Bas P. Hartel

Usher syndrome type IIa

Genotype-phenotype correlations and hearing rehabilitation

Usher syndrome type IIa
Genotype-phenotype correlations and hearing rehabilitation

Bas P. Hartel

Usher syndrome type IIa
Genotype-phenotype correlations and hearing rehabilitation

Thesis Radboud University Nijmegen

ISBN: 978-90-8559-148-1

Layout and printed by: Optima Grafische Communicatie, Rotterdam, the Netherlands

Financial support for the publication of this thesis was provided by:
PENTAX Nederland B.V., Oticon Medical, Olympus Nederland B.V., Meda Pharma B.V., Specsavers, Cochlear Benelux NV, EmiD audiologische apparatuur, Atos Medical, ZEISS, Daleco Pharma B.V., Entercare B.V., Phonak, MED-EL Deutschland GmbH, Dos Medical BV / kno-winkel.nl, Beter horen B.V.

Usher syndrome type IIa
Genotype-phenotype correlations and hearing rehabilitation

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. dr. J.H.J.M. van Krieken,
volgens besluit van het college van decanen
in het openbaar te verdedigen op
vrijdag 29 september 2017,
om 10.30 uur precies

door

Bas Pieter Hartel
geboren op 2 juli 1987
te Gouda

Promotor
Prof. dr. ir. A.F.M. Snik

Copromotoren
Dr. R.J.E. Pennings
Dr. H.A.R. van Wijk

Manuscriptcommissie
Prof. dr. B.J. Klevering
Prof. dr. ir. R. Roepman
Prof. dr. S.E. Kramer (VU Medisch Centrum)

CONTENTS

PREFACE

This thesis is part of the ongoing Nijmegen Usher syndrome research project, initiated in the nineties by Cor Cremers. This is the sixth PhD thesis on Usher syndrome after the theses written by Annelies van Aarem (1996, Heterogeneity in the Usher syndrome), Mariette Wagenaar (2000, The Usher syndrome, a clinical and genetic correlation), Ronald Pennings (2004, Hereditary Deaf-Blindness, clinical and genetic aspects), Erwin van Wijk (2009, Dissection of the molecular pathology of Usher syndrome) and Ferry Kersten (2011, Keeping an eye on novel members of the Usher protein network). Furthermore, two other theses have described performance outcomes of Usher syndrome patients (Godelieve Damen 2007, Cochlear Implantation and Quality of Life Assessment and Rutger Plantinga 2007, Hereditary Hearing Impairment). In October 2012, two Usher syndrome patients, Ivonne Bressers and Gracia Tham, published a report on problematic hearing aid fitting in patients with Usher syndrome, based on interviews with care professionals and patients. This report, together with ongoing questions about the variability in presentation of clinical symptoms in Usher syndrome patients, laid the foundation for this thesis. Unlike the previous theses, this manuscript focuses entirely on patients with two mutations in *USH2A*. Depending on the mutations, such genotype is associated with either Usher syndrome type IIa or nonsyndromic Retinitis Pigmentosa.

The patient played a pivotal role in the realization of this thesis. First, patients were an essential source of inspiration by writing their report on problematic hearing aid fitting. Secondly, Nicole Lo-A-Njoe - Kort, a patient with Usher syndrome type IIa, conducted part of the research described in Chapter 2.1 as part of a scientific internship to obtain her medical degree. Finally, the energy, involvement and enthusiasm of Usher syndrome patients participating in research have been a driving force. Patient involvement in care and research is adopted by our institution, the Radboud university medical centre and the supervisors of this thesis. Because of this, the structure of the introduction and discussion is written as a care program for a patient with Usher syndrome. Detailed descriptions and definitions are presented in grey boxes.

Chapter 1

Introduction

PATIENTS AND SYMPTOMS

In this thesis, patients with pathogenic mutations in *USH2A* in different stages of their care program have been studied. Study objects include patients from the first presentation at the outpatient clinic up to fully genotyped and phenotyped patients who have had optimal hearing rehabilitation.

Usher syndrome is an autosomal recessively inherited condition that is characterized by a combination of sensorineural hearing impairment, vision loss due to Retinitis Pigmentosa (RP), and in some cases vestibular abnormalities (see box 1 and 2). Other symptoms including low odour identification, reduced nasal mucociliary clearance, decreased motility and velocity of sperm, mental deficiencies, cerebral atrophy and ataxia have also been described in Usher syndrome patients.[1-7] However, these symptoms have, so far, not been adopted as part of the clinical diagnosis of Usher syndrome and need further research.

