Genetic Hearing Impairment

Clinical, Genetic and Audiological Aspects of Turner syndrome

and *MYH9*-Related Disease

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Genetic Hearing Impairment

Clinical, Genetic and Audiological Aspects of Turner syndrome

and *MYH9*-Related Disease

Genetisch gehoorverlies

Klinische, Genetische en Audiologische Aspecten van het Syndroom van Turner
en *MYH9*-Gerelateerde Aandoeningen

(met een samenvatting in het Nederlands)

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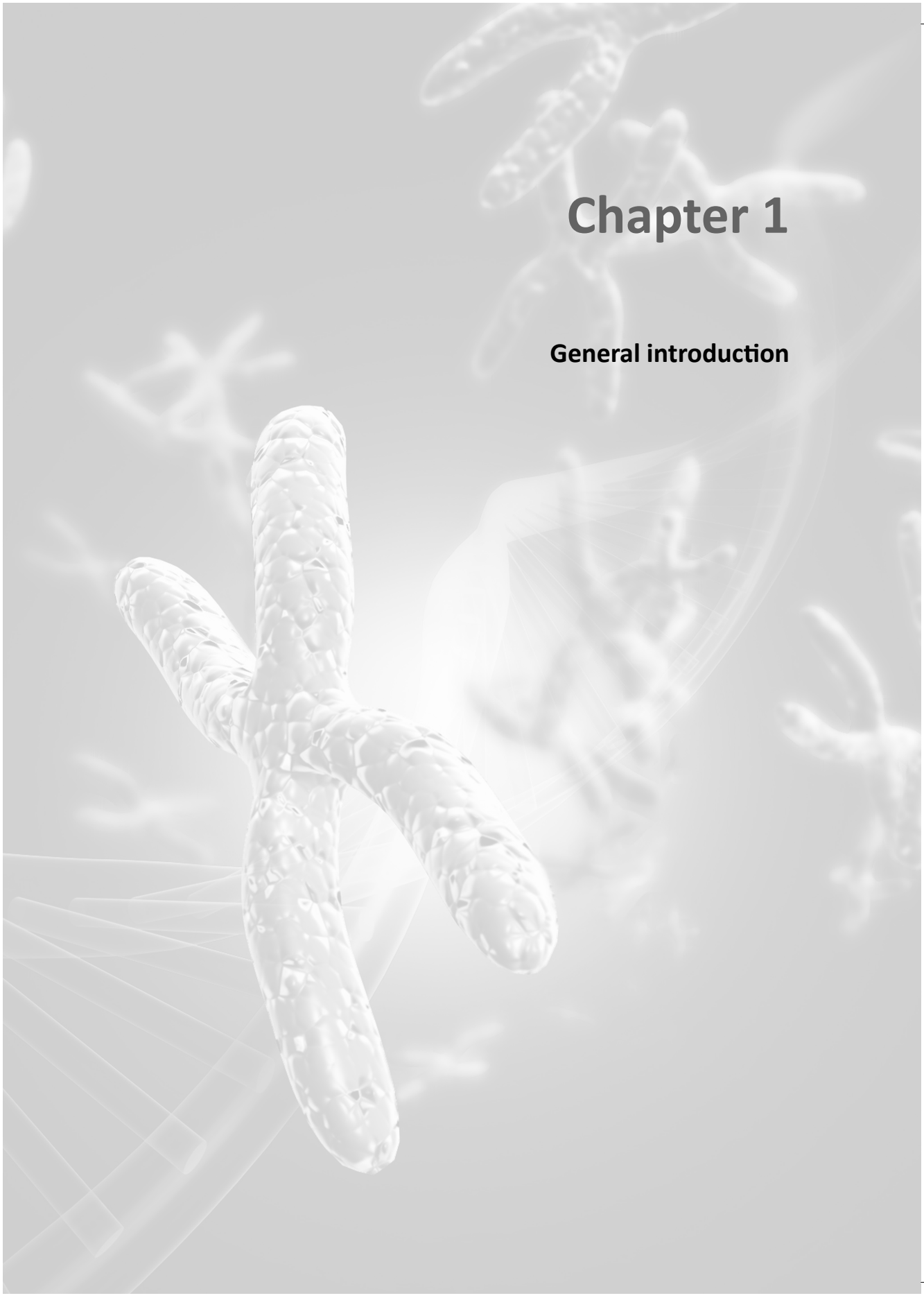

Chapter 1

General introduction

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1.1 General introduction

This first chapter provides a general introduction on genetic hearing impairment. The following topics are described:

- Background
- Clinical characteristics
- Etiology
- Inheritance patterns
- Clinical assessment of the patient with genetic hearing impairment
- Genetic counseling and clinical management
- Aims of this thesis

1.2 Background

Hearing impairment is the most common sensory disorder in developed countries.[1] The reported incidence of hearing impairment in literature is variable, which might be due to differences in diagnostic criteria, screening protocols, follow-up and the population and geographic location studied. In the United States, approximately 1 per 1000 newborn infants has severe to profound bilateral sensorineural hearing impairment.[2–4] In the general population, the prevalence of hearing impairment increases with age. Approximately 50 percent of those who are 75 years and older have disabling hearing impairment (hearing loss of 35 decibels or more in the better ear).[5] Hearing impairment can be described in many different ways. Most commonly it is mentioned as being syndromic or non-syndromic and classified by age of onset, type, severity, audiogram configuration, laterality, symmetry and progression. The etiology of hearing impairment can be genetic or non-genetic (acquired), as well as it can be due to genetic susceptibility and environmental factors acting together (multifactorial inheritance).[4] Genetic hearing impairment can be caused by chromosomal anomalies, a mutation in a single gene (monogenetic or Mendelian inheritance), or by several mechanisms of inheritance that are not explained by traditional Mendelian inheritance patterns. Table 1 gives a short overview of the characteristics of hearing impairment which will be discussed in further detail in the following paragraphs.

Table 1: Characteristics for a clinical description of hearing impairment

Clinical characteristics
- Syndromic or non-syndromic
- Age of onset (prelingual or postlingual)
- Type of hearing impairment (conductive, sensorineural, mixed)
- Severity of hearing impairment (mild, moderate, severe, profound)
- Audiometric configuration (low frequency ascending, mid-frequency U-shaped, high frequency gently/steeply sloping, flat)
- Bilateral (symmetrical or asymmetrical) or unilateral
- Progression
Etiology
- Genetic Chromosomal anomalies Monogenetic (Mendelian inheritance) Non-traditional inheritance
- Genetic and environmental factors acting together (multifactorial inheritance)
- Non-genetic (acquired)

1.3 Clinical characteristics

1.3.1 Syndromic and non-syndromic hearing impairment

Syndromic hearing impairment is characterized by hearing impairment that is associated with other anomalies whereas non-syndromic hearing impairment is not associated with other distinctive clinical features. Manifestations which could suggest a syndromic hearing impairment are for example branchial cleft pits, cysts or fistulae, preauricular pits, telecanthus, heterochroma iridis, white forelock, pigmentary anomalies, high myopia, pigmentary retinopathy, goiter and craniofacial anomalies.[6] Some syndromic features associated with hearing impairment may be clearly recognizable upon physical examination, whilst others are variable or not obviously visible and may require a more specialized physical examination or laboratory analyses, for example thrombocytopenia in an individual with *MYH9*-related disease (*MYH9*-RD). Furthermore it is important to realize that some anomalies may already be present in a newborn, and others are not expressed until later in childhood or in adulthood.[4] For instance, all patients with *MYH9*-RD have thrombocytopenia, platelet macrocytosis and characteristic cytoplasmic inclusions in granulocytes since birth, whilst they can develop additional non-congenital manifestations throughout life such as sensorineural hearing impairment, presenile cataract, proteinuric nephropathy, and/or elevation of liver enzymes.[7,8] Turner syndrome (TS) is characterized by short stature and ovarian dysgenesis, besides dysmorphic features and various anomalies in other organ systems. TS patients can be recognized at birth due to the presence of dysmorphic features (such as lymphedema or a webbed neck) or cardiac anomalies.[9] Though, for TS patients not presenting with classic features at birth, the diagnosis could be delayed until late childhood or adolescence. The eventual diagnosis often presents itself than by the short stature, pubertal delay, amenorrhea or infertility.[10,11] In general, for some syndromes, etiological diagnosis may be straightforward

by an assessment of the patient's physical features. However, syndromes with clinical features that are not externally visible or are variable, incomplete penetrant or age dependent, present a greater challenge to establish a definitive diagnosis. Therefore, thorough delineation of clinical characteristics of the hearing impaired individual is of utmost importance. Detailed description of genotype-phenotype correlations will help to diagnose syndromic hearing impairment more easily.

1.3.2 Age of onset

The age of onset of the hearing impairment is importantly associated with language skills. Prelingual hearing impairment refers to hearing impairment that occurs before speech develops. It also immediately indicates that speech and language development is endangered in individuals with this early onset of hearing impairment. Consequently, postlingual hearing impairment occurs after the development of normal speech and is often easier to habilitate.

1.3.3 Type of hearing impairment

Hearing impairment can be categorized depending on which part of the auditory system is damaged, resulting in different types of hearing impairment.

Conductive hearing impairment results from anomalies of the outer ear and/or the middle ear which prevents sound to be properly conducted to the inner ear. Audiograms show normal bone conduction with abnormal air conduction thresholds resulting in an air-bone gap. Possible causes are for example otitis media with effusion, cholesteatoma, perforation of the eardrum, otosclerosis, and atresia of the outer ear/ear canal. Conductive hearing impairment is likely to be treated medically, surgically, or with hearing aids.

Sensorineural hearing impairment is due to damage or malfunction of inner ear structures (i.e. cochlea) or the nerve pathways from the inner ear to the brain. Audiograms show hearing impairment that equally affects air and bone conduction. Possible causes for sensorineural hearing impairment are for example genetic predisposition, infections, trauma, ototoxicity, noise-induced or age-related. Sensorineural hearing impairment is in most cases a permanent loss and is frequently not medically or surgically treatable. Depending on its severity hearing aids could be advocated and very severe cases can benefit from a cochlear implant.

Mixed hearing impairment is a combination of conductive and sensorineural hearing impairment. Audiograms show abnormal bone conduction with even worse air conduction thresholds resulting in an air-bone gap.

1.3.4 Severity of hearing impairment (averaged over 0.5, 1, 2, and 4 kHz)[12]

Mild:	20-40 dB HL
Moderate:	41-70 dB HL
Severe:	71-95 dB HL
Profound:	>95 dB HL

There consist several classification systems for the degree of hearing impairment, as well as the audiometric configuration, with only minor differences between them.

1.3.5 Audiometric configuration[12]

Low frequency ascending: > 15 dB HL difference between the poorer low frequency thresholds and the better high frequency thresholds.

Mid-frequency U-shaped: > 15 dB HL difference between the poorest mid-frequency thresholds and the better low and high frequency thresholds.

High frequency:

- Gently sloping: 15-29 dB HL difference between the mean of 0.5 and 1 kHz, and the mean of 4 and 8 kHz.
- Steeply sloping: > 30 dB HL difference between the above frequencies.

Flat: < 15 dB HL difference between the mean of 0.25, 0.5 kHz, the mean of 1 and 2 kHz and the mean of 4 and 8 kHz thresholds.

1.3.6 Bilateral, symmetrical or asymmetrical, and unilateral hearing impairment

Hearing impairment can be classified as bilateral, symmetrical or asymmetrical, versus unilateral. Bilateral hearing impairment affects both ears whilst in unilateral hearing impairment one ear is normal hearing though there is hearing impairment in the other ear. Symmetrical hearing impairment means that the degree and configuration of hearing impairment are the same in each ear. An asymmetrical hearing impairment is one in which the degree and/or configuration is different for each ear.

1.3.7 Progression of hearing impairment

An important characteristic to describe hearing impairment is by its propensity for progression. Progressive hearing impairment refers to deteriorating of hearing thresholds over time. Creating age-related typical audiograms (ARTA) plots is a method to characterize progressive hearing impairment phenotypes. Based upon regression analysis, ARTA can be constructed, which show the thresholds predicted for a number of decade steps in age.[13] ARTA can be helpful in providing more rapid genotyping by predicting the possible gene involved in a specific family with hearing impairment.[14] When the genotype in a family is already known, the corresponding ARTA can be valuable in genetic counseling by giving a characterization of the corresponding hearing impairment. This is valuable information concerning questions of the patient on the prognosis of the hearing impairment.

1.4 Etiology

1.4.1 Genetic hearing impairment

Genetic hearing impairment accounts for at least 50% of the cases with congenital hearing impairment. Among children with genetic hearing impairment, approximately 70% is affected with a non-syndromic form and 30% suffers from syndromic hearing impairment.[4,6,15] Non-syndromic prelingual hearing impairment can be transmitted in an autosomal recessive (~75-85%), autosomal dominant (~15-25%), and X-linked (~1-2%) way, or by other, primarily mitochondrial, modes of inheritance (<1%).[2,6,15,16] The Hereditary Hearing Loss Homepage (http://hereditaryhearingloss.org) provides a regularly updated overview of all hearing impairment loci and genes.

1.4.2 Non-genetic, i.e. acquired, hearing impairment

Acquired hearing impairment in children commonly results from prenatal infections ("TORCHES": **t**oxoplasmosis, **o**ther infections, **r**ubella, **c**ytomegalovirus, **he**rpes, **s**yphilis), or postnatal infections, particularly bacterial meningitis.[17] Congenital cytomegalovirus (CMV) infection plays a major etiological role in hearing impairment among children.[18] In the Netherlands, approximately 1000 children per year are born with congenital CMV infection of whom approximately 180 (0.1% of all newborns) will be affected with long term sequelae, with hearing impairment being the symptom encountered the most frequently.[19]

Acquired hearing impairment in adults is often attributed to environmental factors of which noise exposure is the most common one. Other examples of acquired hearing impairment are trauma, infections and ototoxic drugs. Besides genetic or environmental causes of hearing impairment acting independently, hearing impairment can also be a complex disorder, caused by an interplay between genetic and environmental factors (age-related hearing impairment, otosclerosis).[6,20–22]

1.5 Inheritance patterns

1.5.1 Chromosomal anomalies

A regular human cell has 46 chromosomes: 22 pairs of autosomes and one pair of sex chromosomes which specify gender (XX = female and XY = male). Each chromosome has a short arm (p) and a long arm (q) separated by a centromere. A karyotype is a display of chromosomes grouped from large to small. In order to get this picture, the chromosomes are isolated, stained, and examined under the microscope. Characteristic banding patterns and the size of the chromosome permit identification of each individual chromosome.

Structural chromosomal anomalies occur when the chromosome's structure is altered and include for example deletions (portion of the chromosome is missing/deleted), duplications (portion of the chromosome is duplicated), inversions (portion of the chromosome has broken off, turned upside down, and reattached), insertions (portion of one chromosome has

been deleted from its normal place and inserted into another chromosome), translocations (portion of one chromosome is transferred to another chromosome), rings (portion of a chromosome has broken off and formed a ring) or isochromosomes (formed by the mirror image copy of a chromosome segment including the centromere). In numerical anomalies such as aneuploidy there is either a missing (monosomy) or an extra (trisomy) chromosome whereas polyploidy refers to the state in which there are additional sets of chromosomes. Down syndrome is one of the most common chromosomal disorders caused by the presence of an extra chromosome 21 (trisomy 21). There are very few conditions in which an extra or missing whole chromosome is tolerated.[4,23] Monosomy of an autosome is lethal; only loss of a sex chromosome can result in a live-born human. Monosomy 45X, characterized by the presence of a single X-chromosome in all cells, is the most frequent occurring karyotype in TS (Fig. 1).

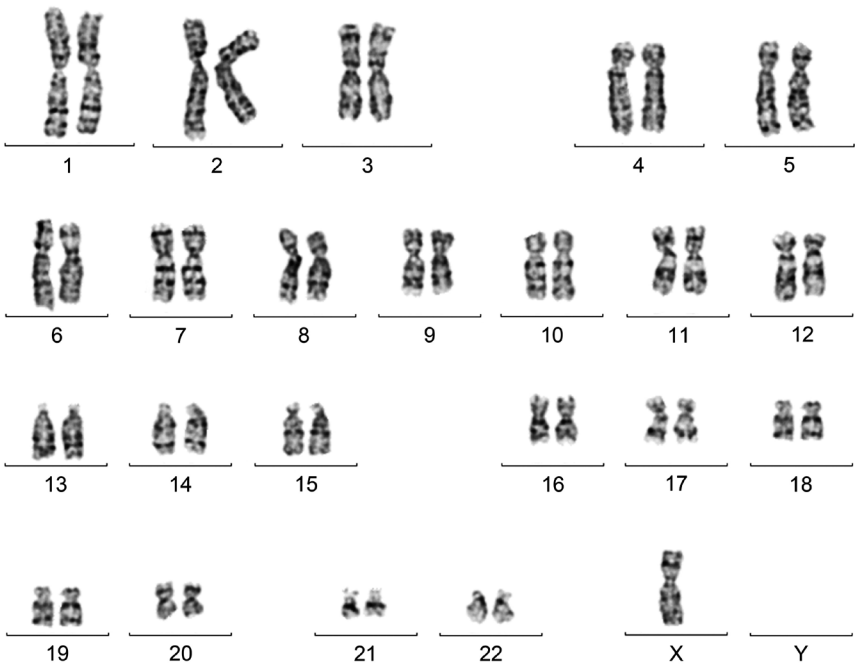

Figure 1. Karyotype of a female with Turner syndrome (TS) having a monosomy 45X, missing one entire X-chromosome in all cells.

1.5.2 Monogenetic inheritance

Monogenetic or Mendelian inheritance refers to the inheritance of conditions that are determined by mutations in a single gene. There are four patterns of monogenetic inheritance: autosomal recessive, autosomal dominant, X-linked, and Y-linked.

Autosomal recessive inherited hearing impairment appears only in individuals who inherited two mutated alleles of a gene located on an autosome, one from each parent. The parents' second allele is typically without a mutation, i.e. a wild-type allele. Therefore, parents are heterozygotes, i.e. carriers, usually having normal hearing (Fig. 2). Two carriers from the same type of autosomal recessive hearing impairment have, with every pregnancy, a 25% chance to both pass on the mutated allele (affected child), a 50% chance to pass on one mutated and one wild-type allele (unaffected carrier child), and 25% chance to both pass on the wild-type allele (unaffected child). Typically, a person with autosomal recessive hearing impairment is the only deaf person in the family or has one or more siblings with hearing impairment. Consanguineous couples, who share one or more common ancestors, are more likely to be carriers for the same autosomal recessive hearing impairment and have an affected child. Most of the autosomal recessive non-syndromic hearing impairment loci are associated with severe to profound prelingual hearing impairment.[4] A particular gap junction protein, GJB2, which codes for the protein connexin 26 is the most common cause of non-syndromic autosomal recessive hearing impairment.[24–27]

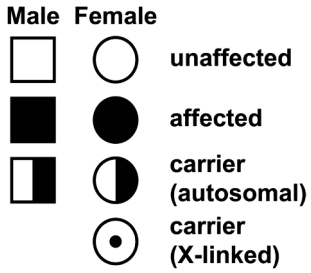

Figure legend for the following pedigree figures 2 to 6.

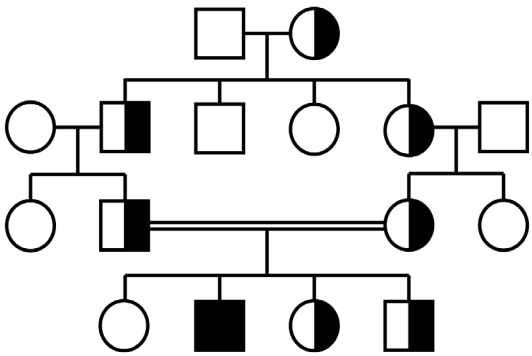

Figure 2. Autosomal recessive inheritance showing unaffected carrier parents. Note that the maternal grandmother and paternal grandfather are siblings, resulting in parental consanguinity indicated by the double horizontal line. For figure legend see above.

In autosomal dominant inheritance, only one mutated allele on an autosome is required to cause hearing impairment and therefore heterozygotes are affected. With every pregnancy, an individual with autosomal dominant inherited hearing impairment has a 50% chance to pass on the mutated allele and have an affected child and a 50% chance to pass on the wild-type allele and have an unaffected child. Pedigrees of families with autosomal dominant hearing impairment usually include multiple affected individuals in successive generations (Fig. 3).[4] However, in rare cases unaffected parents can have an affected child due to a new mutation in the child. Families with autosomal dominant non-syndromic hearing impairment often show a wide variation in the age of onset, progression, configuration and severity of hearing impairment. This is also called variable expression. Another feature of some dominantly inherited conditions is reduced penetrance which refers to the situation in which some individuals with the mutated allele show no features of that condition.

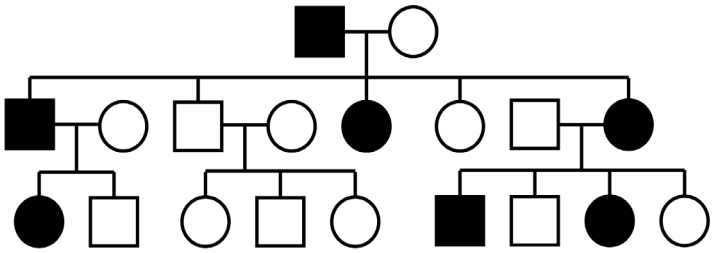

Figure 3. Autosomal dominant pedigree showing vertical transmission independent of the sex of the affected individual. For figure legend see page 15.

X-linked inherited disorders are caused by mutations in genes on the X-chromosome. Males with a mutated gene for hearing impairment on the X-chromosome will be affected because they have no corresponding wild-type gene on the Y-chromosome. There is no male-to-male transmission as affected males will only pass on their Y-chromosome to all of their sons. For an X-linked dominant condition, only one single mutant allele on the X-chromosome, whether in a female with two X-chromosomes or males with one X-chromosome, is necessary for an individual to be affected with the condition (Fig. 4). However, females are usually less severely affected than males because they have a wild-type allele on their other X-chromosome. An X-linked recessive disorder requires two mutant alleles, one on each X-chromosome, to affect the female phenotype. Females with only one mutant allele are unaffected carriers of an X-linked recessive condition (Fig. 5). A female who is a carrier of an X-linked recessive disorder has a 25% chance of having an unaffected son, a 25% chance of having an affected son, a 25% chance of having an unaffected daughter and a 25% chance of having a daughter who is an unaffected carrier.[4]

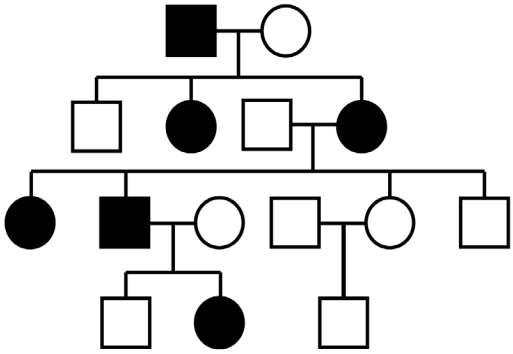

Figure 4. Pedigree showing typical X-linked dominant inheritance. Affected males pass the trait to all their female offspring but to none of their male offspring. For figure legend see page 15.

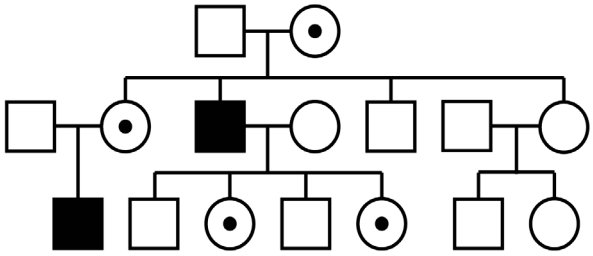

Figure 5. Pedigree showing typical X-linked recessive inheritance. Affected males have all unaffected sons and carrier daughters. For figure legend see page 15.

Y-linked inheritance is expressed in males with a mutated allele on the Y-chromosome as there is no corresponding wild-type allele on the X-chromosome. They will pass on this mutated allele to all of their sons who will be affected, but never to their daughters who will receive the father's X-chromosome (Fig. 6). Only one locus, DNFY1, has been described to show Y-linked inherited non-syndromic hearing impairment.[16]

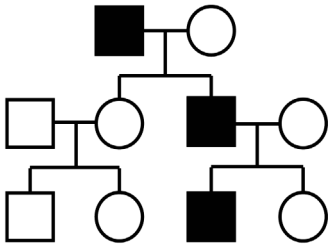

Figure 6. Y-linked inheritance. The pedigree shows only male-to-male transmission. For figure legend see page 15.

1.5.3 Non-traditional inheritance

Non-traditional inheritance includes several unusual inheritance patterns that are not explained by traditional Mendelian inheritance. These include genomic imprinting (phenotype of an affected individual may differ, dependent on whether the maternal or paternal allele is mutated), uniparental disomy (inheritance of two chromosomes, or parts of chromosomes, from one parent, without contribution of the other), inheritance of an unstable repeat expansion, and maternal inheritance of disorders caused by mutations in mitochondrial DNA, which we will not discuss here in detail.[4]

1.5.4 Multifactorial inheritance

Multifactorial inheritance is the result of a combination of genetic and environmental factors. Age-related hearing impairment as well as noise-induced hearing impairment are thought to be caused by an interaction between genetics and environment.[20,28–30] A characteristic feature of a multifactorial trait is that the recurrence in relatives is greater than the frequency of the disorder in the general population, with the closest related relatives being most likely affected. Furthermore, the chance of recurrence of the trait increases as the number of affected relatives increases and the more severe the malformation, the greater the risk will be to the relatives. If sex differences exist in the frequency of the trait in question, a member of the less commonly affected sex has a greater probability of transmitting the disorder.[4]

1.6 Clinical assessment of the patient with genetic hearing impairment

Correctly diagnosing the specific cause of hearing impairment in an individual is essential for accurate genetic counseling and treatment. Diagnosing genetic hearing impairment requires a multidisciplinary team which may include otorhinolaryngologists, audiologists, speech and language therapists, clinical geneticists and pediatricians. Based on the clinical findings or suspicions, various other types of specialists may also be needed, especially in syndromic hearing impairment (e.g., ophthalmologist, cardiologist, nephrologist, endocrinologist, neurologist, etc.).[6,31] The diagnostic approach for each patient should be adapted based on the clinical presentation and/or the suspicion of a particular syndromic disorder, and therefore not all assessments discussed here will be necessary for each patient.[4,6,31,32]

Family history. A three- to four-generation family history should be obtained with attention to consanguinity, inheritance pattern, hearing status of the relatives and any other associated medical conditions.

Medical history. The medical history may be helpful in differentiating between acquired and inherited causes of hearing impairment. This includes the prenatal history (maternal CMV infection, medication exposure, etc.), neonatal history (prenatal birth, hypoxia etc.), postnatal history (infections, trauma, etc.) and earlier performed audiological evaluation. Conditions that could be associated with syndromic hearing impairment such as visual or renal anomalies should be noted.

Clinical examination. All persons with hearing impairment of unknown etiology should be evaluated for features associated with syndromic hearing impairment. As variable expression tends to be a hallmark of autosomal dominant syndromic hearing impairment, sometimes correct diagnosis may depend on physical examination of the patient as well as the relatives. Components of physical examination can include assessment of growth parameters, particular craniofacial features or external ear anomalies, musculoskeletal features, neurological function, pigmentary anomalies or ophthalmological assessment.

Audiologic evaluation. Hearing status can be determined at any age. The age of onset, type of hearing impairment, configuration, severity and progression over time can be helpful to discriminate between different kinds of genetic hearing impairment.

Imaging. Temporal bone computed tomography (CT) or magnetic resonance imaging (MRI) of the temporal bones may be useful for detecting malformations of the inner ear suggestive for the presence of particular syndromes such as Pendred showing Mondini deformity and/or enlarged/dilated vestibular aqueduct (EVA) or CHARGE syndrome (coloboma, heart defects, choanal atresia, retarded growth and development, genital anomalies, and ear anomalies) in which a hypoplasia of the auditory nerve and/or semicircular canals can be found.

Additional examinations. Other examinations can be performed on indication such as renal ultrasound for branchio-oto-renal syndrome (BOR), echocardiogram for Jervell and Lange Nielsen (prolonged QT-interval), electromyelogram when peripheral neuropathy or myopathy is suspected or vestibular examination. A variety of laboratory studies can help as well to determine the underlying etiology of hearing impairment. Testing for CMV infection is recommended in newborns with congenital hearing impairment as CMV is a common cause of pediatric hearing impairment.

Molecular genetic testing. Nowadays it is possible to make genetic diagnosis based on molecular tests in an increasing number of otologic disorders. When single-gene testing is performed, the genes for testing will be chosen based on epidemiologic and/or phenotypic data. For example, it is recommended to offer GJB2 (encoding for protein connexin 26) and GJB6 (encoding for protein connexin 30) mutation screening in individuals with early onset non-syndromic hearing impairment as these mutations account for the majority of autosomal recessive non-syndromic hearing impairment. However, the genetic heterogeneity, wherein many different genes result in phenotypes that cannot be easily distinguished clinically, makes it challenging to identify the responsible mutation in individuals with hearing impairment. Therefore, next generation sequencing techniques (NGS) have been developed. This provides the opportunity to sequence a large panel of genes or the complete exome for particular cases (whole exome sequencing (WES) involves only sequencing of the protein coding regions (exome) of the genome). Even whole genome sequencing (WGS) (determining the complete DNA sequence (genome) of an individual) is possible.[33–36] With NGS there is a potential for discovering incidental findings unrelated to hearing impairment. The patient

should be informed about this and make the decision whether he/she wants to be tested with the risk of receiving unexpected or unwanted information.[37,38]

Cytogenetic testing. The study of chromosomes and their anomalies is known as cytogenetics. Cytogenetic testing such as karyotyping can provide a diagnosis in case of chromosomal anomalies such as TS.

