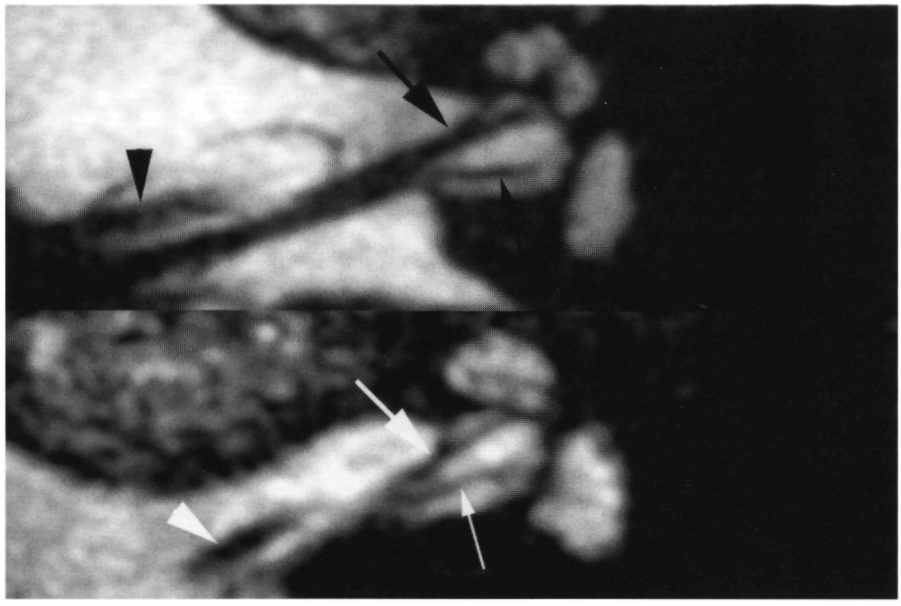


Figure 8: Axial three-dimensional Fourier transformation-constructive interference in steady-state image through the left internal auditory canal of a normal inner ear (A) and the inner ear of patient III-1 (B) showing hypoplasia of the cochlear branch of the VIIIth cranial nerve. Normally, the cochlear branch (black arrow) is only slightly smaller than the facial nerve (black arrowhead). In this patient, the cochlear branch is very hypoplastic (white arrow) and much thinner than the facial nerve (white arrowhead). Small arrows, inferior vestibular branch of the vestibulocochlear nerve.

DISCUSSION

In the literature, radiological studies on the inner ear in genetic syndromes are scarce and most reports are on single cases. Many of these syndromes are genetically heterogeneous, i.e. affected individuals from different families have different causal genes. Thus to determine the variability produced by a single gene, studies should be restricted to a genetically homogeneous population, such as within a kindred, or to individuals in whom molecular means have shown mutations in the same gene[11]. Jackler et al. (1987)[12] proposed a new classification for radiological inner ear malformations that includes more information about the vestibular system than previous classification into Michel deformity, Mondini aplasia, Bing-Siebenmann aplasia and Alexander aplasia. Modifications to this classification system were suggested by Triglia et al.[13].

Computed tomography and MRI studies were performed on the temporal bones of 8 affected members of a four generation family with the BOR syndrome Clinical presentation is indicated in the pedigree (Figure 1) Preliminary gene linkage showed a positive lod score of 1 07 for marker D8S286 This suggests linkage to the 8q mutation of the EYA1 gene Blood samples could only be collected from a part of the family members limiting a higher linkage result A mutation has not yet been found

Pathological, surgical and conventional tomographic studies of the middle ear have reported a wide variety of malformations, including reduced middle ear cavity, hypoplastic or plump malleus and/or incus, ossicular fusion and fixation, footplate fixation and anteriorly displaced ossicles, [4 9 14 22] The findings in the inner ear seem to be more uniform a hypodysplastic cochlea with reduction of the number of turns and developmental anomalies of the semicircular canals A large vestibular aqueduct has also been reported[4 8 10]

Computed tomography of the temporal bone revealed malformations of the external ear, middle ear and inner ear in 20 BOR patients[6 8 10 19 21 22] The findings included stenosis or atresia of the external ear canal, malformed middle ear cavity, hypoplastic incus, malformed ossicles, malleo-incudal fusion, bony fixation, absence of the oval window, hypoplasia or dysplasia of the cochlea with reduction of the number of turns, enlarged vestibule, hypoplastic or aplastic semicircular canals, large vestibular aqueduct, facial nerve anomalies (anomalous course, duplication, overhanging) and upward tilt of the medial end of the petrous bone

Abnormalities of the malleus and incus were found in only 3 cases in this family These were articular fusion and in one case fixation of the head of the malleus to the anterior wall of the epitympanum Such findings were much more frequent in other families Anomalies of the stapes were encountered relatively more frequently in this family (5 patients) This may have been caused by different expression in this family and/or to the equipment and technique used in our CT studies (spiral acquisition, reconstructed slices every 0 2 mm, small FOV, matrix of 512 x 512) In 4 of our BOR patients, hypoplasia of the stapes was constantly associated with flattening of the pyramidal eminence and apparent absence of the stapedius muscle and tendon To our knowledge, this combination has not been described before in BOR patients, but it was a characteristic feature in this family (Figure 2)

In agreement with previous reports all the patients in this family had cochlear abnormalities The basal turn mostly showed only mild anomalies, whereas the second turn and apical turn consistently showed severe hypoplasia/dysplasia

Our MRI studies demonstrated that these cochlear malformations were not consistent with typical Mondini aplasia, i e a normal basal turn and fusion between the second turn and the apical turn, with absence of, or incomplete, interscalar and intrascalar separation In our patients, the second turn was often hypoplastic, but it was never fused to the apical turn The latter was severely hypoplastic in 4 patients and absent in one patient Our findings may be a variant of the Mondini malformation[23], in view of the slight involvement of the basal turn, the severely hypoplastic modiolus, the incomplete separation between the scala tympani and scala vestibuli (demonstrated in 1 case and uncertain in 2 cases (artefacts were present in the 3DFT-CISS sequence because of small head movements)), and the association with labyrinthine malformations and with large vestibular aqueduct The numerous variants of the Mondini malformation are probably the reason why this finding is not mentioned in some pathologic reports

Labyrinthine malformations were mostly located in the LSC, with various degrees of severity, but with constant major involvement of the ampulla, which was narrowed or absent (Figs 6 and 7) We observed a direct correlation between the degree of severity of the cochlear malformations and LSC malformations

LVA has been reported in patients with the BOR syndrome [4 8 10] In our series, this finding was present in 3 patients (1 bilateral, 2 unilateral) In one of the two patients with unilateral LVA, MRI revealed bilateral enlargement of the endolymphatic sacs The two patients with bilateral anomalies showed progression of hearing loss on serial audiograms, which may have been explained by LVA In many of the cases reported by Jackler et al [12], LVA was associated with episodes of sudden hearing loss after even minor trauma None of the patients in the study by Ostri et al [6] had LVA, and none of them had progressive hearing loss In patients with the BOR syndrome, CT is useful to explain the conductive component of the hearing loss, because it can image the bony labyrinth MRI is complementary to CT, because it can image the liquid content of the membranous labyrinth Our 3DFT-CISS sequence (slices of 0 7 mm, matrix > 256 x 256 and small FOV) clearly displayed the interscalar and intrascalar separation inside the cochlea In two of our patients, these structures were not recognizable, because of headmovements in one case and the use of lower spatial resolution in the other (1 0 T equipment) CT displayed confluence of the second turn and apical turn in most of our patients In contrast, MRI demonstrated separation and severe hypoplasia of the apical turn, with better evaluation of the modiolus, which was severely hypoplastic, but less so than was indicated by CT (Fig 3) Intrascalar separation was also visible on CT, but the signs were more subtle In our cases this finding was always confirmed by MRI 5/5 in the basal turn and 2/2 in the

second turn. When it was considered absent on CT or uncertain, the correlation with MRI was lower (4 cases present and 1 case doubtful in the basal turn; 2 cases present, 2 cases incomplete and 4 cases dubious in the second turn) (Figs 4 and 5). However, it should be stressed that all the dubious MRI findings were in the four inner ears of the two patients whose examination was of lower technical quality.

Bilateral hypoplasia of the cochlear branch of the eighth nerve was seen in one patient. Normally this branch has a similar thickness to that of the facial nerve at the level of the internal auditory canal or is only slightly narrower. In this patient the cochlear branch was not even half as thick as the facial nerve. Hypoplasia and aplasia of the cochlear branch were already described in association with congenital inner ear malformations but they can also occur in a normal inner ear[24]. These nerve abnormalities can, of course, be seen only on MR.

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2.4

PROGRESSIVE FLUCTUANT HEARING LOSS, ENLARGED VESTIBULAR AQUEDUCT, AND COCHLEAR HYPOPLASIA IN THE BRANCHIO-OTO-RENAL SYNDROME

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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

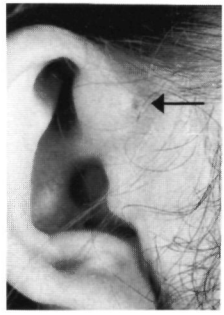

Figure 1: Slightly malformed right auricle and preauricular sinus (ar-row) of patient A.

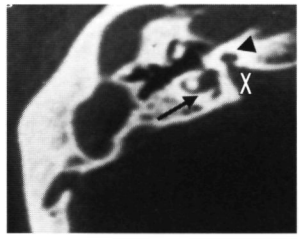

Figure 2: Patient A: CT scan of the right ear in the axial plane. Widened IAC ("X") and hypoplastic cochlea (arrowhead). The vestibule is widened, the lateral SCC is slightly too small (arrow).

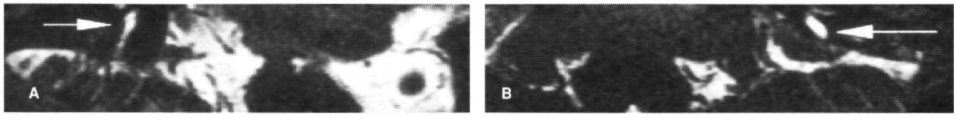

Figure 3: Patient A: Coronal reconstruction MRI through the endolymphatic ducts on the right (A) and left side (B). The left endolymphatic duct is abnormally wide, whereas the right one is of normal size (arrows). No endolymphatic sac could be visualized.

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 visualized (Figure 3).

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.

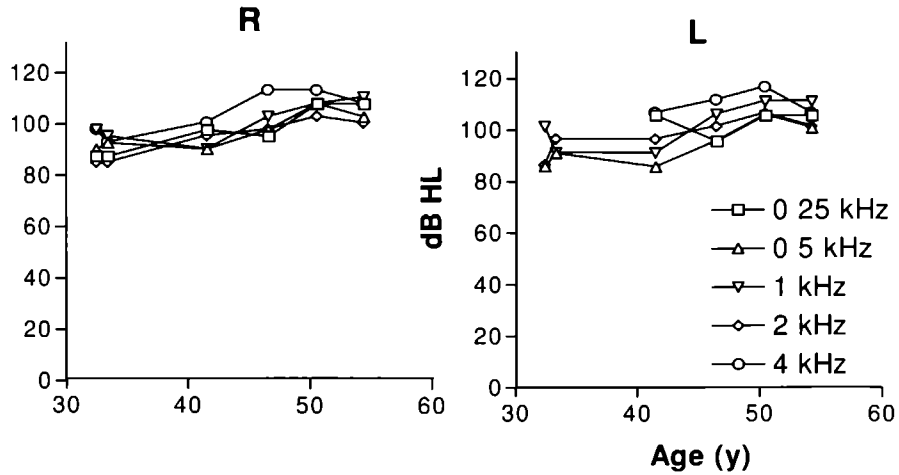

Figure 4 Patient A air conduction thresholds plotted against age (R, right, L, left) These longitudinal threshold data show only limited progression, well within the normal limits of presbyacusis (see text) The data are not suitable for fluctuation analysis

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]

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]

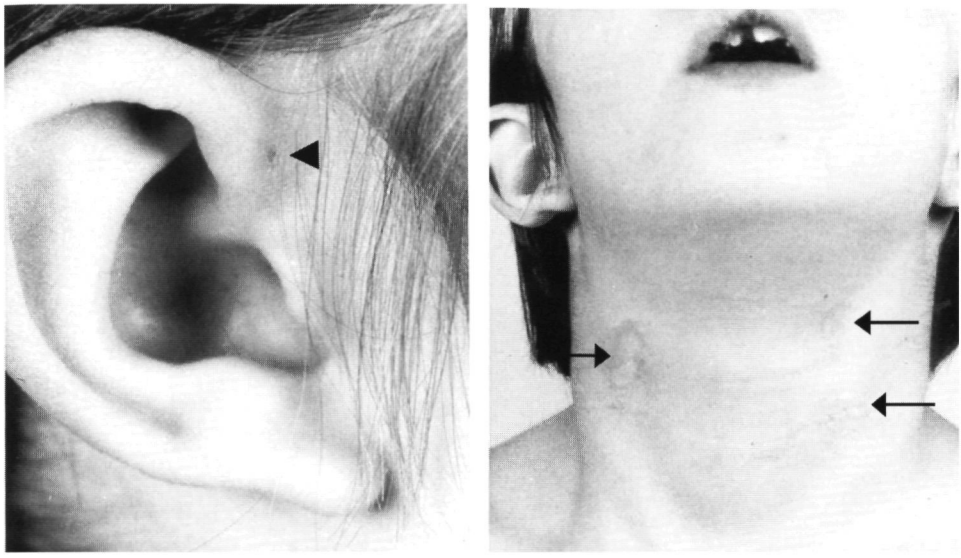

Figure 5: *Left panel*: Slightly malformed right auricle and preauricular sinus (arrowhead) of patient B at 7 years of age. *Right panel*: Bilateral second branchial arch fistulas (arrows) of patient B at 7 years of age.