Box 1. Hearing and sensorineural hearing impairment

In a person with normal anatomy of the hearing organ, a longitudinal sound wave travels through the external auditory canal to the tympanic membrane (1 in figure 1). The middle ear contains three auditory ossicles: the malleus, incus and stapes, which connect the tympanic membrane to the oval window (2 in figure 1). Sound vibrations are transmitted via the ossicles to the inner ear by the movement of the stapes footplate located in the oval window (3 in figure 1). Consequently, in the inner ear, the basilar membrane starts to move in a vertical direction resulting in deflection of the hair cell stereocilia. The sensory inner and outer hair cells are located in the Organ of Corti (4 and 5 in figure 1). These cells are responsible for converting sound vibrations into an electric action potential. This action potential travels via the auditory nerve to the primary auditory cortex (6 in figure 1). Sensorineural hearing impairment is considered as a loss or malfunctioning of the sensory organ in the cochlea and/or hearing associated neural structures. Due to this malfunctioning, the sound is not adequately transmitted to the auditory cortex, causing hearing impairment.

The combination of hearing and visual impairment was first described by the German ophthalmologist Albrecht von Graefe in 1858. However, the syndrome was named after the Scottish ophthalmologist Charles Howard Usher (1865-1942). The prevalence of Usher syndrome is estimated to range from 4.4 to 6.2 per 100,000 inhabitants. Therefore, Usher syndrome is considered to be a rare disease by the definition of the European Commission (see box 3).[8-10] Usher syndrome accounts for 3-6% of all childhood deafness, for 10-20% of the RP population and for 75% of the hereditary deafblindness population.[9, 11, 12] From the first clinical descriptions on Usher syndrome patients, it was clear that hearing impairment and RP varied among patients in both onset, severity and progression. In 1977, Davenport and Omenn proposed the first clinical classification to group the most important variations. This classification, reviewed by Smith and al. in 1994, is shown in Table 1.[13-15]

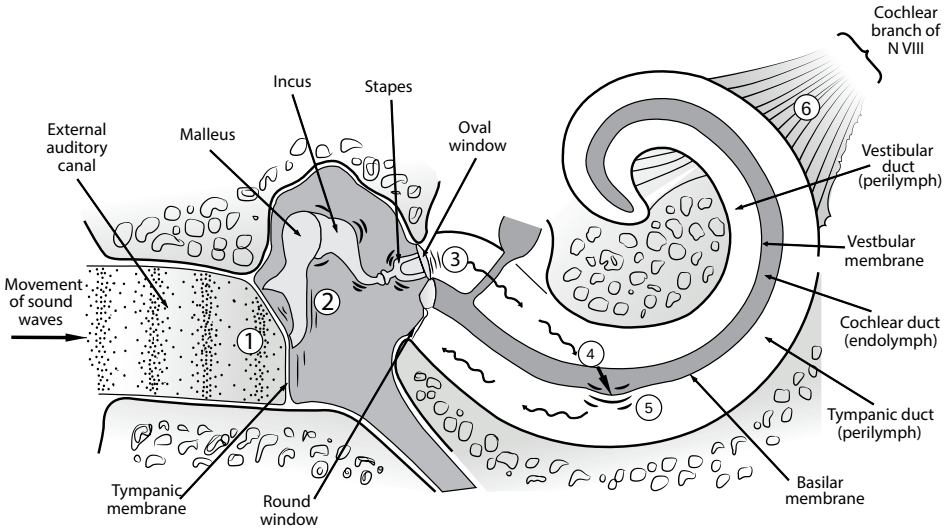


Figure 1. Schematic illustration of a sound wave travelling through the human ear. Abbreviation: N = Nerve

Table 1. Clinical classification of Usher syndrome

	Hearing impairment	Visual impairment	Vestibular function
Usher syndrome type I	Congenital, severe to profound	Diagnosis before puberty, progressive	Absent
Usher syndrome type II	Congenital, moderate to severe	Diagnosis around puberty, progressive	Intact
Usher syndrome type III	Variable onset, progressive	Variable age of diagnosis, progressive	Variable

Box 2. Vision and retinitis pigmentosa

In a healthy individual, light enters the eye through the pupil and is refracted by the cornea and lens before being projected on the retina. The retina contains the light-sensitive photoreceptor cells which use opsins to absorb the light and transmit a signal via a transduction pathway up to the cortex (Figure 2). Retinal photoreceptor cells are divided into rods and cones (Figure 3). Rods are concentrated on the outer edges of the retina and responsible for peripheral vision and vision in dim light, whereas cones are concentrated in the central region of the retina and responsible for colour vision. Synonyms for Retinitis Pigmentosa (RP) are pigmentary retinopathy and tapetoretinal degeneration. The term "retinitis" suggests an inflammatory process, however, this is not the case. RP is characterized by degeneration of the retinal photoreceptor cells avnd leads to formation of pigment deposits (hence the term pigmentosa) in the retina. The deterioration in RP is thought to be caused by a progressive peripheral to central degeneration of these light-sensitive cells.[16]

Box 3. European Commission's definition of Rare Diseases

1. Life-threatening or chronically debilitating disease
2. Affecting fewer than 5 people in 10,000
3. Combined efforts are needed to prevent or reduce the disease and preserve the quality of life and socio-economic potential