1.7 Genetic counseling and clinical management

Genetic counseling is defined as a communication process in which patients and/or their families are informed about the likelihood of developing a particular, genetically determined disease, the risk of transmitting it, possible preventive measures, early diagnosis, and treatment if available. Genetic counseling is important to help the patient in the acceptance and understanding of the information of the genetic evaluation.[39,40] Also, an etiological diagnosis enables the physician to provide an estimation of recurrence risk for relatives which might be helpful in making decisions about having children in the future.[41,42] Furthermore, a correct diagnosis enables accurate prognostic information to be provided to the family such as the prediction whether a hearing impairment will remain the same or whether it will get worse over time. A genetic diagnosis can have its implications on treatment (if available) as well. For example earlier cochlear implantation could be advisable in case of a progressive sensorineural deafness caused by a mutation that is known to have excellent results with cochlear implantation. In addition, a genetic diagnosis can be useful concerning preventive measures which are effective in genetic hearing impairment conditioned by environmental factors, such as mutations conferring sensitivity to aminoglycosides or cisplatin toxicity.[43,44] At last, deafness may often be the first manifestation of a more complex syndrome. Therefore, when the mutation identified may cause syndromic manifestations, it is essential to assess the potential involvement of other organs (loss of vision, cardiac anomalies, kidney failure, etc.).

Still, there are families with hearing impairment in which the exact cause cannot be identified by genetic testing. It is important for counselors to emphasize that many genes for hearing impairment remain unidentified, and a genetic cause is still possible.[4,31,32] These patients should also be offered follow-up. Some subtle signs of a syndromic form of hearing impairment may only become apparent later in life, which prompts additional medical tests or referrals to other specialists. Follow-up visits also offer the opportunity to inform individuals about new genetic tests that may have become available or changes in the interpretation of previous test results as medical knowledge advances.[32]

Treatment of hearing impairment includes determining the appropriate rehabilitation options such as hearing aids. Sequential audiologic and otologic examination is important to evaluate the stability or progression of the hearing impairment so treatment can be adjusted when needed. Cochlear implantation is a potential rehabilitation option for profoundly hearing

impaired patients in which conventional amplification is insufficient. Besides conventional hearing aids and cochlear implantation, there are other treatment options as well which are effective in selected cases. For example, in TS patients, otologic surgery could improve the hearing in case of cholesteatoma with destruction of the ossicular chain.

1.8 Aims of this thesis

The main aim of this thesis is to improve the knowledge about the phenotypic and genotypic characteristics of genetic hearing impairment associated with TS and *MYH9*-RD. Clinical, audiological and management aspects are assessed. There are studies in which we mention hearing loss in terms of ears instead of persons, which is accepted in otorhinolaryngology.

1.8.1 Turner syndrome (TS)

TS is a chromosomal disorder which is caused by complete or partial absence of one X-chromosome. TS patients are phenotypically female. Prominent features are short stature, estrogen deficiency and dysmorphic anomalies such as a webbed neck, low posterior hairline, and edema in the newborn period (Fig. 7).[9,45] TS patients are prone to develop otologic disease and hearing impairment. Girls with TS are usually treated with growth hormone to increase adult height and estrogen to induce puberty. Growth hormone treatment can only partially overcome the growth failure observed in TS. In addition, Oxandrolone, a weak androgen, has been shown to increases growth velocity and adult height in TS.[46–49]

Low set-, cup-, or protruding ears
Auricular malformations
Micrognathia
Short/broad- or webbed neck

Ptosis
Epicanthic fold
Strabismus

Low posterior hairline

Figure 7. Possible clinical features concerning the head and neck region in Turner syndrome (TS) (adapted from https://www.radboudumc.nl/Zorg/Ziektebeelden/Documents/1%20Turner.pdf)

Chapter 2 describes the otologic and audiologic characteristics in a large group of children with TS. We also report whether there is a correlation between the occurrence of hearing impairment and the different karyotypes.

Chapter 3 analyses the otologic and audiologic characteristics, in relation to karyotype, in a group of young adults with TS. This gives us the opportunity to investigate how the otologic problems in TS evolve throughout life. Furthermore, we investigate if previous Oxandrolone treatment, a synthetic anabolic steroid which has been shown to increase adult height in TS, has any effects on hearing.

1.8.2 MYH9-related disease (*MYH9*-RD)

MYH9-RD is an autosomal dominant syndromic disorder due to mutations in *MYH9*, the gene for the heavy chain of non-muscle myosin IIA (NMMHC-IIA). All the *MYH9*-RD patients have congenital hematological alterations, namely platelet macrocytosis, thrombocytopenia, and characteristic NMMHC-IIA inclusions in the cytoplasm of granulocytes. During life, the majority of *MYH9*-RD patients develop additional non-congenital extra-hematological manifestations such as sensorineural hearing impairment, presenile cataract, proteinuric nephropathy, and/or alterations of liver enzymes.[7,8] *MYH9*-RD encompasses four syndromes that have been considered for many years as distinct disorders; May-Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, and Epstein syndrome. The identification of *MYH9* as the gene responsible for all of these disorders demonstrated that May-Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, and Epstein syndrome actually represent a different clinical presentation of the same condition, for which the definition *MYH9*-RD has been introduced.[50,51]

Chapter 4 shows a family study in which individuals had thrombocytopenia and large platelets, alterations that associated with sensorineural hearing impairment led to the diagnostic suspicion of *MYH9*-RD. This study describes if this suspicion was confirmed by finding the causative *MYH9* mutation.

Chapter 5 gives an audiological characterization of a large group of patients with *MYH9*-RD. We describe the genotype-phenotype correlation in order to provide adequate genetic counseling and clinical management.

Chapter 6 reports on the effects and safety of cochlear implantation in patients with *MYH9*-RD.

Chapter 7 includes the discussion and conclusion.

Chapter 8 provides a summary of this thesis.

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Chapter 2

Ear and hearing problems in relation to karyotype in children with Turner syndrome

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ABSTRACT

The aim of the study was to report otologic and audiologic characteristics in a group of children with Turner syndrome (TS) and correlate these findings to karyotype. Additionally, we give recommendations for the otologic care of these children. Sixty children (age 1.7-21.2 years) were included in this retrospective study. Medical history and karyotypes were recorded and otologic and audiologic evaluation was performed. A history of recurrent otitis media was reported in 41/60 (68%) children and 3/60 (5%) had suffered from cholesteatoma. Audiometric data in 56 children revealed that normal hearing was only present in 33/112 (29%) ears. All other ears 79/112 (71%) were classified in five different audiometric categories for hearing loss. Hearing thresholds in general appeared to be about 10-11 dB worse in children with a monosomy 45,X or isochromosome (both have a total deletion of the short (p) arm of the X-chromosome) compared to those having a mosaicism or structural anomaly (partial deletion, or total deletion in only a few cells). Our findings support the hypothesis that hearing can be affected by loss of the p-arm of the X-chromosome. It is for the first time that a relation between hearing problems and karyotype is statistically confirmed in a large group of children with TS.

1. INTRODUCTION

Turner syndrome (TS) is a relatively common chromosomal disorder, occurring in approximately 1 in 2.000 live-born females.[1] TS is caused by a total or partial deletion of one X-chromosome. Monosomy 45,X, having only one X-chromosome in all cells, is the most frequently occurring karyotype (50%). Thirty to forty percent of the patients have a mosaicism, with two or more chromosomally different cell lines, for example 45,X/46,XX ; 45,X/47,XXX or 45,X/46,XY. Other patients have structural sex chromosome anomalies such as deletions or ring chromosomes. There are also karyotypes with a duplication of the long arm of the X-chromosome (isochromosome, 46,X,i(Xq)).[1–3]

The main characteristics of TS are short stature and ovarian dysgenesis. Girls with TS are usually treated with growth hormone to increase adult height by several inches and with estrogen to induce puberty.[1,2] Other possible clinical manifestations are a webbed neck, lymphedema, a broad chest with widely spaced nipples, a low posterior hairline and cubitus valgus. TS patients are at risk for cardiovascular disease, renal malformations, hypothyroidism, diabetes mellitus, dyslipidemia, hypertension, and osteoporosis.[1–4]

Auricular malformations, middle ear disease and hearing impairment are commonly seen in TS. The external ear anomalies have been described as low set ears, cupped auricles, narrowing of the external auditory canal and abnormally protruding ears.[5–7]

Chronic or recurrent otitis media is frequently seen in young and adolescent females with TS, resulting in conductive hearing loss (CHL).[7–9] Many TS patients have an abnormal craniofacial morphology. A retarded development of the cranial skeleton, an unusual downward slope of the external auditory canal and palatal anomalies such as a high arched palate are frequently seen.[8,10–14] These findings have impact on the Eustachian tube function causing recurrent middle ear infections. A subnormal immune response and the effect of X-chromosome related hormonal impact have also been put forward as contributing factors.[7,8]

Women with TS often develop a mid-frequency sensorineural hearing loss (SNHL) (basin-shaped, maximum around 1-2 kHz) in their teens or adolescence. As long as the higher frequencies are still well heard, this usually does not cause hearing problems. The mid-frequency loss usually occurs in women with the karyotype 45,X and 46,X,i(Xq). In these karyotypes, the short (p) arm of the X-chromosome is missing, which suggests that the locus for hearing impairment in TS may be located on this arm.[15–17] Furthermore, a progressive high-frequency SNHL is associated with TS.[16]

The rate of hearing decline is much higher in women with TS than in age-matched women in the general population. It is suggested that the karyotypes 45,X and 46,X,i(Xq) but, even more, the presence of a mid-frequency loss, is a high predictive value for rapid decline in the high-frequencies.[18] CT-scanning could not demonstrate anomalies of the inner ear or vestibular system responsible for the hearing loss.[6] Only one study showed hypoplastic lateral semicircular canals by CT-scanning in two TS patients with SNHL.[12]

The lack of endogenous estrogens has been proposed to be a contributing factor in the cause of SNHL.[19] Estrogen receptors α and β have already been shown to exist in the human inner ear. Although there are indications that estrogens may have a beneficial effect on hearing, this requires further research.[19,20] Concerning body height and serum IGF-1 concentrations, one study showed a positive correlation with hearing function.[21]

The aim of this study was to determine the prevalence and features of ear and hearing problems in TS children and to correlate their audiometric data with different karyotypes. Finally, recommendations for the otologic care of TS children are given.

2. MATERIALS AND METHODS

2.1. Patients

The study was approved for by the medical ethical committee of the Radboud University Nijmegen Medical Centre (RUN MC). Between 2004 and 2008, 77 TS children under regular control of their pediatric endocrinologist received an invitation letter for otologic and audiologic screening. Sixty underwent screening and were included in this study. The mean age was 11.2 years (range 1.7-21.1) for otologic examination and 11.7 years (range 4.1-21.1) for audiologic examination. Because of their young age, 4 children were only tested with a screening test consisting of the head turn response to sound stimuli. Informed consent was obtained prior to participation. Medical history was taken, focusing on previous and current ear infections, hearing loss, karyotype and growth hormone and estrogen substitution therapy.

2.2. Otologic and audiologic examination

The otologic and audiometric data were retrieved from the medical files. We describe the findings at the time of everyone's last evaluation that was performed before the start of this study.

All ears were externally as well as microscopically examined. Hearing measurements were conducted according to standard audiometric methods (ISO 389) in a sound proof room. Pure-tone hearing thresholds (dB hearing level, dB HL) were determined by air conduction (AC) and bone conduction (BC) for the following test frequencies: (0.25) 0.5, 1, 2, 4 and 8 kHz. As always, there can be a 5 dB machine error rate associated with audiogram measurement. Based on pure-tone audiograms, different audiometric categories were developed in order to correlate audiometric findings to the underlying karyotype. The classification of audiometric categories as mentioned below was used as the different types of hearing impairment presented themselves in such a way.

2.2.1. Normal hearing

The AC thresholds were equal to or better than 20 dB HL across the frequency range of 0.25-8 kHz. When there was a dip within the normal range, the subject fell into the mid-frequency dip category (see below). Audiograms with an air-bone gap (ABG) of at least 10 dB at more than one frequency, within the normal range, were categorized as mild CHL (see below).

2.2.2. Mild conductive hearing loss

The AC thresholds were never worse than 20 dB HL, though there was an ABG of at least 10 dB at more than one frequency. By this classification we include this relevant ABGs in the analysis.

2.2.3. Conductive hearing loss (CHL)

AC thresholds were worse than 20 dB HL at one or more frequencies in the range of 0.25-8 kHz. There was an ABG of at least 10 dB at one or more frequencies at which the AC threshold was worse than 20 dB HL. BC thresholds were better than 20 dB HL at any frequency.

2.2.4. Mixed hearing loss (MHL)

The BC thresholds were worse than 20 dB HL at one or more frequencies in the range of 0.25-8 kHz and there was an ABG of at least 10 dB at one or more frequencies.

2.2.5. Pure Sensorineural hearing loss (SNHL)

The AC thresholds were worse than 20 dB HL at one or more frequencies in the range of 0.25-8 kHz and there was no ABG. High-frequency SNHL was defined as AC thresholds worse than 20 dB HL at 4 kHz or higher frequencies or AC thresholds worse than 20 dB HL at frequencies below 4 kHz with an increasing loss with increasing frequency ('down sloping loss').

2.2.6. Sensorineural mid-frequency dip

This category included audiograms showing the typical mid-frequency dip within the range of 0.5-4 kHz. The dip was defined as BC thresholds being at least 10 dB HL worse (at one or two frequencies) than all of the lower and higher frequencies.

2.3. Statistical analysis

We tested the hypothesis that children with a monosomy or isochromosome have a higher occurrence of hearing problems compared to those with a mosaicism or structural chromosomal anomaly. First, we tested whether or not each type of hearing impairment (audiometric category) was distributed by chance alone over the different karyotypes. We used the chi-square test for the goodness of fit in a 2 x 3 table (degrees of freedom d.f. = 2). Fisher's exact test was performed in a 2 x 2 table on the monosomy and mosaicism groups,

comparing the separate prevalence numbers of the right and left ear to test whether or not there was a significant difference between both ears.

Linear regression was used to analyze the influence of age and karyotype on hearing. An F test was used to compare between regression lines (Prism program, GraphPad, San Diego); testing on the slopes between the regression lines was performed first and testing between the 'elevations' was only performed if there was no significant difference in slope. In any statistical test, a level of $p < 0.05$ was considered to indicate statistical significance.

3. RESULTS

3.1. Medical history

The mean age of diagnosis was 4.3 years, with a wide range as some were diagnosed prenatal and others not until the age of 14 years. The majority of children 44/60 (73%) received growth hormone treatment at the moment of screening (mean period 3.7 years). A few children 9/60 (15%) already accomplished their growth-promoting treatment whereas others 7/60 (12%) did not start yet. Estrogens inducing pubertal development were given to 19/60 (32%) of the girls (mean period 3 years). Data concerning otologic history are presented in Figure 1 and this shows that many children had suffered from otologic disease; 41/60 (68%) of the children had a history of recurrent otitis media and 39/60 (65%) had undergone treatment with ventilation tubes. The number of ventilation tubes varied between individuals from once to even twelve, and some of them got T-tubes. Surgical intervention for cholesteatoma was performed in 3/60 (5%) children; one of them had cholesteatoma on both ears. All four ears appeared to have large cholesteatoma and there was destruction of the ossicular chain and parts of it had to be removed. Three out of 4 ears had recurrent or residual cholesteatoma and surgery finally resulted in an open cavity in 3 ears and a bony canalplasty was performed in one ear. Two girls underwent bone-anchored hearing aid (BAHA) surgery because persistent otorrhoea made conventional hearing aids unable to wear. One of them underwent surgery for cholesteatoma in the past.

3.2. Karyotype

Karyotypes were analyzed and categorized according to the amount of loss of the p-arm of the X-chromosome. In monosomy (45,X) and isochromosome (45,X/46,X,i(Xq) or 46,X,i(Xq)) cases, there was a total deletion of the p-arm. In cases with a mosaicism or structural anomaly the p-arm was partially deleted, or totally deleted in only a few cells (Table 1). If an isochromosome was present together with another cell line (except for 45,X) the child was classified in the mosaicism/structural group.

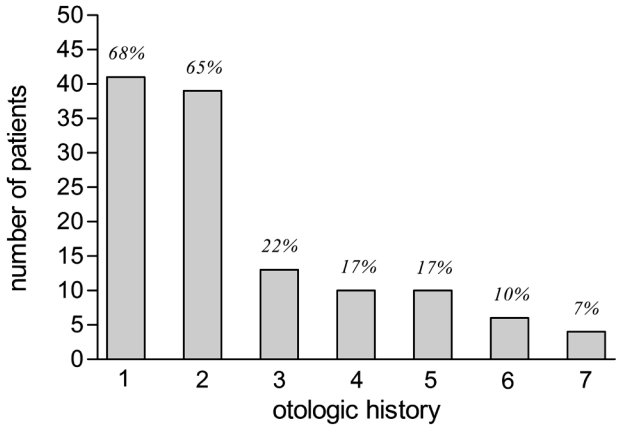

Figure 1. Otologic history (n = 60 children). 1: recurrent otitis media, 2: ventilation tubes, 3: adenotonsillectomy, 4: adenotomy, 5: tonsillectomy, 6: hearing aid/BAHA at present time, 7: otologic surgery.

Table 1. Distribution of individual karyotype in 60 TS children.

Karyotype	N (%)
Monosomy	26 (43%)
45,X	26
Isochromosome	12 (20%)
45,X/46,X,i (Xq)	8
46,X,i (Xq)	4
Mosaicism/structural anomaly	22 (37%)
45,X/46,XX	5
45,X/46,X,r (X)	2
46,X+der,t(X;13)(q13;q12.3)	2
45,X/46,X,dic(Y)(p11.2)	1
45,X/46,X,idic(Y)(p11.2)	1
45,X/46,X,idic(Y)(p11.3)	1
45,X/46,XY	1
45,X/47,XXX	1
45,X/46,X,r(X)/46,X,i(Xq)	1
46,X,i(Xq)/46,XX	1
45,X/46,X,idic(X)(p10)/47,X,idic(X)(p10)X2/48,X,idic(X)(p10)X3	1
45,X/46,X,+mar	1
45,X/46,X,+mar(X)/46,X,der(X)	1
45,X/47,X,idic(Y)(q11.2),idic(Y)(q11.2)	1
45,X/46,XX/46,X,i(Xq)	1
46,X,del(X)(p10)	1

Karyotype distribution, categorized according to loss of p-arm of the X-chromosome. del = deletion, r = ringchromosome, der = derivate, dic = dicentric, idic = isodicentric, mar = marker, t = translocation.

3.3. Ear, Nose and Throat (ENT) examination

3.3.1. External ear findings

The only reported external ear anomalies were a down sloping bony ear canal in 3 ears, an abnormally anteriorly curved ear canal in one ear, and a very narrow external meatus in four other ears. In one of those ears, the narrow external meatus was due to an abnormal position of the conchal cartilage.

3.3.2. Eardrum and middle ear findings

Myringosclerosis, retraction or perforation of the tympanic membrane, ventilation tubes, or middle ear effusion was seen in 59/120 (49%) ears, at the time of the last evaluation. Many ears showed several kinds of eardrum/middle ear pathology. The most common findings were myringosclerosis and middle ear effusion (Fig. 2).

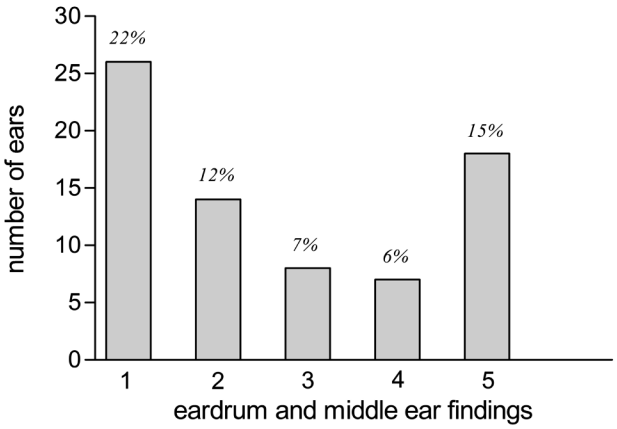

Figure 2. Eardrum and middle ear findings (n = 120 ears). 1: myringosclerosis, 2: retraction, 3: ventilation tube, 4: perforation, 5: middle ear effusion.

3.4. Audiologic examination

As result of their young age, 4 children were only tested with a screening test, not measuring specific bone conduction. The audiometric test results of the remaining 56 children are shown in Table 2. In 10 ears, BC thresholds were not measured at the last audiometric evaluation. Then, if possible BC thresholds from earlier measurements were used.

Normal hearing was found in 33/112 (29%) ears. Consequently, 79/112 (71%) ears showed anomalies that could be classified in one of the remaining audiometric categories. A mild CHL was present in 15/112 (13%) ears.

Table 2. Audiometric results in 56 TS children (n = 112 ears).

Ears (n = 112)	Test results	%
Normal hearing	33/112	29
Mild conductive hearing loss	15/112	13
Conductive hearing loss	37/112	33
Mixed hearing loss	16/112	14
Pure sensorineural hearing loss	5/112	4
Sensorineural mid-frequency dip	19/112[a]	17

[a] 13 ears had CHL as well so those ears were counted twice.

A CHL was found in 37/112 (33%) ears. We divided those ears in two groups according to the degree of hearing loss. Group 1 contained the ears in which the mean AC threshold was 25 dB HL or worse (0.25-8 kHz) and counted 17/112 (15%) ears (Fig. 3). The mean ABG was 28.4 dB. Group 2 contained the ears in which the mean AC threshold was better than 25 dB HL (0.25-8 kHz) and counted 20/112 (18%) ears (Fig. 3). The mean ABG was 9.6 dB.

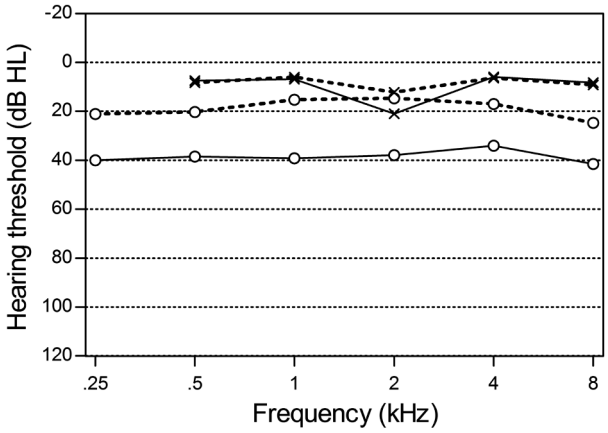

Figure 3. Mean AC and BC threshold for the CHL-group, divided in 2 groups. Group1 (——) AC threshold ≥ 25dB (n = 17 ears), Group 2 (---) AC threshold < 25dB (n = 20 ears). O: AC, X: BC. AC at 0.25 and 8 kHz was in group 1 only known in 10 and 11 ears respectively, and in group 2 in 17 ears. BC at 8 kHz was in group 1 and 2 only known in 9 and 15 ears, respectively. In two ears, in which middle ear effusion was seen, BC was not known at all. In one girl (2 ears) the audiogram was made before cerumen was removed.

MHL was present in 16/112 (14%) ears (Fig. 4). The mean ABG was 14.6 dB. Concerning the BC thresholds of the MHL-group, a high-frequency SNHL and a combined mid- and high-frequency SNHL were the most common audiogram configurations and both counted 7/112 (6%) ears.

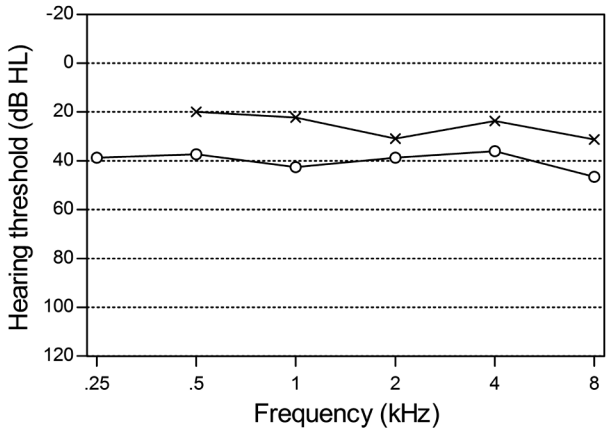

Figure 4. Mean AC and BC threshold for the MHL-group (n = 16 ears). O: AC, X: BC. AC at 0.25 and 8 kHz was only known in 13 and 15 ears, respectively.

A pure SNHL was found in 5/112 (4%) ears, of which 4 had a high-frequency SNHL as shown in Figure 5.

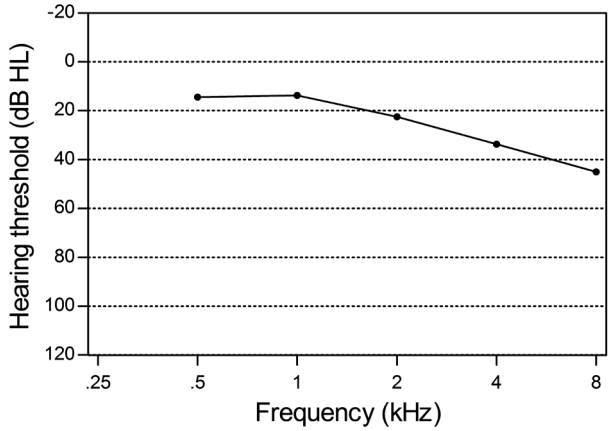

Figure 5. Mean AC threshold for high-frequency SNHL (n = 4 ears) in the SNHL-group.

A sensorineural mid-frequency dip was found in 19/112 (17%) ears. We divided the children in two groups according to their age to evaluate for progression. In the age of 5-9 years a dip was found in 12 ears of 7 children. The dip maximum was at 2 kHz with a mean value of 25.8 dB HL (range 10-45) (Fig. 6). In the age of 14-20 years a dip was present in 7 ears of 7 children. The dip maximum was in the 2 kHz region with a mean value of 24.3 dB HL (range 10-35) (Fig. 7). The mean values of the two age groups were almost identical.

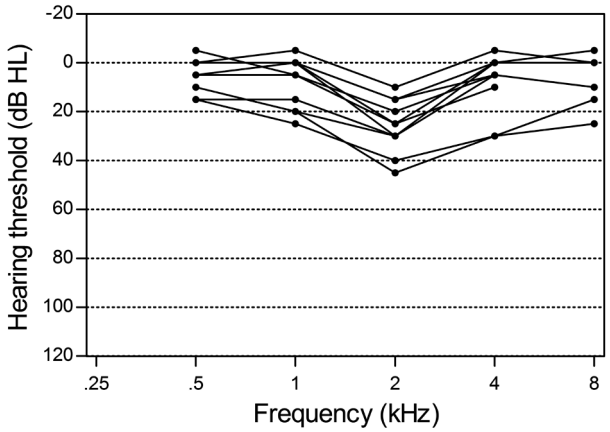

Figure 6. BC thresholds for the sensorineural mid-frequency dip group (n = 12 ears) for children aged 5-9 years.

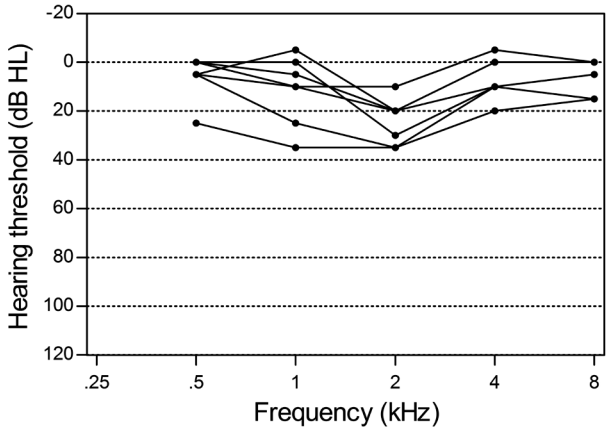

Figure 7. BC thresholds for the sensorineural mid-frequency dip group (n = 7 ears) for children aged 14-20 years.

3.5. Statistical analysis: Hearing problems in relation to karyotype

There were no substantial differences in the occurrence of each type of hearing impairment among the different karyotypes.

Then, we performed statistical analysis concerning pure-tone hearing thresholds in general. The mean AC threshold of every child, for both the monosomy/isochromosomes and the mosaicism/structural anomalies, was plotted at each frequency as a function of age. Linear regression analysis (threshold on age) showed that there was no significant deterioration in hearing thresholds with increasing age (i.e. slopes were not significantly different from zero). Furthermore, there was no significant difference in slopes between both groups.

However, there was a significant difference in elevation at the frequency range of 0.5-4 kHz (a measure that pertains to the group's 'grand mean' threshold) indicating a substantial difference in hearing thresholds, being about 10-11 dB worse for children with a monosomy/isochromosome as compared to those with a mosaicism/structural anomaly. Figure 8 shows the data at 1 kHz as an example.

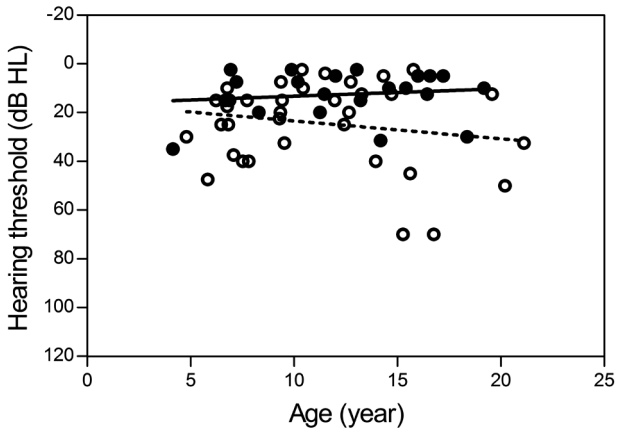

Figure 8. Mean AC thresholds at 1 kHz plotted as a function of age and linear regression line for the cases with a monosomy/isochromosome (○ and---) and those with a mosaicism/structural anomaly (● and ——) (n = 56 children). Linear regression analysis shows a significant difference in elevations between both groups.