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.
The audiometric follow-up data of this patient over 23 years (29 audiograms; age range, 6-29 years) (Figure 7) demonstrated clear progression of hearing loss.

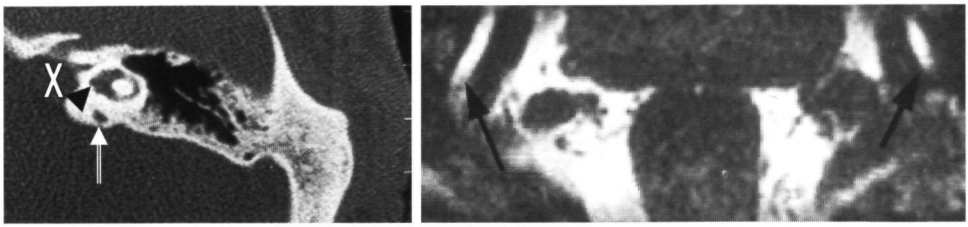

Figure 6: *Left panel*: CT scan of the left ear in the (transverse) axial plane of patient B. A widened internal meatus is visible ("X") as well as a plump vestibule (arrowhead). The vestibular aqueduct (arrow) is widened. *Right panel*: Coronal reconstruction of 3D high-resolution MRI showing an enlarged endolymphatic duct (arrows) on both sides of patient B at the age of 28 years.

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 7).

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. 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.

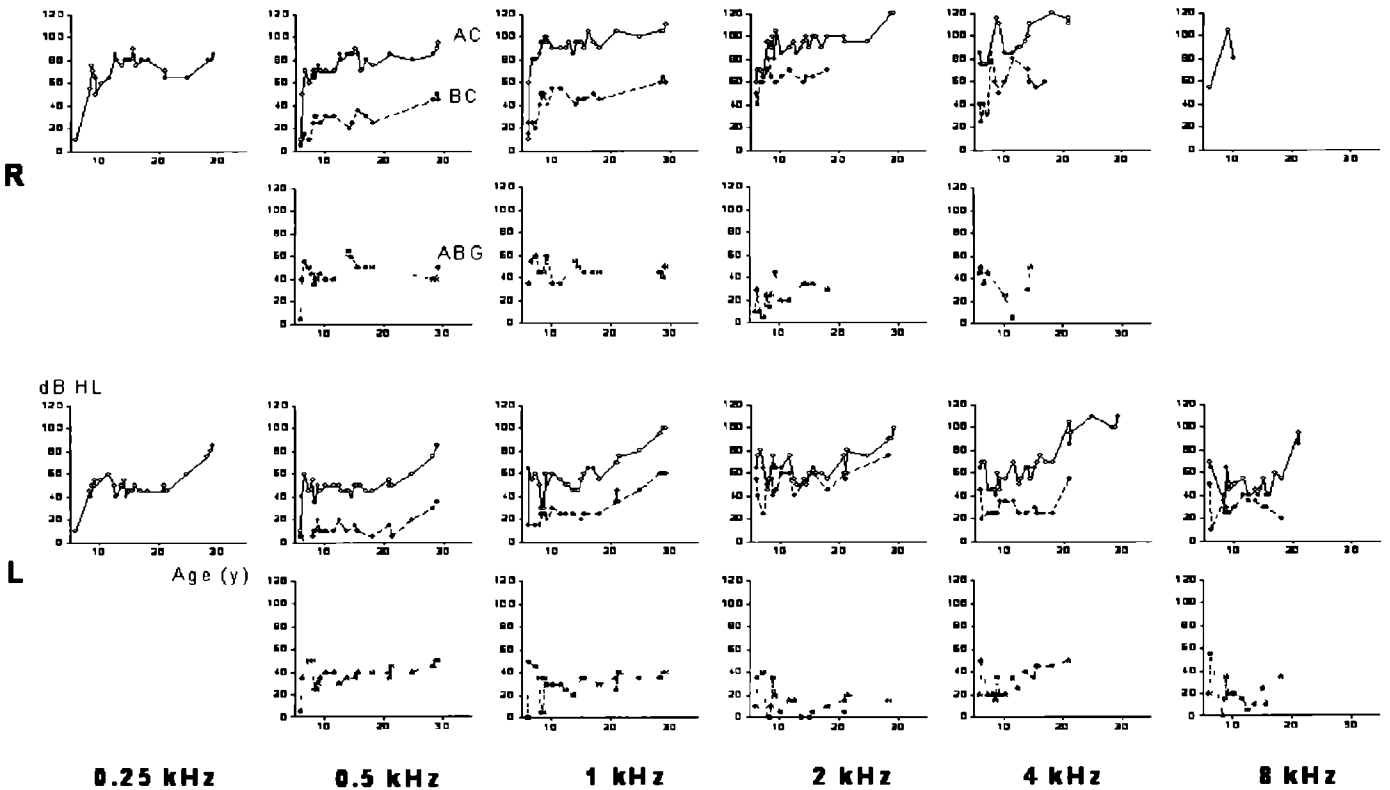

Figure 7 Patient B Plots of 0 25-8 kHz air conduction thresholds (AC, dB HL, o-), bone conduction thresholds (BC, dB HL, ●–) and air bone gaps (ABG, *) in the right (R) and left (L) ear against age (in years) There is clear progression in AC and BC levels at most frequencies, which is lacking in ABG Fluctuation analysis (see text) constituted pairwise intervariable comparison (per ear) or interaural comparison (per variable) at given frequencies (vertically aligned panels)

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 recognized 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 III

The Pendred syndrome

3.1

FLUCTUANT, PROGRESSIVE HEARING LOSS ASSOCIATED WITH MENIÈRE LIKE VERTIGO IN THREE PATIENTS WITH THE PENDRED SYNDROME

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ABSTRACT

Objective To evaluate vestibular and long-term audiometric findings in patients with Pendred syndrome
Study design Retrospective analysis of long-term clinical data
Setting University hospital department
Patients Three patients with Pendred syndrome caused by a mutation in the *PDS* gene
Methods Perchlorate discharge test, mutation analysis of the *SLC26A4* gene, MR imaging of temporal bones, vestibular function test (in 2 cases) and serial audiometry A saturation hyperbola with onset age was fitted to the audiometric threshold-on-age data using a nonlinear regression method The residues remaining after regression were analyzed in a correlation analysis to detect significant ipsilateral or contralateral cofluctuation
Results All three patients had a mutation in the *SLC26A4* gene and bilateral enlarged vestibular aqueduct, two of them had a positive perchlorate discharge test but in one of two siblings this test was negative Hearing loss was significantly progressive with significant ipsilateral and contralateral cofluctutation in all evaluable cases, combined with episodes of vertigo in two cases One case had unilateral caloric areflexia and one had bilateral vestibular hyporeflexia, proven to be progressive in a repeat examination
Conclusions Similar to patients with the enlarged vestibular aqueduct syndrome, also caused by mutations in the *SLC26A4* gene, patients with the Pendred syndrome may exhibit progressive and fluctuant hearing loss with episodes of vertigo

INTRODUCTION

The Pendred syndrome was originally described in 1896 by Pendred[13] It is considered to be a combination of congenital deafness and goitre Thyroid enlargement is caused by impairment of thyroxin synthesis due to a defect in the organification of iodide[14] This defect can be demonstrated by the perchlorate discharge test This test consists of administration of radioactive iodide to the patient One hour later, perchlorate is administered and the release of radioactive iodide taken up by the thyroid gland is measured An abnormal result is defined as a washout of >10 % of radioactive iodide

The Pendred syndrome is inherited as an autosomal recessive trait Recently, the gene responsible for Pendred syndrome has been mapped to chromosome 7q31 [1 20 21] Later on, the gene was identified and designated as the Pendred syndrome gene *SLC26A4*[2] The gene product, pendrin, is a chloride-iodide cotransporter, which has a function in the uptake and organification of iodine in the thyroid gland[11]. Mutations in *SLC26A4* are also responsible for non-syndromic autosomal recessive hearing loss associated with enlarged vestibular aqueduct (EVA)[3 4 12] *Pds*-knockout mice have recently been found to develop endolymphatic dilatation from embryonic day 15 onwards[32] These mice showed hearing impairment, which unfortunately was not evaluated for progression, as well as progressive vestibular impairment

We describe in more detail three patients out of the 12 patients described earlier by Cremers et al [9], paying special attention to the occurrence of progression and fluctuation in hearing loss, as well as episodic vertigo Although more of the 12 patients had a history of fluctuant hearing loss, audiometric follow-up was most elaborate and adequate in the present three cases

MATERIAL AND METHODS

Three patients with Pendred syndrome are described All three of them had a perchlorate discharge test and long-term audiometric follow-up MR imaging studies of the temporal bones were performed Vestibular examination consisted of rotary chair and caloric testing with electronystagmography and computer analysis Blood samples were collected for gene linkage studies and mutation analysis Threshold-on-age plots were fitted with nonlinear regression analysis, using a commercial programme (Prism, version 3 02, GraphPad, San Diego CA, USA) The equation used was that of a saturation hyperbola, similar to our previous report[9], unless a linear regression fitted better to the data The goodness of fit was judged from the residual standard deviation and the sum of squares (i e squared residues) As there was no statistical test available for progression in the case of nonlinear regression, "significant progression" was concluded to exist when the linear regression coefficient (slope) was significantly ($P < 0\ 025$) > 0

Fluctuations in threshold were evaluated by performing correlation analysis between the threshold residues after regression at any frequency, in either ear, for all possible pairwise comparisons Significant positive correlation ($P < 0\ 025$) detected "significant cofluctuation", ipsilaterally - i e between (adjacent) frequencies within one ear, or contralaterally - i e between the two ears In given

cases it was assessed whether the relative frequency of significant correlations relating to ipsilateral or contralateral comparisons was higher than expected ($P < 0.05$) according to binomial distribution statistics

CASE HISTORIES

Case 1

The first patient was a 38-year-old woman, the first child of normally hearing parents She is a former pupil of a school for the hard of hearing and has a younger brother with normal hearing Pregnancy was uneventful and she was born at term by caesarean section At the age of 18 months, her parents noted some retardation in her motor milestones, and hypothyroidism was diagnosed Strumectomy was performed and thyroid hormone substitution therapy was started At the age of 2, her parents noted a decreased response to sounds she used to be able to hear, she no longer responded to ringing bells or passing aeroplanes There also was a decrease in speech At the age of 4, hearing impairment of maximally 60 to 70 dB was found At the age of 4 years and 9 months, hearing loss had increased to about 90 dB in the right ear, which was her best ear Over the years following, hearing impairment in her right ear progressed with fluctuations At the age of 9, she was admitted to the hospital for sudden hearing loss combined with vertigo Thirteen and sixteen years later, she had new episodes of vertigo She described the vertigo as an acute episode of disabling dizziness, with dizzy spells recurring over several months, especially when she had slept on her left ear Then she felt dizzy and nauseous upon awakening, but this was rarely associated with vomiting Sometimes an episode of vertigo occurred during the day, especially in stressing conditions It frequently happened that objects fell out of her hands She was given flunarizine for her dizziness, which she used intermittently One year later, she was admitted because of a new episode of sudden hearing loss and tinnitus without vertigo She was treated with prednisone and vasodilators Vestibular examination showed normal vestibular function in the right ear and caloric areflexia in the left The diagnosis of Pendred syndrome was made within the context of the patient's request for genetic counselling The perchlorate discharge test showed 40% discharge At the age of 30 she again had an episode of vertigo with hearing loss Hearing stabilized, but vertigo persisted Repeat vestibular examination findings were unchanged MR imaging of the temporal bones showed cochlear hypoplasia and a wide vestibular aqueduct bilaterally The large vestibular aqueduct was defined by Valvassori et

al as being more then 1,5 mm in diameter in the mid portion of the descending limb[27] Mutations in the *SLC26A4* gene were found L236P and G139A[15]

Case 2

This 26-year-old woman is the second child of normally hearing parents She is a former pupil of the school for the hard of hearing The earliest data on her report an unexpected retardation of speech development by about two years at the age of 4 At the age of 6, speech development was still 2 years behind schedule The first audiogram obtained at age 5 years and 5 months showed bilateral hearing impairment with pure-tone (air conduction) thresholds of between 50 and 85 dB on both sides Over the years a fluctuant but slowly progressive hearing loss was noted At the age of 9, she presented with sudden deafness following a common cold, which was complete in the right ear and showed a U-shaped audiometric configuration in the left She was treated with steroids Vestibular examination revealed bilateral hyporeflexia At about the same time a first perchlorate discharge test was performed and showed a washout of 27% Hearing recovered, but in the years following fluctuations in hearing occurred repeatedly At the age of 14, she had several periods of vertigo with nausea, which generally recovered after 2 to 3 days Repeat vestibular examination showed increased bilateral hyporeflexia One year later, she had a similar episode, which took about 10 days to recover A repeat perchlorate discharge test was performed and showed a washout of 63% At the age of 26, 4 weeks after parturition, she presented with a feeling of sudden hearing loss There was a slight decrease in hearing compared to three years before, but no treatment was started MR imaging of the temporal bones showed a bilaterally normal cochlea and enlarged vestibular aqueduct (wider in the right ear) Mutation analysis showed mutations in the *SLC26A4* gene FS383 and T416P[15]

Case 3

This 29-year-old woman is the first child of normally hearing parents and the older sister of case 2 She is a former pupil of a school for the hard of hearing Pregnancy and delivery were uneventful Her parents noted somewhat retarded motor milestones, she started walking at the age of 23 months At the age of 18 months, she used one-word sentences Speech development arrested at this age and even deteriorated She could still hear passing aeroplanes and covered her ears with both hands when she heard them At the age of 3, hearing impairment was suspected A hearing level of 55 to 75 dB was found using free-field behavioural audiometry Since then, hearing progressively deteriorated with

fluctuations At the age of 12, she came for examination as the Pendred syndrome had been diagnosed in her younger sister She showed an elevated thyroglobulin concentration The perchlorate discharge test showed a washout of < 10% This test being positive in her sister, the diagnosis of Pendred syndrome was also accepted in her case Regular check-ups were done twice a year, and at the age of 23 thyroid hormone substitution therapy was started MR imaging of the temporal bones showed a bilaterally normal cochlea and enlarged vestibular aqueduct (wider on the right side) Mutation analysis showed mutations in the *SLC26A4* gene FS383 and T416P[15]