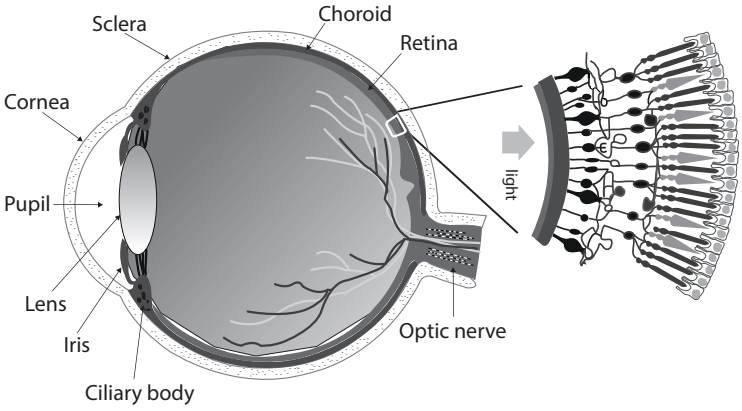


Figure 2. Anatomy of the human eye (adapted with permission from thesis E. van Wijk)

Usher syndrome type II is the most common clinical type of Usher syndrome and accounts for more than half of all patients. As mentioned in Table 1, patients with Usher syndrome type II present with congenital hearing impairment.[17] In addition, patients develop the first clinical signs of RP around puberty. The most frequently reported first visual complaint is nightblindness. From this moment onwards, the average delay before a clinical diagnosis of Usher syndrome type II is about 10 years, at a mean age of 26 years. (For detailed information on ages see chapter 2.2). In contrast to the other two clinical types of Usher syndrome, patients with Usher syndrome type II have an intact vestibular function.

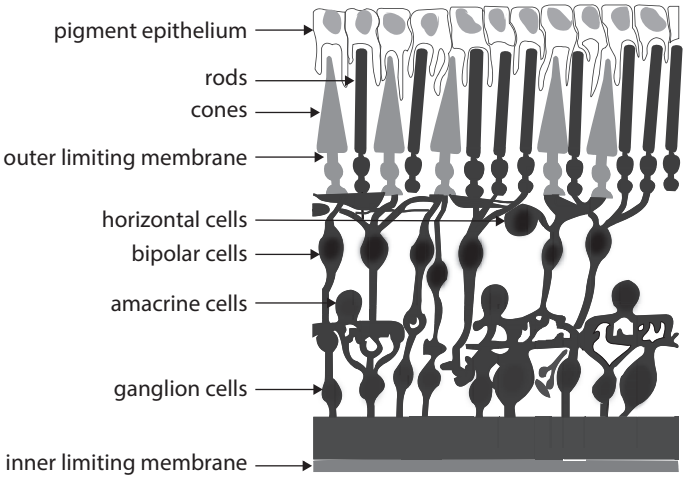


Figure 3. Cellular structure of the retina (adapted with permission from thesis E. van Wijk)

FROM A CLINICAL TO A GENETIC DIAGNOSIS

After the onset of visual complaints, the combination with pre-existing hearing impairment normally leads to a suspicion of Usher syndrome and, often, genetic testing will then be performed. As Usher syndrome is an autosomal recessively inherited disorder, patients must have a pathogenic mutation on both alleles of an Usher syndrome-associated gene to induce a phenotype. Already in 1959, Hallgren et al. suggested that the difference in phenotypic outcome between Usher syndrome patients could be caused by mutations in different genes.[18] So far, mutations in six different genes result in Usher syndrome type I. Mutations in *USH2A*, *ADGRV1* and *WHRN* result in Usher syndrome type II and defects in *CLRN1* lead to Usher syndrome type III. Furthermore, it has been reported that mutations in *PDZD7* can act as a modifier of retinal disease in Usher syndrome type IIa or, together with mutations in *ADGRV1*, act in a model of digenic inheritance resulting in Usher syndrome type II (Table 2, http://hereditaryhearingloss.org, visited 10-11-2016). At a molecular level, Usher syndrome associated genes are translated into proteins which have a specific function in the eye and ear. These proteins function together in a dynamic Usher protein network (see box 4).[17-19]

Table 2. Genes and loci associated with Usher syndrome

Type	Genetic type	Gene	Chomosomal location	Protein	OMIM	Reference
Type I	USH1b	*MYO7A*	11q13.5	myosin VIIa	276903	20
	USH1c	*USH1C*	11p15.1	harmonin	276904	21-23
	USH1d	*CDH23*	10q22.1	cadherin 23	601067	24-26
	USH1e	unknown	21q21		602097	27
	USH1f	*PCDH15*	10q21-22	Protocadherin 15	602083	28, 29
	USH1g	*USH1G*	17q24-25	SANS	606943	30, 31
	USH1h	unknown	15q22-23		312632	32
	USH1j	*CIB2*	15q23-q25.1	CIB2	614869	33
	USH1k	unknown	10p11.21-q21.1		614990	34
Type II	USH2a	*USH2A*	1q41	usherin	276901	35-37
	USH2c	*ADGRV1*	5q14.3-q21.3	ADGRV1	605472	38, 39
	USH2d	*WHRN*	9q32	Whirlin	611383	40
Type III	USH3	*CLRN1*	3q21-q25	Clarin-1	276902	41, 42
Modifier		*PDZD7*	10q24.31	PDZD7		43