We repeated the above regression analysis for BC thresholds (as a superior indicator of the 'real' sensorineural threshold). We found a similar difference in BC thresholds, being worse in the monosomy/isochromosome cases (significant at 0.5-2 kHz, i.e. in 3 out of 5 frequencies, which -according to binomial statistics- is significantly frequent, i.e. cannot reasonably be attributed to chance alone). Figure 9 shows the data at 2 kHz as an example.
The BC thresholds deteriorated significantly with increasing age at 0.5-4 kHz in the monosomy/isochromosome cases and only at 4 kHz in the mosaicism/structural anomalies.

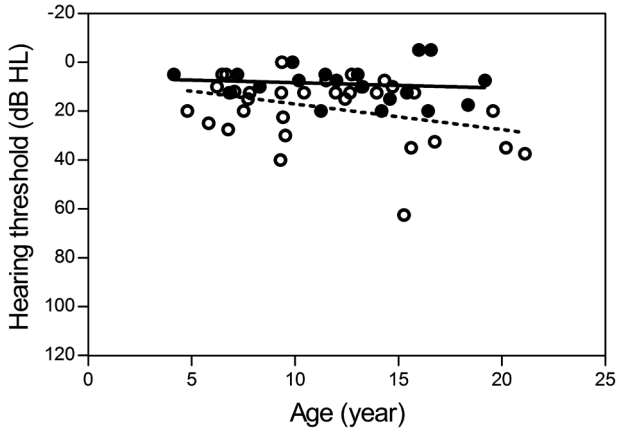

Figure 9. Mean BC thresholds at 2 kHz plotted as a function of age and linear regression line for the cases with a monosomy/isochromosome (○ and ---) and those with a mosaicism/structural anomaly (● and ——) (n = 56 children). Linear regression analysis shows a significant difference in elevations between both groups and there is a significant deterioration of hearing thresholds with increasing age for the monosomy/isochromosome cases.

4. DISCUSSION

4.1. Middle ear disease and otomicroscopic anomalies

The prevalence of recurrent otitis media in this study (68% of the patients) is in agreement with most TS studies.[5–8,11,15–17,22] In literature, a history of recurrent middle ear infections is described in 19.6 to 91% of patients.[5–8,11,14–17,22–26] Abnormal craniofacial morphology, causing Eustachian tube dysfunction, is suggested as a cause for the recurrent otitis media.[7,8,11]

Otomicroscopic anomalies were seen in 49% of the ears. Other TS studies reported percentages varying from 57 to 71%.[6,7,11] These are high percentages compared to children in the normal population. One study in children without TS found pathology of the eardrum in 26.3% of the ears.[27] Another study found middle ear pathology in 4.95% of the children in a normal population.[28]

In our study, 65% of the children had undergone treatment with ventilation tubes. Other TS studies found lower percentages varying from 32 to 57%.[6,7,14,16,24] These are all high percentages compared to a study performed in the normal population where only 13% of the 16 year old had ventilation tubes inserted in the past.[27]

TS children are prone to develop middle ear disease and are at risk for developing speech and language problems and learning disabilities.[29] Based on the results of this study and the reported literature, early ventilation tube insertion in TS children presenting with middle ear disease is recommended. In cases of persistence of active middle ear disease, we suggest to proceed rapidly to the insertion of T-tubes, as recommended by Hall as well.[30]

4.2. Audiometric findings

Hearing problems were common as described in earlier reports. Normal hearing was only found in 29% of all ears. The range of normal hearing in TS literature varies between 17 and 63.7%.[5,6,9,11,14,23,31,32]

4.2.1. Conductive hearing loss (CHL)

We found CHL in 33% of all ears. Taking into account the group with a mild CHL, this number even increases to 46%. Percentages of CHL in TS literature vary from 0 to 44%.[5–9,14,15,22,23,31,32] The young age of our population could explain the considerable amount of ears with CHL.

4.2.2. Mixed hearing loss (MHL)

MHL was found in 14% of all ears. The range of MHL in TS literature varies from 3 to 23%.[6,8,9,14,15,17,23,26,31,32] The different types of SNHL among the children with a MHL are implemented in the two paragraphs below.

4.2.3. Pure sensorineural hearing loss (SNHL)

TS literature shows great variability in the reported prevalence of SNHL varying from 9 to 66. 7%.[5,6,8,9,14,22,23,25,26,31,32] One study even found a high-frequency SNHL (8-18 kHz) in 98.7% of the patients (mean age 20.8 year, N = 38).[11] The mid-frequency dip and the high-frequency losses are the most prominent SNHL configurations. We found pure SNHL in 4% of all ears which is a low percentage compared to other studies. This can partly be explained by the fact that we separated the children with a mid-frequency dip as a different group because the peak of the dip did not always exceed the 20 dB HL. This was also the case in some other studies, though most of them described the dip as part of the SNHL group, enlarging this group.[7,15,17] Apparent discrepancy in percentages of SNHL may also be attributable to different mean ages of the populations studied. High-frequency SNHL is a more common finding in older women than in young children with TS.[6,18] A study in which subjects have been followed longitudinally has shown a decrease in hearing thresholds with age.[18] Furthermore, we must take into account that SNHL was actually found in a higher percentage of ears as SNHL was also present in all ears with a mixed hearing loss. When we add the pure SNHL (4%), sensorineural mid-frequency dip (17%) and the MHL-group (14%), we find SNHL in 35% of all ears.

4.2.4. Sensorineural mid- frequency dip

While all authors refer to the typical sensorineural mid-frequency dip, most literature does not offer a quantitative definition. Assuming our definition, we found a dip in 17% of all ears. The dip maximum was most frequently in the 2 kHz region according to other studies, and the mean depth was 25 dB HL. Stenberg also studied a group of children and found a dip in 58% of the cases with a mean depth of 18.8 dB HL.[7] Hultcrantz found a dip in 69% of

patients aged 4-15 years and the mean depth did not exceed the 20 dB HL.[16] Watkin found a dip in 4 children (17%), with a maximum at 1 kHz and a mean AC threshold of 45 dB HL.[26] The difference in numbers may be due to the variation in definitions used. The youngest person presenting with a dip in our study was 5.8 years of age which confirms that the dip can already develop during childhood. The youngest person with a dip described in literature was 6 years.[7]

Hultcrantz showed that the dip progresses with age.[16,17] Regarding TS patients of all age categories, a dip has been described in 8 to 88% of the patients.[5–7,16–19,26,31,32] Our cross-sectional data showed no obvious difference in depth of the dip between younger and older children. In many of our young children a dip might not have been developed yet and therefore we would need individual follow-up to confirm that the dip progresses with age. Furthermore, some ears demonstrated a dip-configuration though they did not fulfill the requirements, i.e. the dip was not as pronounced. Others showed an obvious dip but there was also a loss in the high-frequencies, so regarding our criteria they were excluded from the mid-frequency dip group. These ears were represented as combined mid- and high-frequency SNHL (6% of all ears) in the MHL group. Barrenäs found a combined mid- and high-frequency loss in 39% of the patients of whom most were adult women.[5] Hultcrantz suggested that the high-frequency loss develops after the dip in the same individual.[15] The mean age of the children in our study with a combined mid- and high-frequency loss was indeed the highest of all groups (15.5 years). However, these are cross-sectional data and therefore we cannot say if the high-frequency loss developed after the dip in the same child. Further longitudinal studies are required and therefore follow-up over time is important.

In our study, 79% of the ears showing a dip were of children with a monosomy or isochromosome. Hederstierna also reported that a high percentage (80%) of the audiograms with a dip configuration were found in children with a monosomy or isochromosome.[18]

4.3. Audiometric findings in correlation to the different karyotypes

Barrenäs postulated the cell cycle delay hypothesis (CCD).[5,21] A prolonged cell cycle and a lack of growth regulating genes, such as the SHOX (short stature homeobox-containing) gene, located on the p-arm of the X-chromosome, would lead to growth disturbances during development of the auricle, temporal bone, cochlea and the Eustachian tube.[5] According to this hypothesis, a higher occurrence of ear and hearing problems is expected in children with a monosomy or isochromosome (greater loss of p-arm of X-chromosome) compared to those with a mosaicism or structural anomaly.

We found indeed that hearing thresholds in general were about 10-11 dB worse in children with a monosomy or isochromosome compared to those with a mosaicism or structural anomaly. This is an indication that karyotype may be used as a predictor for future hearing impairment. We demonstrated that the difference between both groups might already be

present at young age. Barrenäs also found a significant difference in hearing thresholds between both groups, being worse in monosomy/isochromosome cases, however the difference was only obvious in adult age.[5]

We found a significant age-related deterioration in BC thresholds at frequencies of 0.5-4 kHz in the monosomy/isochromosome cases and only at 4 kHz in the mosaicism/structural anomalies; the age-related deterioration in BC thresholds was more prominent in the monosomy/isochromosome cases. Barrenäs also described that hearing seemed to deteriorate more rapidly with increasing age in monosomy/isochromosome cases, however, the significance of this apparent difference was not tested.[5] Another study found that only the AC thresholds at 8 kHz and the BC thresholds at 4 kHz were significantly correlated with age and that age-related high-frequency SNHL was more prominent in cases with a monosomy than in those with a mosaicism.[22] However, the significance of these findings can be questioned because the author (incorrectly) treated the data pertaining to each ear (left and right) as separate entities.

4.4. Otologic surgery

Cholesteatoma was found in 3/60 (5%) children (4 ears) which is a remarkable high number. In a study among 2.664 school aged children (8-13 years) in the normal population, cholesteatoma was only found in 2 children (0.07%).[28] Other studies also suggest that TS patients are prone to develop cholesteatoma. Hall described a population of 178 TS patients of whom 6/178 (3.4%) had cholesteatoma.[30] Dhooge found cholesteatoma in 2.3% of the ears and Sculerati in 9% of TS patients.[6,14]

In the overall population, young children with poor middle ear ventilation, large cholesteatoma and resorption of the ossicular chain are at special risk of developing recurrent cholesteatoma.[33,34] This was also the case in the present study and a high recurrence rate of 3/4 ears (75%) was seen. Recurrence rates of cholesteatoma in the normal population are reported to vary from 5 to 71%.[35]

Although we prefer to perform a canal wall up procedure, a canal wall down procedure was performed in 3 ears. In a cavity, the tendency to develop recurrent ear infections can be problematic when hearing aids are needed and therefore two children were fitted with a BAHA. The need for hearing aids in TS patients is high as percentages of 5 to 27.3% have been described.[5,6,16,17,19,21] In our study 10% of the children already had some kind of hearing aid at relatively young age.

4.5. Recommendations

Neonatal hearing screening in the Netherlands usually takes place within two weeks after delivery by otoacoustic emission (OAE) testing. Then, we recommend an otologic and audiometric evaluation in TS children at the age of 9-12 months, in case that the diagnosis

TS is already known by then. When no anomalies are found at that time, we suggest follow up at the age of 3, 5, 8, 12 and 16 years. When ear or hearing problems are present, we recommend a highly individualized approach and follow-up depending on the problems. In TS children with recurrent middle ear disease, early insertion of ventilation tubes, with rapid progression to T-tubes, is advocated. Regarding the surgical management in case of cholesteatoma, we prefer an intact canal wall technique. Because of a high recurrence rate, children should be observed many years after surgery.

4.6. Conclusion

Otologic disease is an important characteristic in TS. Each type of hearing impairment can already be present at young age. Hearing impairment has an undeniable influence on the development and performance of a child. Therefore, careful follow-up during childhood is necessary to detect early ear and hearing problems and in some cases active interventions will be necessary. Furthermore, our findings support the hypothesis that hearing can be affected by a loss of the p-arm of the X-chromosome as we found statistically significant differences in hearing thresholds among the different karyotypes. A correlation with karyotype could help to predict future hearing problems.

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Chapter 3

Karyotype-specific ear and hearing problems in young adults with Turner syndrome and the effect of Oxandrolone treatment

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ABSTRACT

Objective: To evaluate karyotype-specific ear and hearing problems in young adult patients with Turner syndrome (TS) and assess the effects of previous treatment with Oxandrolone (Ox).

Study design: Double-blinded follow-up study.

Setting: University hospital.

Patients: Sixty-five TS patients (mean age, 24.3 year) previously treated with growth hormone combined with placebo, Ox 0.03 mg/kg per day, or Ox 0.06 mg/kg per day from the age of 8 years and estrogen from the age of 12 years.

Intervention: Ear examination was performed according to standard clinical practice. Air and bone conduction thresholds were measured in decibel hearing level.

Main outcome measures: We compared patients with total monosomy of the short arm of the X-chromosome (Xp) -monosomy 45,X and isochromosome 46,X,i(Xq)- with patients with a partial monosomy Xp -mosaicism or other structural X-chromosomal anomalies-. We assessed the effect of previous Ox treatment.

Results: Sixty-six percent of the patients had a history of recurrent otitis media. We found hearing loss in 66% of the ears, including pure sensorineural hearing loss in 32%. Hearing thresholds in patients with a complete monosomy Xp were about 10 dB worse compared with those in patients with a partial monosomy Xp. Air and bone conduction thresholds were not different between the placebo and Ox treatment groups.

Conclusions: Young adult TS individuals frequently have structural ear pathology, and many suffer from hearing loss. This indicates that careful follow-up to detect ear and hearing problems is necessary, especially for those with a monosomy 45,X or isochromosome 46,X,i(Xq). Ox does not seem to have an effect on hearing.

INTRODUCTION

Turner syndrome (TS), a chromosomal disorder with a prevalence of approximately 1 in 2000 live-born girls, is caused by complete or partial absence of one X-chromosome. Prominent features are short stature, estrogen deficiency, and dysmorphic abnormalities.[1] Monosomy 45,X, characterized by the presence of a single X-chromosome in all cells, is the most frequent karyotype. Other patients have a karyotype with a duplication of the long arm of the X-chromosome (isochromosome, 46,X,i(Xq)), mosaicism or another structural X-chromosomal anomaly. In general, women with monosomy 45,X have a higher disease burden than those with a mosaicism.[2,3]

Ear and hearing problems are frequently reported in TS. In childhood, middle ear infections resulting in conductive hearing loss are common.[4,5] Abnormal craniofacial morphology and Eustachian tube dysfunction are thought to be the main contributing factors. TS patients are also prone to develop sensorineural hearing loss (SNHL).[6,7] A typical sensorineural mid-frequency dip is often already seen in adolescence. Progressive sensorineural high-frequency loss manifests earlier in TS women than in healthy women, especially in those patients already having a mid-frequency dip.[8]

TS patients with monosomy 45,X or isochromosome 46,X,i(Xq), that is, those with monosomy of the complete short arm of the X-chromosome (Xp), are supposed to be more prone to hearing problems than those with mosaicism or other structural X-chromosomal anomalies, in which monosomy of Xp is only partial.[9,10] This suggests that the locus for hearing impairment may be located on Xp. Furthermore, estrogen deficiency is suggested to be a contributing factor in hearing problems.[7,11,12]

Although growth hormone (GH) has been shown to improve final height, such treatment can only partially overcome the growth failure observed in TS. It is for this reason that adjunctive therapy has been tried, notably with Oxandrolone (Ox), a synthetic anabolic steroid derived from dihydrotestosterone. In previous studies, we investigated the growth-enhancing effect of Ox, a weak androgen, in addition to GH in TS girls.[13] We, and others found that low-dose treatment with Ox increases growth velocity and adult height.[13–15] The effect of Ox on hearing has not been previously investigated. Theoretically, Ox may also positively affect the growth and development of the mastoid bone and middle ear. This, in turn, could lead to prevention of recurrent otitis media and conductive hearing loss. In addition, Ox could also positively affect inner ear function through a suspected decrease in sex hormone-binding globulin and the resulting higher free estrogen levels.[16] However, the negative effects of androgens on hearing are suggested as well.[17,18]

We recently carried out a follow-up study to determine the long-term effects of previous Ox treatment on growth and body composition in young adult women with TS.[19] This provided the unique opportunity to analyze the otologic and audiologic features of a well-characterized TS

population. In addition to the evaluation of karyotype-specific ear and hearing problems, the aim of the study was to investigate whether hearing is influenced by previous Ox treatment.

MATERIALS AND METHODS

Patients

All of the 133 participants of the original randomized, double-blind, placebo-controlled trial were invited for a medical assessment including otologic and audiologic examination. Participants of the original trial were recruited between 1991 and 2003 in 10 pediatric endocrine centers in the Netherlands and randomized to receive Ox in a dosage of 0.03 mg/kg per day (Ox 0.03) or 0.06 mg/kg per day (Ox 0.06) or placebo (Pl) after reaching the age of 8 years.[13] Furthermore, girls were treated with GH (1.33 mg/m^2 per day) from baseline and, when spontaneous puberty was absent, with estrogen from the age of 12 years. For more details see Menke et al.[13] and Freriks et al.[19]

This follow-up study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the ethics committee of the Radboud University Nijmegen Medical Centre. Written informed consent was obtained for each participant. The study is registered at www.trialregister.nl (NTR1934).

Otologic and audiologic examination

All ears were macroscopically and micro-otoscopically examined according to standard clinical practice. Medical history was taken, focusing on ear and hearing problems, including questions about confounding factors, such as meningitis, head injury, and problems related to birth.

Pure-tone hearing thresholds were measured according to standard audiometric methods (ISO 389) in a sound proof room. Air conduction (AC) thresholds and bone conduction (BC) thresholds were measured in decibel hearing level (db HL) at 0.25, 0.5, 1, 2, 4, and 8 kHz.

We defined the following audiometric categories:

Normal hearing

The AC thresholds were equal to or better than 20 dB HL across the frequency range of 0.25 to 8 kHz.

Mild conductive hearing loss

The AC thresholds were never worse than 20 dB HL, although there was an air-bone gap (ABG) of at least 10 dB at more than one frequency within normal range. By this classification we include these relevant ABGs in the analyses.

Conductive hearing loss
AC thresholds were worse than 20 dB HL at one or more frequencies in the range of 0.25 to 8 kHz. There was an ABG of at least 10 dB at one or more frequencies at which the AC threshold was worse than 20 dB HL. BC thresholds were better than 20 dB HL at any frequency.

Mixed hearing loss
The BC thresholds were worse than 20 dB HL at one or more frequencies in the range of 0.25 to 8 kHz, and there was an ABG of at least 10 dB at one or more frequencies at which the BC threshold was worse than 20 dB HL.

Pure SNHL
The AC thresholds were worse than 20 dB HL at one or more frequencies in the range of 0.25 to 8 kHz, and there was no ABG at the frequencies at which the AC threshold was worse than 20 dB HL.

Based on the BC thresholds of the mixed hearing loss (MHL) and SNHL groups, we categorized the following subgroups:

Sensorineural mid-frequency dip
Audiograms show a typical sensorineural mid-frequency dip at 1 and/or 2 kHz. The dip was defined as BC thresholds being at least 10 dB HL worse than all of the lower and higher frequencies.

Sensorineural mid- and high-frequency loss
Audiograms show a typical sensorineural mid-frequency dip at 1 and/or 2 kHz in combination with a dip at 8 kHz. The dip at 8 kHz was only scored when worse or not more than 10 dB better as the maximum of the mid-frequency dip.

Sensorineural high-frequency loss
High-frequency SNHL was defined as BC thresholds worse than 20 dB HL at 4 kHz or higher frequencies or BC thresholds worse than 20 dB HL at frequencies below 4 kHz with an increasing loss with increasing frequency (down sloping).

Statistical analysis
We tested the hypothesis that patients with a monosomy 45,X or isochromosome 46,X,i(Xq) karyotype (complete monosomy Xp) have a higher prevalence of hearing problems compared with those with a mosaicism or other structural X-chromosomal anomaly (partial monosomy Xp). Linear regression was used to analyze the influence of age and karyotype on hearing.

An *F* test was used to compare between regression lines. Testing on the slopes between the regression lines was performed first, and testing between the *elevations* (measure that pertains to the group's *offset* threshold) was only performed if there was no significant difference in slope. The total effect of all frequencies was considered significant when the *p* value was < 0.11 at three or more frequencies. Concerning the influence of previous Ox treatment, we tested the differences in mean hearing thresholds between different dosage groups and placebo by testing linear regression using two dummies (for Ox 0.03 and Ox 0.06). We performed a modified intention-to-treat analysis, that is, including only those patients who took at least one dose of the study medication. We used the Statistical Package for the Social Sciences version 16.0 (SPSS, Inc., Chicago, IL, USA) and the Prism program (GraphPad, San Diego, CA, USA) where applicable.

RESULTS

Patients

Of the 133 original participants in the trial, 68 could be included in the general follow-up study. Besides lost to follow-up (n = 9), excluded (n = 7), and deceased (n = 2), 47 women refused to participate. Main reasons for refusal were the burden of the investigations besides regular medical follow-up and travel distance. For the specific ear and hearing analyses, we excluded one extra patient because of age just under 18 years. Furthermore, in two patients, no ear and hearing evaluation took place for logistic reasons. Table 1 shows the karyotype differentiation of the 65 patients who participated in the study (Pl, n = 22; Ox 0.03, n = 26; Ox 0.06, n = 17). The mean age was 24.3 ± 3.6 years (range, 18-32 year). One patient did not undergo otoscopic examination. For the analysis of the effects of previous Ox use, we excluded two more patients who did not take any dose of the study medication.

Ear, Nose, and Throat history

Data concerning history are presented in Table 1. Many patients had suffered from otologic disease. Two of the five patients with previous surgery for cholesteatoma suffered from cholesteatoma in both ears. Recurrent cholesteatoma occurred in five ears, and surgery finally resulted in a modified radical cavity in five of seven ears.

External ear findings

Forty-two of the 65 patients (65%) had one or more external ear anomalies, including dysmorphic auricles, posteriorly rotated ears, low-set ears and/or abnormally protruding ears varying from only mild to more severe forms.

Table 1. Karyotype distribution in relation to ear, nose, and throat history, eardrum and middle ear findings, and audiometric results.

	Monosomy 45,X (n = 32 patients, 49%)	Isochromosome[a] (n = 10 patients, 15%)	Mosaicism/structural anomaly[b] (n = 23 patients, 35%)	Total (n = 65 patients or 130 ears)
Ear, nose, and throat history (n = 65 patients)				
Recurrent otitis media	25 (78%)	6 (60%)	12 (52%)	43 (66%)
Adenotomy/tonsillectomy/adenotonsillectomy	21 (66%)	7 (70%)	12 (52%)	40 (62%)
Ventilation tubes	24 (75%)	5 (50%)	10 (43%)	39 (60%)
Otologic surgery[c]	7 (22%)	1 (10%)	2 (9%)	10 (15%)
Cholesteatoma	3 (9%)	0 (0%)	2 (9%)	5 (8%)
Hearing aid/BAHA at present time	3 (9%)	0 (0%)	1 (4%)	4 (6%)
Eardrum and middle ear findings (n = 128 ears)[d]				
Myringosclerosis	21 (34%)	5 (25%)	16 (35%)	42 (33%)
Retraction	10 (16%)	0 (0%)	6 (13%)	16 (13%)
Conservative radical cavity	3 (5%)	0 (0%)	2 (4%)	5 (4%)
Atelectasis	1 (2%)	2 (10%)	1 (2%)	4 (3%)
T-tube	2 (3%)	0 (0%)	1 (2%)	3 (2%)
Perforation	2 (3%)	0 (0%)	1 (2%)	3 (2%)
Otorrhoea	2 (3%)	1 (5%)	0 (0%)	3 (2%)
Postmyringoplasty	1 (2%)	0 (0%)	0 (0%)	1 (1%)
Erosion of malleus/incus	0 (0%)	0 (0%)	1 (2%)	1 (1%)
Audiometric results (n = 130 ears)				
Normal hearing	18 (28%)	2 (10%)	24 (52%)	44 (34%)
Mild conductive hearing loss	1 (2%)	0 (0%)	2 (4%)	3 (2%)
Conductive hearing loss	5 (8%)	1 (5%)	4 (9%)	10 (8%)
Mixed hearing loss	21 (33%)	4 (20%)	6 (13%)	31 (24%)
Pure sensorineural hearing loss	19 (30%)	13 (65%)	10 (22%)	42 (32%)

[a] Pure 46,X,i(Xq) (n = 4) or in combination with 45,X: 45,X/46,X,i(Xq) (n = 6).
[b] 45,X/46,XX (n = 5), 45,X/46,X,r(X) (n = 4), 45,X/46,X,del(X) (n = 3), 45,X/47,XXX (n = 3), 45,X/46,X+mar (n = 2), 46,X,del(X) (n = 1), other (n = 5, including 4 patients with mosaicisms containing more than two cell lines and one patient with a complex mosaicism: 46,X,Xp-/46,X,i(Xq)).
[c] Including mastoidectomy, tympanoplasty, myringoplasty, and operations for cholesteatoma resulting in a modified radical cavity.
[d] Excluding one patient with monosomy 45,X who did not underwent otoscopic examination.
BAHA: bone-anchored hearing aid.

Eardrum and middle ear findings

Myringosclerosis and tympanic membrane retraction were the most common findings, occurring in 42 (33%) of 128 and 16 (13%) of 128 ears, respectively. An overview of all eardrum and middle ear findings is shown in Table 1. Some ears showed multiple pathology.

Audiologic examination

Normal hearing was present in 44 (34%) of 130 ears. The other ears could be classified in one of the categories of abnormal audiometric results (Table 1). Conductive hearing loss (CHL) was found in 10 (8%) of 130 ears. Figure 1A shows the hearing thresholds of the ears in which the mean AC was worse than 25 dB, counting 5 (4%) of 130 ears. The other five ears showed CHL at one or more frequencies, although the mean hearing threshold of all frequencies was better than 25 dB. MHL was present in 31 (24%) of 130 ears (Fig. 1B). Pure SNHL was found in 42 (32%) of 130 ears (Fig. 1C). Figure 1C only shows the ears in which the mean AC threshold was worse than 25 dB, counting 15 (12%) of 130 ears.

Concerning only the BC thresholds of the MHL and SNHL group, the following audiogram configurations were seen (Fig. 1D-F): a typical sensorineural mid-frequency dip (19/130 ears, 15%), a combined mid-and high-frequency sensorineural hearing loss (14/130 ears, 11%), and high-frequency sensorineural hearing loss (27/130 ears, 21%). The remaining 13 (10%) of 130 ears showed BC threshold configurations that could not be classified in one of these categories.

Audiometric findings in relation to karyotype

Linear regression showed no significant deterioration in mean BC thresholds with increasing age of each patient, for both the patients with a complete monosomy Xp and those with a partial monosomy Xp. Furthermore, there was no significant difference in slopes between both groups. However, there was a significant difference in elevation indicating a substantial difference in hearing thresholds, being about 10 dB worse for patients with a complete monosomy Xp compared with those with a partial monosomy Xp. Figure 2 shows an example at 2 kHz.

Regression analysis for AC thresholds showed no progression with age. The differences in AC thresholds were similar, being about 10 dB worse in the patients with complete monosomy Xp compared to those with a partial monosomy Xp. Figure 3 shows an example at 1 kHz.

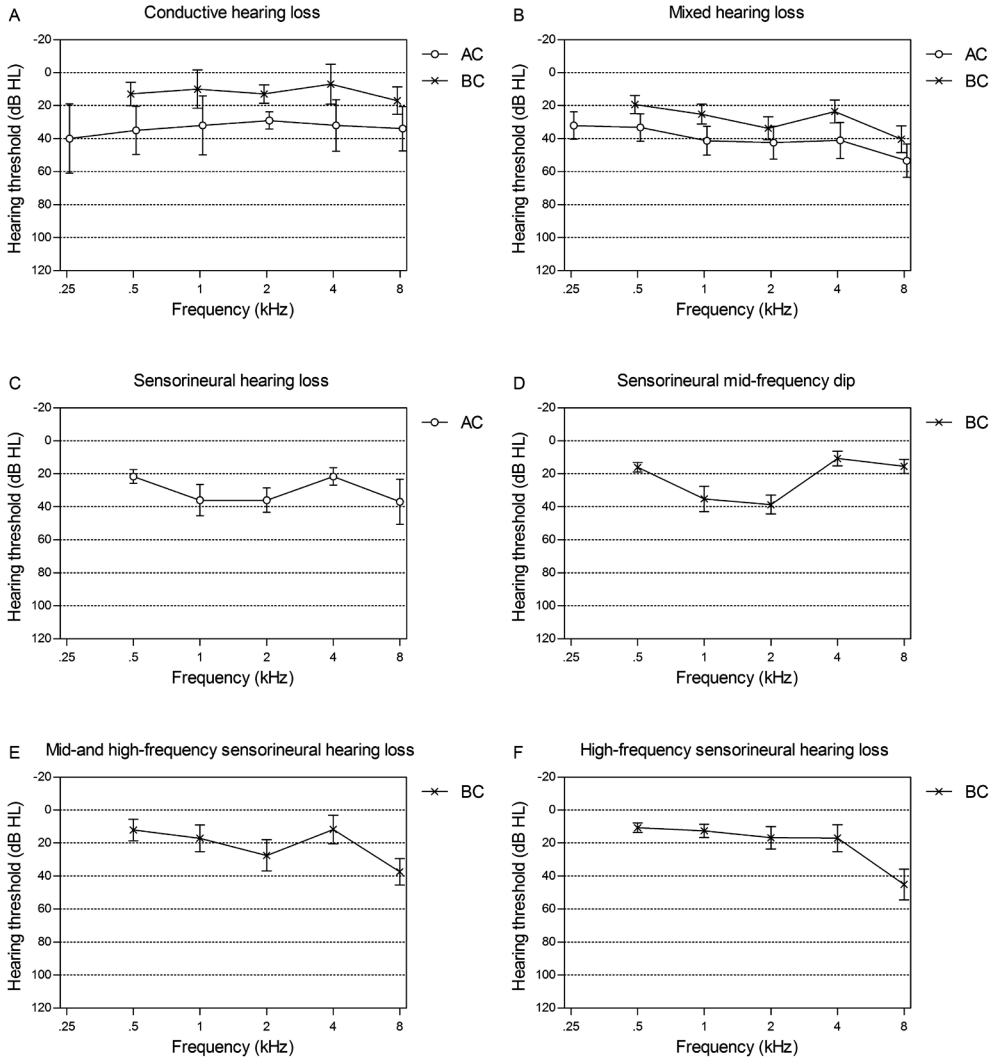

Figure 1. -O- indicates air conduction;-X- indicates bone conduction. (A) Mean air conduction (AC) and bone conduction (BC) thresholds for the conductive hearing loss (CHL) group, only showing the ears with a mean AC threshold worse than 25 dB (n = 5 ears). (B) Mean AC and BC thresholds for the mixed hearing loss (MHL) group (n = 31 ears). (C) Mean AC threshold for the sensorineural hearing loss (SNHL) group, only showing the ears with a mean AC threshold worse than 25 dB (n = 15 ears). Six audiograms of the SNHL group had an ABG of 10 to 20 dB at more than one frequency at which the BC was within normal range. (D) Mean BC threshold for the sensorineural mid-frequency dip group (n = 19 ears). (E) Mean BC threshold for the group showing combined mid- and high-frequency sensorineural hearing loss (n = 14 ears). (F) Mean BC threshold for the group with high-frequency sensorineural hearing loss (n = 27 ears).