Table 1 Relative frequencies of the findings significant (S) progression (assessed by linear regression analysis for each frequency and ear) and S cofluctuation (between two ipsilateral or contralateral frequencies) in cases 1-3

Feature	Case 1	Case 2	Case 3
Best fitting equation	linear	hyperbolic*	linear
S progression			
R	*3/6*[a]	*2/5*	*2/5*
L	na	*3/5*	1/5
S cofluctuation			
ipsilateral R	*10/15*	*6/10*[b]	*5/10*
ipsilateral L	na	*10/10*	*5/10*
S cofluctuation contralateral	na	*6/25*	*3/25*

Italic values indicate significantly high relative frequency in the appropriate binomial distribution L left ear, na not available, R, right ear

Notes *, see ref 9 for definitions and details, [a], significant slope (of linear regression line) at three out of a total number of six frequencies evaluated [b] significant cofluctation (correlation coefficient) in six out of ten pairwise comparisons (=correlation analyses)

RESULTS

Individual audiograms and threshold-on-age plots pertaining to the present cases can be found in our previous report (ref 9) that included part of the threshold data the present cases 1, 2 and 3 correspond to the previous cases 6, 10 and 9, respectively All three cases showed significant progression of hearing impairment at a significantly high number of frequencies (Table 1) Progression in case 2 can be appreciated from Figure 1 For a general impression of the fitted regression parameter values, see Table 2 of the previous report[9] As described in the case histories, all three cases had shown early progressive hearing impairment Cases 1 and 2 showed further progression during the observation interval, but case 3 at some frequencies did not show any substantial further progression All three cases showed substantial threshold fluctuations suitable for further analysis

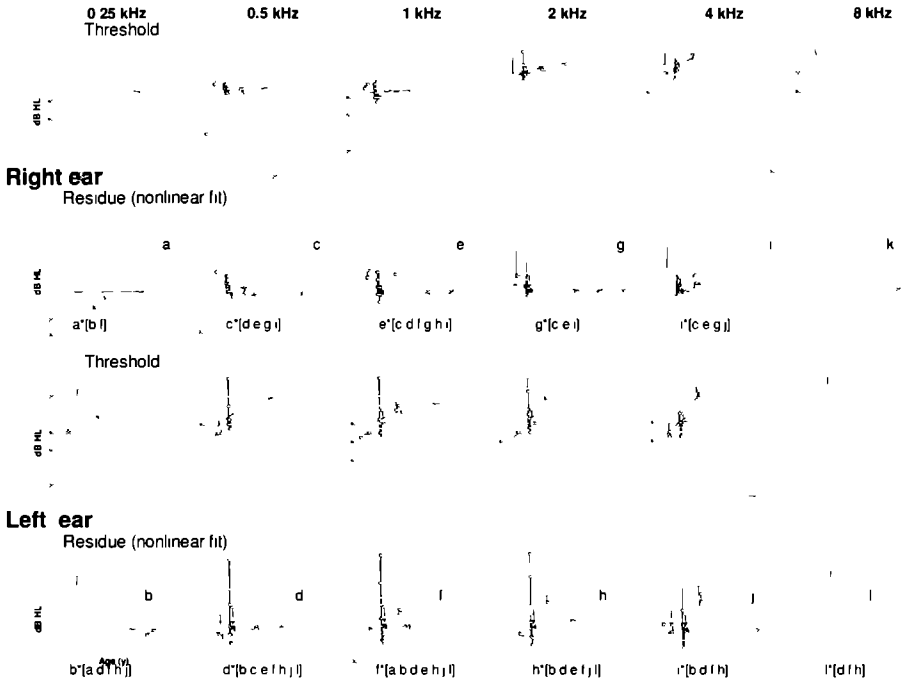

Figure 1: Plots of thresholds with the fitted hyperbolas (upper panels) and threshold residues (lower panels, labeled a-l) against age (y=year) are shown for all frequencies (0.25-8 kHz) in the right ear (top two rows of plots) and the left ear (bottom two rows of panels) of case 2. See text for further details

Figure 1 shows all the plots relating to the threshold-on-age data (top and third row panels) in case 2. The residue panels (second and bottom row) are labeled a-l. The expressions shown in these panels indicate the significant correlations found. For example, the expression e*[c,d,f,g,h,i] in panel e (residues at 1 kHz in the right ear), indicates the finding of significant positive correlations between the residues in panel e and those in the ipsilateral panels (frequencies) c, g and i, and the contralateral panels (frequencies) d, f and h. In this case, 6 out of the 10 correlations between ipsilateral frequencies in the right ear were significant, as were all the 10 correlations between ipsilateral frequencies in the left ear and 6 out of the 25 correlations between contralateral frequencies (Table 1). All these relative frequencies were significantly high according to binomial distribution statistics, which means that this case showed significant ipsilateral and contralateral fluctuation. Clear examples of cofluctation are shown in Figure 2.

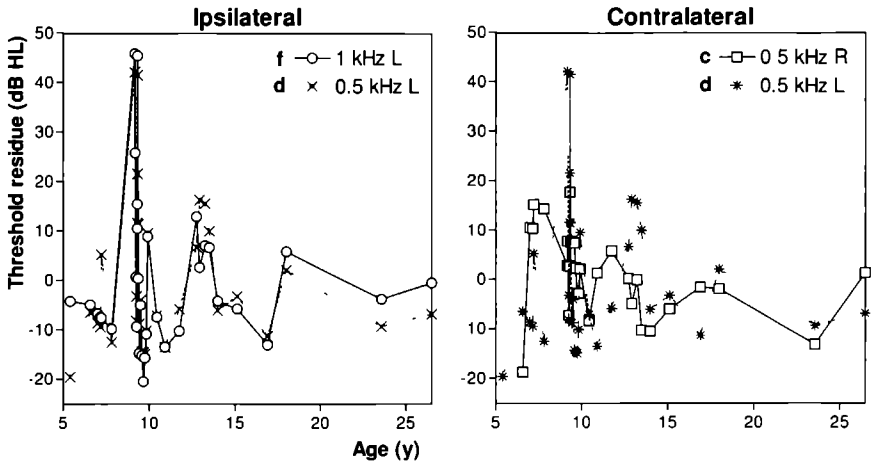

Figure 2: Superposition plots of ipsilateral (left panel) and contralateral (right panel) threshold residues at 0.5 and/or 1 kHz in case 2. Same data as in corresponding residue panels (c, d and f) in Figure 1.

The residues selected from Figure 1 (0.5 kHz R and 0.5-1 kHz L, panels c, d and f) are now superimposed with their connection lines. Although the presence of ipsilateral cofluctuations in this case is pretty clear, it was formally tested for significance in all cases (Methods and Table 1). Comparison between the two panels of Figure 2 illustrates the general trend that ipsilateral cofluctuations (left panel) were more impressive than contralateral cofluctuations (right panel), i.e. more often significant (Table 1). Significant ipsilateral cofluctuation was found in all 3 cases. Significant contralateral cofluctuation was found in the 2 cases in which this could be evaluated.

DISCUSSION

Until recently, only few clinical data have been reported concerning progression and fluctuation of hearing loss in Pendred syndrome. The classical phenotype of Pendred syndrome was described as deafness combined with an enlarged thyroid gland and a positive perchlorate discharge test. Deafness was considered to be congenital and profound. Expressivity of the syndrome seems though to be variable, as was shown by Masmoudi et al. and Reardon et al.[30,31].

Progression in hearing loss was described in a small number of cases[5 6 7 24] Reports with long-term audiometric follow-up show severe, progressive hearing loss, most progressive in early childhood[6 9 24] In a few patients fluctuant hearing loss is mentioned[8 23 24] In three of the previously described four patients with fluctuant hearing loss, audiometric follow-up was published

Only recently, it became clear that an enlarged vestibular aqueduct, endolymphatic sac and endolymphatic duct are common features in Pendred syndrome[9 10] Less frequently, cochlear hypoplasia is found in the Pendred syndrome[10]

The gene involved in Pendred syndrome is located on chromosome 7q31[1 20 21] and is designated as the *SLC26A4* gene[22 25] *SLC26A4* encodes a putative transmembrane protein, which is designated pendrin[22] Initially, this protein was supposed to be a sulfate transporter, but recent studies have demonstrated that pendrin functions as a sodium-independent cotransporter of chloride and iodide[11] These findings, coupled with the known organification defect in Pendred syndrome, suggested that pendrin might function in the thyroid to transport iodine into the follicular lumen before its incorporation into thyroglobulin This hypothesis has been supported by studies of Bidart et al and Royaux et al [28 29] Further investigation into the expression pattern of the mouse ortholog of the Pendred syndrome gene is suggesting a key role for pendrin in the inner ear Pendrin is predominantly expressed in the endolymphatic duct and sac, which are thought to play an important role in endolymph resorption Additional areas of pendrin expression include nonsensory regions of the utricle, saccule and cochlea[25] It is postulated that anion (chloride) transport depending on normal pendrin is critical for maintaining endolymphatic ionic homeostasis, which is essential to normal inner ear function This strongly suggests that deafness in Pendred syndrome is caused by malfunction or absence of pendrin and is not a secondary effect of thyroid dysfunction, as has been postulated earlier [also see ref 32]

The *SLC26A4* gene is also mutated in DFNB4, a non-syndromic autosomal recessive type of childhood deafness and in non-syndromic hearing loss associated with the enlarged vestibular aqueduct (EVA) syndrome[3 4 12] Recently, the possible relationship between Pendred syndrome and DFNB4 has been reviewed In the family in whom DFNB4 was originally diagnosed, no perchlorate discharge test had been performed Later on, however, the affected persons were found to have goitre and therefore the Pendred syndrome, rather than non-syndromic deafness[3] In one family reported to have the EVA syndrome and a mutation in *SLC26A4*, no perchlorate discharge test has been performed, as there was no sign of thyroid dysfunction[3] Usami et al [4] found seven mutations in

SLC26A4 in six families with one or two siblings with bilateral EVA, who did not met the classical criteria for Pendred syndrome Scott et al showed an association between SLC26A4 gene product and phenotypic variation in patients with Pendred syndrome and patients with non-syndromic hearing loss, enlarged vestibular aquaeduct and SLC26A4 mutations, which they called DFNB4[27]

The most common clinical picture associated with EVA is progressive, discretely stepwise, down-sloping and often fluctuant sensorineural hearing loss combined with episodic vertigo Most of the cases do not show any additional cochlear malformation[16 19] The reason why the EVA syndrome had not been associated for a long period of time with the Pendred syndrome, is the apparent lack of typical associated clinical features in patients with the Pendred syndrome As yet, only four patients with that syndrome have been described with fluctuant hearing loss and/or episodic vertig[1 8 23 24]

The present case histories and those previously reported by Cremers et al [8] suggest that the clinical distinction between the EVA and Pendred syndromes can be questioned In the present report we describe two patients with typical clinical features of Pendred syndrome Both are deaf, have goitre and a positive perchlorate discharge test Remarkably, the sister of one of them (case 3), who has the same *SLC26A4* mutation as her sister, had fairly similar clinical features, except for her negative perchlorate discharge test All three cases showed progressive hearing loss The rate of progression was at a maximum in early childhood and gradually declined thereafter All three patients had an enlarged vestibular aqueduct and showed remarkable fluctuations in hearing threshold, while two of them also experienced associated episodes of vertigo The progressive fluctuant hearing loss and the Meniere like vestibular episodes of vertigo are considered to have a common etiology in the homeostasis of endolymph As argued later on, this is supported by recent findings regarding the function of the Pendrin gene[29 30 33] One of them, in addition, showed cochlear hypoplasia It is intriguing but puzzling to note that there was significant cofluctuation not only ipsilaterally, i e involving (adjacent) frequencies, but also contralaterally The only case that did not show significant contralateral cofluctuation was case 1, whose hearing and vestibular function on the left side was very poor or absent, which can be a trivial explanation for the lack of contralateral cofluctuation As far as we know, the present cases (1 and 3) are the first in whom it could be proven in a formal way that their threshold fluctuations were significantly simultaneous, as well as bilateral. In cases with bilateral enlarged vestibular aqueduct, variations in cerebrospinal fluid pressure have been previously suggested to underly the simultaneous bilateral presentation of hearing

impairment[8]. Recent experimental evidence strongly suggests that the profound dilatation of innerear structures involved is secondary to altered osmotic content of endolymph caused by a failure in pendrin's normal anion-transporting function[25,32]. The occurrence of bilateral cofluctation is suggestive of bilaterally coupled, or perhaps systemic, variations in the physiological consequences of *SLC26A4* mutations. Perhaps, there is systemic, regulatory involvement of endocrine factors relating to substances such as, for example, vasopressin.

The clinical picture in our patients is quite similar to that in patients with the EVA syndrome. This raises the question as to whether some cases of EVA might in fact have a milder variant of Pendred syndrome rather than a different disorder. Of the present two sisters sharing the same mutation in *SLC26A4*, one has a positive, but the other a negative perchlorate discharge test. As there is no question about the validity of the diagnosis of Pendred syndrome in the latter case, it is clear that a positive perchlorate discharge test is not obligatory for the diagnosis of Pendred syndrome.