OMIM: Online Mendelian Inheritance in Man

Box 4. Usher protein network

Usher syndrome type I and Usher syndrome type II proteins function together in a multiprotein scaffold: the Usher protein network. This network is present in different compositions at different subcellular locations during different stages of inner ear and retinal development. Although its precise function is still unknown, several proposed functions have been attributed to the protein network. First, it has been shown to be crucial for the elongation of stereocilia and organisation of hair bundles.[44-46] Secondly, in the photoreceptor periciliary region, it has been proposed to function in the transport and docking of vesicles that contain essential proteins for outer segment formation, maintenance and function.[47] Finally, there are indications that the Usher protein network is involved in synapse formation and synaptic transmission in the hair cells and photoreceptor cells.[47, 48] The Usher protein network is illustrated in figure 4.

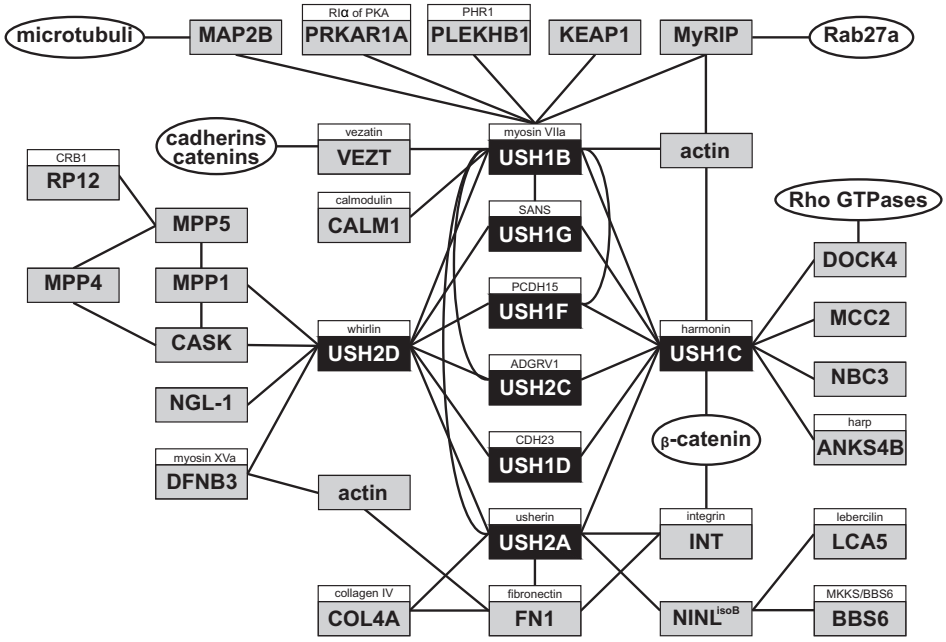


Figure 4. The Usher protein network. Identified protein-protein interactions are indicated. The Black boxes represent Usher syndrome-associated proteins. The grey boxes indicate other know proteins interacting with the Usher proteins. (Adapted with permission from thesis E. van Wijk)

Box 5. Genetic testing

Individuals are genetically discriminated from each other by thousands of DNA variations. Most of the time these variations do not directly affect general health directly. These variants are called Single Nucleotide Polymorphisms (SNP, often pronounced SNIPs) and occur in at least 1% of the population. If variations do affect health, they are called mutations. Modern diagnostic strategies include, amongst other, Copy Number Variation analyses, linkage analyses, and multiple methods for sequence analysis. Currently, the two most frequently used strategies for sequence analysis are Sanger sequencing and Whole Exome Sequencing (WES). With Sanger sequencing the precise order of nucleotides within a specific gene is determined, whereas with WES, the nucleotide sequence of all known exons (EXpressed regiON) within the human genome is determined in one assay. These sequences are compared with that of unaffected controls (e.g. the Exome Variant Server, http://evs.gs.washington.edu/EVS/) in order to find abnormal variants or mutations.[49, 50]

In 1995, Kimberling et al. identified the first locus for Usher syndrome localised at chromosome 1q41 (long arm of chromosome 1).[36] Three years later, in 1998, Eudy et al. identified mutations in *USH2A* to be causative for Usher syndrome type II.[35] In 2004, van Wijk et al. identified 51 additional exons of *USH2A* that altogether encode a novel, long isoform of usherin (see box 6 and figure 5).[37] Usher syndrome type IIa (USH2a) is the most common genetic type of Usher syndrome and has been reported to represent 72 to 82% of Usher syndrome type II cases.