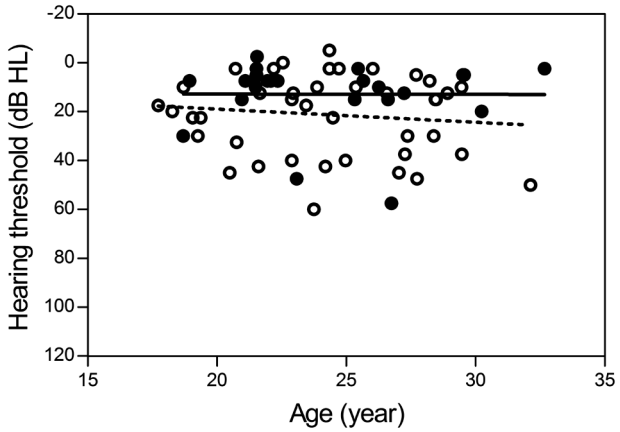

Figure 2. Mean bone conduction (BC) thresholds at 2 kHz plotted as a function of age and linear regression line for the patients with a monosomy/isochromosome (○ and ---) and those with a mosaicism/structural anomaly (● and ——) (n = 65 patients). Linear regression analysis shows a significant difference in elevation between both groups.

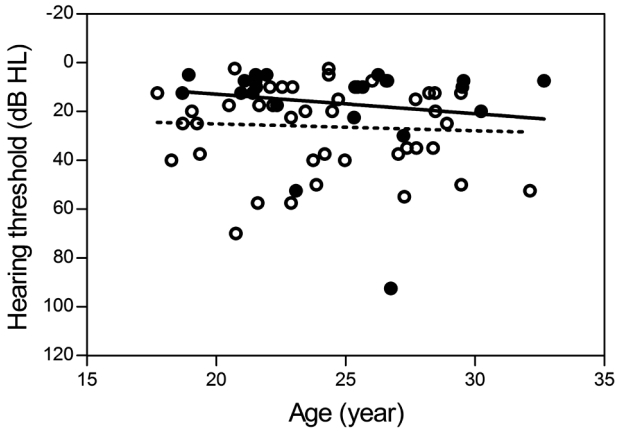

Figure 3. Mean air conduction (AC) thresholds at 1 kHz plotted as a function of age and linear regression line for the patients with a monosomy/isochromosome (○ and ---) and those with a mosaicism/structural anomaly (● and ——) (n = 65 patients). Linear regression analysis shows a significant difference in elevation between both groups.

Oxandrolone and hearing

Regarding the influence of Ox on hearing, we found that mean AC and BC thresholds were not significantly different in the three treatment groups (Pl, Ox 0.03 and Ox 0.06; Table 2). All *p* values were > 0.11 at all frequencies. We found no relevant correlation with height, adult height gain, and duration of Ox use (data not shown).

Table 2. Mean hearing thresholds related to Oxandrolone use (n = 63)[a]

	GH + Pl (n = 22)	GH + Ox 0.03 (n = 24)	GH + Ox 0.06 (n = 17)
Air conduction[b]			
0.25 kHz	18.3 ± 10.3	18.6 ± 12.7	20.3 ± 21.9
0.5 kHz	17.7 ± 10.3	20.1 ± 11.6	22.5 ± 22.1
1.0 kHz	19.0 ± 15.0	24.3 ± 16.7	25.3 ± 25.3
2.0 kHz	21.6 ± 19.8	20.0 ± 14.7	23.7 ± 28.3
4.0 kHz	18.6 ± 16.5	17.8 ± 17.3	22.5 ± 23.3
8.0 kHz	28.4 ± 20.7	28.5 ± 21.9	29.7 ± 24.3
Bone conduction[b]			
0.5 kHz	12.2 ± 9.4	14.0 ± 9.7	12.6 ± 11.4
1.0 kHz	12.7 ± 13.6	18.8 ± 14.9	15.9 ± 15.1
2.0 kHz	19.7 ± 18.0	17.2 ± 13.5	18.2 ± 18.7
4.0 kHz	11.8 ± 13.4	8.5 ± 14.0	10.9 ± 11.3
8.0 kHz	21.9 ± 16.8	23.8 ± 21.7	22.6 ± 17.2

[a] Excluding those two patients who never took any dose of the study medication.
[b] All *p* values for the comparison of GH + Ox 0.03 and GH + Ox 0.06 to placebo are > 0.1.
GH indicates growth hormone; Pl, placebo; Ox 0.03, Oxandrolone 0.03 mg/kg per day; Ox 0.06, Oxandrolone 0.06 mg/kg per day.

DISCUSSION

Middle ear disease

The prevalence of a history of recurrent otitis media of 66% is consistent with previous reports on TS.[4,6,9,10,20–25] Ventilation tubes were placed in 60% of the patients. Other studies showed lower percentages varying from 32 to 57%.[4,6,21,26,27]

Middle ear infections in TS are thought to be related to an abnormal craniofacial morphology, including a high arched palate and an abnormal orientation of the Eustachian tube. Rizell et al. showed that TS patients have a short posterior and flattened cranial base, retrognathy, short and posteriorly rotated maxilla and mandible, increased ramus height, and relatively shorter posterior facial height.[28] These abnormalities are probably the result of a prolonged cell cycle in the brachial arches and neck region and a lack of transacting growth-regulating genes, such as the *SHOX* gene.[29] A subnormal immune response could also be a contributing factor in recurrent infections.[4,20]

Audiometric findings

Normal hearing was found in only 34% of the ears. CHL was found in 8% of the ears. In other TS populations, percentages of CHL vary between 0 and 44%, depending on the age group included.[4,5,9,20,21,23,25,27,30,31] In our previous report on TS children (age 4.1-21.1 year), CHL was more common with a percentage of 33.[10] The relatively low percentage of CHL found in the current adult group might be explained by the lower prevalence of middle ear pathology at increasing age. Others found no pure CHL in an adult population.[23]

MHL was present in 24% of the ears, which is more common than in our pediatric cohort (14%).[10] The range of MHL described in the literature varies from 3 to 23%.[5,20,21,23,24,27,30–32]

We found a high percentage of pure SNHL in 32% of the ears. The percentages of SNHL described in the literature are highly variable in a range of 4 to 66%.[5,9,10,20,21,25,27,30–33] The prevalence of pure SNHL was much higher than the 4% in our pediatric population.[10]

Regarding the BC thresholds of the MHL and SNHL group, we found typical audiograms with a mid-frequency dip (15%), combined mid- and high-frequency loss (11%), and high-frequency loss (21%). In the literature, percentages of a mid-frequency dip vary between 8 and 88%.[4,6,8–10,21,24,30,32,33] This wide range might be caused by different definitions and age categories.[6,24] Our previous study on TS children showed a mid-frequency dip in 17% of the ears. The mean depth of the dip in the current adult patients is 38.7 dB, which is more pronounced compared with 25 dB reported in TS children.[10] The dip maximum exceeded 20 dB in all adult cases in contrast with the findings in TS children where the dip maximum was within normal range as well.[4,6,10] In the present study, 11% of the ears showed a combined mid- and high-frequency loss, which is more than was found in TS children (6%).[10] It is likely that the high-frequency loss develops after the dip in the same individual.[23] A high-frequency loss was the most common finding in 21% of the ears of the adult TS patients. Our study on TS children showed a high-frequency loss in 4% of the ears. This confirms that sensorineural hearing levels in TS decrease with age, as we and others demonstrated previously.[6,8,10,23]

The cause of SNHL in TS is not clear. Specific cochlear malformations have not yet been revealed.[21,34,35] In two TS patients with SNHL, hypoplastic lateral semicircular canals were found.[36] Furthermore, the cell cycle delay hypothesis might be an explanation for SNHL.[29] In general, the middle turn of the cochlea has the highest hair cell density, and this is where the cell cycle needs to be upregulated the most during development to acquire a sufficient number of sensory hair cells for signal analysis.[37] In TS, it is speculated that this middle part of the cochlear duct is most prone to growth disturbances because of a delayed cell cycle that might cause a mid-frequency dip.[29] The early onset of the high-frequency hearing loss in TS is also suggested to be the result of a reduced number of sensory hair cells.[29]

Low estrogen levels in TS women could be a possible contributing factor to SNHL because, in women without TS, more rapid hearing deterioration occurs after the menopause when circulating levels of endogenous estrogen drop rapidly.[38] Estrogen receptors have been shown to exist in human inner ear.[12] However, since almost all women used adequate estrogen replacement therapy at the moment of audiometric measurement, this could not be an explanation for the differences found in this study.

Oxandrolone and hearing

We and others have shown that, when given at a low dose, the weak androgen steroid Ox has a growth-enhancing effect in girls with TS.[13–15] This is the first study to investigate whether

there is an association between previous Ox use and hearing. We hypothesized that Ox would have a beneficial impact on hearing.

First, along with the growth-stimulating effect of Ox on body height, Ox may also enhance the growth and development of the mastoid bone, middle ear cavity, eardrum, and outer ear canal. This might occur through increase in insulin-like growth factor 1 (IGF-1) observed in TS patients treated with Ox.[13,16] Low IGF-1 levels are associated with hearing loss.[39] Both middle ear infections and SNHL were related to low IGF-1 levels in TS.[29] Subtle improvements in growth and development of the ear resulting in a more physiological anatomy could to some extent prevent recurrent otitis media and hearing loss. Our current findings indicate, however, that there were no differences in mean hearing thresholds between the groups treated with Pl, Ox 0.03 and Ox 0.06. Unfortunately, audiologic examinations were not performed during treatment, so effects may have been transient.

Second, Ox could also positively influence hearing through estrogen-mediated effects on the inner ear. Estrogen deficiency may have deleterious effects on hearing, which was suggested to be caused by lack of stimulation of the estrogen receptors ER-α and ER-β, which are abundantly present in the inner ear.[11,12] Ox treatment is expected to decrease estrogen protein binding and in turn increase free active estrogen levels, which could positively affect inner ear function. In our study, however, we did not discern any differences in hearing thresholds between the treatment groups. This might be related to the fact that all patients received proper estrogen replacement therapy, with no additional effects of Ox on the availability of estrogen in the ear.

In theory, beneficial effects might also be counterbalanced by disadvantageous effects of androgens. Previous studies suggested that prenatal exposure to androgens negatively influences the auditory system by weakening of the cochlear mechanisms that underlie the production of otoacoustic emissions.[17] In addition, there is evidence that circulating androgen levels postnatally might negatively influence otoacoustic emissions as well.[40,41] In men, higher levels of circulating testosterone were found to be associated with smaller click-evoked otoacoustic emission amplitudes, and male monkeys produced click-evoked otoacoustic emissions of lower amplitude during the breeding season, when testosterone levels are higher.[40,41] In human females, data are controversial. One study reported that females with polycystic ovary syndrome (PCOS)-having higher androgen levels- showed significantly more high-frequency hearing loss compared with women without PCOS.[18] On the other hand, a recent study in women with PCOS showed that hyperandrogenism did not seem to influence otoacoustic emission levels in adult female subjects.[42]

Audiometric findings in relation to karyotype

We found that hearing thresholds in general were about 10 dB worse in adults with a complete monosomy Xp compared with those with a partial monosomy Xp. This agrees with previous

findings in TS children and adults.[9,10] These results support the hypothesis that genes located on the short arm of the X-chromosome (Xp) are of importance in hearing.

Otologic surgery

Cholesteatoma was found in as many as 8% of the patients (five patients, seven ears). A canal wall down procedure was necessary in five of seven ears. This can be problematic in the hearing aid-dependent TS patient with a tendency to develop recurrent ear infections. In those patients, a bone-anchored hearing aid may offer a solution. A previous study in young adults reported cholesteatoma in 2.3%.[21] Previous studies in TS children showed percentages ranging from 3 to 5.[10,34] Abnormal craniofacial morphology and Eustachian tube dysfunction could be contributing factors in developing cholesteatoma. Early insertion of ventilation tubes should be considered in TS patients who suffer from middle ear disease.

CONCLUSION

This systematic otologic and audiologic evaluation in a well-described young adult TS population treated with Ox or placebo in addition to GH in childhood shows that TS women frequently have eardrum and middle ear pathology and that many of them suffer from hearing loss. SNHL, in contrast to CHL, is more pronounced in young adult TS women than in TS children. Hearing thresholds in patients with a monosomy 45,X or isochromosome 46,X,i(Xq) (complete monosomy Xp) are significantly worse compared with those having a mosaicism or other structural X-chromosomal anomaly (partial monosomy Xp). These findings indicate that careful follow-up is necessary to detect ear and hearing problems, especially for those with monosomy 45,X or isochromosome 46,X,i(Xq). Ox, which at a low dose has a positive effect on growth, has no long-term effects on hearing.

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Chapter 4

p.R705H mutation of *MYH9* is associated with *MYH9*-related disease and not only with non-syndromic deafness DFNA17

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ABSTRACT

MYH9-related disease (*MYH9*-RD) is a rare autosomal dominant disease caused by mutation of *MYH9*, the gene encoding for the heavy chain of non-muscle myosin IIA (NMMHC-IIA). *MYH9*-RD patients have macrothrombocytopenia and granulocyte inclusions (pathognomonic sign of the disease) containing wild-type and mutant NMMHC-IIA. During life they might develop sensorineural hearing loss, cataract, glomerulonephritis, and elevation of liver enzymes. One of the *MYH9* mutations, p.R705H, was previously reported to be associated with DFNA17, an autosomal dominant non-syndromic sensorineural hearing loss without any other features associated. We identified the same mutation in two unrelated families, whose four affected individuals had not only hearing impairment but also thrombocytopenia, giant platelets, leukocyte inclusions, as well as mild to moderate elevation of some liver enzymes. Our data suggest that DFNA17 should not be a separate genetic entity but part of the wide phenotypic spectrum of *MYH9*-RD characterized by congenital hematological manifestations and variable penetrance and expressivity of the extra-hematological features.

INTRODUCTION

MYH9-related disease (*MYH9*-RD) is an autosomal dominant syndromic disorder caused by mutations in *MYH9*, the gene encoding for the heavy chain of non-muscle myosin IIA (NMMHC-IIA).[1,2] Myosin IIA is a cytoplasmic, non-sarcomeric myosin expressed in most cell types and tissues, where it participates in several processes requiring generation of chemomechanical forces by the cytoskeleton.[3] As the other conventional myosins, it exists as a hexameric complex containing a dimer of NMMHC-IIA moieties and two pairs of regulatory and essential light chains.[4]

MYH9-RD is characterized by a complex clinical phenotype. All the *MYH9*-RD patients have congenital hematological alterations, namely platelet macrocytosis, thrombocytopenia and characteristic inclusions in the cytoplasm of granulocytes containing both mutant and wild-type NMMHC-IIA. These inclusions are always detectable by immunofluorescence staining for NMMHC-IIA and, when large, they are also visible as basophilic inclusions (also known as Döhle-like bodies) upon conventional staining of blood slides.[5–7] The majority of *MYH9*-RD patients develop additional non-congenital extra-hematological manifestations: sensorineural hearing loss (SNHL), presenile cataract, proteinuric nephropathy, and/or a chronic or intermittent elevation of liver enzymes.[7,8] Each of these non-hematological manifestations can occur alone or variably associated with the other ones.

For many years, patients with *MYH9*-RD were diagnosed as having different disorders, such as May-Hegglin anomaly (MHA, OMIM 155100), Sebastian (SBS, OMIM 605249), Epstein (EPTS: OMIM 153650) or Fechtner syndrome (FTNS: OMIM 153640). After the cloning of *MYH9* and identification of mutations in these patients, it was clear that MHA, SBS, EPTS, and FTNS represented different clinical presentations of the same disease.[7,9–11]

At least 70 different mutations causing *MYH9*-RD have been identified so far.[12,13] Only one *MYH9* alteration, p.R705H, was not associated with *MYH9*-RD but with DFNA17 (OMIM 603622), an autosomal dominant SNHL described in two large unrelated pedigrees.[14,15] DFNA17 was reported as a non-syndromic form of SNHL, without any of the other features associated with *MYH9*-RD. Therefore, the current nosography of disorders caused by *MYH9* mutations consists of two entities, the non-syndromic DFNA17 hearing loss because of p.R705H and the syndromic *MYH9*-RD caused by all the other mutations identified in *MYH9*.

We studied four individuals from two unrelated families who also carried the p.R705H substitution. After a comprehensive clinical characterization, they had not only SNHL but also the hematological defects and elevation of liver enzymes typical of *MYH9*-RD, suggesting that DFNA17 should not be considered a separate entity.

PATIENTS AND METHODS

Patients

All the patients or their legal guardians gave written informed consent for this investigation, which was performed according to the Declaration of Helsinki.

Family 1

The proband (III-1; Fig. 1a) was a male suffering from progressive SNHL since the age of 3 years, who became a candidate for cochlear implantation at the age of 12. Both his 36 year old mother (II-2) and 62 year old grandmother (I-2) had received a cochlear implant for progressive SNHL, which was associated with chronic thrombocytopenia. Moreover, individual II-2 had received a clinical diagnosis of FTNS syndrome for the finding of Döhle-like bodies at examination of a bone marrow aspirate.

Family 2

The proband (II-1; Fig. 1a) was a 13 year old female referred for evaluation of congenital thrombocytopenia. *MYH9*-RD was suspected for the association of giant platelets with SNHL. There was no family history with clinical features of *MYH9*-RD.

Mutational screening and phenotype studies

Mutational screening of *MYH9* was carried out as previously described.[6] The effect of the missense variations was evaluated using four pathogenicity prediction programs, such as PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), Mutation Taster (http://www.mutationtaster.org/) Mutation Assessor (http://mutationassessor.org/), and SIFT (http://sift.jcvi.org).

The methods used for investigation of the patients' phenotypes are summarized in the notes of Table 1 and in Figures S1 and S2, Supporting Information.

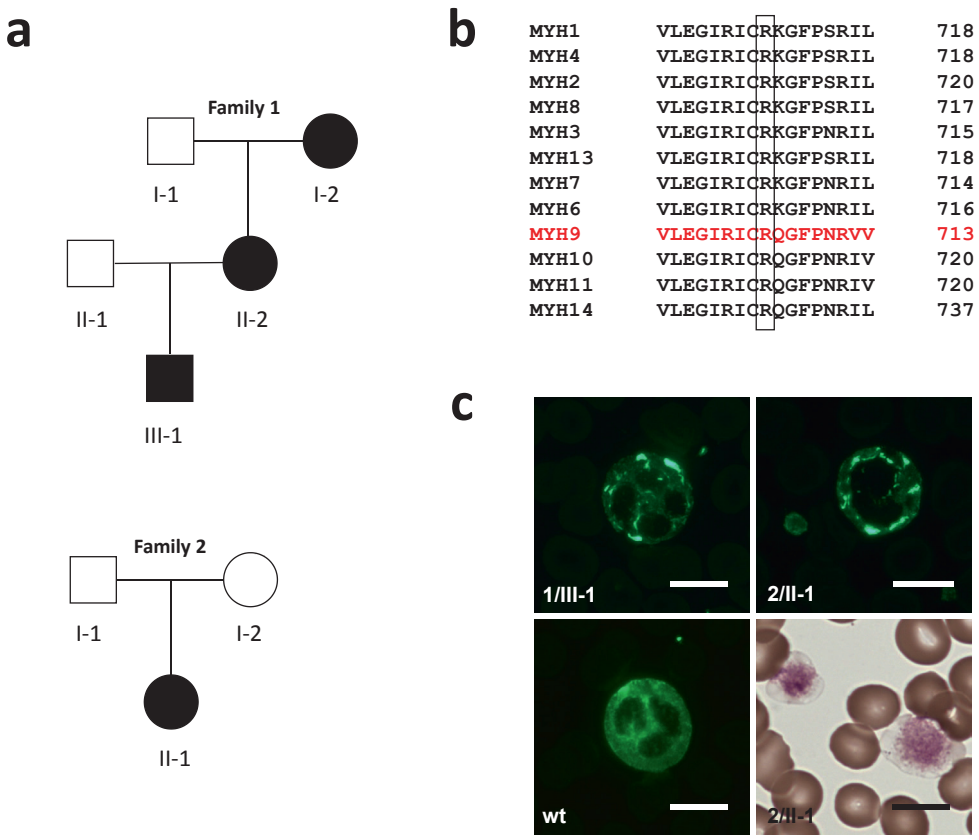

Figure 1. Identification of the p.Arg705His mutation in families 1 and 2. (a) Family pedigrees with all the affected individuals carrying a heterozygous c.2114G > A (p.R705H) mutation in exon 17 of *MYH9*. (b) Alignment of all human muscle and non-muscle myosins of class II with the conserved 705 residue boxed. MYH1 (NM_005963), MYH4 (NM_017533), MYH2 (NM_017534), MYH8 (NM_002472), MYH3 (NM_002470), MYH13 (NM_003802), MYH7 (MN_000257), MYH6 (NM_002471), MYH9 (AB191263), MYH10 (NM_005964), MYH11 (NM_002474), and MYH14 (AY165122). (c) Hematological phenotypes in probands of families 1 and 2. In upper panels, immunofluorescence staining for NMMHC-IIA in individuals 1/III-1 (family 1) and 2/II-1 (family 2). Immunofluorescence analysis of a granulocyte from a healthy individual (wild-type (wt)) is also shown for comparison. In the bottom right panel, representative example of platelet macrocytosis of proband 2/II-1 (family 2). At examination of blood smears stained by May-Grünwald-Giemsa, platelets appear exceedingly large, with some platelets even larger than red blood cells. Scale bars correspond to 10 µm.

Table 1. Summary of the results of phenotype investigation of four patients carrying the p.R705H mutations of NMMHC-IIA.

Family/ patient	Age/ gender	Platelet count[a] (x10^9/L)	MPD[b] (µm)	Spontaneous bleeding	NMMHC-IIA leukocyte inclusions[c]	Döhle-like basophilic inclusions[d]	Sensorineural hearing loss[e]	Proteinuria/ kidney failure[f]	Cataract	Liver enzymes elevation[g]
1/III-1	12/M	96	4.0	None	Yes	Yes	Yes	No / No	No	Yes
1/II-2	36/F	115	4.1	Easy bruising	Yes	Yes	Yes	No / No	No	Yes
1/I-2	62/F	142	3.8	Easy bruising	Yes	Yes	Yes	No / No	No	Yes
2/II-1	13/F	107	4.3	None	Yes	No	Yes	No / No	No	Yes

[a]Normal range of platelet count: 150-350 x 10^9/L.
[b]MPD (mean platelet diameter) calculated by software-assisted image analysis on blood slides stained by May-Grünwald-Giemsa (MGG) as previously described.[19] The MPD median (25th-75th percentiles) obtained in a series of 17 consecutive *MYH9*-RD patients and in 50 consecutive healthy individuals is 4.2 (3.8-4.5) and 2.4 (2.2-2.6), respectively.[16]
[c]According to immunofluorescence analysis of NMMHC-IIA (non-muscle myosin heavy chain IIA) performed as previously reported.[6]
[d]According to examination of blood smears stained by MGG.
[e]Pure-tone audiograms were obtained according to standard clinical practice (ISO-389).
[f]Kidney involvement was evaluated by measurement of the protein/creatinine ratio on morning urine samples and by calculation of estimated glomerular filtration rate using the CKD-EPI creatinine equation[20], on at least two different occasions.
[g]Liver involvement was assigned by ratios of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT) levels with respect to the upper normal limits (UNL) of the laboratories where the analyses were carried out.[8]

RESULTS

Identification of the p.R705H mutation

In the probands of both families, mutational screening of the *MYH9* gene identified a heterozygous c.2114G > A mutation in exon 17. The substitution changes an arginine (R) to a histidine (H) at position 705 (p.R705H), a residue located in the head domain of NMMHC-IIA. The same mutation was identified in members I-2 and II-2 of family 1 (Fig. 1a). The parents of the proband of family 2 did not carry c.2114G > A, showing that the substitution occurred as a *de novo* mutation. The amino acid alignment of the 13 human myosin heavy chains of class II showed conservation of arginine 705 in all the proteins (Fig. 1b). Arginine 705 is also conserved among orthologs (Canis lupus familiaris, Bos Taurus, Mus musculus, Rattus norvegicus, Gallus gallus, and Danio rerio; at http://www.ncbi.nlm.nih.gov/homologene; data not shown), suggesting that it exerts a fundamental role in structure and function of the class II myosins. Consistent with conservation data, the bioinformatics tools assigned a pathogenetic score to the mutation (data not shown).

Individuals carrying p.R705H have the congenital hematological features of MYH9-RD

The individuals with the p.R705H substitution presented the complete typical hematological picture of *MYH9*-RD (Table 1). The immunofluorescence study identified the pathognomonic NMMHC-IIA inclusions in granulocytes in all the four affected individuals but not in their healthy relatives (Fig. 1c). In the three affected subjects of family 1, the inclusions were also evident as basophilic Döhle-like bodies after conventional staining (data not shown). The four patients also had mild thrombocytopenia and marked platelet macrocytosis with giant platelets (Table 1 and Fig. 1c). The degree of platelet macrocytosis was similar to that reported in a cohort of 17 consecutive *MYH9*-RD patients.[16]

Non-congenital extra-hematological features of MYH9-RD

All the affected individuals had SNHL (Table 1). The patients from family 1 underwent several serial audiometric examinations prior to cochlear implantation, allowing us to characterize the progression of their hearing defect in more detail (Fig. S1). The self-reported ages at onset of hearing loss were 3, 4, and 19 years for individuals III-1, II-2 and I-2, respectively. Hearing defect was fairly symmetrical, starting in the high frequencies and rapidly deteriorating with age and eventually resulting in profound deafness affecting all frequencies. In individual III-1 the middle and high frequencies were already nearly equally affected at relatively young age. At presentation in our clinic (24 years old), individual I-2 already had severe SNHL (binaural mean AC Pure Tone Average at 0.5, 1, 2, and 4 kHz > 70 dB). The individuals II-2 and III-1 developed severe deafness at the age of 19 and 8 years, respectively. All the affected individuals of family 1 showed significant progression at all frequencies, except for I-2 at 4

and 8 kHz (Fig. S2); hearing level at 4 kHz was already at 130 dB since the age of 26 years and hearing level at 8 kHz was already 130 dB since the first audiometric measurement in our clinic at the age of 24 years.

All four affected individuals did not present any signs of kidney damage or cataract (Table 1). Instead, they had mild or moderate elevations of liver alanine aminotransferase (ALT), aspartate aminotransferase (AST) or gamma-glutamyltransferase (GGT), which could not be explained by any concurrent other possible causes of liver damage. The proband of family 2 had ALT 2.2-fold more elevated than the upper normal limit (UNL), whereas AST and GGT were normal. In family 1, individuals I-2 and III-1 had slightly elevated ALT (1.2 and 1.15-fold the UNL, respectively). Individual II-2 only showed elevated GGT of 1.6-fold the UNL. Consistent with the other phenotypic features, the liver enzyme abnormalities were interpreted as part of the *MYH9*-RD syndrome.[8,17]

DISCUSSION

Before the cloning of the disease-causing gene, patients with *MYH9*-RD were diagnosed as having MHA, SBS, EPTS or FTNS, four distinct disorders characterized by associations of the different manifestations of *MYH9*-RD, including macrothrombocytopenia, Döhle-like bodies in granulocytes, SNHL, cataract, and glomerulonephritis.[7] MHA and SBS were hematologic diseases with platelet and leukocytes defects. In addition to macrothrombocytopenia, patients with EPTS had the hearing and kidney anomalies (but not Döhle-like bodies and cataract), whereas those with FTNS had the most severe phenotypes with all the features of *MYH9*-RD associated.

However, as patients with *MYH9* mutations were identified, MHA, SBS, EPTS and FTNS lost their distinctive peculiarities.[9–11] First of all, finding that Döhle-like bodies were aggregates of NMMHC-IIA allowed the recognition of the granulocyte inclusions in all *MYH9*-RD individuals.[18] Independently of their size, they are always detectable using immunofluorescence analysis on peripheral blood smears.[5,7] Moreover, platelet macrocytosis and thrombocytopenia are also present in all the affected individuals, although there are rare exceptions of platelet counts at the lower limit of the normal range.[12] Moreover, individuals with *MYH9* mutations, including those previously classified as having MHA or SBS, are at risk of developing SNHL, kidney damage, and/or cataract during life. Finally, studies of large cohorts allowed us to further extend the phenotypic spectrum of *MYH9*-RD finding that more than 50% of affected individuals have elevated liver enzymes.[8,17]

DFNA17 remained the last distinct clinical entity because of *MYH9* mutations. Reported in two families affected by an apparently non-syndromic form of progressive SNHL, DFNA17 was specifically associated with the p.R705H mutation.[14,15] To the best of our knowledge, it

was not reported whether the affected individuals had defective platelet count and size, or bleeding tendency despite they underwent cochlear implantation.