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3.2

PENDRED SYNDROME REDEFINED. REPORT OF A NEW FAMILY WITH FLUCTUATING AND PROGRESSIVE HEARING LOSS

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In 1896, Vaughan Pendred (figure 1) described a combination of congenital deafness and goitre that developed during puberty in 2 sisters[1]. In 1927, four new families were again reported[2]. In 1956, the autosomal recessive patterns of inheritance were recognized[3]. Thyroid enlargement is not always present and in 1958 it was reported that it is caused by a defect in organification of iodide, which results in impairment of thyroxin synthesis[4]. The test that until now has been used to diagnose Pendred syndrome is the perchlorate discharge test. Following perchlorate administration, radioactive iodide is given to the patient and the release of radioactivity (washout) is then recorded: a washout of > 10% is diagnostic for Pendred syndrome.

Pendred syndrome is an autosomal recessive disorder. Regular features in Pendred syndrome are an enlarged vestibular aqueduct (EVA) and, less commonly, hypoplasia of the cochlea[5] (figure 2,3).

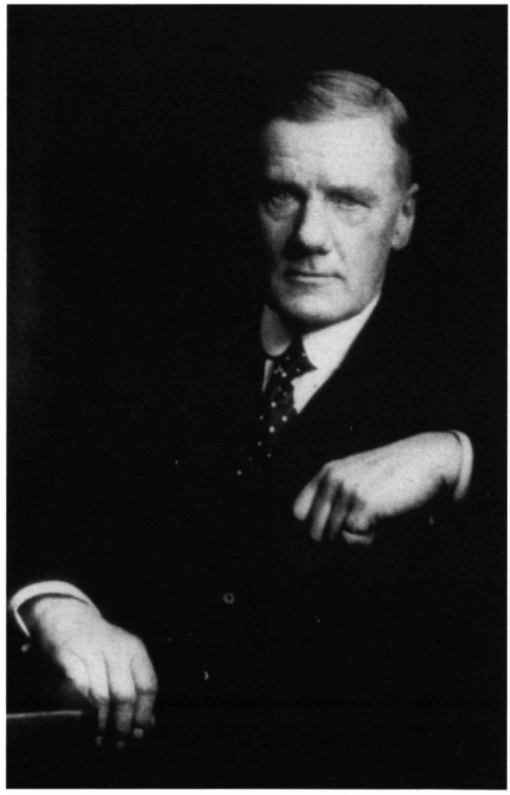

Figure 1: Vaughan Pendred (1869-1964

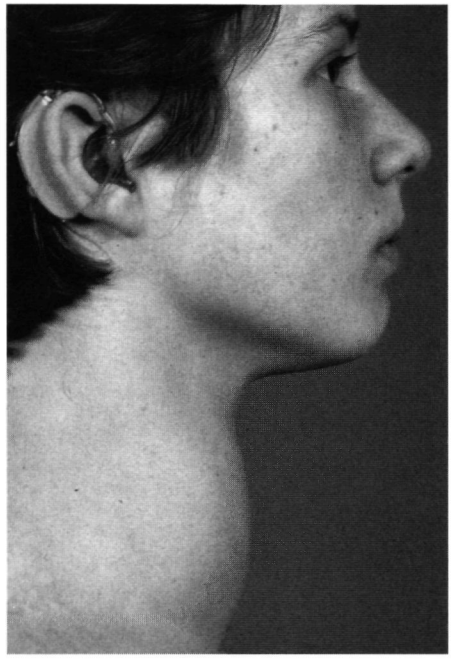

Figure 2: Patient with the Pendred syndrome. Clinical features as the enlarged thyroid.

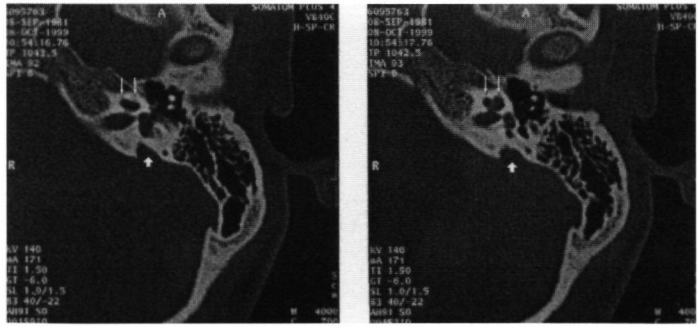

Figure 3a, b: CT scans of the inner ear in the Pendred syndrome. The widened vestibular aqueduct is shown by a broad white arrow. The hypoplasia of the cochlea is shown by two small white arrows.

The responsible gene has been mapped to chromosome 7q31 and designated PDS (SLC26A4)[6 8] The gene product, pendrin, is a transmembrane transporter protein that was originally thought to carry sulphate, but later turned out to be a chloride-iodide co-transporter[9] The function of pendrin in the thyroid gland is probably to transport iodine into the follicular lumen prior to its incorporation into thyroglobulin[10 11] In the inner ear, pendrin is predominantly expressed in the endolymphatic sac and duct and, to a lesser extent, in nonsensory regions of the utricle, saccule and cochlea It has been postulated that pendrin-dependent chloride transport is critical for maintaining endolymphatic ion homeostasis, which is essential to normal inner ear function[12 13]

Disease-causing mutations in [SLC26A4] have also been found in non-syndromic deafness with EVA, which has been called DFNB4 in some reports, and the EVA syndrome in others The first reported family whose hearing impairment trait was designated as DFNB4 later was found to involve thyroid enlargement and thus can be diagnosed as Pendred syndrome rather than a non-syndromic type of deafness[14] Later, more families with traits of non-syndromic deafness and EVA were identified and found to have mutations in [SLC26A4], again, both the terms EVA syndrome and DFNB4 have been applied to such traits by different authors[15 17] It is doubtful whether the label DFNB4 can be maintained

Report of a new Pendred syndrome family with fluctuating hearing loss

Figure 4 shows longitudinal audiometric data (air conduction threshold) in 2 of our most recent Pendred patients (A and B) to illustrate the features of progression and fluctuation Mutation analysis was reported by Van Hauwe et al [18] (family 1) Patient A is a 27-year-old woman, born as the third of normal hearing and healthy parents Pregnancy and delivery were normal and birth weight was 4100 g At the age of 2 months she was hospitalized for breathing problems and hypothyroidism was diagnosed At that age, the parents noticed that their daughter had hearing loss

Her hearing deteriorated progressively and with threshold fluctuations (figure 4a, left ear) At the age of 16, she presented with an euthyroid multinodular goitre, and diagnosis of Pendred syndrome was made Cytogenic examination showed normal female karyotype MRI of the temporal bones showed bilateral EVAs and bilateral normal cochlea and cochlear nerve Genetic analysis showed mutations FS 634 and V138F in [SLC26A4] in this patient

Patient B is patient A's 4 years' old cousin Pregnancy was complicated by bleeding at 3 months and a streptococcal infection at 8 months Both parents and his older sister have normal hearing

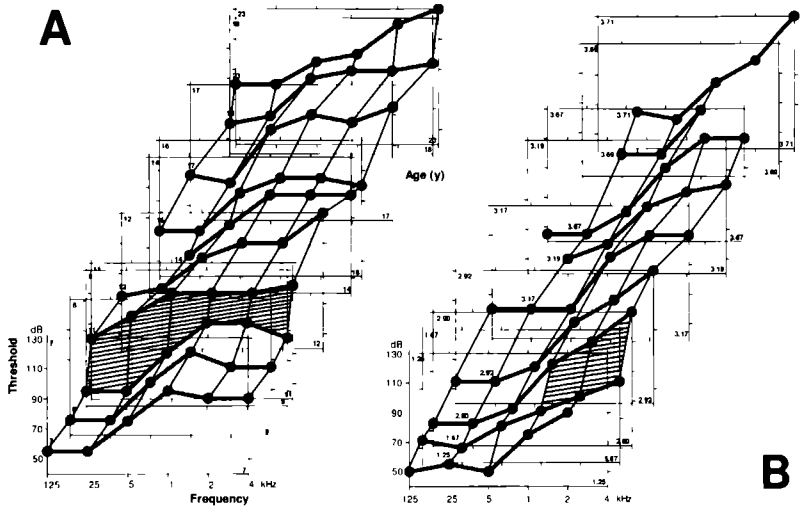

Figure 4: Pseudo-3D surface view (i.e. with an unscaled age axis and without perspective) of serial hearing threshold data for one ear in 2 Pendred patients, followed between age 7 and 23 (A) and age 1.25 and 3.71 years (B). The 3D upper surface shown is outlined by the data points connected by threshold lines (bold) and lines connecting consecutive measurements at the same frequency at different ages. "Roughness" of that surface is accentuated by the presence of substantial threshold fluctuations. Surface "steepness" relates to progression in threshold. For example, the largest across-frequency mean threshold shifts between consecutive threshold planes (age indicated on planes) occur at the crosshatched areas (from age 9 to 11 in panel A and from age 1.67 to 2.90 in panel B). Please note that placement of the consecutive threshold planes along the age axis is in the correct order but at unscaled positions. This is because interplane distance was manipulated in such a way that at each frequency the threshold measured at a given age was always plotted at a higher position than all previously measured thresholds at that frequency It was thus possible to maintain a clear view on all separate parts of the upper surface.

At the age of 6 months he still reacted to noises, but at the age of 10 months, his parents (his father is the brother of patient A) first noticed a decrease in hearing ability. BERA was performed and showed on the left side a hearing level of 60 dB SPL and on the right side a hearing level of 80 dB SPL. Because of a subacute otitis media, tympanic drains were placed. In the postoperative period he fell seriously. Postoperative BERA did not show different hearing level on the right side, on the left side no reactions were measured anymore. Hearing gradually deteriorated with threshold fluctuations (figure 4b, right ear). At the age of 2, he

presented with episodes of vertigo with a falling tendency and vomiting Neurological examination, including EEG, was normal He was euthyroid and had no goitre A perchlorate discharge test was negative. Imaging studies revealed the bilateral presence of an EVA, as well as an enlarged vestibulum and shortening of the posterior semicircular canal. There was an abnormal modiolus and probably a beginning Mondini dysplasia. Mutation analysis of [SLC26A4] revealed the presence of FS 634 and G209V mutations

Radiological findings of the inner ear in Pendred syndrome and EVA syndrome
EVA syndrome is defined as non-syndromic deafness with EVA An EVA is also a very common feature in Pendred syndrome Often, a Mondini-type cochlea with a deficiency of the interscalar septum is present The first to report a radiologically confirmed Mondini-type cochlea in a patient with Pendred syndrome was Jensen[19] in 1967 This was confirmed by Illum[20] in 1972 The first description of a large vestibular aqueduct shown by imaging was by Valvassori and Clemis[21] in 1978 They defined the EVA as being > 1.5 mm in diameter in the mid-portion of the descending limb In 1998, Phelps et al [22] examined 40 patients with Pendred syndrome by high-resolution computed tomography. In 8 of the 40 cases they found the presence of a Mondini-type cochlea with a deficiency of the interscalar septum An EVA (according to the criteria of Valvassori and Clemis[21]) was shown bilaterally in 31 of the 40 patients, and unilaterally in 2 cases The enlarged vestibular aqueduct was usually asymmetrical, with a diameter varying from 1 8 to 5 8 mm. Twenty cases underwent MRI and all were found to have an enlarged endolymphatic sac. Phelps et al [22] were also able to demonstrate a certain radiological discordance between different siblings In the same year, Cremers et al.[5] examined 12 consecutive Pendred patients Seven of them underwent CT or MRI. all had bilateral EVA and 4 had a hypoplastic cochlea They concluded that EVA and hypoplasia of the cochlea are very common features in Pendred's syndrome

Mutation analysis in Pendred syndrome and EVA syndrome (DFNB4)
In 1995, Baldwin et al [14] examined three large Druze families with multiple deaf members Deafness was congenital an no other additional features were observed They localized DFNB4 to a 5-cM region between D7S501 and D7S523 on chromosome 7q Affected members of this family were later found to have goitres and thus Pendred syndrome. Coyle et al.[6] and Sheffield et al.[23] found linkage between Pendred syndrome and markers on chromosome 7q31, and Coucke et al [8] reduced the Pendred candidate region to 1.7 cM. In 1997, Everett

et al [24] reported the identification of the Pendred gene [SLC26A4], and identified three deleterious mutations in five Pendred families They found that [SLC26A4] encodes for a putative sulphate transporter The protein encoded by the [SLC26A4] gene was predicted to be a 780-amino-acid protein and was named pendrin There was a statistically significant homology to 13 other proteins, which are most known to function as sulphate transporters Different mutations in [SLC26A4] gene were identified[18 25 26] In 1999, Scott et al [9] were able to demonstrate that [SLC26A] does not encode a sulphate transporter, but a chloride-iodide transport protein In a mouse model, Everett et al [12] demonstrated a [SLC26A4] expression throughout the endolymphatic sac and duct, in distinct areas of the utricle and saccule and in the external sulcus region within the cochlea One year later, Royaux et al [11]demonstrated that pendrin has a likely role as an apical porter of iodide in the thyrocyte Recently, Everett et al [13] have developed a Pds knockout mouse Those mice develop early onset, profound deafness, as well as pronounced signs of vestibular disease with variable expressivity They do not have biochemical or histological evidence of thyroid disease The lack of pendrin in these mice leads to a profound dilatation of inner-ear structures, associated with degeneration of outer and inner hair cells in the organ of Corti and of the maculae in the utricle and saccule

Simultaneously to these investigations, in different families with nonsyndromic hearing loss with EVA and normal perchlorate test, mapping to 7q31 was found[15] In six families with non-syndromic hearing loss and EVA, seven mutations of [SLC26A4] have been found[16] The postulation was made that mutations in [SLC26A4] cause both syndromic and non-syndromic hearing loss This hypothesis was supported by Masmoudi et al [27] who found phenotypic variability in two families carrying the same [SLC26A4] missense mutation Scott et al [28] demonstrated that mutations in [SLC26A4] associated with Pendred syndrome had complete loss of pendrin-induced chloride and iodide transport, while alleles unique to people with DFNB4 were able to transport both iodide and chloride, but at a lower level than wild-type pendrin