Box 6. Usherin
USH2A is translated into the protein usherin. In the inner ear, usherin is part of a complex, forming transient basal links, or ankle links of the hair bundle. These links are thought to be essential for the proper organization and development of the cochlear hair bundle (see Box 1). Besides that, usherin is found at the synaptic region of hair cells and in spiral ganglia.[51] In the photoreceptor cells of the retina, usherin has been found at the apical region of the inner segment, the so called "periciliary region", and at the synaptic region of these sensory cells. At the periciliary region, usherin is thought to be part of a protein that is involved in the docking and transportation of vesicles from the inner towards the outer segment of the photoreceptor cell.[52]

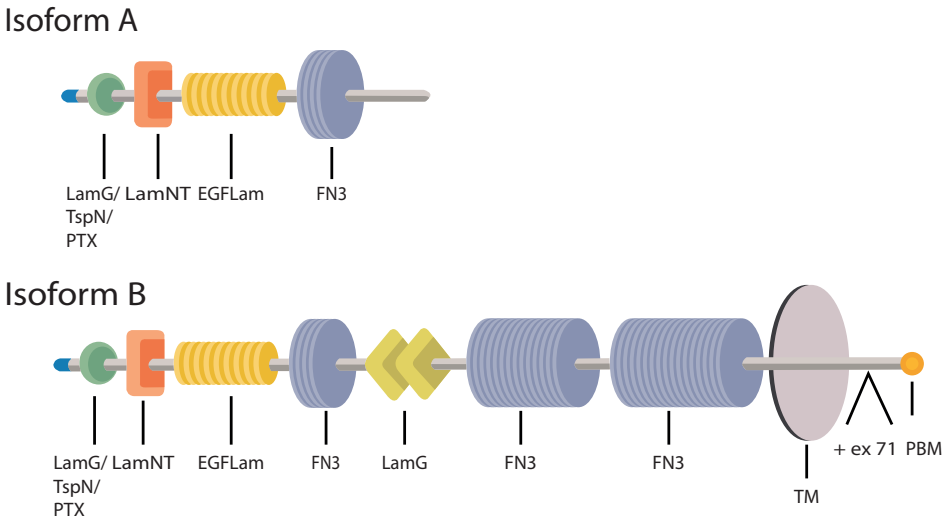


Figure 5. Schematic representation of the architecture of usherin isoform A and isoform B, the protein involved in Usher syndrome type IIa. (Adapted with permission from thesis E. van Wijk)

THE CONSEQUENCE OF A GENETIC DIAGNOSIS OF USH2a

The clinical and genetic diagnosis of USH2a has a big impact on patients as they are confronted with an uncertain future. Much is still unknown about the natural course of the disease.

Usher syndrome type II is characterized by a congenital, moderate to severe bilateral hearing impairment which is more prominent in the higher frequencies. Originally, progression of hearing impairment was not considered to be part of the Usher syndrome type II phenotype. Both Davenport and Omenn (1977) and Smith et al. (1994) considered the absence of progression as minimal diagnostic criteria for Usher syndrome type II.[13, 14] However, previous studies suggested a progressive nature of the hearing impairment in USH2a patients.[53-56] In chapter 2.1 of this thesis, hearing impairment in a large international sample of 110 genetically confirmed USH2a patients was evaluated. This study was performed to evaluate the severity and progression of hearing impairment and to identify potential genotype-phenotype correlations.

Box 7. Truncating versus nontruncating mutations
As a consequence of the large amount of unique disease causing mutations in *USH2A* (>500; LOVD-USHbases [57]), the effect of only a handful mutations has been studied at a molecular level. Mutations can be differentiated into predicted protein truncating or nontruncating mutations. These predictions are made by the current knowledge of the effect of certain mutations on protein translation and by bio-informatic prediction algorithms. Truncating mutations result in a shortened protein or an absent protein as a consequence of Nonsense Mediated mRNA Decay (NMD).[58] In contrast, nontruncating mutations presumably lead to (misfolded) proteins with reduced function. This might have advantages (e.g. residual function) and disadvantages (e.g. unwanted interactions with other proteins and toxic aggregation and accumulation of misfolded proteins).

The visual impairment caused by RP is known to be progressive. Initially, the degeneration of rods leads to a gradual loss of peripheral vision resulting in tunnel vision.[59] This is followed by degeneration of cones in the central retina leading to a decrease in visual acuity. Eventually, the majority of USH2a patients become legally blind around the age of 54. In chapter 2.2 of this thesis, the onset and progression of visual symptoms in patients with two pathogenic mutations in *USH2A* was evaluated. In this chapter, another disorder associated with mutations in *USH2A* is introduced: nonsyndromic retinitis pigmentosa (nsRP). *USH2A*-associated nsRP presents without congenital hearing impairment. In chapter 2.2 the onset and progression of visual symptoms were compared between patients with USH2a and patients with *USH2A*-associated nsRP. In chapter 2.3, the audiological profile of *USH2A*-associated nsRP patients was studied in detail. This is considered important to properly differentiate the clinical presentation of patients with USH2a versus *USH2A*-associated nsRP.