On the contrary, the patients in this study had thrombocytopenia and large platelets, alterations that associated with SNHL led to a diagnostic suspicion of *MYH9*-RD. The diagnosis was confirmed by finding the NMMHC-IIA aggregates in their granulocytes and identifying the same *MYH9* mutation as that described in the DFNA17 families.[14,15] Consistent with the phenotypic spectrum of *MYH9*-RD, our patients also had mild to moderate elevation of some liver enzymes. Of note, the p.R705H mutation was also found to be associated with macrothrombocytopenia, granulocyte inclusions, and SNHL in two patients from the French *MYH9* network.[13]

These data indicate that patients with the p.R705H mutation have the syndromic phenotype of *MYH9*-RD. Indeed, the association of macrothrombocytopenia with SNHL, as the only extra-hematological feature, is one of the most frequent presentations of *MYH9*-RD, being reported in 18% of patients at a mean age at evaluation of 35 years.[12] Therefore, we conclude that there is no significant evidence to consider DFNA17 as a separate nosological entity from *MYH9*-RD.

ACKNOWLEDGEMENTS

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SUPPORTING INFORMATION

Material and Methods

Hearing evaluation. Pure-tone audiograms were obtained according to standard clinical practice (ISO-389). Air conduction (AC) and bone conduction (BC) thresholds were measured at 0.25, 0.5, 1, 2, 4 and 8 kHz. The binaural mean AC threshold at a given frequency was included in the data analysis only if the mean air-bone gap (ABG) averaged for 0.5-2 kHz was 15 dB or less, and if the thresholds were fairly symmetric. A difference between left and right ear AC of >25 dB for at least 3 consecutive frequencies was labeled as asymmetric. Binaural mean AC thresholds (decibel hearing level, dB HL) were plotted against age for each frequency for each family member. A commercial program (Graph Pad Prism version 3.0, 1999) was used for linear regression analyses.

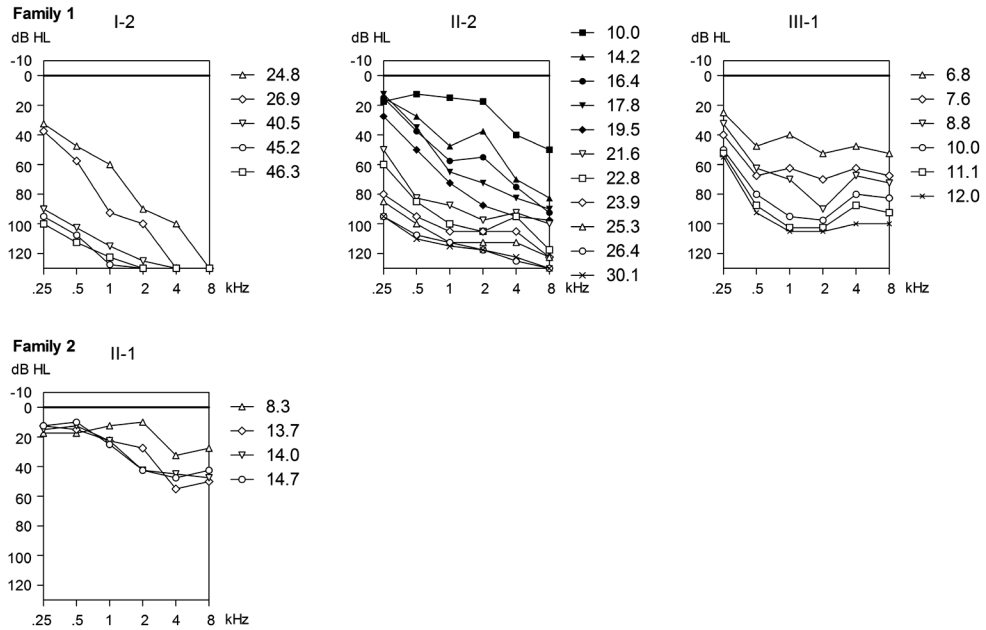

Figure S1. Longitudinal binaural mean air conduction threshold data of the 4 affected individuals of family 1 and 2. Age in years is shown by symbols next to each graph. For the clarity of the figure not all audiometric measurements are shown.

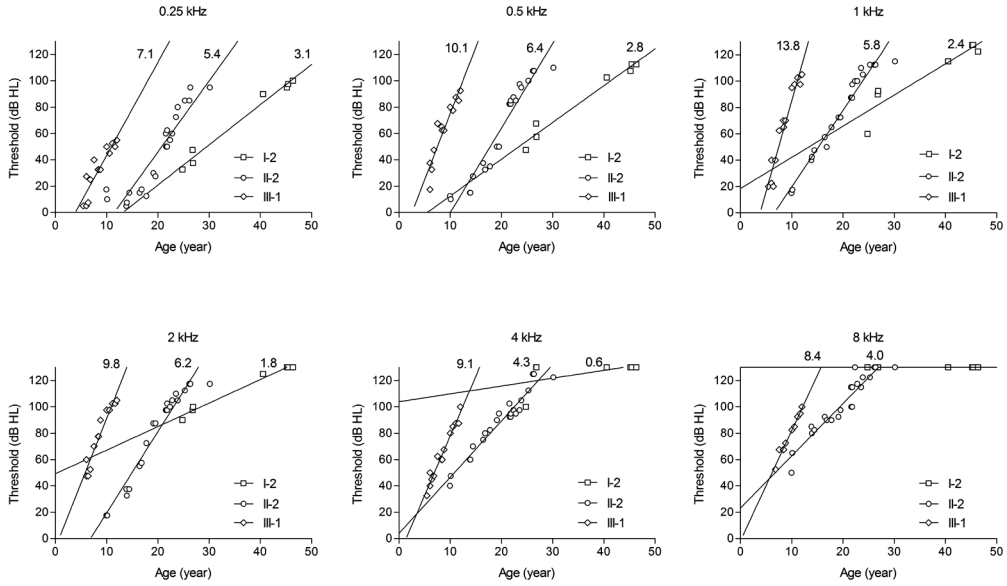

Figure S2. Longitudinal individual measurement for the 3 affected individuals (I-2, II-2, and III-1) of family 1, with individual linear regression lines (solid lines) are shown for each frequency separately. The annual threshold deterioration (ATD, in decibels per year) is shown above each individual linear regression line.

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Chapter 5

Non-muscle myosin heavy chain IIA mutation predicts severity and progression of sensorineural hearing loss in patients with *MYH9*-related disease

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ABSTRACT

Objectives: *MYH9*-related disease (*MYH9*-RD) is an autosomal dominant disorder deriving from mutations in *MYH9*, the gene for the non-muscle myosin heavy chain IIA (NMMHC-IIA). *MYH9*-RD has a complex phenotype including congenital features, such as thrombocytopenia, and non-congenital manifestations, namely sensorineural hearing loss (SNHL), nephropathy, cataract, and liver abnormalities. The disease is caused by a limited number of mutations affecting different regions of the NMMHC-IIA protein. SNHL is the most frequent non-congenital manifestation of *MYH9*-RD. However, only scarce and anecdotal information is currently available about the clinical and audiometric features of SNHL in *MYH9*-RD subjects. The objective of this study was to investigate the severity and propensity for progression of SNHL in a large series of *MYH9*-RD patients in relation to the causative NMMHC-IIA mutations.

Design: This study included the consecutive patients diagnosed with *MYH9*-RD between July 2007 and March 2012 at 4 participating institutions. A total of 115 audiograms were analyzed from 63 patients belonging to 45 unrelated families with different NMMHC-IIA mutations. Cross-sectional analyses of audiograms were performed. Regression analysis was performed, and age-related typical audiograms (ARTA) were derived to characterize the type of SNHL associated with different mutations.

Results: Severity of SNHL appeared to depend on the specific NMMHC-IIA mutation. Patients carrying substitutions at the residue R702 located in the short functional SH1 helix had the most severe degree of SNHL, whereas patients with the p.E1841K substitution in the coiled-coil region or mutations at the non-helical tailpiece presented a mild degree of SNHL even at advanced age. The authors also disclosed the effects of different amino acid changes at the same residue: for instance, individuals with the p.R702C mutation had more severe SNHL than those with the p.R702H mutation, and the p.R1165L substitution was associated with a higher degree of hearing loss than the p.R1165C. In general, mild SNHL was associated with a fairly flat audiogram configuration, whereas severe SNHL correlated with downsloping configurations. ARTA plots showed that the most progressive type of SNHL was associated with the p.R702C, p.R702H, and the p.R1165L substitutions, whereas the p.R1165C mutation correlated with a milder, nonprogressive type of SNHL than the p.R1165L. ARTA for the p.E1841K mutation demonstrated a mild degree of SNHL with only mild progression, whereas the ARTA for the mutations at the non-helical tailpiece did not show any substantial progression.

Conclusions: These data provide useful tools to predict the progression and the expected degree of severity of SNHL in individual *MYH9*-RD patients, which is especially relevant in young patients. Consequences in clinical practice are important not only for appropriate patient counseling but also for development of customized, genotype-driven clinical management. The authors recently reported that cochlear implantation has a good outcome in *MYH9*-RD patients; thus, stricter follow-up and earlier intervention are recommended for patients with unfavorable genotypes.

INTRODUCTION

MYH9-related disease (*MYH9*-RD) is a rare autosomal dominant syndromic disorder deriving from mutations in *MYH9*, the gene for the non-muscle myosin heavy chain IIA (NMMHC-IIA).[1–3] Patients with *MYH9*-RD present with macrothrombocytopenia and characteristic inclusions of the mutant protein in leukocytes since birth. The degree of thrombocytopenia and bleeding tendency is variable among different subjects. During infancy or adult life, most of them develop additional non-congenital manifestations, namely sensorineural hearing loss (SNHL), nephropathy, presenile cataract, and/or alterations of liver enzymes.[3,4] *MYH9*-RD encompasses four syndromes that have been considered for many years as distinct disorders, May-Hegglin anomaly (MIM 155100), Sebastian syndrome (MIM 605249), Fechtner syndrome (MIM 153640), and Epstein syndrome (MIM 153650). After the identification of *MYH9* as the gene responsible for all of these disorders, analyses of large series of patients demonstrated that May-Hegglin anomaly, Sebastian, Fechtner, and Epstein syndrome actually represented some of the different possible clinical presentations of the same condition, for which the definition of *MYH9*-RD has been introduced.[5–7] The estimated prevalence of *MYH9*-RD is around 1/400,000 although the actual prevalence is expected to be higher since this disorder is still underdiagnosed.[8] To date, more than 300 *MYH9*-RD pedigrees have been reported worldwide.[3,9,10]

SNHL is the most frequent non-congenital manifestation of *MYH9*-RD: a recent study showed that SNHL was present in about 50% of patients at a mean evaluation age of 35 years and was expected to develop over time in almost all cases.[9] Sporadic observations on small case series suggest a high degree of variability in the severity and propensity for progression of the SNHL among different *MYH9*-RD patients. In fact, some subjects presented with a progressive SNHL leading to profound deafness before the age of 40, whereas other individuals had comparatively stable disease with mild SNHL even at an advanced age.[11,12]

Non-muscle myosin-IIA is a cytoplasmic myosin participating in several processes requiring generation of chemomechanical forces by the cytoskeleton, such as cell motility, cytokinesis, and maintenance of cell shape.[13] It is expressed in most cell types and tissues, including some structures of the inner ear, such as the organ of Corti, spiral ligament, and spiral limbus.[14,15]

As all conventional myosins, myosin IIA presents a hexameric structure formed by a heavy chain (NMMHC-IIA) dimer and two pairs of light chains. Each NMMHC-IIA molecule comprises two distinct domains: the amino-terminal head domain and the carboxy-terminal tail domain.[16] The head domain has a globular three-dimensional structure and contains the highly conserved functional regions essential for actin binding, ATPase activity, and generation of the chemomechanical forces.[17,18] The tail domain comprises a long alpha-helical coiled-coil and a short carboxy-terminal non-helical tailpiece (NHT) (Fig. 1A).[16] The coiled-coil is responsible for formation of heavy chain dimers and myosin assembly in functional filaments, whereas the NHT is a phosphorylation site with regulatory functions.[16,19]

MYH9-RD is caused by a limited spectrum of *MYH9* mutations that affect different regions of the NMMHC-IIA protein.[3] Mutations affect either the head or the tail domain. Almost all mutations in the head domain are point substitutions hitting one of two specific regions of the globular head: a distinct hydrophobic interface between the SH3-like motif and the motor domain (SH3/MD interface) or the arginine at residue 702 (R702) located in the short functional SH1 helix (Fig. 1A).[9,20] Mutations in the tail domain affect either the coiled-coil or the NHT. The coiled-coil mutations mainly consist of point substitutions, even if in rare pedigrees short in-frame deletions or duplications have been identified. Importantly, three coiled-coil residues are most frequently affected: R1165, D1424 and E1841 (Fig. 1A). Finally, all the mutations affecting the NHT are nonsense or frameshift alterations of the last exon (exon 41), resulting in deletion of a variable fragment (13-37 residues) of the NHT.[3] Previous genotype-phenotype studies showed that patients with mutations in the head domain have a higher risk of developing non-congenital manifestations and more severe thrombocytopenia with respect to patients with mutations affecting the tail domain.[21] A more recent investigation also showed that substitutions in the SH1 helix of the head domain were associated with the highest risk of having SNHL and nephropathy, whereas the deletions at the NHT correlated with a significantly lower risk compared to mutations in the other NMMHC-IIA regions.[9]

Given that SNHL is very frequent among *MYH9*-RD patients, an otologist and audiologist are usually involved in clinical management of these patients. However, very scarce and anecdotal data are currently available about the clinical and audiometric features of SNHL in *MYH9*-RD subjects, including descriptions of the severity and rate of progression. To fill this gap, we investigated these features in a large series of *MYH9*-RD patients in relation to their NMMHC-IIA mutations. Age-related typical audiograms (ARTA) were constructed to characterize the progression of SNHL associated with different genotypes. These data provide essential tools to predict the evolution of the SNHL in individual *MYH9*-RD subjects and therefore contribute to the opportunity to offer more personalized patient care.

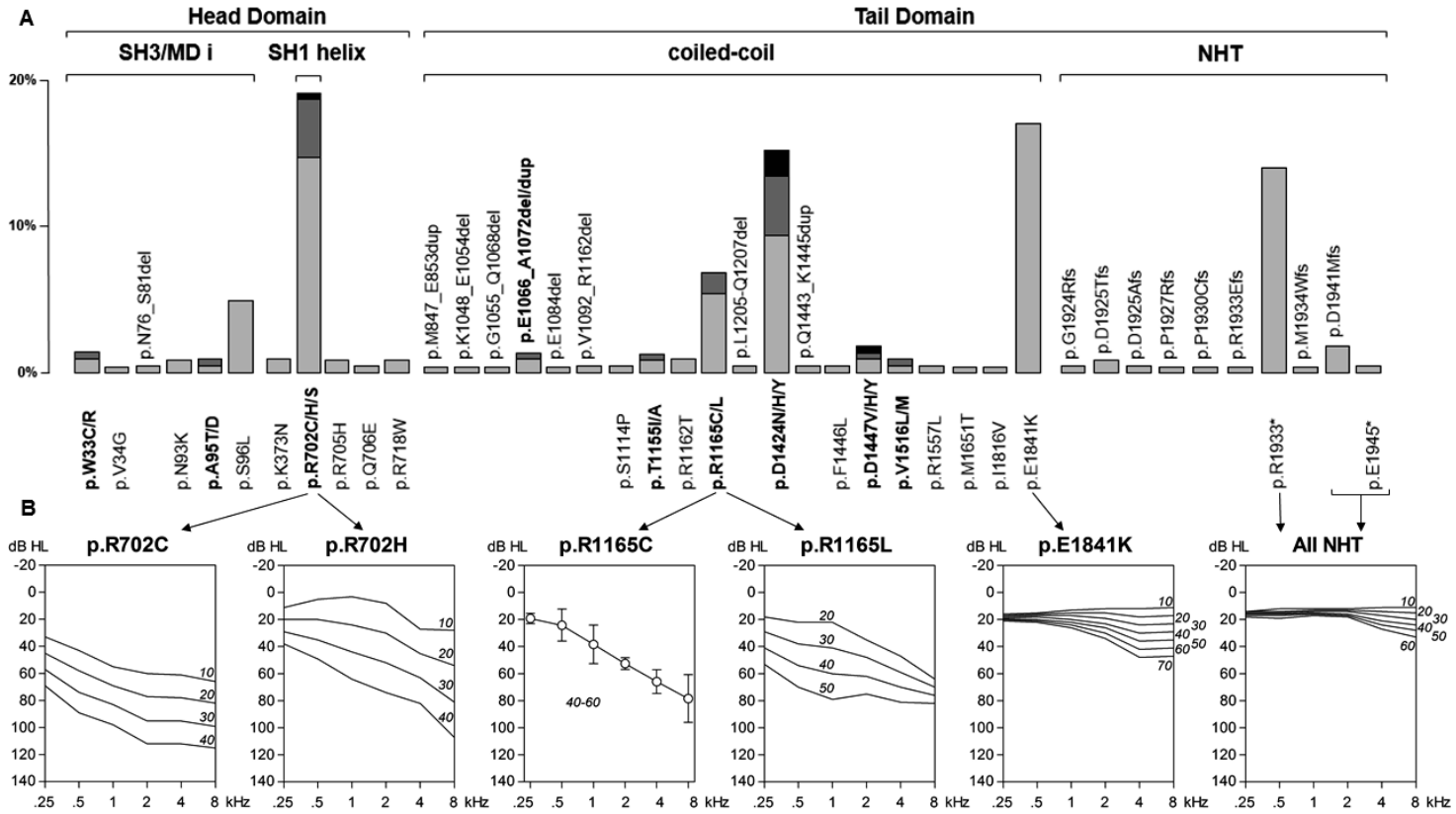

Figure 1. Spectrum of non-muscle myosin heavy chain-IIA (NMMHC-IIA) mutations identified in the *MYH9*-related disease pedigrees so far.
A. NMMHC-IIA mutations are shown together with their relative frequency, which is indicated by the bars' height, and their localization along the different domains (head or tail domain) and regions (SH3-like motif/motor domain interface [SH3/MD i], SH1 helix, coiled-coil, and non-helical tailpiece [NHT]) of the NMMHC-IIA protein. Missense and nonsense mutations are represented below the bars, while in frame deletions or duplications, and frameshift alterations are represented above the bars. The residues indicated in bold are those hit by more than one mutation. Scale on the Y axis indicates the relative frequency of the different mutations in the *MYH9*-related disease pedigrees reported so far.
B. Age-related typical audiograms (ARTA) for some selected NMMHC-IIA mutations correlated to the schematic representation of the NMMHC-IIA protein. Audiograms at different ages (italics) in decade steps. The p.R1165C panel shows the mean ± 1SD for threshold in absence of age-dependent progression.

MATERIALS ANDS METHODS

Patients

This study included all the consecutive patients diagnosed with *MYH9*-RD between July 2007 and March 2012 at 4 different participating institutions for whom audiograms were available. All the audiograms available for each patient were collected and retrospectively analyzed. Audiometric examinations were performed between June 1992 and July 2013. In all cases, the diagnosis was based on the immunofluorescence assay for the identification of pathognomonic NMMHC-IIA leukocyte aggregates and on the identification of the causative mutation by molecular screening of *MYH9*.[22] Both analyses were centralized (Department of Internal Medicine, IRCCS Policlinico San Matteo Foundation, Pavia, Italy, and Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Trieste, Italy, respectively) and were carried out as previously described.[22] All the patients and mutations have been previously reported.[9] All patients or their legal guardians provided written informed consent. The investigation was approved by the Institutional Review Board of the IRCCS Policlinico San Matteo Foundation.

Audiometric examination

Pure-tone audiometry was performed at each referring institution according to current clinical standards to determine hearing thresholds at least at six frequencies (0.25, 0.5, 1, 2, 4, and 8 kHz). The binaural mean air conduction (AC) threshold at any given audio frequency was included in the data analysis only if the mean air-bone gap (ABG) averaged for 0.5 to 2 kHz was 15 dB or less and if the thresholds were fairly symmetric. A difference between left and right ear AC threshold of > 20 dB for at least three consecutive frequencies was labeled as asymmetric SNHL. When an ear was excluded from analysis because of an intolerable asymmetry or an ABG in excess of 15 dB, pure-tone thresholds from the better ear was used instead of the binaural mean threshold.

Statistical testing for hearing impairment beyond ISO 7029 estimates of presbyacusis

Hearing is known to deteriorate with age (presbyacusis). Hearing impairment develops more rapidly at high than at low frequencies, and men are more severely affected than women. However, the effect of age-related hearing impairment varies considerably among individuals. Furthermore, a decrease in hearing ability may not necessarily be caused by aging itself but may also be influenced by environmental as well as genetic factors. The ISO 7029 (International Organization for Standardization, ISO 7029 1984) established the expected pure-tone hearing thresholds distributions of otologically normal subjects aged between 18 and 70 years who have not been exposed to known risk factors for hearing impairment. Apart from median (P50) values by age and gender, ISO 7029 also specifies several percentiles: P75, P90, and P95, or the percentiles 75, 90, and 95, that is, the 75%, 90%, and 95% tail

probabilities, respectively, for the sample distribution at a given age to characterize the variability in hearing impairment that results from presbyacusis. These data can be used to estimate the amount of hearing impairment caused by a specific agent or genetic factor in a population. In this study of patients with *MYH9*-RD, pure-tone thresholds were compared to the age- and gender-related distributions as defined by the ISO 7029 standard. Thus, for each frequency, the threshold can be expressed as beyond the P95, P90, P75, or P50 of the age- and gender-dependent control curve of the general population.

For each ear, four tests were performed to determine whether there was significant hearing impairment beyond presbyacusis and if it was beyond the P95, P90, P75, or P50 of ISO 7029 estimates of presbyacusis (ISO 7029). Hearing impairment in a given ear was considered to represent impairment beyond estimates of presbyacusis if two of six (2/6) (or more) audiometric frequencies had a threshold beyond the P95, three of six (or more) beyond the P90, four of six (or more) beyond the P75, or six of six beyond the P50 of ISO 7029 estimates of presbyacusis. When the P95 criterion was fulfilled, hearing impairment in that ear was considered to be present beyond the P95 level of presbyacusis. When the P95 test failed but the P90 test was positive, hearing impairment was considered to be present beyond the P90 level of presbyacusis, etc. When the P50 test failed, it was concluded that there was no significant hearing impairment beyond presbyacusis.

Regression analysis and ARTA

Binaural mean AC thresholds (dB HL), obtained from the last-visit audiogram of each patient, were plotted against age for each frequency. A commercial program, Prism version 3.0 (Graph-Pad, San Diego, CA), was used for linear regression analyses (threshold on age). The regression coefficient, that is, the slope, was called annual threshold deterioration (ATD, in decibels per year). We evaluated the significance of hearing loss progression at each frequency; this required that the 95% confidence interval for the regression coefficient, or the ATD, did not include zero. Hearing impairment was labeled as progressive when two or more of six frequencies showed significant progression ($p = 0.0088$ according to the binomial distribution with N = 6, $p = 0.025$, $q = 0.075$).

Based on the regression analysis, ARTA were constructed, which show the predicted thresholds for a number of decade steps in age.[23] Results of the linear regression analysis of the group cross-sectional last-visit data were compared with those of the linear regressions of the available individual longitudinal thresholds from serial audiograms to ensure that there was general agreement.

RESULTS

Patients

We obtained 123 audiometric examinations of 65 patients with *MYH9*-RD belonging to 47 unrelated families. Because of asymmetric hearing loss (11 ears), ABG (17 ears), both asymmetric hearing loss and ABG (2 ears), or incomplete hearing assessments (6 ears), data from one ear (n = 20) and both ears (n = 8) were excluded. Therefore, our data analysis included 115 audiometric examinations of 63 patients from 45 families. There were 25 males and 38 females with a mean age at the time of the last audiometric examination of 33.8 years (range, 4 to 72). Table 1 details the genotypic features of the investigated subjects.

Table 1. Genotypic features of 63 investigated patients with *MYH9*-related disease.

***MYH9* exon**	***MYH9* mutation***	**NMMHC-IIA domain**	**NMMHC-IIA region**	**NMMHC-IIA mutation**	**No. patients (No. families)**	**No. audiograms**
2	c.101T>G	HD	SH3/MD i	p.V34G	1 (1)	1
2	c.284C>A	HD	SH3/MD i	p.A95D	2 (1)	5
2	c.287C>T	HD	SH3/MD i	p.S96L	2 (1)	2
17	c.2104C>T	HD	SH1 helix	p.R702C	7 (7)	20
17	c.2105G>A	HD	SH1 helix	p.R702H	6 (5)	11
21	c.2539_2559dup	TD	coiled-coil	p.M847_E853dup	2 (1)	4
25	c.3195_3215dup	TD	coiled-coil	p.E1066_A1072dup	2 (2)	3
25	c.3195_3215del	TD	coiled-coil	p.E1066_A1072del	2 (2)	5
26	c.3464C>T	TD	coiled-coil	p.T1155I	1 (1)	1
27	c.3493C>T	TD	coiled-coil	p.R1165C	3 (2)	8
27	c.3494G>T	TD	coiled-coil	p.R1165L	4 (2)	9
31	c.4270G>A	TD	coiled-coil	p.D1424N	3 (2)	8
31	c.4270G>C	TD	coiled-coil	p.D1424H	2 (2)	8
31	c.4270G>T	TD	coiled-coil	p.D1424Y	2 (2)	5
31	c.4340A>T	TD	coiled-coil	p.D1447V	3 (2)	4
32	c.4546G>A	TD	coiled-coil	p.V1516M	1 (1)	1
33	c.4670G>T	TD	coiled-coil	p.R1557L	1 (1)	1
39	c.5521G>A	TD	coiled-coil	p.E1841K	5(3)	13
41	c.5797C>T	TD	NHT	p.R1933*	9 (5)	9
41	c.5821delG	TD	NHT	p.D1941Mfs*7	2 (1)	2
41	c.5833G>T	TD	NHT	p.E1945*	3 (1)	3
Total					63 (45)	115

***** Nucleotide numbering reflects the *MYH9* complementary DNA with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (RefSeq NM_002473.4). The initiation codon is codon 1.

HD, head domain; TD, tail domain; SH3/MD i, SH3-like motif/motor domain interface; NHT, non-helical tailpiece.

Cross-sectional analyses of audiograms

The obtained audiograms are shown in Figure 2. Each panel of Figure 2 combines the last-visit audiograms (binaural mean AC threshold in dB HL) for the subjects specified in the panel title. Patients were grouped in each panel according to the specific NMMHC-IIA mutation, and panels were clustered according to the NMMHC-IIA region affected by mutation (SH3/MD interface, SH1 helix, coiled-coil, or NHT, see Table 1). For example, the bottom right panel shows these audiograms obtained from patients 14, 15, and 52, all carrying the p.E1945* mutation located in the NHT at the ages of 30.07, 34.38 and 56.75 years, respectively.

Hearing impairment beyond ISO 7029 standards for age-related hearing loss

All the investigated patients showed significant hearing impairment beyond the P95, P90, P75, or P50 estimates of age-related hearing loss (ISO 7029), with the single exception of patient 12, aged 56.68 years, who carried the p.E1841K mutation in the coiled-coil.

Variability in level of hearing impairment within subgroups

Much of the variability in the severity of SNHL shown in Figure 2 seemed to depend on age. However, there were some notable exceptions. Among the patients 30, 45, and 46 with the p.D1447V mutation in the coiled-coil, the youngest patient 46 had the poorest threshold at relatively young age (35.45 years), whereas patient 45 had the best threshold at the highest age (66.65 year). Patient 61 with the p.R702C mutation in the SH1 helix had severe SNHL by the age of 13.20 years (Fig. 2). For the cross-sectional analysis discussed below, we evaluated whether this measurement might be interpreted as an outlier, but it appeared that this measurement fitted within the 95% and 90% prediction bands for the regression line (threshold on age) at each frequency. The two top-right panels in Figure 2 demonstrate that a distinctive feature of the thresholds in the carriers of the p.R702C or p.R702H mutation was the large variability in their degree of SNHL, which could be associated with wide prediction bands for the associated cross-sectional regression lines if a substantial part of this variability was not strictly age-dependent. Indeed, a lack of significant progression found in the cross-sectional analysis of the thresholds in the p.R702C carriers (see below, Fig. 4) was an associated finding.