Audiometric presentation of Pendred and EVA syndrome

Originally, hearing loss in in Pendred syndrome was mainly considered as being congenital sensorineural hearing loss Figure 5 shows the mean hearing threshold in 141 ears of 71 Pendred patients described in literature[5 14 29 40] Hearing loss is profound and the audiogram has a steeply downsloping configuration Given the fact that it is the mean threshold that is shown in figure 5, many patients ($\leq$ 50%) have or will eventually have residual hearing

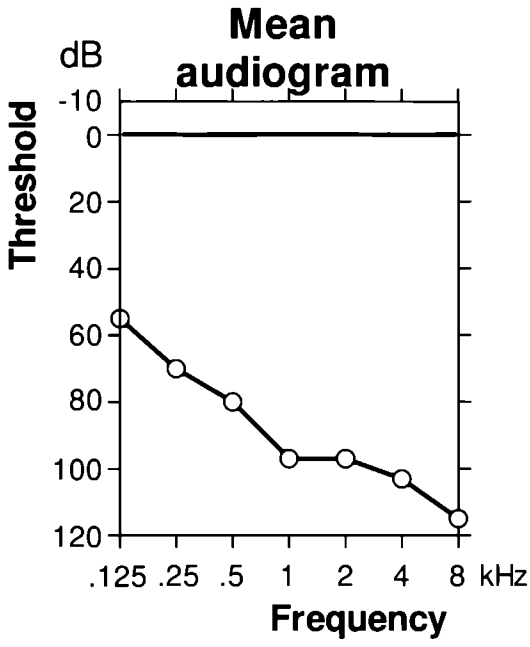

Figure 5: Mean audiogram of 71 previously reported Pendred patients (141 ears)

Although progressive hearing loss in Pendred syndrome has been previously mentioned[41,42], the first reports demonstrating progression and/or fluctuation of hearing loss with audiograms in patients with Pendred syndrome date from 1980. Hörmann et al.[29] described a patient with progressive hearing loss and Pendred syndrome, who had episodes of sudden deterioration, which (partially) recovered during therapy. In a family with seven affected persons, Bergstrom[30] found one sibling with unilateral fluctuations in hearing threshold, while all other affected members of the family showed congenital moderate to severe sensorineural hearing loss. In 1987 Johnsen et al.[31] described 17 unrelated Pendred patients. In fifteen patients, hearing loss had been diagnosed before the age of three years. Three patients had noticed some progression of hearing loss, and in one of those this amounted to about 20 dB. In the same year Das[32] reported on a patient with Pendred syndrome and severe bilateral sensorineural deafness at the age of three years. Later she had episodes of vertigo accompanied by nausea and occasional

vomiting Hearing loss had slightly progressed by 10-15 dB Cremers et al [5 35] found significant progression in hearing loss in several patients, progression was most rapid in early childhood We found fluctuant hearing loss by extensive serial audiometry in some patients, with significant ipsilateral and contralateral cofluctuation, two of them had recurrent episodes of vertigo[43]

Because an EVA seems to be one of the main features in Pendred syndrome, which is often associated with progression and/or fluctuation in hearing loss, a link can be made to the EVA syndrome (sometimes labeled DFNB4) Both syndromes have important clinical features in common and are caused by mutations in [SLC26A4] Hearing loss in the EVA syndrome has been described to show stepwise progression and threshold fluctuations as characteristic features Levenson et al [44] described 22 ears in 12 patients Four ears showed profound sensorineural hearing loss and ten ears severe to profound down-sloping loss with fluctuations Eight ears had initially normal to serviceable hearing but six of these developed progressive loss featuring as sudden hearing loss in five cases Jackler and De La Cruz[45] examined 17 patients with 33 abnormal ears The audiometric configuration was down-sloping in 23 ears, midfrequency in 4 ears, flat in 1 ear and profound in 3 ears Significant progression was present in 15 ears and was characterized by stepwise decrement Hearing fluctuations were noted in 2 patients Five patients also had vestibular problems adults mentioned episodic vertigo, in children it was more some type of imbalance[45] Other authors also described vertigo[15 16 46 47]

DISCUSSION

Based on the clinical features of both syndromes, one could question if DFNB4 is not a milder form of Pendred syndrome As previous mentioned Masmoudi et al [27] studied two large Southern Tunesian families with Pendred syndrome All 19 affected individuals were found to have the same mutation (L445W) in [SLC26A4] Only 11 of them had palpable goitre and the perchlorate discharge test was negative in all 8 individuals tested The authors concluded that the perchlorate discharge test is not as suitable for diagnosing Pendred syndrome as was previously thought Variable expression of the syndrome is probable, ranging from non-syndromic hearing loss with EVA to typical Pendred syndrome with thyroid enlargement This hypothesis is supported by Scott et al [28], who demonstrated that affected persons having mutations associated with Pendred syndrome showed a complete loss of pendrin-controlled iodide and chloride transport, while alleles

unique to persons with DFNB4 were associated with transport of both iodide and chloride, but at a far lower level than in persons expressing wild-type pendrin. They hypothesized that the residual level of anion transport is sufficient to eliminate or postpone the onset of goitre in individuals with DFNB4.

CONCLUSIONS

Mutations in [SLC26A4] are responsible for several clinical conditions with overlapping features, ranging from classical Pendred syndrome to non-syndromic hearing loss with EVA. The perchlorate discharge test is not as sensitive as was previously thought and can be negative in patients with clinically obvious Pendred syndrome. Hearing loss in these clinical conditions associated with [SLC26A4] mutations is predominantly down-sloping en progressive, sometimes fluctuating with episodes of sudden hearing loss. Progression is particularly rapid in early childhood. Episodic vertigo can be present in a number of cases.

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Chapter IV

NON-SYNDROMIC AUTOSOMAL RECESSIVE HEARING LOSS DFNB1 (Connexin 26 and Connexin 30)

4.1

LONGITUDINAL PHENOTYPIC ANALYSIS IN PATIENTS WITH Connexin 26 (*GJB2*) (DFNB1) AND Connexin 30 (*GJB6*) MUTATIONS

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ABSTRACT

In 15 Belgian subjects with prelingual sensorineural hearing impairment, the connexin 26 *(GJB2)* gene and the connexin 30 *(GJB6)* gene were analyzed for the presence of the 35delG mutation and the Δ(*GJB6*-D13S1830) deletion first described by del Castillo et al in 2002 Seven patients were found to be homozygous for the 35delG mutation, 7 were combined heterozygotes for the 35delG mutation and the *GJB6* deletion In 11 subjects, phenotype and genotype were correlated Significant, transient progression, in the range of 1 7 to 2 7 dB/year, was only found in 2 patients in the first part of the second decade of life Hearing impairment was otherwise stable, with mean thresholds of 75, 90 and 100 dB at 0 125, 0 25 and 0 5 kHz, respectively, and 100 dB or higher at 1 to 4 kHz There was no significant difference in hearing impairment between the patients with the homozygous 35delG mutation in *GJB2* and those who are heterozygous for both the 35delG mutation and the deletion encompassing part of *GJB6*

INTRODUCTION

Congenital sensorineural hearing loss has a general prevalence of 1 to 2 per 1,000 newborns About 50 % of the cases of hearing impairment have genetic causes, and most cases are autosomal recessive[1] Mutations in the gene coding for connexin 26 (CX26) *(GJB2)* are responsible for about half of the cases of autosomal recessive deafness[2 3]. A deletion of a guanosine (G) in a sequence of 6 Gs extending from position 30 to position 35 (35delG) accounts for the majority of the CX26 mutant alleles Other mutations in *GJB2* have been identified, and compound heterozygotes (in each allele a different *GJB2* mutation) have been described[2 13]

Several authors have reported on families with hearing loss linked to the DFNB1 locus and only 1 mutated *GJB2* allele[2 4 6 8 12 14] Therefore, it was postulated that another gene close to *GJB2* might be responsible for these cases The gene encoding CX30 *(GJB6)* was an obvious candidate It is expressed in the same inner ear structures as *GJB2* and the expressed proteins are functionally related[15 17] Del Castillo et al succeeded in identifying a deletion Δ(*GJB6*-D13S1830) in 9 individuals with positive linkage of the families to DFNB1 and only one *GJB2* mutant allele[18] In a Belgian family covering 4 generations with several hearing-impaired individuals, 15 subjects were further analyzed In 11 individuals, covering generations IV and V of the pedigree, we were able to compare the phenotype of

the individuals with homozygous 35delG mutations to the phenotype of those heterozygous for both the 35delG and Δ(*GJB6*-D13S1830) mutations

PATIENTS AND METHODS

The subject of the clinical study was a Belgian family containing 16 individuals showing bilateral nonsyndromal prelingual hearing loss Eleven patients were pupils or former pupils of the Royal Institute for the Deaf, Spermalie (Brughes) In order to investigate homogeneity in the causes of obviously autosomal recessive deafness in this family, we analyzed genotype and, where possible, phenotype
All individuals participating in this clinical study agreed to a genetic examination The individuals whose audiometric results were available agreed to have their audiograms evaluated for further analysis A medical history was taken Blood samples were obtained for genetic analysis

Serial audiometry and statistical analysis
In 11 patients in generations IV and V of the pedigree , different consecutive pure-tone audiograms (air conduction thresholds) were obtained of every individual, with a mean number of measurements of 12 64 each and a mean follow-up of 18 6 years
Bone conduction was measured incidentally to check that hearing impairment was purely sensorineural It was checked that the patients did not have any features of syndromic hearing impairment Individual longitudinal data were analyzed per frequency, per ear, by use of linear regression analysis (threshold on age) performed with a commercial program (Prism 3, GraphPad, San Diego, California) Slope (regression coefficient) was designated as significant if its 95% confidence interval did not include zero A given ear was considered to show significant slope (positive or negative) only if the prevalence of frequencies showing significant slope among all frequencies evaluated was higher than expected (P = 0 025) according to the appropriate binomial distribution Regression lines were compared by an analysis similar to analysis of covariance One-way analysis of variance and Student's *t*-tests were performed to detect significant differences (P < 05) between any (sub)groups of patients Frequency distributions and confidence intervals were also evaluated

Mutation analysis
Genomic DNA was isolated from peripheral blood as previously described[19]

The presence of the 35delG mutation in the GJB2 gene was studied by sequence analysis of a polymerase chain reaction (PCR) fragment after amplification with the primers 5'-GTGTTGTGTGCATTCGTCTTCTCC-3' and
5'-GTCGGCCTGCTCATCTCCCC-3' under standard PCR conditions.
For sequencing of the entire coding region of the GJB2 gene, exon 1 was amplified with the primers 5'-TCCGTAACTTTCCCAGTCT-3' and
5'-GGGTTCCTGCACACAACCAGGTCGGGG-3'.
Exon 2 was amplified with the primers 5'-GTGTTGTGTGCATTCGTCTTCTCC-3' and 5'-CGGAGTAGGGAGAGTACGACAG-3'. Sequence analysis was performed with the ABI Prism Big Dye Terminator cycle sequencing V2.0 ready reaction kit and the ABI Prism 3700 DNA analyzer (Applied Biosystems, Breda, the Netherlands).

The presence of the deletion encompassing part of the GJB6 gene[18] was analyzed by use of 3 primers to amplify the break-point-containing fragment and the normal GJB2 allele simultaneously. The primer sequences are as follows
5'-AGTGATCCATCTGCCTCAGC-3' (primer 1, Fig 1);
5'-GTCTGTGCTCTCTTTGATCTC-3' (primer 2, Fig 1) and
5'-GGAAGGTGTGGATCACAGTC-3' (primer 3, Fig 1).
Amplification was performed with Accu Prime Buffer II (Accu Prime kit, Invitrogen, Nieuwerkerk aan de Yssel, the Netherlands). The cycling conditions were 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute, 35 cycles in a PTC200 thermal cycler (MJResearch Inc, Waltham, Massachusetts). The PCR fragments were analyzed on a 1.5% agarose gel.

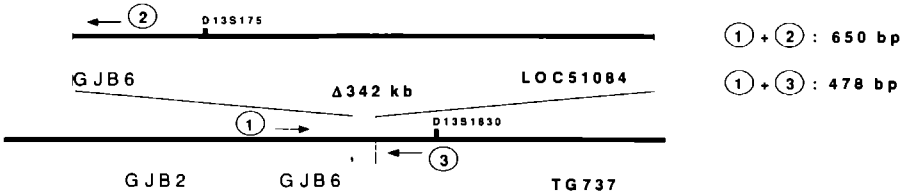

Figure 1: Schematic representation of genomic region of *GJB2* and *GJB6* genes and 342 kb deletion. Primers are represented by circled numbers.

RESULTS

Genetic analysis

All patients, and when possible their parents, were tested for the presence of the 35delG mutation in the *GJB2* gene by sequence analysis. In generations III through V, 7 patients were homozygous for this mutation (Fig 2, genotype a/a; y/y, where a indicates the 35delG *GJB2* mutation and y wild-type *GJB6*), which explains the hearing loss in these cases. In 7 other patients the 35delG mutation was heterozygously detected (Fig 2, genotype a/x;...; with x indicating ...; wild-type *GJB2*). Sequencing the entire coding region of the *GJB2* gene for the patients IV:4, V:5, and V:6 did not reveal any additional mutations.

After a partial deletion of the *GJB6* gene had been described to cause hearing loss when present in combination with a heterozygous 35delG allele of the *GJB2* gene[18], patients heterozygous for the latter mutation were tested for the presence of this deletion of part of *GJB6* in the second chromosome. These cases were all heterozygous (genotype indicated as ...;b/y) for this deletion (Figs 2 and 3). Subsequently, all remaining individuals of the family from whom DNA was available were tested for the *GJB6* deletion (Fig 3). These tests revealed that the deletion is likely to have been inherited directly or indirectly from individual II:3.