THE WORLD AFTER THE DIAGNOSIS OF USH2a

Full participation in family and society is considered to be very important by all patients with Usher syndrome. They demonstrate an enormous ambition to contribute to family,

working life and research (for examples see the Dutch websites http://www.ushersyndroom.nl and http://www.swodb.nl). To enable this, proper hearing rehabilitation is of utmost importance. As a consequence of their congenital hearing impairment, patients with USH2a usually use bilateral hearing aids from a young age.[12] The most basic principle of hearing aids is to receive sounds through a microphone, to amplify the sound and to present it to the patient through a small speaker placed in the external ear canal. Today's sophisticated hearing aids, however, offer multiple complex settings combined in programs in which the incoming sounds can be manipulated. Owing to these complex settings and programs, fitting procedures are prolonged to find the best setting, especially for patients with multiple impairments. Difficulties with hearing aid fitting were described by two patients with Usher syndrome, Gracia Tham and Ivonne Bressers. In their report "*Extremely soft and yet incredibly close*", patients and care professionals were interviewed to investigate fitting procedures and difficulties with hearing aids in patients with Usher syndrome.[60] One of the most important reported difficulties was the inability to localise sounds and to estimate the distance of the sound source while using hearing aids. In chapter 3.1 of this thesis, patients with USH2a were fitted with new hearing aids after which sound localisation and speech perception performances of different hearing aid programs were compared. The programs used different algorithms to amplify the incoming sounds and therefore affected normal sound localisation cues in different ways (see Box 8).

Box 8. Sound localisation

In humans, sound localisation in the horizontal plane is mainly achieved by using two sound cues. For the lower frequencies (<1.5 kHz), the difference in sound arrival at both ears, the Interaural Time Difference is used. To localise higher frequency sounds (> 3 kHz), humans use the difference in sound level between both ears, the Interaural Level Difference.[61]

In a select group of USH2a patients, severe progression eventually results in severe to profound hearing impairment for which hearing aids provide insufficient rehabilitation. In these cases, cochlear implantation is the designated choice of rehabilitation. A cochlear implant is a semi-implantable technical device which receives sound through a microphone, encodes the sound with a speech processor and stimulates the auditory nerve directly through a set of electrodes implanted in the cochlea. At the Radboud university medical centre in Nijmegen, cochlear implants have been implanted in children and in adults with different types of Usher syndrome. The performances after cochlear implantation have been described for patients with deafblindness and in particular two other types of Usher syndrome, Usher syndrome type I and III. In chapter 3.2 of

this thesis, the performance and quality of life of patients with USH2a after cochlear implantation is evaluated.

Concluding this introduction, the aims of this thesis were

1. To extensively evaluate the hearing and visual phenotype in patients with USH2a and *USH2A*-associated nsRP. (chapters 2.1, 2.2 and 2.3)
2. To identify genotype-phenotype correlations in patients with USH2a and *USH2A*-associated nsRP. (chapters 2.1, 2.2 and 2.3)
3. To optimize hearing aid fitting in patients with USH2a for a better performance in communication and sound localisation (chapter 3.1)
4. To report the performances of patients with USH2a after cochlear implantation. (chapter 3.2)