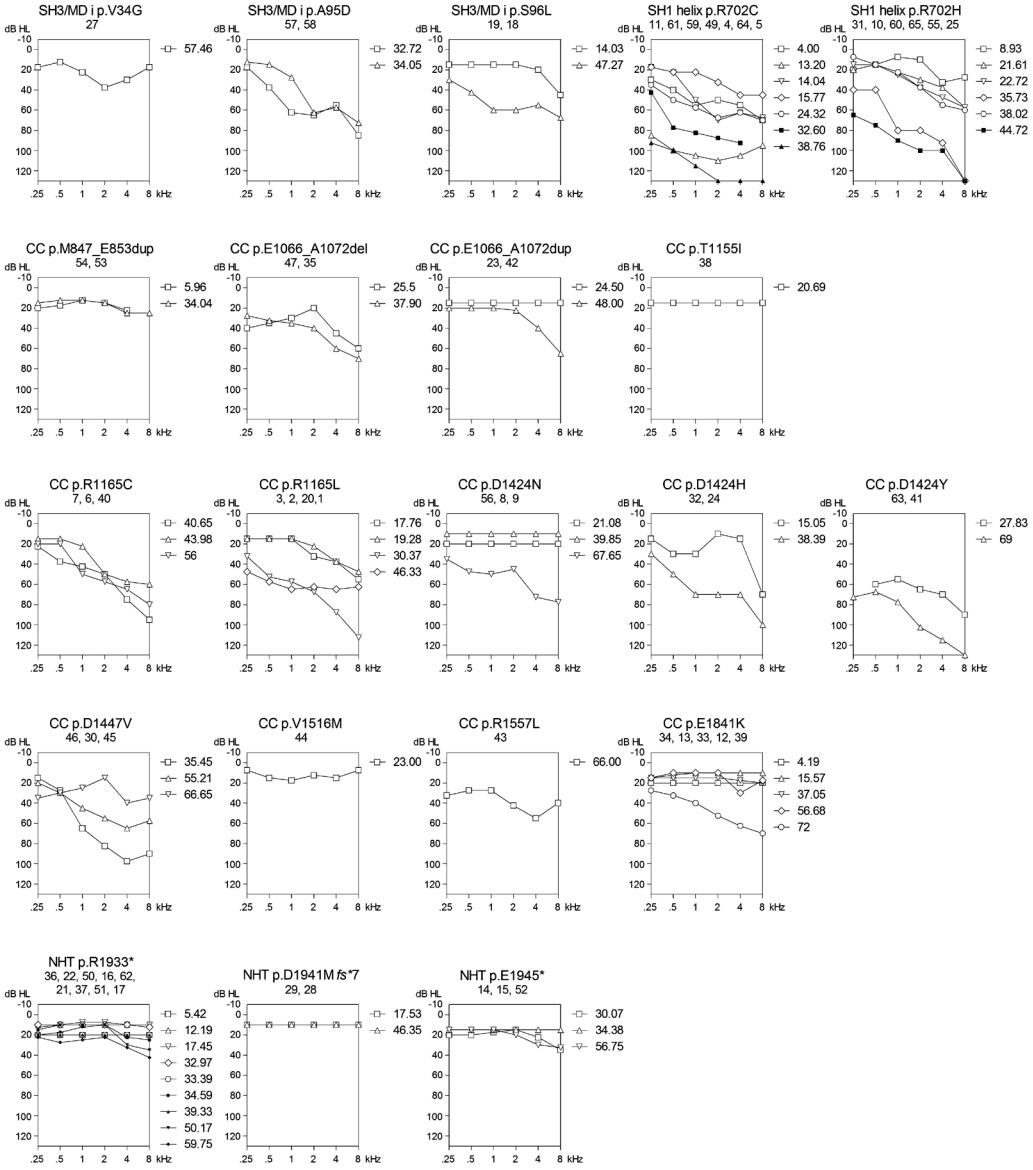

Figure 2. All last-visit audiograms (binaural mean air conduction threshold in dB HL plotted against frequency in kHz) obtained for the various subgroups of patients with different mutations. The panel titles indicate the non-muscle myosin heavy chain II-A (NMMHC-IIA) region, mutation, and the individual ID numbers of the individuals covered. Panels were clustered according to the NMMHC-IIA region affected by mutation (SH3-like motif/motor domain interface [SH3/MD i], SH1 helix, coiled-coil [CC], or non-helical tailpiece [NHT]). The key to each panel specifies the individual's age at the last-visit audiometry (in years) in the same order (top to bottom) as the individual ID numbers (left to right) in the panel title.

Hearing impairment evaluated by progression, cross-sectional analyses

Figure 3A shows all the available threshold data in a mixed single-snapshot plot, that is, individual longitudinal data mixed with cross-sectional last-visit data, for the mutations in the SH1 helix, p.R702C and p.R702H. This plot suggests that hearing in patients with the p.R702C substitution is more severely affected than in those with the p.R702H. In fact, in the plots for 0.5 to 4 kHz (Fig. 3A only shows 1-kHz plot as example), it is possible to divide the plotting area into an (irregularly shaped) upper/left part and a lower/right part in such a way that the upper/left part includes only the seven p.R702C patients and the lower/right part includes only the six p.R702H patients. These counts, when tested in a 2x2 table using Fisher exact test, disclosed significantly different distributions of the data points pertaining to these two different mutations (p = 0.00058, inset in plot). Similarly, the upper/left part in the 8-kHz panel includes the seven p.R702C patients and one p.R702H patient, and the lower/right part includes five p.R702H subjects (data not shown). These counts were also associated with significantly different data point distributions pertaining to these two mutations (p = 0.0045).

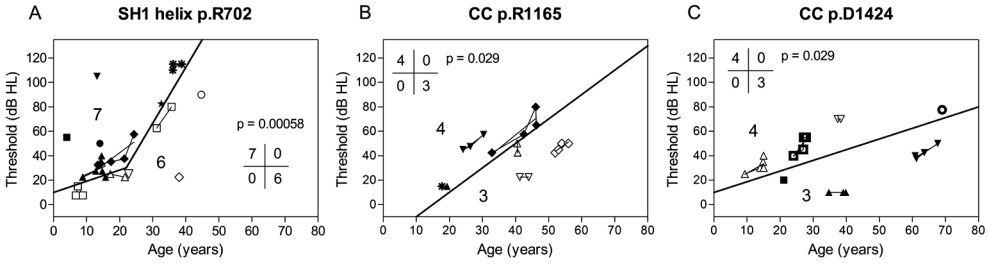

Figure 3. Mixed single-snapshot for the SH1 helix R702 mutation (A), coiled-coil (CC) R1165 mutation (B), and coiled-coil D1424 mutation (C). For each mutation, only the thresholds at 1 kHz are shown as an example. Threshold in dB HL plotted against age in years. In each plot, data points for each individual are shown by a different symbol. Consecutive thresholds for one and the same person are connected by lines. Different mutations are indicated by different types of symbols (solid/open/bold) (see below for symbol keys). Dividing lines are shown with the number of patients counted on each side of the line. The inset shows the corresponding 2 x 2 table and the p value for the associated Fisher exact test. Keys to symbols: (A) solid symbols p.R702C, open symbols p.R702H; (B) solid symbols p.R1165L, open symbols p.R1165C; (C) solid symbols p.D1424N, open symbols p.D1424H, open bold symbols p.D1424Y.

Figure 4 shows the regression analyses of the cross-sectional threshold data for the p.R702C/H mutations. The plots for patients with the p.R702C mutation as well as for those with the p.R702H shown in Figures 3A and 4 are suggestive of progression. However, ATD reached statistical significance only for the patients with the p.R702H mutation at 2 to 8 kHz, by 1.8 to 2.6 dB/year.

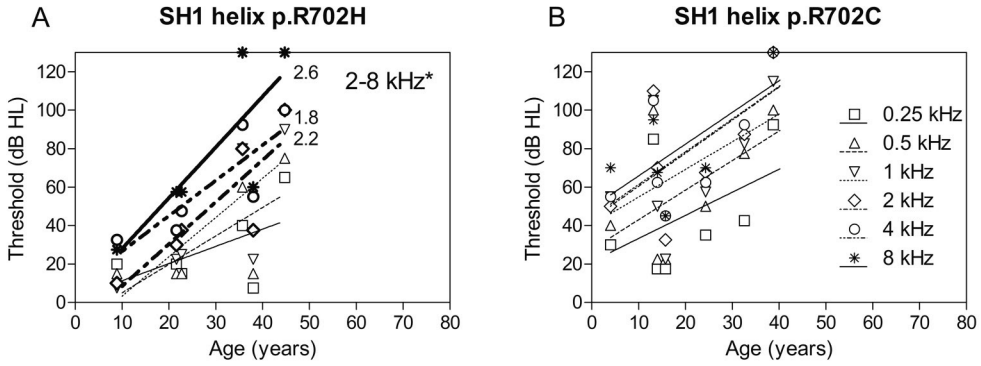

Figure 4. Cross-sectional analysis of pure-tone thresholds of persons with SH1 helix mutations p.R702H (A) and p.R702C (B). Threshold in dB HL plotted against age in years. Annual threshold deterioration (ATD in dB/year) is shown for frequencies with significant progression (*****) as indicated by bold lines and symbols.

We created mixed single-snapshots plots also for the coiled-coil mutations p.R1165C and p.R1165L. At 0.25 to 1 kHz, it was possible to divide the plotting area into an upper/left part (associated with higher thresholds at predominantly younger ages) and a lower/right part (associated with lower thresholds at predominantly more advanced ages) in such a way that the upper/left part includes only the four p.R1165L patients and the lower/right part includes only the three p.R1165C (Fig. 3B only shows 1-kHz plot as example). These counts resulted in a significantly different distribution (p = 0.029), suggesting that SNHL is more severe in patients carrying the p.R1165L mutation than in patients with the p.R1165C. The p.R1165L substitution was associated with significant progression (last-visit data only in cross-sectional analysis) at 0.25 to 1 kHz by 1.2 to 1.8 dB/year (regression data not shown).

Concerning the mutations affecting the D1424 residue, mixed single-snapshot plots for 1 to 8 kHz suggested that the hearing levels in p.D1424Y patients are fairly in line with those in p.D1424H patients, at levels that are worse than found in the p.D1424N patients, possibly by 30 dB or more at any age. In fact, it was possible to divide the plotting area of the panels for 0.5 to 8 kHz (Fig. 3C only shows 1-kHz plot as example) into an upper/left part that includes only the four p.D1424H or p.D1424Y patients and a lower/right part that includes only the three p.D1424N patients. These counts also disclosed significantly different distributions (p = 0.029). SNHL was therefore more severe in patients carrying the p.D1424H or p.D1424Y mutation than in patients carrying the p.D1424N.

Patients with the NHT mutation p.R1933* showed minimal but significant progression at 4 to 8 kHz by as low as 0.4 to 0.5 dB/year; there was no appreciable progression found among carriers of the p.D1941Mfs* or p.E1945* mutations in the NHT (data not shown).

ARTA

The cross-sectional regression analyses pertaining to only a few domains or mutations were suitable for deriving ARTA. The relevant ARTA are shown in Figure 1B correlated to the schematic representation of the NMMHC-IIA protein. Given the findings illustrated in Figure 3A-B, there are separate ARTA for the SH1 helix p.R702C and p.R702H mutations, as well as for the coiled-coil p.R1165C and p.R1165L mutations. As the threshold data for the p.R1165C patients did not suggest progression, an average, that is, age-independent, audiogram is presented for these patients. Finally, the separate data for the mutations in the NHT region could be combined into a single regression analysis (data not shown).

The ARTA plots show a more severe and progressive SNHL associated with the SH1 helix p.R702C and p.R702H mutations and with the coiled-coil mutation p.R1165L, whereas the p.R1165C mutation appeared to be associated with a milder SNHL than the p.R1165L. The ARTA for the coiled-coil p.E1841K substitution demonstrated very mild levels of SNHL with only mild progression, whereas the NHT mutations had minimal progression (see also Fig. 2). In general, relatively mild SNHL was associated with a fairly flat audiogram configuration (p.E1841K mutation, all NHT mutations), whereas more severe SNHL was associated with downsloping configurations (p.R702C/H, p.R1165C/L).

Carriers of the SH1 helix p.R702C/H mutations or the coiled-coil p.R1165L mutation suggested progression similar to, or perhaps even beyond, presbyacusis. This is derived from the observation that the average P50 age-related change in hearing sensitivity at 8 kHz for men and women increases by on the order of 5 dB from age 30 to 40 (reference data not shown). Between the same ages, the average P95 increase in age-related hearing sensitivity for men and women is on the order of 10 dB. Figure 1B shows threshold increases between age 30 and age 40 of ~15 dB and ~25 dB for the p.R702C and p.R702H mutations, respectively; for the p.R1165L mutation, a corresponding increase of ~5 dB can be seen at 8 kHz. Given the variability in threshold in these groups of carriers, we did not attempt to perform any statistical test on progression compared to the ISO 7029 norm. Remarkably, the progression shown by the patients with the coiled-coil p.R1165L mutation was more concentrated in the lower frequencies, unlike the pattern of progression that is commonly associated with presbyacusis.

DISCUSSION

MYH9-RD is a syndromic disorder with a complex clinical phenotype, which includes congenital features, such as thrombocytopenia and leukocyte inclusions, and non-congenital manifestations, namely SNHL, kidney damage, cataract, and/or alterations of liver enzymes.[3,4,7] *MYH9*-RD is caused by a limited number of *MYH9* mutations that affect different regions

of the NMMHC-IIA protein.[3] Previous genotype-phenotype studies investigated the risk of developing the non-congenital manifestations (including SNHL) associated with the specific NMMHC-IIA alterations. An earlier study demonstrated that patients with mutations in the head domain, taken together, have a higher risk of developing SNHL and nephropathy as compared to patients with mutations hitting the tail domain.[21] A recent analysis of a larger case series provided a more accurate hierarchical prognostic model, as it disclosed a different risk of developing SNHL deriving from different mutations hitting the same domain, and even the same residue. In particular, this analysis identified three genotype subgroups with significantly different risks. (1) Substitutions at residue R702 (SH1 helix) were associated with a significantly higher prevalence of SNHL; (2) mutations hitting the SH3/MD interface, the p.R1165C/L, and p.D1424H/Y substitutions resulted in an intermediate-high risk; and (3) finally, the p.D1424N and p.E1841K substitutions and NHT mutations correlated with the significantly lower risk of developing SNHL over time.[9]

SNHL is the markedly most frequent non-congenital manifestation of the *MYH9*-RD, as it is expected to develop over time in almost all patients.[9] Notwithstanding this, there is very limited information about the clinical and audiometric features of the SNHL manifested by individuals with *MYH9*-RD, including descriptions of hearing loss severity and progression. Here, we systematically investigated these features in a series of 63 *MYH9*-RD patients with known NMMHC-IIA mutations.

All the investigated patients but one showed significant SNHL beyond the P95, P90, P75, or the P50 derived from the ISO 7029 standard for age-related hearing loss. Concerning the severity of the SNHL, subjects with substitutions affecting the residue R702 in the SH1 helix had the most severe degree of SNHL, while patients with NHT mutations or the p.E1841K mutation in the coiled-coil presented a very mild SNHL even at an advanced age. Moreover, we demonstrated a different effect deriving from different substitutions at the residue D1424, as the p.D1424N substitution was associated with a lower degree of SNHL compared to the p.D1424H or p.D1424Y.

This genotype-phenotype picture is globally consistent with that derived from the previous study evaluating the risk of developing SNHL, indicating that the NMMHC-IIA alterations associated with the higher prevalence of SNHL also result in the higher severity of the SNHL, probably reflecting a higher degree of NMMHC-IIA loss-of-function.[9] However, the present study disclosed novel and important genotype-phenotype correlations that were not identified by previous investigations. In particular, we showed that patients with the p.R702C substitution had more severe SNHL than subjects with the p.R702H substitution, while previous studies reported that the risk of developing SNHL was not different between the p.R702C and p.R702H mutations.[9] This suggests that even if patients with the p.R702C or p.R702H mutation have the same probability of developing a SNHL, the subjects with the p.R702C mutation may develop a more severe degree of SNHL, as compared with individuals

carrying the p.R702H mutation. A similar comment can be made for the substitutions at the residue R1165. In fact, we observed that patients with the p.R1165L had a more severe degree of SNHL than patients carrying the p.R1165C, while the relative prevalence of SNHL was similar between these two mutations.

Concerning the audiogram configuration, we showed that, in general, relatively mild SNHL was associated with a fairly flat configuration most common for the coiled-coil p.E1841K mutation and NHT mutations. Instead, more severe SNHL was associated with downsloping configurations as seen in most mutations in the head domain and some mutations in the coiled-coil.

Concerning the progression of SNHL, a few mutations were suitable for constructing ARTA plots. This analysis showed that the most severe and progressive SNHL was associated with the SH1 helix mutations and with the mutation p.R1165L, whereas the p.R1165C mutation appeared to be associated with a milder, nonprogressive, SNHL compared to the p.R1165L mutation. The ARTA for the coiled-coil p.E1841K mutation demonstrated very mild levels of SNHL combined with very mild progression, whereas the ARTA for the NHT mutations, at similar starting levels of SNHL, did not show any substantial progression (Fig. 1).

Altogether, these data provide useful tools to predict the propensity for progression and the expected degree of severity of SNHL in individual *MYH9*-RD patients, especially in young subjects. For example, a patient presenting with SNHL and the p.R702C mutation is expected to experience a rapid deterioration over time leading to a severe SNHL at a relatively young age, compared with a patient with SNHL and a mutation in the NHT, who is expected to have a mild SNHL even at a more advanced age, without substantial deterioration. Consequences in clinical practice are relevant for development of personalized, genotype-driven clinical management. We recently reported that cochlear implantation is effective in restoring hearing function and verbal communication ability in patients with *MYH9*-RD and severe to profound SNHL.[24] We also observed that as with other forms of postlingual SNHL, the duration of deafness before implantation negatively affects cochlear implant performance.[25,26] Thus, a stricter follow-up and earlier surgery should be considered for patients with unfavorable genotypes, such as substitutions of R702 in the SH1 helix or the p.R1165L mutation in the coiled-coil.

The mechanism by which *MYH9* dysfunction causes SNHL is still unknown. Studies in rodents showed that NMMHC-IIA is expressed in the hair cells of the organ of Corti, spiral ligament and spiral limbus, with only minimal expression within the spiral ganglion.[14,27] In hair cells, NMMHC-IIA was localized along the length of the stereocilia, in the cuticular plate, and the plasma membrane.[27,28] On this basis, it was hypothesized that *MYH9* mutations cause SNHL by altering the structural integrity of stereocilia, similarly to what has been observed in mouse models of deafness deriving from mutations in three other members of the myosin superfamily, namely myosin VI, VIIA and XVA.[29–33] Concerning the prominent NMMHC-IIA

expression in the spiral ligament, fibrocytes belong to the major cellular components of this connective tissue. These cells are supposed to play a role in providing anchorage for surrounding cells or developing or reacting to tension generated in the basilar membrane-spiral ligament complex.[34] Mhatre et al.[27] hypothesized that dysfunction of *MYH9* could contribute to SNHL by disrupting the functions mediated by the fibrocytes of the spiral ligament. Indeed, the observation that cochlear implantation has a good outcome in *MYH9*-RD patients is consistent with these pathogenetic hypotheses, as the cochlear implant directly stimulates the spiral ganglion and thereby bypasses hair cell stereocilia and spiral ligament.

In conclusion, we reported for the first time systematic information on the clinical and audiometric features of SNHL in patients with *MYH9*-RD. We showed that severity and progression of SNHL are largely dependent on the causative NMMHC-IIA mutation, and we provided useful findings for patient counseling and clinical management.

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Chapter 6

Cochlear implantation is safe and effective in patients with *MYH9*-related disease

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ABSTRACT

Background: *MYH9*-related disease (*MYH9*-RD) is a rare syndromic disorder deriving from mutations in *MYH9*, the gene for the heavy chain of non-muscle myosin IIA. Patients present with congenital thrombocytopenia and giant platelets and have a variable risk of developing sensorineural deafness, kidney damage, presenile cataract, and liver abnormalities. Almost all *MYH9*-RD patients develop the hearing defect, which, in many individuals, progresses to severe to profound deafness with a high impact on quality of life. These patients are potential candidates for cochlear implantation (CI), however, no consistent data are available about the risk to benefit ratio of CI in *MYH9*-RD. The only reported patient who received CI experienced perisurgery complications that have been attributed to concurrent platelet defects and/or *MYH9* protein dysfunction.

Methods: By international co-operative study, we report the clinical outcome of 10 patients with *MYH9*-RD and severe to profound deafness who received a CI at 8 institutions.

Results: Nine patients benefited from CI: in particular, eight of them obtained excellent performances with restoration of a practically normal hearing function and verbal communication abilities. One patient had a slightly worse performance that could be explained by the very long duration of severe deafness before CI. Finally, one patient did not benefit from CI. No adverse events attributable to *MYH9*-RD syndrome were observed, in particular no perisurgery bleeding complications due to the platelet defects were seen. Patients' perioperative management is described and discussed.

Conclusions: CI is safe and effective in most patients with *MYH9*-RD and severe to profound deafness and should be offered to these subjects, possibly as soon as they develop the criteria for candidacy.

BACKGROUND

MYH9-related disease (*MYH9*-RD) is an autosomal dominant syndromic disorder deriving from mutations in *MYH9*, the gene for the heavy chain of non-muscle myosin IIA (NMMHC-IIA).[1,2] *MYH9*-RD is characterized by a complex phenotype. All patients present at birth with thrombocytopenia, platelet macrocytosis, and pathognomonic cytoplasmic inclusions of the mutant protein in leukocytes; most of them subsequently develop sensorineural hearing loss, proteinuric nephropathy, presenile cataract, and/or alterations of liver enzymes.[3,4] *MYH9*-RD encompasses four syndromes that have been considered for many years as distinct disorders, May-Hegglin Anomaly (MHA, MIM 155100), Sebastian syndrome (SBS, MIM 605249), Fechtner syndrome (FTNS, MIM 153640), and Epstein syndrome (EPTS, MIM 153650). The identification of *MYH9* as the gene responsible for all of these syndromes led to demonstration that MHA, SBS, FTNS, and EPTS actually represented different clinical presentations of the same condition, for which the definition of *MYH9*-RD has been introduced.[5–7]

Sensorineural hearing loss is the most frequent non-congenital manifestation of the *MYH9*-RD. A recent analysis of 255 patients showed that deafness was present in about one-half of cases at a mean age at evaluation of 35 years, and was expected to develop over time in almost all cases.[8] The severity of hearing impairment is variable among different patients: while in some *MYH9*-RD subjects the hearing defect is mild or moderate even at advanced age, in other patients hearing loss presents during childhood and progresses to profound deafness within the first decades of life.[8–10] Thus, in many *MYH9*-RD patients deafness greatly contributes to patients' disability.

Non-muscle myosin-IIA is a cytoplasmic myosin expressed in most cell types and tissues, including the inner ear.[11–14] As all conventional myosins, it has a hexameric structure formed by one heavy chain (NMMHC-IIA) dimer and two pairs of light chains. Each NMMHC-IIA molecule recognizes an N-terminal head domain (HD) responsible for enzymatic activity and a C-terminal tail domain (TD) mainly responsible for myosin assembly.[15] Genotype-phenotype studies showed that patients with mutations in the HD have a higher risk of early-onset and severe deafness than subjects with mutations in the TD.[8–10] Of note, the *MYH9* gene was identified as responsible also for a non-syndromic form of autosomal dominant deafness, designated DFNA17 (MIM 603622), in two pedigrees carrying the same HD mutation, p.R705H.[12,16]

Cochlear implantation (CI) is a potential option for patients with *MYH9*-RD and severe to profound deafness, however, no consistent data are available about the risk to benefit ratio of CI in this condition. Reduced platelet counts of *MYH9*-RD patients obviously result in an increased risk of bleeding complications during or after surgery, and decision to perform CI and perioperative management requires the co-operation of the hematologist with the ENT surgeon. To date, only one patient with a clinical diagnosis of EPTS who had received

CI has been reported.[17] This patient benefited from CI, but surgery was complicated by delayed wound healing and subsequent severe chronic infection, which were attributed to the chronic thrombocytopenia and defective tissue repair due to the impaired NMMHC-IIA function.[17] The efficacy of CI in *MYH9*-related deafness is made even more uncertain in view of the discordant results obtained in non-syndromic deafness DFNA17: members of both the reported DNFA17 pedigrees received CIs, with good outcomes in one family and very poor results in the other one.[16,18]

Here we report the clinical outcome of CI in 10 patients with *MYH9*-RD and severe to profound deafness. Our results provide evidence that CI should be offered to patients affected by this condition.

PATIENTS AND METHODS

This study includes 10 *MYH9*-RD patients who received CI between 1987 and 2009 at 8 different ENT centres in The Netherlands (1 centre/3 patients), Italy (3 centres/3 patients), France, Germany, Greece, and Argentina (1 patient each).[19–22] Mutational screening of the *MYH9* gene was performed at 3 different institutions by previously reported methods.[19,20] Immunofluorescence assay for the identification of NMMHC-IIA leukocyte inclusions was carried out as previously described.[19,23] Severity of bleeding was graded according to the WHO bleeding score, except for severity of intraoperative bleeding during CI surgery, which was described according to Boezaart et al.[24] Pure-tone audiometric examinations were performed at the different ENT centers by standard methods. Pure tone average (PTA) was calculated using air conduction thresholds at 500, 1000, 2000 and 4000 Hz. Speech discrimination tests were administered at the different ENT centers too. Despite some differences in the utilized tests, all of them included the assessment of the percentages of discrimination of words and sentences at a conversation voice from an open list, which were therefore used to describe the CI outcomes. Unless otherwise specified, the speech perception scores are mentioned without the use of visual support. To describe short-term outcome of CI, evaluation at 6 or 12 months after switch-on of the implanted device was reported; for long-term outcome, evaluation at the last follow-up visit was used. The investigation was approved by the Institutional Review Board of the IRCCS Policlinico San Matteo Foundation, Pavia, Italy. All the patients or their legal guardians gave written informed consent for this retrospective study, which was conducted according to the declaration of Helsinki.

RESULTS

Patients

The main clinical and laboratory features of the 10 enrolled patients are summarized in Table 1. In all the cases, diagnosis of *MYH9*-RD was based on the findings of congenital thrombocytopenia, giant platelets, and identification of pathognomonic NMMHC-IIA leukocyte inclusions by immunofluorescence assay[19], and was confirmed by identification of the causative *MYH9* mutation. In 8 cases the mutation affected the HD of NMMHC-IIA and in two cases the TD. Finally, for 7 patients the *MYH9*-RD was transmitted in an autosomal dominant manner, while three patients had sporadic forms deriving from *de novo* mutations (Table 1). Table 2 reports the management of bleeding risk on the occasions of previous surgery and respective outcomes.

Sensorineural deafness

All the patients had bilateral sensorineural hearing loss that at diagnosis affected only or predominantly the high frequencies and subsequently progressed toward severe to profound deafness involving all frequencies. Mean age at onset was 16.6 years and speech development was normal in all the cases.

Table 1. Essential clinical features of 10 *MYH9*-RD patients who received a cochlear implant.

Patient/ family	Age[1]/ gender	Inheritance	NMMHC-IIA mutation (domain)[2]	Leukocyte inclusions, MGG/IF	Platelet count x10^9/L , automated/ microscopic	Spontaneous bleeding WHO[3] (type of bleeding)	Kidney involvement	Cataract	Other relevant information	ref.
1/1	34/F	Sporadic	p.R702C (HD)	Yes/Yes	8/14	2 (easy bruising, menorrhagia)	Kidney transplantation	No	Chronic immunosuppressive drugs administration[4]	A
2/2	40/M	Sporadic	p.R702C (HD)	No/Yes	24/31	1 (easy bruising)	Nephrotic range proteinuria, CRF	No	Previous splenectomy; history of recurrent otitis media (bilateral); HCV hepatitis	A
3/3	43/M	Sporadic	p.R702S (HD)	No/Yes	21/25	2 (easy bruising, epistaxis)	Proteinuria	No	Previous splenectomy; history of chronic otitis media (bilateral)	A
4/4	72/M	AD	p.A95D (HD)	Yes/Yes	70/nd	0	No	No	History of chronic otitis media (left ear) with TM perforation	A
5/5	27/F	AD	p.D1424Y (TD)	Yes/Yes	10/80	0	No	No	None	A
6/6	50/F	AD	p.W33R (HD)	Yes/Yes	19/nd	1 (easy bruising)	No	No	None	B
7/7	12/M	AD	p.R705H (HD)	Yes/Yes	96/nd	0	No	No	None	C
8/7	30/F	AD	p.R705H (HD)	Yes/Yes	115/nd	1 (easy bruising)	No	No	None	C
9/7	46/F	AD	p.R705H (HD)	Yes/Yes	142/nd	1 (easy bruising)	No	No	None	C
10/8	54/F	AD	p.D1424N (TD)	Yes/Yes	39/nd	2 (easy bruising, menorrhagia)	No	No	None	D

Notes: [1] = age at time of cochlear implant. [2] = NMMHC-IIA domain affected by mutation. [3] = according to WHO bleeding scale: grade 0, no bleeding; grade 1, only cutaneous bleeding; grade 2, mild blood loss; grade 3, gross blood loss, requiring transfusion; grade 4, debilitating blood loss, retinal or cerebral associated with fatality. [4] = immunosuppressive treatments (tacrolimus, mycophenolate mofetil, and steroids) for previous kidney transplantation. Abbreviations: NMMHC-IIA, non-muscle myosin heavy chain IIA; HD, head domain; TD, tail domain; MGG, May-Grünwald-Giemsa; IF, immunofluorescence; ref., reference, for previously reported patients; AD, autosomal dominant; CRF, chronic renal failure; TM, tympanic membrane; nd, not determined. References: A = Pecci et al.[8], B = Saposnik et al.[20], C = Verver et al.[21], D = Greinacher et al.[22].

Table 2. Surgical history of 10 *MYH9*-RD patients who received a cochlear implant.

Patient/ family	Platelet count [x10^9/L], automated/microscopic	Type of surgery	Prophylaxis for bleeding	Perioperative bleeding (WHO grade)
1/1	8/14	Kidney transplantations (twice), nephrectomy, laparotomy (peritonitis)	Platelet transfusions before surgery, on all interventions	None
2/2	24/31	Splenectomy	Platelet transfusions	None
3/3	21/25	Tonsillectomy, splenectomy, removal of pilonidal cyst	Platelet transfusions before surgery, on all interventions except tonsillectomy	Bleeding after tonsillectomy (2)
4/4	70/nd	None	-	-
5/5	10/75	None	-	-
6/6	19/nd	Tonsillectomy, removal of tympanic glomus tumor, reduction of calcaneous fracture, ankle arthrodesis	Platelet transfusions before surgery, on all interventions	None
7/7	96/nd	Positioning of ventilation tubes	None	None
8/7	115/nd	Appendectomy, ovariectomy, laparoscopic removal of adhesions, episiotomy	None	Bleeding after episiotomy (2)
9/7	142/nd	Fixation of ankle fracture	None	None
10/8	45/nd	Adenoidectomy, nasal septoplasty, hysterectomy	Tranexamic acid before and after surgery, on all interventions	None

Abbreviations: nd = not determined.

Data about progression of deafness and the findings at the last available audiometric examination before CI are reported in Table 3 and Figure 1. Mean duration of deafness before CI was 24.2 years (range, 7-55), and mean duration of severe deafness (PTA >70 dB HL) before CI was 8.0 years (range, 2-22). In all the subjects CI was performed because of poor benefit deriving from conventional hearing aids: all the patients had an open list speech recognition score lower than 50% in the best aided conditions without lip reading.

Table 3. Age at onset, progression, and severity of sensorineural deafness before CI in 10 *MYH9*-RD patients.