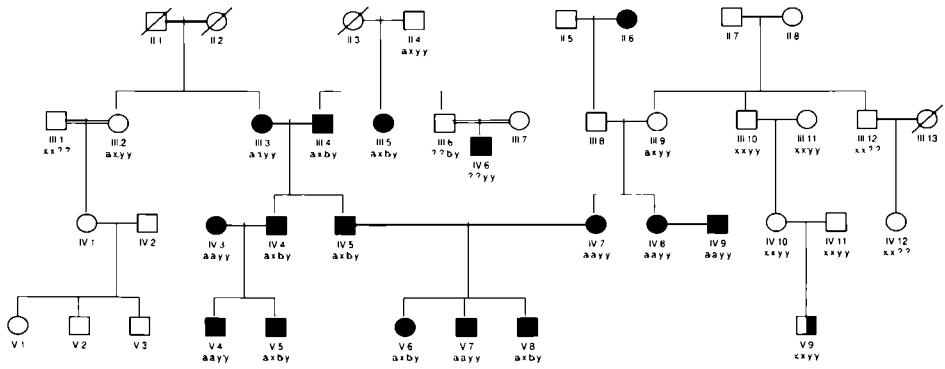

Figure 2: Pedigree. Roman numerals indicate generations. Square-man; Circle-woman; filled symbols-bilateral hearing impairment; half-filled symbol-unilateral hearing impairment; slashed symbol-deceased person; x-wild-type (normal) *GJB2*; y-wild-type *GJB6*; a-*GJB2* 35delG; b-*GJB6* deletion; ?-unknown.

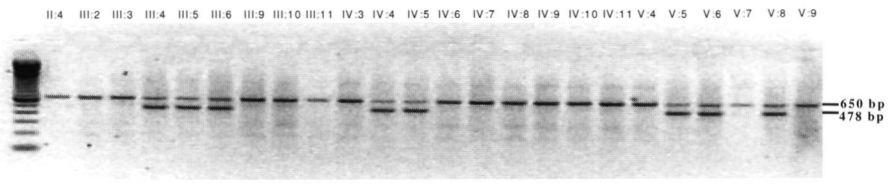

Figure 3: Segregation of 342 kb deletion in family. Presence of 478 bp fragment indicates 342 kb deletion. 650 bp fragment represents normal allele.

Audiometric analysis

Hearing impairment was evaluated in 11 of the 12 affected persons in generations IV and V; no audiogram could be obtained from person IV:6 (Fig 2)The audiograms obtained at one of the last visits are shown in Fig 4. The patients showed residual hearing, ie, functional hearing only at the lower frequencies, with a steeply down-sloping threshold (by about 10 dB per octave) from about 75 dB at 0.125 kHz up to 120 dB or more at the higher frequencies (details below). Most of the patients dated their first symptoms of hearing loss as early as they could remember. All had delayed speech and language development. None of the patients had any symptoms that seemed to be related to the vestibular system. No formal vestibular testing was performed.

The longitudinal threshold data are shown (ie, only for the right ear) in Fig 5. Two patients (IV:4 and IV:7) showed significant progression in both ears, whereas patient IV:5 showed this feature only in the right ear and patient IV:8 only in the left one (data not shown). Remarkably, there were also patients who showed a significant negative slope: patients V:5 and V:6, each in one ear. The latter finding appeared to be associated with the presence of thresholds measured at a very young age, where threshold evaluation can be notoriously difficult. For this reason we excluded data obtained at the age of 6 year or below and repeated the regression analyses (data not shown); significant negative slopes were no longer found.

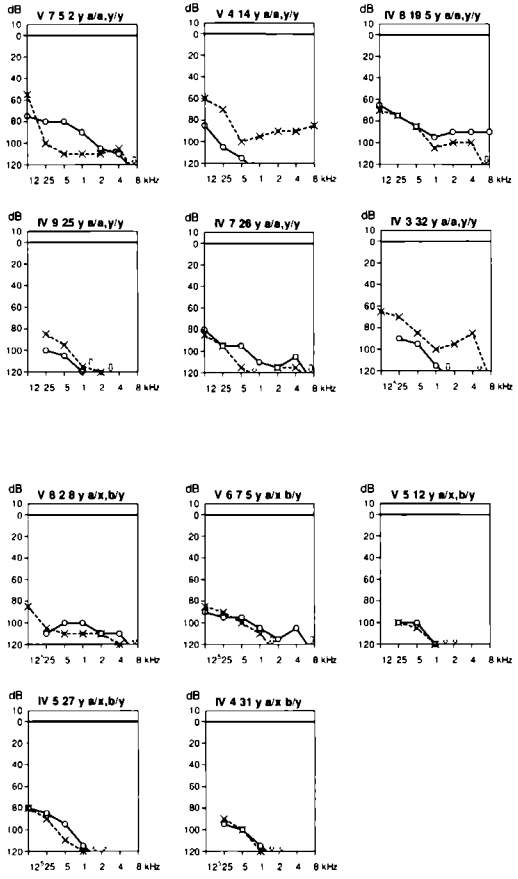

Figure 4: Individual audiograms (air conduction threshold in decibels hearing level) shown for right (circles) and left ear (crosses) of 11 affected individuals in generations IV and V. Downward arrows indicate out-of-scale measurements. Patient's age is given in years (y). Audiogram at last visit was selected, or previous one if that was more informative. Patients are ordered by genotype and age at last visit. Upper 6 panels are patients with homozygous 35delG (genotype a/a; y/y). Lower 5 panels are patients with combined heterozygous mutations (genotype a/x; b/y).

It appeared that the exclusion of measurements at the age of 6 years and below caused the loss of significant progression in patient IV:8 (left ear, data not shown), but not in patients IV:4, IV:5 and IV:7 (right ear, Fig 5). In patient IV:5, significance was lost after the additional exclusion of ages 6.5 and 7 years (Fig 5, dashed vertical line). Significant progression in patients IV:4 and IV:7 (both ears; right ear shown in Fig 5) was specifically linked to a single episode at age 11 to 14 years (1.7-2.0 dB/y) and age 7 to 13.5 years (2.2-2.7 dB/y), respectively. The upper age interval (Table 1) used for the calculation of individual threshold statistics (mean and SD) is indicated in Fig 5. It will be clear from the above-described selection

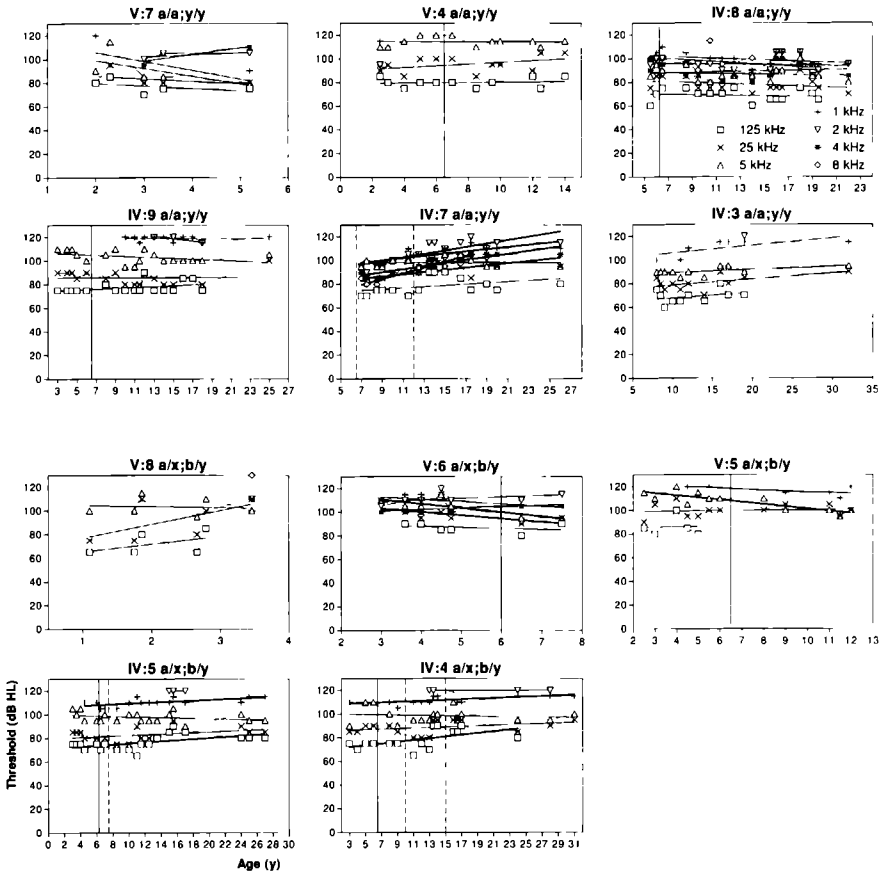

Figure 5: Longitudinal records of threshold (decibels hearing level, measurable thresholds only) against age for right ear (left ear not shown). Patients and ordering are similar to those in Fig 4. Connecting hairlines and regression lines are included. Bold regression lines show significant (non-zero) slope. Negative slopes were no longer significant (data not shown) after exclusion of ages of 6 years and below (continuous vertical line). Pair of dashed vertical lines in panels of patients IV:4 and IV:7 pinpoint age interval showing significant progression (separate regression lines not shown). Only threshold data on right side of rightmost vertical line were used for calculation of individual threshold statistics (Table 1).

procedure that no significant progression (or negative slope) was observed within this age interval, and hearing could reasonably be assumed to be stable. Reliable longitudinal evaluation was possible in 8 patients. Patients V:6, V:7, and V:8 were unsuitable for such an evaluation ($n < 3$) after exclusion of age 6 years and below. Student's *t*-tests did not reveal any substantial interaural threshold difference per frequency at any age; 67% of the patients had an interaural SD at any frequency of < 7 dB. The thresholds in the right and left ear were therefore averaged for the calculation of the intersubject threshold statistics (mean, SD and 95% confidence

intervals, Table 2). Student's *t*-tests and analysis of variance or analysis of covariance disclosed significant differences between the lower frequencies (0.125, 0.25 and 0.5 kHz), but not between the frequencies 1-4 kHz, which were therefore pooled. The mean threshold rounded to the nearest multiple of 5 dB, was 75, 90 and 100 dB hearing level (HL) at 0.125, 0.25, and 0.5 kHz, respectively, and > 100 dB at 1 to 4 kHz (Table 2).

Table 1: Mean thresholds (decibels hearing level) in eight evaluable cases.

	Right ear							Left ear						
	0 125 kHz	0 25 kHz	0 5 kHz	1 kHz	2 kHz	4 kHz	8 kHz	0 125 kHz	0 25 kHz	0 5 kHz	1 kHz	2 kHz	4 kHz	8 kHz
Genotype a/a; y/y														
IV:3 (age 8-32y)														
N	10	11	11	7*	-			11	11	11	11	11	9*	4*
Mean	69	81	90	110				60	66	78	99	97	91	91
SD	6	6	4	7				9	8	7	5	7	6	2
IV:7 (age 12 5-26y)														
N	9	9	9	9	9	9	6*	9	9	9	7*	9	9	
Mean	83	96	99	108	113	101	98	82	93	111	118	118	117	
SD	7	5	4	4	4	5	5	4	2	3	3	2	3	
IV:8 (age 6.5-22y)														
N	13	14	14	14	14	14	8*	13	14	14	14	14	14	8*
Mean	69	78	86	99	95	89	97	78	80	87	103	96	94	91
SD	5	6	6	7	7	7	8	6	6	5	2	6	7	9
IV:9 (age 8-25y)														
N	6	7	7	-		-	-	12	12	13	13	8*	-	-
Mean	80	96	114					80	83	99	116	118		
SD	4	7	3					6	6	3	4	4		
V:4 (age 7-14 y)														
N	6	7	7	-	-			7	7	7	7	7	7	2*
Mean	80	96	114					68	80	98	94	88	85	85
SD	5	7	3					8	8	6	7	4	5	0
Genotype a/x,b/y														
IV:4 (age 16-31 y)														
N	4	6	6	6	2*	-	-	4	6	6	6	4*		
Mean	85	92	99	114	120			90	94	99	116	120		
SD	4	4	6	4	0			4	7	4	2	0		
IV:5 (age 8.5-27y)														
N	12	12	12	12	3*			12	12	12	12	5*		-
Mean	78	84	97	111	120			78	83	99	112	118		
SD	7	6	4	3	0			7	5	6	5	4		
V:5 (age 8-12y)														
N		5	5	4*		-	-	1	5	5	5	-	-	-
Mean		101	101	115				75	98	101	119			
SD		4	5	4					3	4	2			

Genotypes a/a,x/x and a/x,b/y indicate homozygous 35delG mutations and combined heterozygous (*GJB2/GJB6*) mutations, respectively

*, out of scale thresholds excluded (mean biased downwards)

Table 2 Intersubject binaural mean thresholds (decibels hearing level) of eight cases in table 1

	0 125 kHz	0 25 kHz	0 5 kHz	1 kHz	2 kHz	4 kHz	8 kHz
N	7	8	8	8	4	3	-
Mean	77	88	98	109	99	95	
SD	7	9	9	9	12	13	
Round mean	75	90	100		>100		
95%CI	60-95	70-110	80-120		>80		

CI – confidence interval

It should be emphasized that the mean thresholds at 4 kHz may have been higher, as only measurable thresholds could be included and many thresholds at this frequency were out of scale (Fig 4) The 95% confidence intervals at 0 125, 0 25 and 0 5 kHz were 60 to 95, 70 to100 and 80 to120 dB HL, respectively The fifth percentile (P_5) level (lower confidence limit) at 1 to 4 kHz was > 80 dB HL

Using Student's *t*-test to compare mean binaural thresholds according to frequency between subgroups of patients, we failed to find any significant difference between the subgroup of patients with homozygous 35delG mutations (genotype a/a,y/y) and that of patients with the combined heterozygous genotype (genotype a/x,b/y)

DISCUSSION

Previous reports have appeared on the phenotype of DFNB1 and genotype analysis for homozygous and compound heterozygous *GJB2* mutations Estivill et al[2] described hearing losses in patients homozygous for the 35delG mutation that were severe to profound for the middle and high frequencies, in a few cases with some sparing of the low frequencies Denoyelle et al[3] described symmetric hearing impairment varying from mild to profound, associated with flat or downsloping audiometric curves in patients with prelingual onset Intrafamilial variation in the severity of hearing impairment was common, and hearing impairment was generally not, or only slightly, progressive These findings also apply to the studies on 29 homozygous and compound *GJB2* heterozygous hearing-impaired individuals by Sobe et al[9], the 52 hearing-impaired individuals with CX26 mutations examined by Dahl et al[11] and the 34 patients with CX26 mutations described by Kenna et al[10] Progression was present in 10 individuals studied by Cohn et al[20], the hearing impairment was comparable to that in the

studies mentioned above. A difference in the degree of hearing impairment between homozygotes and compound *GJB2* heterozygotes was noticed by Orzan et al[6]; the relative frequency of homozygotes for the 35delG mutation was lower in the category of severe impairment than in profound or mild impairment. Compound *GJB2* heterozygotes, on the other hand, tended to occur more often among the severely impaired individuals.