REFERENCES

1. Bonneau D, Raymond F, Kremer C, et al. Usher syndrome type I associated with bronchiectasis and immotile nasal cilia in two brothers. J Med Genet 1993;30(3):253-254.
2. Drouet A, Swalduz B, Guilloton L, et al. [Usher syndrome: a case report]. Rev Neurol (Paris) 2003;159(3):323-325.
3. Hess-Rover J, Crichton J, Byrne K, et al. Diagnosis and treatment of a severe psychotic illness in a man with dual severe sensory impairments caused by the presence of Usher syndrome. J Intellect Disabil Res 1999;43 (Pt 5):428-434.
4. Hunter DG, Fishman GA, Mehta RS, et al. Abnormal sperm and photoreceptor axonemes in Usher's syndrome. Arch Ophthalmol 1986;104(3):385-389.
5. Mangotich M, Misiaszek J. Atypical psychosis in Usher's syndrome. Psychosomatics 1983;24(7):674-675.
6. Schaefer GB, Bodensteiner JB, Thompson JN, Jr., et al. Volumetric neuroimaging in Usher syndrome: evidence of global involvement. Am J Med Genet 1998;79(1):1-4.
7. Zrada SE, Braat K, Doty RL, et al. Olfactory loss in Usher syndrome: another sensory deficit? Am J Med Genet 1996;64(4):602-603.
8. Boughman JA, Vernon M, Shaver KA. Usher syndrome: definition and estimate of prevalence from two high-risk populations. J Chronic Dis 1983;36(8):595-603.
9. Rosenberg T, Haim M, Hauch AM, et al. The prevalence of Usher syndrome and other retinal dystrophy-hearing impairment associations. Clin Genet 1997;51(5):314-321.
10. Spandau UH, Rohrschneider K. Prevalence and geographical distribution of Usher syndrome in Germany. Graefes Arch Clin Exp Ophthalmol 2002;240(6):495-498.
11. Lentz J, Keats B. Usher Syndrome Type II. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews(R). Seattle (WA); 1993.
12. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. Lancet 2006;368(9549):1795-1809.
13. Davenport SLH, Omenn GS. The heterogeneity of Usher syndrome. In: Fifth International Conference on Birth Defects; 1977; Amsterdam: Excerpta Medica; 1977. p. 87-88.
14. Smith RJ, Berlin CI, Hejtmancik JF, et al. Clinical diagnosis of the Usher syndromes. Usher Syndrome Consortium. Am J Med Genet 1994;50(1):32-38.
15. Plantinga RF, Kleemola L, Huygen PL, et al. Serial audiometry and speech recognition findings in Finnish Usher syndrome type III patients. Audiol Neurootol 2005;10(2):79-89.
16. Liu X, Bulgakov OV, Darrow KN, et al. Usherin is required for maintenance of retinal photoreceptors and normal development of cochlear hair cells. Proc Natl Acad Sci U S A 2007;104(11):4413-4418.
17. Bonnet C, El-Amraoui A. Usher syndrome (sensorineural deafness and retinitis pigmentosa): pathogenesis, molecular diagnosis and therapeutic approaches. Curr Opin Neurol 2012;25(1):42-49.
18. Hallgren B. Retinitis pigmentosa combined with congenital deafness; with vestibulo-cerebellar ataxia and mental abnormality in a proportion of cases: A clinical and genetico-statistical study. Acta Psychiatr Scand Suppl 1959;34(138):1-101.
19. Kremer H, van Wijk E, Marker T, et al. Usher syndrome: molecular links of pathogenesis, proteins and pathways. Hum Mol Genet 2006;15 Spec No 2:R262-270.
20. Weil D, Blanchard S, Kaplan J, et al. Defective myosin VIIA gene responsible for Usher syndrome type 1B. Nature 1995;374(6517):60-61.
21. Smith RJ, Lee EC, Kimberling WJ, et al. Localization of two genes for Usher syndrome type I to chromosome 11. Genomics 1992;14(4):995-1002.

22. Bitner-Glindzicz M, Lindley KJ, Rutland P, et al. A recessive contiguous gene deletion causing infantile hyperinsulinism, enteropathy and deafness identifies the Usher type 1C gene. Nat Genet 2000;26(1):56-60.
23. Verpy E, Leibovici M, Zwaenepoel I, et al. A defect in harmonin, a PDZ domain-containing protein expressed in the inner ear sensory hair cells, underlies Usher syndrome type 1C. Nat Genet 2000;26(1):51-55.
24. Wayne S, Der Kaloustian VM, Schloss M, et al. Localization of the Usher syndrome type ID gene (Ush1D) to chromosome 10. Hum Mol Genet 1996;5(10):1689-1692.
25. Bolz H, von Brederlow B, Ramirez A, et al. Mutation of CDH23, encoding a new member of the cadherin gene family, causes Usher syndrome type 1D. Nat Genet 2001;27(1):108-112.
26. Bork JM, Peters LM, Riazuddin S, et al. Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene CDH23. Am J Hum Genet 2001;68(1):26-37.
27. Chaib H, Kaplan J, Gerber S, et al. A newly identified locus for Usher syndrome type I, USH1E, maps to chromosome 21q21. Hum Mol Genet 1997;6(1):27-31.
28. Ahmed ZM, Riazuddin S, Bernstein SL, et al. Mutations of the protocadherin gene PCDH15 cause Usher syndrome type 1F. Am J Hum Genet 2001;69(1):25-34.
29. Alagramam KN, Yuan H, Kuehn MH, et al. Mutations in the novel protocadherin PCDH15 cause Usher syndrome type 1F. Hum Mol Genet 2001;10(16):1709-1718.
30. Mustapha M, Chouery E, Torchard-Pagnez D, et al. A novel locus for Usher syndrome type I, USH1G, maps to chromosome 17q24-25. Hum Genet 2002;110(4):348-350.
31. Weil D, El-Amraoui A, Masmoudi S, et al. Usher syndrome type I G (USH1G) is caused by mutations in the gene encoding SANS, a protein that associates with the USH1C protein, harmonin. Hum Mol Genet 2003;12(5):463-471.
32. Ahmed ZM, Riazuddin S, Khan SN, et al. USH1H, a novel locus for type I Usher syndrome, maps to chromosome 15q22-23. Clin Genet 2009;75(1):86-91.
33. Riazuddin S, Belyantseva IA, Giese AP, et al. Alterations of the CIB2 calcium- and integrin-binding protein cause Usher syndrome type 1J and nonsyndromic deafness DFNB48. Nat Genet 2012;44(11):1265-1271.
34. Jaworek TJ, Bhatti R, Latief N, et al. USH1K, a novel locus for type I Usher syndrome, maps to chromosome 10p11.21-q21.1. J Hum Genet 2012;57(10):633-637.
35. Eudy JD, Yao S, Weston MD, et al. Isolation of a gene encoding a novel member of the nuclear receptor superfamily from the critical region of Usher syndrome type IIa at 1q41. Genomics 1998;50(3):382-384.
36. Kimberling WJ, Weston MD, Moller C, et al. Gene mapping of Usher syndrome type IIa: localization of the gene to a 2.1-cM segment on chromosome 1q41. Am J Hum Genet 1995;56(1):216-223.
37. van Wijk E, Pennings RJ, te Brinke H, et al. Identification of 51 novel exons of the Usher syndrome type 2A (*USH2A*) gene that encode multiple conserved functional domains and that are mutated in patients with Usher syndrome type II. Am J Hum Genet 2004;74(4):738-744.
38. Pieke-Dahl S, Moller CG, Kelley PM, et al. Genetic heterogeneity of Usher syndrome type II: localisation to chromosome 5q. J Med Genet 2000;37(4):256-262.
39. Weston MD, Luijendijk MW, Humphrey KD, et al. Mutations in the VLGR1 gene implicate G-protein signaling in the pathogenesis of Usher syndrome type II. Am J Hum Genet 2004;74(2):357-366.
40. Ebermann I, Scholl HP, Charbel Issa P, et al. A novel gene for Usher syndrome type 2: mutations in the long isoform of whirlin are associated with retinitis pigmentosa and sensorineural hearing loss. Hum Genet 2007;121(2):203-211.