Patient/ family	Age at onset [1]	Age at first use of hearing aids	Age at evolution toward PTA>70 dB HL	Age at CI	PTA before CI right/left [2]
1/1	20	23	24	34	87 / 82
2/2	20	30	32	40	115 / 120
3/3	8	34	34	43	91 / 110
4/4	17	64	70	72	107 / 106
5/5	20	25	25	27	78 / 91
6/6	25	48	48	50	118 / 116
7/7	3	5	8	12	97 / 104
8/7	4	13	19	30	121 / 111
9/7	19	21	24	46	126 / 121
10/8	30	33	44	54	100 / 85
Mean (SD)	16.6 (9)	29.6 (17)	32.8 (17)	40.8 (16)	

Notes: [1] = self-reported. **[2]** = the respective audiometric tracings are showed in Figure 1.

CI surgery

Nine patients received unilateral CI, whereas patient 5 received a sequential bilateral implantation at the ages of 27 (left) and 28 (right). In all patients preoperative CT scan and/or MRI showed no anomalies of the temporal bone at the implanted side. Table 4 reports prophylaxis for bleeding complications and perioperative bleeding in each subject. All patients received prophylactic intravenous antibiotics for 1 to 10 days (median, 3 days) and no infectious episodes were reported. Perisurgery adverse events occurred in two patients. In patient 8, a small hematoma was found at the site of the surgical wound. One stitch was then removed and by giving pressure the hematoma was drained without any further complications. In patient 9, cochleostomy was complicated by massive gusher that was treated by placing a lumbal drain and application of gelfoam at the cochleostomy. Nevertheless, all the electrodes could be correctly introduced. Moreover, postoperatively she developed an eardrum perforation for which a myringoplasty was successfully performed. Finally, normal wound healing was reported for all the patients.

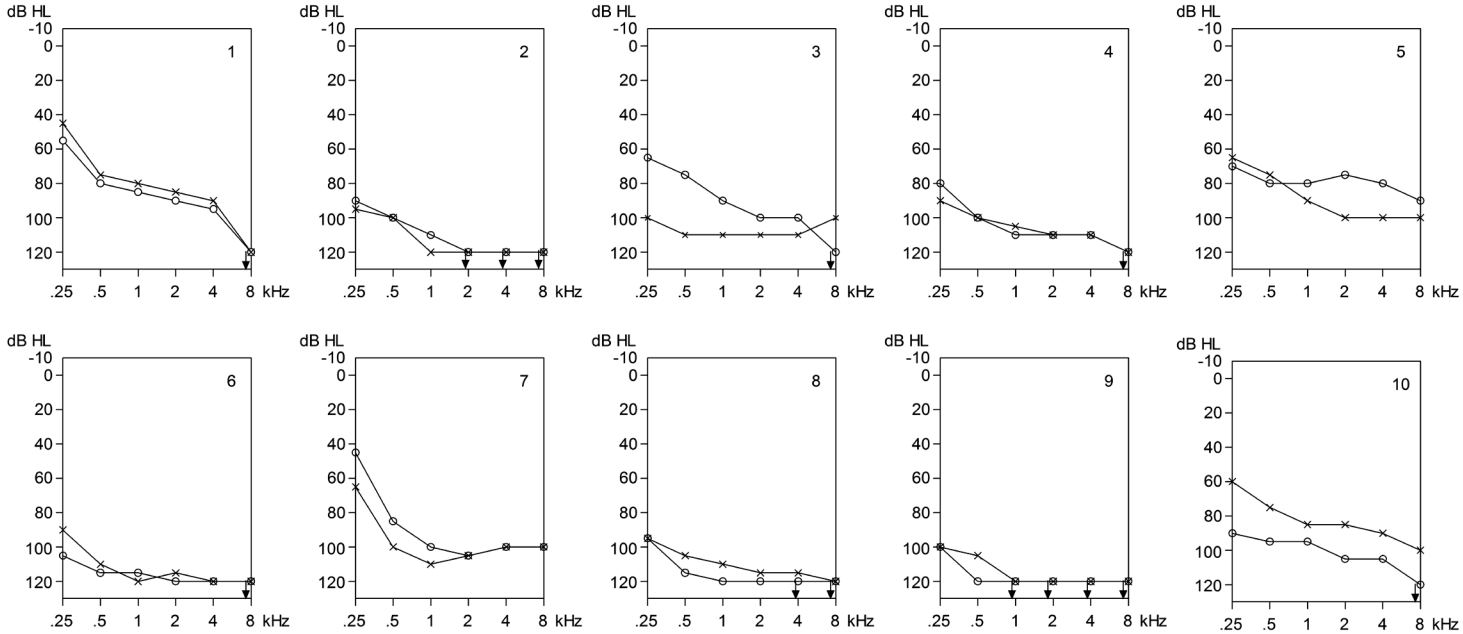

Figure 1. Audiometric tracings prior to CI in 10 patients with *MYH9*-RD. Each audiogram reports the air conduction hearing thresholds of the analyzed patients. Patients are identified by numbers on top right, which correspond to identification numbers reported in Tables. Hearing threshold is defined as the minimum sound intensity (presented at different frequencies) that can be still perceived by the individual during the audiometric examination. Hearing thresholds were measured in decibel hearing level (dB HL) and frequencies ranged from 0.25 to 8 kHz. The right ear is indicated by the symbol "O" and the left ear by the symbol "X". Severe deafness is defined by mean hearing thresholds worse than 70 dB HL, profound deafness by mean hearing thresholds worse than 90 dB HL. Arrows indicate hearing thresholds worse than 120 dB HL.

Outcome of CI

Mean follow-up after CI was 9.3 years (range, 1-25). Table 5 summarizes the results of speech perception tests administered 6 or 12 months after the switch-on of the device and at the last follow-up evaluation. The overall clinical outcome of the CI in the 10 patients can be summarized as follows. (i) Eight patients obtained excellent short-term results, with restoration of a practically regular hearing function and speech perception at the evaluation at 6-12 months. For seven of them, this performance was maintained unchanged until the last follow-up examination carried out at 1 to 9 years after CI (mean, 4.6). For patient 5, such an excellent performance was maintained for about 10 years. Subsequently, she experienced a progressive deterioration of her hearing ability and speech perception. Unfortunately, the specific reasons for this deterioration of CI performance could not be clarified, as the patient refused any further investigations. (ii) Patient 9 obtained worse short-term results with respect to the majority of other responders (Table 5). However, her ability in speech discrimination progressively increased over time, until reaching a maximum sentence score without visual support of 94% at 13 years follow-up (contextual word score 50%), and a maximum word score of 64% at 17 years follow-up (sentence score 85%). However, about 13 years after CI, she developed a progressive cognitive defect due to Alzheimer disease that probably affected the results of speech perception tests and the standard follow-up had to be stopped for severe dementia 19 years after CI. (iii) Patient 6 experienced no significant short- or long-term improvement of hearing ability after CI. On the last evaluation, this patient was able to discriminate 66% and 85% of words (open and closed list, respectively) and 100% of sentences by lip reading and CI, that was a better performance than by lip reading alone (data not shown), suggesting that CI brought some additional information to her. However, her recognition score was 0% for both words and sentences by using the CI alone. The subsequent examinations did not identify a definite cause for this poor outcome.

Table 4. CI surgery in 10 patients with *MYH9*-RD.

Patient/ family	CI side	Surgical approach [1]	Device	Prophylaxis for bleeding	Intraoperative bleeding [2]	Postoperative bleeding
1/1	R	Round window	Digisonic SP (Neurelec)	Platelet apheresis, 1U pre + 1U post	1	None
2/2	R	Round window	Nucleus CI24RE (Cochlear)	Platelet apheresis, 1U pre	3	None
3/3	L	Round window	Maestro Concerto Flex 28 (Med-el)	Platelet apheresis, 1U pre + 1U post	1	None
4/4	R	Round window	Nucleus Freedom CI24RE (Cochlear)	None	1	None
5/5	L + R	Cochleostomy	Nucleus Mini22 (Cochlear)	nd	1	None
6/6	L	Round window	Digisonic SP (Neurelec)	Platelet apheresis, 1U pre phenocompatible	1	None
7/7	L	Cochleostomy	Nucleus CI512 (Cochlear)	None	1	None
8/7	R	Cochleostomy	Nucleus CI24R (CA) (Cochlear)	None	2	Hematoma at the site of surgical wound
9/7	L	Cochleostomy	Mini-system 22 CI22M (Cochlear)	None	1	None
10/8	R	Round window	Maestro Concerto Flex 28 (Med-el)	Tranexamic acid 1 g i.v. pre, then 1500 mg/day p.o. post for 7 days	1	None

Notes: [1] = use of cochleostomy or round window for positioning of the device. [2] = bleeding score adapted according to Boezaart et al.[24]: Grade 1: minimal suction. Grade 2: slight bleeding, infrequent suction. Grade 3: brisk bleeding, frequent suction. Grade 4: strong bleeding, bleeding covers surgical field after removal of suction before instrument can perform manoeuver; Grade 5: uncontrolled bleeding, bleeding out of mastoid cavity on removal of suction. Abbreviations: pre = preoperative. post = postoperatively. nd = not determined.

Table 5. Results of speech discrimination tests administered 6 or 12 months after the switch-on of the device (short-term outcome) and at the last follow-up evaluation (long-term outcome) in 10 *MYH9*-RD patients. The speech perception scores are intended without the use of visual support.

	Short-term outcome		Long-term outcome		
Patient/ family	**Discrimination of words (-HA)[1] [%]**	**Discrimination of sentences (-HA)[1] [%]**	**Time after CI (years)**	**Discrimination of words (-HA)[1] [%]**	**Discrimination of sentences (-HA)[1] [%]**
1/1	90 (88)	95 (95)	5	100 (95)	100 (100)
2/2	90 (nd)	90 (nd)	4.5	92 (nd)	90 (nd)
3/3	95 (90)	95 (95)	4.5	98 (97)	100 (100)
4/4	80 (nd)	100 (nd)	5	90 (nd)	100 (nd)
5/5	90 (nd)	98 (nd)	25	nd	nd
6/6	0 (0)	0 (0)	17	0 (0)	0 (0)
7/7	97 (88)	100 (nd)	3	100 (85)	100 (nd)
8/7	90 (75)	99 (nd)	9	nd (85)[2]	nd (100)[2]
9/7	4 (nd)	24 (nd)	19	nd (42)[2]	nd (65)[2]
10/8	95 (nd)	82 (nd)	1	100 (nd)	82 (nd)

Notes: [1] = whenever recorded, results obtained without the contralateral external hearing aid (HA) are reported in parentheses (-HA). [2] = At the time of their last follow-up evaluations, patients 8 and 9 did not usually utilize a hearing aid: therefore, they were tested with CI alone. Abbreviations: CI = cochlear implant. nd = not determined. The speech perception scores are intended without the use of visual support.

DISCUSSION

Patients affected by *MYH9*-RD with severe to profound deafness are potential candidates for CI. To date the only information about outcome of this procedure in *MYH9*-RD derives from a single case report. This subject benefited from CI, but surgery was complicated by delayed wound healing attributed to the chronic thrombocytopenia and/or NMMHC-IIA dysfunction. Moreover, the effectiveness of CI in *MYH9*-related deafness was questioned in view of the discordant results obtained in two families with the non-syndromic deafness DFNA17.[16,18] In order to provide consistent information on the risk to benefit ratio of CI in *MYH9*-RD, we have gathered the data of 10 patients who received CI at 8 different institutions.

CI was effective in improving the hearing ability in 9 out of 10 *MYH9*-RD patients, while one subject did not take advantage from the procedure. In particular, 8 responders obtained excellent performances, with restoration of a practically regular hearing function since evaluation at 6-12 months after the switch-on of the implant. All of them referred restoration of the ability to engage in a normal conversation even in a noisy environment; they also reported good performance with phone conversations or listening to devices such as radio or television. These excellent responders were characterized by durations of severe deafness

prior to CI ranging from 2 up to 11 years (mean, 7.0). Moreover, CI performance was similarly good in patients with different total durations of deafness before CI (7 to 55 years) and with different ages at implantation (childhood up to 72 years). Finally, similarly good performances have been obtained in 6 patients with mutations hitting the HD of NMMHC-IIA or in 2 patients with mutations in the TD, suggesting that CI outcome was independent on the specific *MYH9* alteration. One subject (patient 9) benefited from CI, but performance was not as good as in the other responders. In fact, results of her speech perception tests were poor at the short-term evaluation after switch-on; however, her speech discrimination scores ameliorated over time until reaching fairly good levels that were maintained until 17 years after implantation. This patient differed from the other ones for the markedly longer duration of severe deafness before CI, i.e. 22 years. In CI responders affected by other forms of postlingual deafness, duration of severe deafness prior to implantation was a major predictor of CI performance.[25,26] We therefore hypothesize that this feature affected the CI performance also in this subject and we suggest that CI should be offered to *MYH9*-RD patients shortly after they develop criteria for candidacy.

On the other hand, the reasons why the patient 6 did not benefit from CI remain undetermined. Among the different factors that could potentially affect CI outcome, we could not identify any feature of this patient explaining her poor response.[25,27,28] The patient carried the p.R33W mutation of the HD of NMMHC-IIA, which represents a rare variant described in only one other *MYH9*-RD patient.[29] However, the clinical pictures of both patients with p.W33R do not suggest that this mutation induces a particularly severe NMMHC-IIA dysfunction with respect to other HD mutations.

CI surgery was carried out without major bleeding complications. In 4 cases with severe thrombocytopenia, bleeding risk was managed by prophylactic transfusion of 1-2 apheresis platelet concentrates. One center with experience in the care of *MYH9*-RD patients routinely uses tranexamic acid to prepare for surgery in patients with moderate thrombocytopenia, and successfully used this drug for prophylaxis of patient 10.[30] In 4 patients with automated platelet counts ranging from 70 to 142 $x10^9$/L prophylaxis for bleeding was not deemed necessary. On the whole, intraoperative bleeding was minimal in 8 patients and moderate in two cases; no postoperative bleedings occurred, with the exception of the formation, in one patient, of a hematoma at the site of surgical wound, which was drained by removing one stitch without any clinically relevant consequences. In clinical practice, two aspects should be considered for management of perioperative bleeding risk of *MYH9*-RD patients. First, routine automated cell counters usually underestimate platelet counts of these patients. In fact, electronic instruments identify platelets mainly based on their size and fail to recognize very large platelets typical of *MYH9*-RD.[31] Thus, microscopic counting should be used to assess the actual platelet counts of *MYH9*-RD patients for their proper management. Secondly, since *in vitro* platelet function in *MYH9*-RD is normal or only slightly reduced, the indication for

prophylactic transfusions can be reasonably based on the general recommendations for thrombocytopenias. Recent guidelines recommend prophylaxis for patients with platelet counts below 100 x10^9/L before surgery at critical sites, and this threshold should be considered for CI.[32] On another line, none of the patients experienced complications related to delayed wound healing. It is therefore unlikely that the complications observed in the previously reported *MYH9*-RD patient who received CI were dependent on factors specific to the disease.[17] Finally, three of our patients had conditions leading to increased risk of infection that are rather frequent among *MYH9*-RD patients[3]: two patients had been splenectomized because of a previous misdiagnosis with immune thrombocytopenia and one patient was on immunosuppressive treatment after kidney transplantation. None of them experienced infectious complications after administration of standard antimicrobial prophylaxis. We therefore conclude that CI is a safe procedure in *MYH9*-RD patients whenever adequate prophylactic interventions are carried out.

Pathogenesis of *MYH9*-related deafness is still unclear. Studies on mouse inner ear showed that NMMHC-IIA is extensively localized in the hair cells of the organ of Corti, the spiral ligament and the spiral limbus, with only minimal expression within the spiral ganglion.[12,13] In hair cells, NMMHC-IIA is abundantly expressed in stereocilia.[14] Given that CI bypasses hair cells by directly stimulating the spiral ganglion, the finding that most *MYH9*-RD patients have excellent CI performances is consistent with the NMMHC-IIA expression pattern observed in animals and with the conclusion that *MYH9* mutations primarily damage the hair cells. Altogether, these observations strengthen the notion that CI outcome is better in patients with deafness caused by defects of genes primarily expressed in the hair cells/membranous labyrinth as opposed to mutations causing spiral ganglion pathology, and further point out the importance of genetic testing in CI candidates.[33,34] The introduction of massively parallel sequencing technology led to recent development of approaches for an efficient screening of all known deafness genes simultaneously.[35–37] The identification of definite correlations between genotype and CI outcome will pave the road to a tailored patients' management and reduce the likelihood of ineffective CIs and unnecessary costs in healthcare.

CONCLUSIONS

This study provides evidence that CI is safe and highly effective in restoring hearing ability in most patients with *MYH9*-RD and severe to profound deafness. This procedure should therefore be offered to these subjects, possibly as soon as they develop the criteria for candidacy.

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Chapter 7

Discussion and Conclusion

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Thorough descriptions of genotype-phenotype correlations in genetic hearing impairment facilitates accurate genetic counseling and allows early diagnosis and intervention. The aim of this thesis on genetic hearing impairment was to further delineate the knowledge about the clinical, genetic and audiological aspects of patients with Turner syndrome (TS) and *MYH9*-related disease (*MYH9*-RD).

7.1 Turner syndrome (TS)

We noticed that for many TS children who received regular pediatric care at the Radboud University Medical Center Nijmegen there was a lack of regular checkups by an otorhinolaryngologist. This seems remarkable since TS patients are prone to develop ear and hearing problems which can be present at young age. Early diagnosis of TS is important for timely detection and treatment of medically significant features such as congenital heart disease.[1] Though, there are still girls with TS who are not correctly diagnosed until adolescence (10-17 year) when short stature and delayed pubertal development finally lead to cytogenetic testing.[2] Many of these girls may present with distinctive otologic signs and symptoms even prior to the diagnosis of TS. Therefore, recurrent otitis media or hearing loss in combination with other manifestations of TS, for example short stature, should alert the otorhinolaryngologist to the possible diagnosis of TS. This would facilitate earlier detection and management of the significant medical and psychological manifestations of this disorder.[3] We invited the parents of children who received regular pediatric care to combine the first following checkup with a visit to the otorhinolaryngologist as well. Thereby, we provided not only further insight into the otologic problems of TS children, but it also allowed us to make recommendations for better patient care. Consequently, we investigated a group of young adult TS patients as well to describe how these problems evolve throughout life.

7.1.1 TS and otologic disease

The main reason why an otorhinolaryngologist should be consulted in TS patients is because of the manifestations and involvement of the ear and hearing. This thesis substantiates a high percentage (66-68%) of TS patients with a history of recurrent otitis media, as well in children (mean age 11.2 years) as in young adults (mean age 24.3 years) as previously reported.[4–12] The etiology of the high occurrence of otologic disease in TS is still not clear. In the general population, Eustachian tube dysfunction and the immunological response to environmental pathogens are considered to be one of the etiological factors in the development of otitis media with effusion.[13] Middle ear infections in TS are thought to be related to an abnormal craniofacial morphology, including an abnormal orientation of the Eustachian tube.[4] In addition, it is thought that the increased susceptibility to middle ear disease could also be the effect of the cell cycle delay hypothesis which was postulated by Barrenäs.[14] This hypothesis states that a prolonged cell cycle in the brachial arches and neck

region and a lack of transacting growth-regulating genes, such as the SHOX (short stature homebox containing) gene located on the short arm of the X-chromosome (Xp), would result in insufficient up-regulation of the cell cycle in the branchial arches and neck region. This causes growth disturbances in the mastoid and cranial base, influencing the placement and function of the Eustachian tube. A subnormal immune response has also been suggested to be a contributing factor in recurrent infections. Diseases related to autoimmunity, such as hypothyroidism, are frequently reported in TS which might indicate an imbalance of the immune system.[4] However, no major immunological deficiency has been found that could explain the increased incidence of otitis media in girls with TS.[15]

As recurrent otitis media occurred frequently, we also found a high percentage of otomicroscopic anomalies in about 50% of the ears in our studied TS patients, which is fairly similar to the reported 57 to 59% found in other TS studies.[4,8] Only one study reported that otomicroscopic anomalies were seen in 76% of the ears.[9] Myringosclerosis was the most common finding in both the children and young adult group, followed by otitis media with effusion in the children and retraction of the tympanic membrane in the young adults. Compared to the normal population, in which Stangerup et al. showed that at the age of 5 years, pathology of the eardrum was found in 19% of the ears and at the age of 16 years, the prevalence increased to 33%, we could conclude that TS patients are more prone to develop middle ear disease.[16] Lim et al. showed that a history of recurrent acute otitis media, chronic otitis media, perforation and particularly tympanic membrane retraction were powerful predictors of cholesteatoma development in TS.[17]

Indeed, five percent (3/60 patients, 4 ears) of the studied TS children and eight percent (5/65 patients, 7 ears) of the TS young adults had a medical history of cholesteatoma. The occurrence of cholesteatoma in the studied TS patients is remarkably high compared to the normal population. In a study among 2.664 school aged children (8–13 years) in the normal population, cholesteatoma was only found in 2 children (0.07%).[18] Other studies also suggest that TS patients are prone to develop cholesteatoma with reported percentages of 2.3 (mean age 24 year)[8], 3.4 (mean age 4.7 years)[19], 3.9 (age range 1.2-44.3 years)[17], and even 9[20].

Cholesteatoma is a lesion formed by keratinizing squamous epithelium and most commonly involves the middle ear but it can occur in all portions of the petrous bone including the mastoid, petrous apex, and external auditory canal. It can form a progressively enlarging and destructive lesion, resulting in erosion of adjacent bony structures. The primary aim of cholesteatoma surgery is the eradication of the disease with a complete removal of the cholesteatoma matrix. Although a canal wall up procedure is preferable, this is not always achievable. The canal wall up technique preserves the normal bony anatomy, avoids the disadvantages associated with cavities, and has shown better hearing results, but it has a significantly higher recurrence rate. A canal wall down procedure provides lower recurrence rates, though a (modified) radical cavity is not desirable because of the tendency to develop

recurrent ear infections when hearing aids are needed, need for regular cavity cleaning and water intolerance. A bone-anchored hearing aid (BAHA) could offer a solution when conventional hearing aids are not tolerated.

7.1.2 TS and hearing impairment

This thesis shows that TS is associated with a high rate of hearing impairment, in childhood as well as adulthood, as normal hearing was only present in 29-34% of the ears. It is not a surprise that in a population prone to recurrent otitis media we substantiated a high percentage (33% of the ears) of conductive hearing loss in the TS children. Adults are less prone to recurrent otitis because here we found a low percentage (8%) of conductive hearing loss. The conclusion seems that this indicates that some of the effects of eardrum and middle ear disease completely resolve in adulthood. In this thesis, we did not specifically study this observation as we did not perform individual longitudinal analysis. We did find that the percentage of conductive hearing loss is within the range of the reported 0-44% in TS literature.[4,6–8,10,20–23] Most striking is the difference in occurrence of pure sensorineural hearing loss (SNHL) in many young adults (32% of the ears) compared to only few children (4% of the ears). The percentage of SNHL reported in literature is highly variable in a range from 9 to 66.7, which is possibly attributable to different classification systems of hearing loss and different age categories included.[3,6–8,12,20–26]

When only bone conduction thresholds were analyzed, the most common configurations were a typical sensorineural mid-frequency dip (15% of all ears), a sensorineural high-frequency loss (21% of all ears) or a combination of both (11% of all ears), which could already present at young age. Although we did not have enough individual longitudinal audiograms, it seems like the sensorineural mid-frequency dip progresses during life and patients will finally also develop a high-frequency SNHL resulting in a downsloping audiogram. The low percentage of SNHL in our children group could to some extent possibly be explained by the fact that we separated the sensorineural mid-frequency dip (17%) apart from the SNHL loss group because the dip maximum did not always exceed the 20 dB HL. The mean depth of the dip in our young adult patients was 38.7 dB, which was more pronounced compared to the mean depth of 25 dB in the children. Hultcrantz et al. also showed that the dip progresses with age.[5] Hederstierna et al. showed that young and middle-aged women with TS have a progressive type of hearing impairment, consisting of a mid-frequency dip or a high-frequency loss, deteriorating at a very rapid pace in adult age. The rate of hearing decline is much higher in women with TS than in age-matched women in the general population and is most prominent in the high-frequency region.[27]

The cause of the SNHL in TS is not known. Specific cochlear malformations have not yet been discovered.[8,19,28] Only in two TS patients with SNHL, hypoplastic lateral semicircular canals were found and one case report showed a Mondini malformation.[3,29] Barrenäs et al.

postulated the cell cycle delay hypothesis as a possible explanation for the SNHL.[14] In general, the middle turn of the cochlea has the highest hair cell density, and this is where the cell cycle needs to be upregulated the most during development to acquire a sufficient number of sensory hair cells for signal analysis.[30] In TS, it is speculated that this middle part of the cochlear duct is most prone to growth disturbances because of a delayed cell cycle that might cause a mid-frequency sensorineural dip.

Low estrogen levels in TS women have also been proposed to be a possible contributing factor to SNHL because in healthy women in the normal population, a more rapid hearing deterioration occurs after menopause when circulating levels of endogenous estrogen decline.[31] Estrogen receptors have been shown to exist in the human inner ear.[32] However, because almost all women used adequate estrogen replacement therapy at the moment of audiometric measurement, this could not be an explanation for the findings in our study.

7.1.3 Hearing impairment in relation to karyotype

We found that hearing thresholds in general were about 10 dB worse in TS children as well as young adults with a complete monosomy Xp (monosomy 45,X or isochromosome 46,X,i(Xq)), compared to those with a partial monosomy Xp (mosaicism or other structural X-chromosomal anomalies). This agrees with previous findings of Barrenäs who showed that hearing function was poorer in patients with a complete monosomy Xp compared to those having a partial monosomy Xp.[6] These results support the hypothesis that a lack of growth regulating genes, such as the SHOX-gene located on the Xp, causes growth disturbances of the temporal bone, the auricle and the cochlea which are of importance in ear and hearing problems in TS.[14]

7.1.4 Oxandrolone (Ox) and hearing

We hypothesized that the weak androgen steroid Ox, given to TS patients in addition to growth hormone to increase final height, could have a beneficial impact on hearing.[33–35] We speculated that Ox might possibly also enhance the growth and development of the mastoid bone, middle ear cavity, eardrum, and outer ear canal by increasing the level of insulin-like growth factor 1 (IGF-1) which could be observed in TS patients treated with Ox.[33,36] Barrenäs et al. found that both middle ear infections and SNHL were related to low IGF-1 levels in TS.[14] Subtle improvements in growth and development of the ear resulting in a more physiological anatomy could to some extent prevent recurrent otitis media and hearing loss. Furthermore, we speculated that Ox might decrease levels of sex hormone-binding globulin and in turn increases free active estrogen levels, which might positively affect inner ear function by stimulation of the estrogen receptors α and- β in the inner ear.[32,37] Hultcrantz et al. suggested that low estrogen levels could negatively influence hearing.[37] However, we could not find differences in mean hearing thresholds between the groups treated with placebo, Ox 0.03

mg/kg per day, and Ox 0.06 mg/kg per day. Notably, all patients received proper estrogen replacement therapy, with no additional effects of Ox on the availability of estrogen in the ear. We must notice that the above mentioned theories can only be speculated upon. There is no consistency in literature about the impact of estrogen and growth hormone on hearing as for example Ostberg et al. stated that the current regimens of estrogen and growth hormone therapy have no impact on adult hearing loss in TS, independent of age.[38]

In theory, beneficial effects might also be counterbalanced by disadvantageous effects of androgens. McFadden et al. suggested that prenatal exposure to androgens could negatively influence the auditory system by weakening of the cochlear mechanisms that underlie the production of otoacoustic emissions.[39] Also, circulating androgen levels postnatally might negatively influence otoacoustic emissions as higher levels of circulating testosterone were found to be associated with smaller click-evoked otoacoustic emission amplitudes in men.[40] In human females, data are controversial. One study reported that females with polycystic ovary syndrome (PCOS), having higher androgen levels, showed significantly more high-frequency hearing loss compared to women without PCOS.[41] However, another study in women with PCOS showed that hyperandrogenism did not seem to influence otoacoustic emission levels.[42]

7.2 MYH9-related disease (MYH9-RD)

7.2.1 p.R705H mutation in association with MYH9-RD

In the work-up towards cochlear implantation at the University Medical Center Utrecht we perform genetic counseling. We encountered a family with 3 individuals from consecutive generations who suffered from a severe SNHL. The finding of thrombocytopenia and large platelets in association with SNHL resulted in the diagnostic suspicion of *MYH9*-RD. At that time no audiological genotype-phenotype correlations were available for *MYH9*-RD and at least 70 different mutations causing *MYH9*-RD were identified so far.[43,44] We were able to confirm the diagnosis of *MYH9*-RD in all individuals by finding the NMMHC-IIA aggregates in their granulocytes and identifying a *MYH9* mutation, namely p.R705H. Meanwhile, we identified a female from another family, with macrotrombocytopenia and SNHL, who appeared to have the p.R705H mutation as well. Consistent with the phenotypic spectrum of *MYH9*-RD, our individuals also had mild to moderate elevation of some liver enzymes. Till now, the p.R705H was the only one *MYH9* mutation that was not associated with *MYH9*-RD but with DFNA17, an autosomal dominant non-syndromic form of progressive SNHL described in two large unrelated pedigrees.[45,46] Therefore, the p.R705H mutation in association with the syndromic phenotype of *MYH9*-RD is found to be a new mutation for syndromic SNHL. This indicates that DNFA17 should possibly not be considered as a separate genetic entity but as part of the wide phenotypic spectrum of *MYH9*-RD. Noteworthy, the p.R705H mutation was also found to be associated with macrothrombocytopenia, granulocyte inclusions, and SNHL in two patients from the French *MYH9* network.[44]

7.2.2 MYH9-RD and hearing impairment

Most of the *MYH9*-RD patients develop SNHL, which could progress to severe to profound deafness. During the studies on our three generation family members with *MYH9*-RD, a first genotype- phenotype correlation for *MYH9* RD and hearing loss was described.[43] Mutations in the SH1 helix of the head domain were associated with the highest risk of having SNHL and nephropathy, whereas the deletions at the NHT correlated with a significantly lower risk compared to mutations in the other NMMHC-IIA regions. Although SNHL is the most frequent non-congenital manifestation of *MYH9*-RD, we noticed that only limited information was available about the audiometric features in these individuals. Therefore, we systematically investigated the audiological traits in a large series of *MYH9*-RD patients with known NMMHC-IIA mutations. Each NMMHC-IIA molecule comprises two distinct domains: the amino-terminal head domain and the carboxy-terminal tail domain.[47] Almost all mutations in the head domain are point substitutions hitting one of two specific regions of the globular head: a distinct hydrophobic interface between the SH3-like motif and the motor domain (SH3/MD interface), or the arginine at residue 702 (R702) located in the short functional SH1 helix.[43,48] Mutations in the tail domain affect either the coiled-coil or the non-helical tailpiece (NHT). Age-related typical audiograms (ARTA) showed that the most severe and progressive type of SNHL was associated with the p.R702C, p.R702H, and p.R1165L substitutions, whereas the p.R1165C mutation correlated with a milder, nonprogressive type of SNHL than the p.R1165L. ARTA for the p.E1841K mutation demonstrated a mild degree of SNHL with only mild progression, whereas the ARTA for the mutations at the NHT did not show any substantial progression. We also described the effects of different amino acid changes at the same residue: for instance, individuals with the p.R702C mutation had more severe SNHL than those with the p.R702H mutation, and the p.R1165L substitution was associated with a higher degree of hearing loss than the p.R1165C. Mild SNHL was associated with a fairly flat audiogram configuration most common for the coiled-coil p.E1841K mutation and NHT mutations, whilst more severe SNHL was associated with downsloping configurations as seen in most mutations in the head domain and many mutations in the coiled-coil. We reported for the first time systematic information on the genotype-phenotype correlations concerning SNHL in patients with *MYH9*-RD. These data provide essential tools to predict the evolution of the SNHL in individual *MYH9*-RD subjects and therefore contribute to the opportunity to offer more personalized patient care.