Wilcox et al[8], who compared hearing impairment in 8 individuals with only 1 allele with a CX26 mutation to that in 6 individuals with two alleles with a CX26 mutation, could not demonstrate any difference in hearing loss between the groups, and neither did Marlin et al[12].
As far as we know, the phenotype of individuals with both CX26 and CX30 mutations has not been previously described. In our patients, there was a high proportion of combined CX26/CX30 heterozygosity. This is remarkable, given the suggestion by Van Camp that this feature might play only a minor role in Belgium[21].

We failed to find any significant difference in the degree of hearing impairment between 35delG homozygotes (genotype a/a;y/y) and heterozygotes for both the CX26 and CX30 mutation (genotype a/x;b/y). In all of these patients, the hearing loss was severe to profound, with a steeply down-sloping audiometric configuration, and showed prelingual onset. In contrast to previously described phenotypes relating to CX26 mutations, none of our patients showed mild or moderate hearing loss. No substantial, significant progression in hearing impairment was observed in any of our patients, except for 2 of them, in whom it occurred only temporarily, in the first part of the second decade of their life (1 case with the genotype a/a;y/y, and 1 case with a/x;b/y). It should be emphasized that we only examined a small number of patients and that all of our patients were younger than 40 years, so presbycusis was not a factor Further studies are needed to extend our knowledge about the prevalence of and variations in severity or progression of hearing loss of patients with homozygous CX26 mutations and those with the combination of a CX26 mutation and a deletion encompassing part of CX30.

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Chapter V

DISCUSSION

DISCUSSION

During the last 10 years the scientific progress made in the field of syndromic and non-syndromic genetic hearing impairment has been enormous The start was made one decade earlier by gene-linkage and gene-identification studies in syndromic types of genetic hearing impairment This new knowledge provided the possibility to study the inner ear metabolism at a molecular level Such knowledge is needed to develop strategies for possible new treatments To evaluate in the future such new treatments, a good knowledge is needed about the natural course of the diseases involved including genotype-phenotype correlation studies

This Ph D study was started in Leuven to look for opportunities to have a more regular access to the clinical and genetic studies in the field of genetic hearing impairment Not having available a tradition locally to study genetic hearing impairment the help of an institute for the hearing impaired/deaf in Brughes proved to be helpful to get in touch with selected families with non syndromic profound childhood deafness (Chapter IV) and with the Flemish BOR family (Chapter II-2) The cooperation with the Nijmegen ORL department proved to be of value in the diseases studied in this Ph D thesis The CX-30 analysis in chapter IV was done by the Nijmegen otogenetic laboratory and so it became possible to finalize that study and to publish these data in chapter IV

In the study of the Flemish BOR family the outcome of especially the MRI study showed to be very valuable in showing up hyplasia of the cochlear nerve and secondly in showing presence of an enlarged vestibular aqueduct The first is a clinical fact valuable to be informed on related to the possibility of cochlear implantation This stimulated the Nijmegen ENT department to start up a similar and more extensive study on the radiological aspects in BOR syndrome and to relate those findings to the degree and progression of the hearing impairment
Regarding the Pendred syndrome the Nijmegen ENT department provided the opportunity to report in more detail on the presence of a fluctuant and progressive hearing impairment associated with Menière like attacks in this Pendred series Having this experience we succeeded to do the same for a Belgian family with 2 Pendred patients
So in all three diseases it proved to be helpful to be in connection with a number of other centres to be able to run the studies incorporated in this Ph D thesis And as it is in a successful cooperation this cooperation brought also benefit to those other centres

The studies on the BOR syndrome and especially on the findings of CT scanning and MRI scanning are the most extensive and thorough ones ever published in this syndrome in the literature. Most important is the prove that hypoplasia of the cochlear nerve can be there. It is most difficult to prove a fluctuating inner ear hearing loss as part of a mixed hearing loss where the conductive component can also be fluctuant. Nevertheless evidence of a progressive (inner ear) hearing loss is given.
Related to the presence of a widened vestibular aquaduct and the presence of malformations of the cochlear the question rises whether these malformations can be responsible for the progressive innear ear hearing loss and secondly whether the widened vestibular acqueduct in itself plays a role in this. The descriptions given are helpful in bringing up this question and to see where else in other diseases this question can be raised.
In the Pendred syndrome a widened vestibular aqueduct has shown to be almost an obligate feature. Dysplasia of the cochlea is also a feature. New knowledge about the genetic background including EVA syndrome made a redefinition of the clinical picture of Pendred syndrome necessary It raised the question whether fluctuant and progressive hearing loss combination or not with Menière like attacks of vertigo were also present in classical cases of Pendred syndrome. This proved again to be so and up till now scientific contributions into the literature are very scarce. This knowledge again helps to raise the question about the inner ear metabolism and whether the anatomical anomalies like the widened vestibular aqueduct and the malformation/hypoplasia of the cochlea are responsible for this fluctuant and progressive course of the hearing impairment combined with Menière like attacks. There is some analogy with the findings in BOR syndrome but the genetic background is quite different. Especially in Pendred syndrome an inner ear metabolism anomaly, based at the presumed function of the gene in the inner ear, might be held responsible to.

So the clinical and genetic contributions to the knowledge in BOR syndrome and Pendred syndrome included in this Ph.D. thesis provided additional and new knowledge helpful to solve in due time questions to be raised about the etiology of this progressive and fluctuant hearing loss. These chapters fulfilled the earlier raised questions to report on the clinical presentation and natural course of the disease in those patients related tot the deafness genotype.
The problem posed in chapter IV proved to be the most difficult one since mutations in connexine 26 could not elucidate fully the genetic background of the hearing impairment in those 15 people with a profound childhood deafness. The

genotype phenotype study could only be finalized after confirming the role of connexion 30 in all heterozygous 35delG hearing impaired subjects. It is also helpful to know that mutations in connexion 30 occur in Flandres. This has stimulated making available mutation analysis as a routine diagnostic procedure (Antwerp) not only for CX26 but also for CX30. In the same way the value for the quality of genetic counselling of this new knowledge does not need any further elucidation.

Performing this Ph.D. study it showed to be again a journey with unpredictable events. The outcomes of this study add like so many other studies to the fast increasing knowledge of genetic hearing impairment.

The design of the study included to look for cooperation with other in this topic specialised centres like an institute for the deaf, otogenetic laboratories especially in Omaha (USA) and Nijmegen (The Netherlands) and in oto-radiology specialised departments in Brughes (Belgium) and Nijmegen (The Netherlands). Having now completed this Ph.D. study it proved to be that this cooperation was essential and that the open scientific and cooperative attitude by those centres made it possible to complete this study.

Chapter VI

SUMMARY AND CONCLUSIONS

SUMMARY

Sensorineural hearing loss has important implications on our nowadays so communicative society, as it affects about 1 in 750 children and about 50 % of the people who reach the age of 80 It can as well be caused by genetic as by environmental (e g intra-uterine infections, noise exposure, blood-antagonism, meningitis, mediation causing deafness) factors

The majority of early childhood sensorineural deafness is caused by a genetic defect This can result in a syndromic or non-syndromic type of hearing loss Of all types of congenital hearing loss, the autosomal recessive defects are the most frequent causes of hearing loss

Before the start of molecular genetic research, syndromic and to a lesser extent autosomal dominant inherited hearing loss were the most investigated forms of hearing loss Investigations were based on the clinical description of the phenotype, and after the application of audiometry in clinical practice also on features in the audiogram related to age of onset, progression and severity of the hearing impairment

About twelve years ago, the first linkage results on non-syndromic autosomal dominant hearing loss were published, followed by linkage results on non-syndromic autosomal recessive and X-linked hearing loss Based on these results, a new classification of non-syndromic hearing loss was proposed DFNA for the autosomal dominant types, DFNB for the autosomal recessive types and DFN for the X-linked types Today, 51 loci for non-syndromic autosomal dominant, 39 for non-syndromic autosomal recessive and 8 for non-syndromic X-linked hearing loss are known, in which the causative gene has been cloned in 20, 20 and 2 loci respectively Also in syndromic hearing loss, different causative genes have been identified (e g EYA 1 in BOR syndrome, SLC26A4 in Pendred syndrome)

Because of the rapid evolution in understanding the genetics of hearing loss, there starts to be a serious gap in knowledge between clinicians and the genetic researchers of genetic hearing impairment

In the light of genetic counseling, it is therefore important that clinicians keep up in gaining new knowledge on this revolution in the increase of understanding of the function of hearing, by starting to understand the malfunction in genetically determined hearing loss

This Phd study therefore wants to describe in more detail the hearing loss in two syndromic (BOR-syndrome and Pendred syndrome) types and one non-syndromic autosomal recessive (DFNB1) type of hearing loss

CHAPTER 2 focuses on the Branchio-oto-renal syndrome
The first subsection gives a review on the classical features and genetics of BOR syndrome The EYA1 gene has been found to be the causative gene in BOR syndrome, but only in 25% of the patients diagnosed with BOR syndrome mutations in this gene have been detected
In the second subsection, a new Belgian family with BOR-syndrome is described Linkage analysis was carried out in 8 of the 12 affected members, and showed a positive lod score of 1 07 for marker D8S286 As the amount of blood samples was poor, it was not possible to get a higher lod score In nine individuals detailed long term follow up audiometry was available, and in two of them progression of hearing loss was found, in one individual results of audiometry were suggestive for unilateral progressive hearing loss The binaural median air conduction threshold in these patients was 100 dB and the range was 75-120 dB
In 8 patients detailed radiological investigation was performed all 8 had CT scanning of the temporal bones and five of them were also investigated by MRI The three individuals who were mentioned above having progressive hearing loss, appeared to have enlarged vestibular aqueducts, two of them bilaterally and one unilaterally More detailed radiographic description of this family is given in the third subsection Hypoplasia and dysplasia of the cochlea were consistent findings, and in one patient bilateral hypoplasia of the cochlear branch of the eight nerve was diagnosed As already mentioned, a widened vestibular aqueduct and sac were frequent but not obligatory features Other malformations of the middle ear included malformations of the ossicular chain
The 4th subsection describes a father and son with BOR syndrome Audiometric follow up of the father was not sufficient to give any information about progression or fluctuation of hearing loss, although he mentioned subjective progression of hearing loss MRI scan showed a marginally widened left vestibular aqueduct The endolymphatic sac could not be identified The son had bilateral enlarged vestibular aqueducts with normal sized endolymphatic sacs On long-term audiometric follow up, there was evidence for progression of the hearing loss All frequencies showed considerable threshold fluctuations with unilateral and binaural cofluctuations in several frequencies

CHAPTER 3 focuses on Pendred syndrome and the associated hearing loss
The first subsection gives an overview on the clinical and radiological features, as well as the genetics of Pendred syndrome An enlarged vestibular aqueduct and hypoplasia of the cochlea show up to be very common features in Pendred's

syndrome Thyroid enlargement and hypothyroidy are not always present The perchlorate discharge test was used in the past to diagnose Pendred syndrome, but is not positive in all cases Pendred syndrome and the enlarged vestibular aqueduct syndrome have important clinical features in common and they are both caused by mutations in the Pendred syndrome gene [SLC26A4]
Two new (related) patients with Pendred syndrome are presented Both had progressive down-sloping hearing loss with threshold fluctuations, and on radiological evaluation both had bilateral enlarged vestibular aqueducts One patient had episodes of vertigo with falling tendency and vomiting and one had an euthyroid multinodular goitre Both had mutations in [SLC26A4], in one patient the perchlorate discharge test was negative
The second subsection describes three patients (two sisters and one not related) with Pendred syndrome, all three had mutations in [SLC26A4] (identical mutations in the two sisters) Perchlorate discharge test was positive in two patients (in one sister it was negative), all three had bilateral enlarged vestibular aqueducts Hearing loss was significantly progressive with significant ipsilateral and contralateral cofluctuation in all three cases, and two patients had several episodes of Menière like vertigo

CHAPTER 4 reports on the results of mutation-analysis for the non-syndromic type of hearing loss DFNB1, with mutations in Connexin 26 and Connexin 30
As part of an etiological evaluation of the causes of deafness in patients at the Royal Institute Spermalie for the deaf and hard of hearing in Brughes, Belgium, a composition of to each other related families with obviously autosomal recessive inherited hearing loss was encountered In order to investigate the causes of deafness, the genotype was analyzed and where possible the phenotype
In generations III to V, 7 individuals were homozygous for the 35delG mutation in the GJB2 gene and 7 were heterozygous for this mutation All individuals were also tested for the partial deletion of GJB6, that had been described by del Castillo et al All 7 individuals with heterozygous 35delG mutations in the GJB2 gene were heterozygous for the deletion in GJB6 In 11 of 12 affected persons of generations IV and V hearing impairment was evaluated They all had residual hearing with a steeply down-sloping threshold from about 75 dB at 125 Hz to 120 dB or more at the higher frequencies In two patients there was significant progression in both ears and in one patient in one ear We failed to find significant difference in the degree of hearing impairment between 35delG homozygotes and heterozygotes for both the Connexin 26 and Connexin 30 mutations