7.2.3 MYH9-RD and cochlear implantation

After having studied the audiological characteristics of *MYH9*-RD patients we also wanted to investigate the outcome of cochlear implantation for patients with *MYH9*-RD and severe to profound SNHL. Since *MYH9*-RD involves thrombocytopenia, attention should be paid to bleeding tendencies during and after surgery. Till now, only a single case study was reported

to have a cochlear implant for SNHL in *MYH9*-RD.[49] This subject benefitted from cochlear implantation, but surgery was complicated by delayed wound healing which could possibly be attributed to the chronic thrombocytopenia and/or NMMHC-IIA dysfunction. Also, the effectiveness of cochlear implantation in *MYH9*-related deafness was questioned in view of the discordant results obtained in the two families with the non-syndromic deafness DFNA17, who had the same p.R705H mutation as our earlier described family members with severe to profound SNHL in association with *MYH9*-RD. The first DFNA17 family reported one individual who received a cochlear implant with poor response whilst the second mentioned five persons who received a cochlear implant with good result.[45,46]

This thesis shows that cochlear implantation was effective in improving the hearing ability in 9 out of 10 *MYH9*-RD patients. Eight patients had an excellent performance and were characterized by durations of severe deafness prior to cochlear implantation ranging from 2 up to 11 years. Similarly good performances were obtained in 6 patients with mutations hitting the head domain of NMMHC-IIA and in 2 patients with mutations in the tail domain, suggesting that cochlear implantation outcome was independent on the specific *MYH9* mutation. For one of these patients, having a mutation in the tail domain, deterioration of hearing ability was mentioned after 10 years, for which the reason remains unclear as the patient refused any further investigations. One other patient did benefit from the cochlear implantation, though performance was poorer compared to the others which might be explained by the very long duration of severe deafness before implantation, i.e. 22 years, and later on she also suffered from Alzheimer disease. In cochlear implantation responders affected by other forms of postlingual deafness, duration of severe deafness prior to implantation has been shown to be a predictor of cochlear implantation performance.[50,51] Eventually, one patient did not benefit from cochlear implantation for unknown reason. We could not identify any factors that might potentially affected the poor outcome of cochlear implantation.[50,52,53] The patient carried the p.W33R mutation of the head domain of NMMHC-IIA, which represents a rare variant described in only one other *MYH9*-RD patient.[54] However, the clinical pictures of both patients with p.W33R do not suggest that this mutation induces a particularly severe NMMHC-IIA dysfunction with respect to other head domain mutations.

The pathogenesis of *MYH9*-related SNHL is still unclear. Studies in rodents showed that NMMHC-IIA is extensively localized in the hair cells of the organ of Corti, the spiral ligament and the spiral limbus, with only minimal expression within the spiral ganglion.[45,55] NMMHC-IIA is distributed within distinct regions of the cochlear sensory hair cell, including the stereocilia as well as the cuticular plate, cytoplasm, plasma membrane and the mitochondrial matrix.[55,56] It was hypothesized that *MYH9* mutations cause SNHL by altering the structural integrity of stereocilia, similarly to what has been observed in mouse models of deafness deriving from mutations in three other members of the myosin superfamily, namely myosin VI, VIIA and XVA.[57–61] Fibrocytes belong to the major cellular components of the connective

tissue from the spiral ligament, in which NMMHC-IIA is prominently expressed. These cells are supposed to play a role in providing anchorage for surrounding cells, or developing or reacting to tension generated in the basilar membrane-spiral ligament complex.[62] Mhatre et al. hypothesized that dysfunction of *MYH9* could contribute to SNHL by disrupting the functions mediated by the fibrocytes of the spiral ligament.[55] Indeed, the observation that cochlear implantation has a good outcome in *MYH9*-RD patients is consistent with these hypotheses, as the cochlear implant directly stimulates the spiral ganglion and thereby bypasses hair cell stereocilia and spiral ligament. These observations strengthen the notion that cochlear implantation outcome is better in patients with deafness caused by defects of genes primarily expressed in the hair cells/membranous labyrinth as opposed to mutations causing spiral ganglion pathology, and further point out the importance of genetic testing in cochlear implantation candidates.[63,64]

Cochlear implantation surgery was carried out without major complications, including those related to thrombocytopenia. In 4 cases with severe thrombocytopenia, bleeding risk was managed by prophylactic transfusion of 1–2 apheresis platelet concentrates, one patient with moderate thrombocytopenia received tranexamic acid and in 4 patients prophylaxis for bleeding was not deemed necessary. Two aspects should be realized for management of perioperative bleeding risk in *MYH9*-RD patients. First, routine automated cell counters usually underestimate platelet counts in *MYH9*-RD by not recognizing the very large platelets typical of *MYH9*-RD.[65] Thus, microscopic counting should be used to assess the actual platelet counts of *MYH9*-RD patients. Secondly, since in vitro platelet function in *MYH9*-RD is normal or only slightly reduced, the indication for prophylactic transfusions can be reasonably based on the general recommendations for thrombocytopenias. Recent guidelines recommend prophylaxis for patients with platelet counts below 100×10^9/L before surgery at critical sites, and this threshold should be considered for cochlear implantation.[66] Finally, three of our patients had conditions leading to increased risk of infection that are rather frequent among *MYH9*-RD patients[67]: two patients had been splenectomized because of a previous misdiagnosis with immune thrombocytopenia and one patient was on immunosuppressive treatment after kidney transplantation. None of them experienced infectious complications after administration of standard antimicrobial prophylaxis. Our study provides evidence that cochlear implantation is a safe procedure in *MYH9*-RD patients whenever adequate prophylactic interventions are carried out.

7.3 Conclusion

This thesis emphasizes the importance of a multidisciplinary approach, including the care of an otorhinolaryngologist, in the evaluation and treatment of genetic deafness. Firstly, it substantiates that otologic disease, including middle ear pathology and hearing loss, is an important characteristic in TS. TS patients frequently suffer from recurrent otitis media and

are prone to develop cholesteatoma. SNHL is more pronounced in young adult TS women, whilst conductive hearing loss is most commonly seen in TS children. Ox, which has a growth enhancing effect in TS, has no effects on hearing. Hearing thresholds in general appeared to be about 10 dB worse in patients with a complete monosomy Xp compared to those having a partial monosomy Xp. Careful follow-up during childhood as well as adulthood is necessary to detect ear and hearing problems, especially for those with complete monosomy Xp.

Secondly, this thesis focuses on *MYH9*-RD and hearing loss. It shows that the p.R705H mutation of the *MYH9* gene is associated with *MYH9*-RD and not only with the non-syndromic deafness DNFA17 as was previously reported. Furthermore, this thesis reports thorough characterization of the clinical and audiometric features of SNHL in patients with *MYH9*-RD for the first time. We showed that severity and progression of SNHL are largely dependent on the causative NMMHC-IIA mutation. These data provide useful tools to predict the propensity for progression and the expected degree of severity of SNHL in individual *MYH9*-RD patients, especially in young subjects. Consequences in clinical practice are relevant not only for appropriate patient counseling, but also to set up personalized clinical management. This thesis shows that cochlear implantation is safe and effective in restoring hearing ability in most patients with *MYH9*-RD and severe to profound SNHL. A stricter follow-up should be considered for patients with unfavorable genotypes, such as substitutions of R702 in the SH1 helix or the p.R1165L mutation in the coiled-coil, and cochlear implantation should be offered to these patients when they develop the criteria for candidacy.

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Chapter 8

Summary - Samenvatting

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SUMMARY

Chapter 1 provides a general introduction concerning different aspects of genetic hearing impairment with special attention for Turner syndrome (TS) and *MYH9*-related disease (*MYH9*-RD).

Chapter 2 describes the otologic and audiologic characteristics in relation to karyotype in a group of 66 children with TS (mean age 11 years). Many children had a history of recurrent otitis media (68%). Otomicroscopic examination showed anomalies in 49% of the ears. Five percent of the children (3 children/4 ears) had a history of cholesteatoma which resulted in a canal wall down procedure in 3 ears. Two children were fitted with a bone-anchored hearing aid (BAHA). Normal hearing was only present in 29% of all ears. Conductive hearing loss (33%) was most commonly found, followed by a sensorineural mid-frequency dip (17%), mixed hearing loss (14%), mild conductive hearing loss (13%), and pure sensorineural hearing loss (4%). Hearing thresholds in general were about 10-11 dB worse in children with complete monosomy of the short arm of the X-chromosome (Xp) (monosomy 45,X or isochromosome 46,X,i(Xq)) compared to those with only partial monosomy Xp (mosaicism or structural anomalies). Our findings support the hypothesis that hearing can be affected by a loss of the Xp. We showed that otologic disease is an important characteristic in TS and careful follow-up during childhood is necessary to detect early ear and hearing problems and in some cases active interventions will be necessary.

Chapter 3 presents karyotype-specific ear and hearing problems in 65 young adults (mean age 24 years) with TS and the effect of treatment with the anabolic steroid Oxandrolone (Ox) which has a growth enhancing effect in TS. Patients were previously treated with growth hormone combined with placebo, Ox 0.03 mg/kg per day, or Ox 0.06 mg/kg per day from the age of 8 years and estrogen from the age of 12 years. A history of recurrent otitis media was mentioned in 66% of the patients. Eight percent of the patients (5 patients/7 ears) had a history of cholesteatoma, resulting in a canal wall down procedure in 5 ears. Normal hearing was only present in 34% of the ears. Pure sensorineural hearing loss (32%) was most commonly found, followed by mixed (24%)-, conductive (8%)-, and mild conductive (2%) hearing loss. When only bone conduction thresholds were analyzed, the most common audiogram configurations were a typical sensorineural mid-frequency dip, sensorineural high-frequency loss, or a combination of both. Hearing thresholds in general were about 10 dB worse in adults with a complete monosomy Xp compared to those with a partial monosomy Xp. Ox does not seem to have an effect on hearing. This study shows that adult TS women frequently have eardrum and middle ear pathology and many of them suffer from hearing loss. Sensorineural hearing loss, in contrast to conductive hearing loss, is more pronounced

in adult TS women than in TS children. Our findings indicate that careful follow-up remains necessary after reaching adulthood to detect ear and hearing problems, especially for those with a complete monosomy Xp.

Chapter 4 focuses on patients with *MYH9*-RD, a rare autosomal dominant syndromic disorder caused by mutation of *MYH9*, the gene encoding for the heavy chain of non-muscle myosin IIA (NMMHC-IIA). *MYH9*-RD patients have macrothrombocytopenia and characteristic NMMHC-IIA inclusions in granulocytes since birth. During life most of them develop sensorineural hearing loss, cataract, glomerulonephritis, and/or elevation of liver enzymes. Till now, only one *MYH9* mutation, p.R705H, was not associated with *MYH9*-RD but with DFNA17, an autosomal dominant non-syndromic sensorineural hearing loss without any other features associated. We identified the same mutation in two unrelated families, whose four affected individuals had not only sensorineural hearing loss but also thrombocytopenia, giant platelets, leukocyte inclusions, as well as mild to moderate elevation of some liver enzymes. These data indicate that patients with the p.R705H mutation have the syndromic phenotype of *MYH9*-RD. This could possibly suggest that DFNA17 should not be considered as a separate genetic entity but as part of the wide phenotypic spectrum of *MYH9*-RD characterized by congenital hematological manifestations and variable penetrance and expressivity of the extra-hematological features.

Chapter 5 gives a description of the severity and propensity for progression of sensorineural hearing loss in a large series of 63 *MYH9*-RD patients (mean age 33 years) belonging to 45 unrelated families of which 115 audiograms were analyzed. Severity of sensorineural hearing loss appeared to depend on the specific NMMHC-IIA mutation. Age-related typical audiograms (ARTA) showed that the most severe and progressive sensorineural hearing loss was associated with the SH1 helix mutations p.R702C and p.R702H and the coiled-coil mutation p.R1165L, whereas the p.R1165C mutation appeared to be associated with a milder, nonprogressive, sensorineural hearing loss compared to the p.R1165L mutation. The coiled-coil p.E1841K mutation demonstrated a mild degree of sensorineural hearing loss with only mild progression, whereas mutations at the non-helical tailpiece (NHT) hardly showed any substantial progression. We also disclosed the effects of different amino acid changes at the same residue: for instance, individuals with the p.R702C mutation had more severe sensorineural hearing loss than those with the p.R702H mutation, and the p.R1165L substitution was associated with a higher degree of hearing loss than the p.R1165C. Mild sensorineural hearing loss was associated with a fairly flat audiogram configuration (coiled-coil p.E1841K mutation, NHT mutations), whilst more severe sensorineural hearing loss was associated with downsloping configurations (p.R702C/H, p.R1165C/L). These data provide useful tools to predict the progression and the expected degree of severity of sensorineural

hearing loss in individual *MYH9*-RD patients, which is especially relevant in young patients. Consequences in clinical practice are important for appropriate patient counseling and contribute to the opportunity to offer more personalized patient care.

Chapter 6 reports on the clinical outcome of 10 patients with *MYH9*-RD and severe to profound sensorineural hearing loss who underwent cochlear implantation at 8 different institutions. Cochlear implantation was effective in improving the hearing ability in 9 patients. Eight of them obtained excellent performances with restoration of a practically normal hearing function and verbal communication abilities. One of these persons experienced a progressive deterioration of her hearing ability and speech perception about 10 years after implantation. This could not be clarified, as she refused any further investigations. One patient, who did benefit from the cochlear implantation, had a poorer performance compared to the others which might be explained by the very long duration of severe deafness before cochlear implantation. Finally, for unknown reason, 1 patient did not benefit from cochlear implantation. No adverse events attributable to *MYH9*-RD syndrome were observed, except for one postoperative hematoma at the site of surgical wound without any clinically relevant consequences. We conclude that cochlear implantation is safe to perform and effective in most patients with *MYH9*-RD and severe to profound sensorineural hearing loss. It should be offered to these patients, as soon as they develop the criteria for candidacy.

Chapter 7 provides a general discussion and conclusion which includes the most recent knowledge about the otologic problems in TS and *MYH9*-RD, and contributes to the optimization of the otologic care for these patients.

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SAMENVATTING

Hoofdstuk 1 geeft een algemene introductie betreffende de verschillende aspecten van gehoorverlies met een genetische oorzaak met bijzondere aandacht voor het syndroom van Turner (SvT) en *MYH9*- gerelateerde aandoeningen (*MYH9*-RD).

Hoofdstuk 2 beschrijft de otologische en audiologische kenmerken, in relatie tot karyotype, bij 66 kinderen met het SvT (gemiddelde leeftijd 11 jaar). De voorgeschiedenis van 68% van de kinderen vermeldde recidiverende otitis media. Otoscopie toonde afwijkingen in 49% van de oren. Bij 5% van de kinderen werd eerder reeds een cholesteatoom gediagnosticeerd (3 kinderen/4 oren) wat resulteerde in een conservatieve radicaalholte in 3 oren. Twee kinderen ontvingen een BAHA (bone-anchored hearing aid). Een normaal gehoor was slechts aanwezig in 29% van alle oren. Conductief gehoorverlies (33%) was het meest voorkomend, gevolgd door een perceptieve dip in de midden-frequenties (17%), gemengd gehoorverlies (14%), mild conductief gehoorverlies (13%) en zuiver perceptief gehoorverlies (4%). Gehoordrempels waren over het algemeen 10-11 dB slechter bij kinderen met een complete monosomie van de korte arm van het X-chromosoom (Xp) (monosomie 45,X of isochromosoom 46,X,i(Xq)) dan bij kinderen met enkel een partiële monosomie van de Xp (mozaïek of structuurafwijking). Deze bevindingen steunen de hypothese dat het gehoor beïnvloed zou kunnen worden door een verlies van de Xp. Concluderend zijn otologische aandoeningen een belangrijk kenmerk van het SvT. Follow-up gedurende de kinderjaren is belangrijk om oor- en gehoorproblemen op te sporen en tijdig te behandelen.

Hoofdstuk 3 geeft een weergave van de oor- en gehoorproblemen, in relatie tot karyotype, bij 65 jongvolwassenen met het SvT (gemiddelde leeftijd 24 jaar) en beschrijft het effect van Oxandrolon (Ox), een anabole steroïde, dat een groeibevorderend effect heeft bij het SvT. De vrouwen waren behandeld met groeihormoon gecombineerd met placebo, Ox 0.03 mg/kg per dag, of Ox 0.06 mg/kg per dag vanaf de leeftijd van 8 jaar en oestrogenen vanaf de leeftijd van 12 jaar. Recidiverende otitis media werd vermeld in de voorgeschiedenis van 66% van de vrouwen. Bij 8% van de vrouwen werd in het verleden een cholesteatoom gediagnosticeerd (5 patiënten/7 oren), resulterend in een conservatieve radicaalholte in 5 oren. Een normaal gehoor was slechts aanwezig in 34% van de oren. Een zuiver perceptief gehoorverlies (32%) was het meest voorkomend, gevolgd door gemengd (24%)-, conductief (8%)-, en mild conductief (2%) gehoorverlies. Alleen de beengeleidingdrempels in acht nemende, waren een perceptieve dip in de midden-frequenties, perceptief hoge tonen verlies, of een combinatie van beide de meest voorkomende audiogram configuraties. Gehoordrempels waren in het algemeen 10 dB slechter bij vrouwen met een complete monosomie van de Xp dan bij vrouwen met een partiële monosomie van de Xp. Ox behandeling in het verleden bleek geen effect

te hebben op het gehoor. Deze studie toont dat middenoorproblematiek en gehoorverlies frequent voorkomen bij volwassen vrouwen met het SvT. Perceptief gehoorverlies is meer uitgesproken bij volwassenen met het SvT, terwijl conductief gehoorverlies meer voorkomend is op de kinderleeftijd. Follow-up blijft belangrijk na het bereiken van de volwassen leeftijd om oor- en gehoorproblemen op te sporen, met name voor vrouwen met een complete monosomie van de Xp.

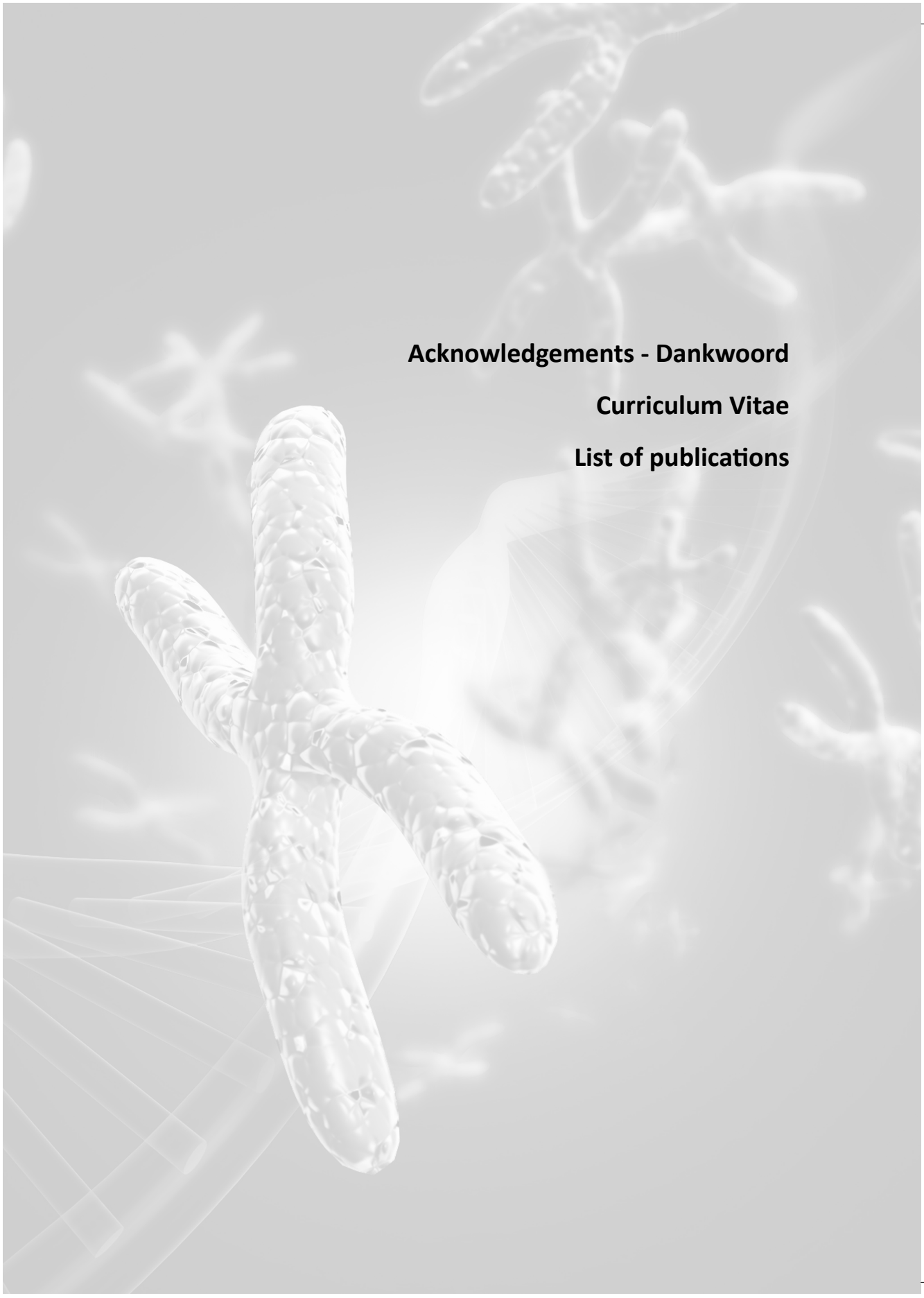
Hoofdstuk 4 gaat in op *MYH9*-RD, een zeldzame autosomaal dominante syndromale aandoening welke wordt veroorzaakt door een mutatie in *MYH9*, het gen dat codeert voor de 'heavy chain of non-muscle myosin IIA' (NMMHC-IIA). Patiënten met *MYH9*-RD hebben een macrotrombocytopenie en karakteristieke NMMHC-IIA inclusies in granulocyten vanaf de geboorte. Daarnaast ontwikkelt de meerderheid van hen gedurende de jaren afwijkingen zoals perceptief gehoorverlies, cataract, glomerulonephritis en/of een verhoging van lever enzymen. Eén van de *MYH9* mutaties, p.R705H, werd eerder niet geassocieerd met *MYH9*-RD maar met DNFA17, een autosomaal dominante niet-syndromale vorm van perceptief gehoorverlies zonder bijkomende symptomen. Wij identificeerden dezelfde mutatie in 2 niet gerelateerde families, waarvan de 4 aangedane personen niet alleen perceptief gehoorverlies hadden, maar ook een macrotrombocytopenie, typische leukocyteninclusies en verhoging van enkele leverenzymen. Dit impliceert dat patiënten met de p.R705H mutatie wel het syndromale fenotype van *MYH9*-RD hebben. Dit zou kunnen suggereren dat DNFA17 niet als een aparte genetische entiteit beschouwd dient te worden, maar als onderdeel van het brede fenotypische spectrum van *MYH9*-RD, gekarakteriseerd door congenitale hematologische manifestaties en variabele penetrantie en expressie van de niet-hematologische kenmerken.

Hoofdstuk 5 beschrijft de ernst en progressie van perceptief gehoorverlies in een groep van 63 patiënten met *MYH9*-RD (gemiddelde leeftijd 33 jaar) behorend tot 45 niet gerelateerde families waarvan 115 audiogrammen werden geanalyseerd. De ernst van het gehoorverlies bleek afhankelijk van de NMMHC-IIA mutatie. Er werden ARTA (age-related typical audiograms) vervaardigd welke toonden dat het meest ernstige en progressieve gehoorverlies geassocieerd was met de SH1 helix mutaties p.R702C en p.R702H en de coiled-coil mutatie p.R1165L, terwijl de p.R1165C mutatie geassocieerd bleek met een milder, niet progressief gehoorverlies in vergelijking tot de p.R1165L mutatie. De coiled-coil p.E1841K mutatie toonde een mild perceptief gehoorverlies met slechts milde progressie, terwijl mutaties in de non-helical tailpiece (NHT) nauwelijks progressie toonden. We vonden ook effecten van verschillende aminozuurveranderingen binnen eenzelfde residu: bijvoorbeeld individuen met de p.R702C mutatie hadden een ernstiger perceptief gehoorverlies dan diegenen met de p.R702H mutatie en de p.R1165L mutatie toonde een ernstiger gehoorverlies dan de p.R1165C mutatie. Mild perceptief gehoorverlies was geassocieerd met een relatief vlakke

audiogram configuratie (coiled-coil p.E1841K mutatie, NHT mutaties) en een ernstiger gehoorverlies met een aflopende audiogram configuratie (p.R702C/H, p.R1165C/L). Deze data zijn waardevol voor het voorspellen van de progressie en verwachte ernst van het gehoorverlies bij individuele patiënten met *MYH9*-RD, wat met name relevant is voor jongere patiënten. Dit is zowel van belang voor patiënten counseling als voor het opzetten van gepersonaliseerde klinische zorg.

Hoofdstuk 6 presenteert de uitkomst van 10 patiënten met *MYH9*-RD welke cochleaire implantatie ondergingen in 8 verschillende klinieken. Negen patiënten hadden baat bij een cochleair implantaat. Acht van hen hadden een uitmuntende uitkomst resulterend in praktisch normale gehoorfunctie en verbale communicatiemogelijkheden. Eén van deze personen ondervond een progressieve verslechtering van het vermogen tot horen en spraakverstaan ongeveer 10 jaar na implantatie. Dit kon helaas niet verder worden gespecificeerd omdat de patiënt geen verder onderzoek wenste. Eén patiënt, welke wel baat had bij cochleaire implantatie, presteerde minder goed in vergelijking tot de anderen, wat verklaard kon worden door de erg lange duur van het bestaan van ernstig gehoorverlies voor implantatie. Tot slot had 1 patiënt om onduidelijke redenen geen enkele baat bij een cochleair implantaat. Er traden geen ongewenste effecten op welke waren toe te schrijven aan *MYH9*-RD, behoudens 1 hematoom ter plaatse van de operatiewond zonder klinische consequenties. Concluderend is cochleaire implantatie veilig en effectief bij de meeste patiënten met *MYH9*-RD en ernstige perceptieve slechthorendheid. Deze procedure zou daarom moeten worden aangeboden zodra patiënten aan de criteria voor implantatie voldoen.

Hoofdstuk 7 bevat zowel een algemene discussie als conclusie welke bijdragen aan de kennis van de otologische problemen bij het SvT en *MYH9*-RD en het optimaliseren van de otologische zorg voor deze patiënten.



Acknowledgements - Dankwoord

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CURRICULUM VITAE

Eva July Johanna was born on March 12th, 1985 in Nijmegen, The Netherlands. She attended secondary school in Nijmegen where she graduated in 2003. In that same year she started her medical studies at the Radboud University Nijmegen. During medical school she became interested in otorhinolaryngology and completed her last internships in otorhinolaryngology at the Gelre Hospital Zutphen and the Radboud University Medical Center Nijmegen. She also performed her research internship on genetic hearing loss at the otorhinolaryngology department of the Radboud University Medical Center Nijmegen.
After graduating from medical school she started to work at the General Surgery department at the Radboud University Medical Center Nijmegen in 2010. Meanwhile, she continued her research on genetic hearing loss which resulted in the present thesis. After one year at the General Surgery department she started her residency in Otorhinolaryngology and Head & Neck surgery at the University Medical Center Utrecht (Prof. dr. W. Grolman) in 2011. Parallel to her residency, she worked on her PhD research under supervision of Prof. dr. W. Grolman, Dr. V. Topsakal and Dr. H.P.M. Kunst. During her residency she also worked at the St. Antonius Hospital, Nieuwegein (supervisor: Dr. M.P. Copper), Gelderse Vallei Hospital, Ede (supervisor: Dr. M.H.J.M. Majoor), Gelre Hospital, Apeldoorn (supervisor: Dr. P.P.G. van Benthem) and the Radboud University Medical Center, Nijmegen (supervisor: Dr. F.J.A. van den Hoogen).

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