CONCLUSIONS

As already mentioned above, in the past 10 years genetic investigations concerning causes of hearing loss have made major progress As before clinical descriptions of individuals with hearing loss were the most important way to distinguish different kinds of genetic hearing loss, nowadays the genotype can be determined and based on this the phenotype can be redefined For genetic counseling and for good clinical guidance it is very important to have detailed knowledge about the phenotype of the different forms of genetic hearing loss that occur This elucidates the value for clinicians to keep up with the evolution in clinical genetic research in the field of genetic hearing impairment

An enlarged vestibular aqueduct with or without an enlarged vestibular sac is one of the important associated findings in BOR syndrome but much more frequently in Pendred syndrome/DFNB4 Hearing loss in those cases is generally progressive with often significant threshold fluctuations and is sometimes associated with Meniere like episodes of vertigo

A non syndromic form of hearing loss, DFNB1, can be caused by homozygous mutations in connexin 26 (most frequently 35delG) or compound heterozygous mutations in connexin 26 and connexin 30 We were able to demonstrate that in our study population hearing loss in both genotypes showed steeply down-sloping hearing thresholds and in some cases progression of hearing loss There was no significant difference in severity of hearing loss between the 35delG homozygotes and heterozygotes for both the Connexin 26 and Connexin 30 mutations

Chapter VII

SAMENVATTING EN CONCLUSIES

SAMENVATTING

Perceptief gehoorverlies heeft belangrijke implicaties in onze sterk communicatieve samenleving, daar het ongeveer 1 op 750 kinderen aantast, en later zelfs 50 % van de bevolking ouder dan 80 jaar Het kan zowel veroorzaakt worden door genetische factoren als door omgevingsfactoren (b v intra-uteriene infecties, lawaaitrauma, bloedgroep antagonisme, meningitis, doofheid veroorzakende geneesmiddelen)

De overgrote meerderheid van vroegkinderlijk perceptief gehoorverlies wordt veroorzaakt door een genetisch defect Dit kan resulteren in een syndromaal of een niet-syndromaal type gehoorverlies De autosomaal recessieve vormen zijn de meest frequente oorzaak van congenitaal gehoorverlies

In de periode voor de ontwikkeling van het moleculair genetisch onderzoek waren het syndromaal en in mindere mate het autosomaal dominant overervend niet-syndromaal gehoorverlies de meest onderzochte vormen van gehoorverlies Onderzoek was vooral gebaseerd op de klinische beschrijving van het fenotype en na de toepassing van de audiometrie ook op de kenmerken van het audiogram, gerelateerd aan de leeftijd waarop het gehoorverlies optreedt, de progressiviteit en de ernst van het gehoorverlies

Ongeveer twaalf jaar geleden werden de eerste genkoppelingsresultaten in verband met niet-syndromaal autosomaal dominant gehoorverlies gepubliceerd, snel gevolgd door de genkoppelingsresultaten van niet-syndromaal autosomaal recessief en X-gebonden gehoorverlies Op basis van deze resultaten werd een nieuwe classificatie van niet-syndromaal gehoorverlies voorgesteld DFNA voor de autosomaal dominante vormen, DFNB voor de autosomaal recessieve vormen en DFN voor de X-gebonden vormen Tot op heden zijn er 51 loci bekend voor niet-syndromaal autosomaal dominant gehoorverlies, 39 voor niet-syndromaal autosomaal recessief gehoorverlies en 8 voor niet-syndromaal X-gebonden gehoorverlies Het oorzakelijke gen werd gekloond voor respectievelijk 20, 20 en 2 loci

Ook voor syndromaal gehoorverlies werden verschillende oorzakelijke genen geïdentificeerd (b v EYA 1 in het BOR syndroom, SLC26A4 in het Pendred syndroom)

Omwille van de snelle evolutie in de kennis van de genetica op moleculair niveau bij gehoorverlies, begint zich een grote afstand te manifesteren in kennis over genetisch bepaald gehoorverlies tussen de clinici en de onderzoekers In het licht van genetische counseling is het daarom zeer belangrijk dat de clinicus deze evolutie in het genetisch onderzoek van gehoorverlies op de voet blijft volgen

Deze doctoraatsstudie probeert daarom meer in detail het gehoorverlies te beschrijven in twee syndromale vormen (BOR-syndroom en Pendred syndroom) en in 1 niet-syndromaal autosomaal recessieve (DFNB1) vorm van gehoorverlies

HOOFDSTUK II behandelt het Branchio-oto-renaal syndroom. Het eerste onderdeel geeft een overzicht van de typische kenmerken en de genetica van het BOR-syndroom Het EYA1 gen werd geïdentificeerd als het oorzakelijk gen in het BOR-syndroom, doch slechts in 25% van alle patienten die werden gediagnosticeerd met het BOR-syndroom werden mutaties in dit gen gevonden.
In het tweede onderdeel wordt een nieuwe Belgische familie met BOR-syndroom beschreven In 8 van de 12 aangetaste familieleden werd genkoppelingsanalyse uitgevoerd en deze toonde een positieve lod score van 1 07 voor merker D8S286 Aangezien het aantal beschikbare bloedstalen beperkt was, was het niet mogelijk een hogere lodscore te bekomen Van negen personen uit deze familie hadden we een lange termijn follow up audiometrie ter beschikking Bij twee van hen vonden we bilaterale progressie van het gehoorverlies, bij 1 persoon waren de resultaten van de audiometrie suggestief voor een unilateraal progressief gehoorverlies
De binaurale mediane luchtgeleidingsdrempel van de onderzochte patienten was 100 dB met een spreiding van 75 tot 120 dB Bij 8 patienten werd een gedetailleerd radiologisch onderzoek uitgevoerd· bij alle 8 werd een CT scan van de ossa temporalia uitgevoerd en bij vijf van hen eveneens een MRI De drie personen bij wie een progressief gehoorverlies werd vastgesteld, bleken een verwijde vestibulaire aquaeduct te hebben waarvan twee bilateraal en één unilateraal
Een meer gedetailleerde radiologische beschrijving van deze familie wordt gegeven in het derde onderdeel van dit hoofdstuk
Hypoplasie en dysplasie van de cochlea waren consistente bevindingen en in één patient werd een bilaterale hypoplasie van de cochleaire tak van de nervus cochleovestibularis gediagnosticeerd Zoals reeds vermeld waren een verwijde ductus en saccus vestibularis oftewel endolymfaticus frequent doch niet obligatoir aanwezig Andere malformaties van het middenoor omvatten vooral malformaties van de beentjesketen.
Het vierde onderdeel van dit hoofdstuk beschrijft een vader en een zoon met BOR-syndroom Audiometrische follow-up van de vader voldeed niet om enige informatie te geven over progressie of fluctuatie van het gehoorverlies, hoewel hij een subjectieve progressie van zijn gehoorverlies vermeldde MRI toonde een marginaal verwijde linker ductus vestibularis De saccus endolymphaticus kon niet worden geïdentificeerd

Zijn zoon had bilateraal een verwijde ductus vestibularis met een saccus endolymphaticus van normale afmetingen Op basis van lange-termijn audiometrische follow-up vonden we evidentie voor progressief gehoorverlies Alle frequenties vertoonden belangrijke fluctuaties van de gehoordrempel met unilaterale en bilaterale cofluctuaties voor verschillende frequenties

HOOFDSTUK III handelt over het Pendred syndroom en het geassocieerd gehoorverlies
Het eerste onderdeel geeft een overzicht van de klinische en radiologische kenmerken, evenals de genetica van het Pendred syndroom Een verwijde ductus vestibularis en hypoplasie van de cochlea blijken zeer frequent voorkomende kenmerken te zijn in het Pendred syndroom Schildklierhypertrofie en hypothyroidie zijn niet altijd aanwezig De perchloraat belastingstest werd in het verleden gebruikt om het Pendred syndroom te diagnosticeren, maar is in sommige gevallen vals negatief Het Pendred syndroom en het verwijde vestibulaire aquaduct syndroom hebben belangrijke klinische kenmerken gemeen en ze worden beide veroorzaakt door mutaties in het Pendred syndroom gen [SLC26A4]
We stelden twee nieuwe (verwante) patienten met het Pendred syndroom voor Beiden hadden een progressief gehoorverlies, meer uitgesproken voor de hoge dan voor de lage tonen, met fluctuaties van de gehoordrempel Bij radiologisch onderzoek hadden beide patienten een bilaterale verwijde ductus vestibularis Een patient had episodes van vertigo met valneiging en braken en een patient had een euthyroide multinodulaire struma Beiden hadden mutaties in [SLC26A4], in een patient was de perchloraatbelastingstest negatief
Het tweede onderdeel geeft een beschrijving van drie patienten (twee zusters en een niet-verwant) met Pendred syndroom, alle drie hadden mutaties in [SLC26A4] (identieke mutaties in de twee zusters) De perchloraatbelastingstest was positief in twee patienten en negatief in een van de twee zusters, en alledrie vertoonden ze een bilateraal verwijde ductus vestibularis Het gehoorverlies was significant progressief met significante ipsilaterale en contralaterale cofluctuaties, en twee van hen hadden verschillende episodes van vertigo van het Meniere type

HOOFSTUK IV bespreekt de resultaten van de mutatie-analyse bij een niet syndromale vorm van gehoorverlies, namelijk DFNB1, met mutaties in connexine 26 en connexine 30
Als onderdeel van een etiologisch onderzoek naar de oorzaken van slechthorendheid bij patienten van het Koninklijk Instituut Spermalie voor doven en slecht-

horenden in Brugge, België, werd een groep aan elkaar verwante families met een duidelijk vermoeden van autosomaal recessief overervend gehoorverlies onderzocht Met als doel de oorzaken van doofheid na te gaan, werden in deze familie genotype en waar mogelijk ook fenotype geanalyseerd
In de generaties III tot V waren 7 personen homozygoot voor de 35delG mutatie in het GJB2 gen en 7 andere waren heterozygoot voor deze mutatie Alle individuen werden ook getest voor de partiele deletie van GJB6, zoals die werd beschreven door del Castillo et al Alle zeven personen met heterozygote 35delG mutaties in het GJB2 gen waren eveneens heterozygoot voor de deletie in GJB6
Bij 11 van 12 aangedane individuen van generatie IV en V werd het gehoorverlies nagegaan Allen hadden ze een restgehoor met een steil afdalende gehoorsdrempel van ongeveer 75 dB op 125 Hz tot 120 dB of meer op de hogere frequenties Bij twee patienten was er significante progressie in beide oren en bij een patient in een oor We konden geen significant verschil vaststellen in de ernst van gehoorverlies tussen de 35delG homozygoten en degenen die heterozygoot waren voor zowel de connexine 26 als de connexine 30 mutatie

CONCLUSIES

Zoals hoger reeds vermeld werd, werd de voorbije tien jaar grote vooruitgang geboekt in het genetisch onderzoek naar oorzaken van gehoorverlies Waar vroeger de klinische beschrijving van personen met gehoorverlies de belangrijkste manier was om de verschillende soorten van erfelijk gehoorverlies te onderscheiden, kan tegenwoordig het genotype bepaald worden en gebaseerd hierop kan het fenotype opnieuw gedefinieerd worden Nochtans blijft het vooral voor genetische counseling en goede klinische begeleiding zeer belangrijk om een goede kennis te hebben over het fenotype van de verschillende vormen van gehoorverlies die voorkomen Dit toont het belang aan voor clinici om de evolutie in het genetisch onderzoek op de voet te volgen
Een verwijde ductus vestibularis, al dan niet gecombineerd met een verwijde saccus endolymfaticus, is een van de belangrijke geassocieerde bevindingen bij sommige patienten met het BOR-syndroom maar komt vooral frequent voor bij patienten met het Pendred syndroom/DFNB4 Het gehoorverlies is in deze gevallen meestal progressief met vaak significante fluctuaties van de gehoordrempel en is regelmatig geassocieerd met episodes van vertigo van het Meniere type

Een niet-syndromale vorm van gehoorverlies, DFNB1, kan veroorzaakt worden door homozygote mutaties in connexine 26 (meest frequent 35delG) of samengesteld heterozygote mutaties in connexine 26 en connexine 30. Wij konden aantonen dat in onze studiepopulatie het gehoorverlies in beide genotypes een steil afdalende gehoorcurve vertoonde met in sommige gevallen progressie van het gehoorverlies. Er was geen significant verschil in ernst van gehoorverlies tussen de 35delG homozygoten en heterozygoten voor de connexine 26 en connexine 30 mutaties.

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Christel Stinckens werd op 3 september 1971 geboren te Hasselt. In 1989 behaalde zij haar eindexamen aan het Ursula Instituut te Herk-de-Stad. In datzelfde jaar begon zij haar studies geneeskunde aan de Katholieke Universiteit Leuven In het laatste jaar hiervan werd tijdens haar co-assistentschap op de dienst NKO in het UZ Leuven de samenwerking opgestart met Prof Dr C Cremers, die uiteindelijk resulteerde in dit proefschrift. Haar arts-examen behaalde ze in 1996 met grootste onderscheiding. Aansluitend startte zij haar opleiding tot NKO-arts in het UZ Leuven, waarvan ook één jaar in het AZ Maria Middelares te Gent. Haar opleiding tot NKO-arts, die met zes maanden werd verlengd naar aanleiding van de geboorte van haar drie oudste kinderen, werd beeindigd op 31 januari 2002. Op 1 februari 2002 startte ze als NKO-arts in het AZ Diest Van februari 2002 tot juli 2003 bleef zij als toegelaten geneesheer een dag per week werkzaam op de dienst Rhinologie in het UZ Leuven