41. Sankila EM, Pakarinen L, Kaariainen H, et al. Assignment of an Usher syndrome type III (USH3) gene to chromosome 3q. Hum Mol Genet 1995;4(1):93-98.
42. Joensuu T, Hamalainen R, Yuan B, et al. Mutations in a novel gene with transmembrane domains underlie Usher syndrome type 3. Am J Hum Genet 2001;69(4):673-684.
43. Ebermann I, Phillips JB, Liebau MC, et al. PDZD7 is a modifier of retinal disease and a contributor to digenic Usher syndrome. J Clin Invest 2010;120(6):1812-1823.
44. Tilney LG, Connelly PS, Vranich KA, et al. Why are two different cross-linkers necessary for actin bundle formation in vivo and what does each cross-link contribute? J Cell Biol 1998;143(1):121-133.
45. Kikkawa Y, Mburu P, Morse S, et al. Mutant analysis reveals whirlin as a dynamic organizer in the growing hair cell stereocilium. Hum Mol Genet 2005;14(3):391-400.
46. Lefevre G, Michel V, Weil D, et al. A core cochlear phenotype in USH1 mouse mutants implicates fibrous links of the hair bundle in its cohesion, orientation and differential growth. Development 2008;135(8):1427-1437.
47. Maerker T, van Wijk E, Overlack N, et al. A novel Usher protein network at the periciliary reloading point between molecular transport machineries in vertebrate photoreceptor cells. Hum Mol Genet 2008;17(1):71-86.
48. Reiners J, Marker T, Jurgens K, et al. Photoreceptor expression of the Usher syndrome type 1 protein protocadherin 15 (USH1F) and its interaction with the scaffold protein harmonin (USH1C). Mol Vis 2005;11:347-355.
49. Fokkema IF, Taschner PE, Schaafsma GC, et al. LOVD v.2.0: the next generation in gene variant databases. Hum Mutat 2011;32(5):557-563.
50. Sanger F, Coulson AR. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. J Mol Biol 1975;94(3):441-448.
51. van Wijk E, van der Zwaag B, Peters T, et al. The DFNB31 gene product whirlin connects to the Usher protein network in the cochlea and retina by direct association with *USH2A* and VLGR1. Hum Mol Genet 2006;15(5):751-765.
52. Cosgrove D, Zallocchi M. Usher protein functions in hair cells and photoreceptors. Int J Biochem Cell Biol 2014;46:80-89.
53. Pennings RJ, Huygen PL, Weston MD, et al. Pure tone hearing thresholds and speech recognition scores in Dutch patients carrying mutations in the *USH2A* gene. Otol Neurotol 2003;24(1):58-63.
54. van Aarem A, Pinckers AJ, Kimberling WJ, et al. Stable and progressive hearing loss in type 2A Usher's syndrome. Ann Otol Rhinol Laryngol 1996;105(12):962-967.
55. Wagenaar M, van Aarem A, Huygen P, et al. Hearing impairment related to age in Usher syndrome types 1B and 2A. Arch Otolaryngol Head Neck Surg 1999;125(4):441-445.
56. Sadeghi M, Cohn ES, Kelly WJ, et al. Audiological findings in Usher syndrome types IIa and II (non-IIa). Int J Audiol 2004;43(3):136-143.
57. Roux AF, Faugere V, Vache C, et al. Four-year follow-up of diagnostic service in USH1 patients. Invest Ophthalmol Vis Sci 2011;52(7):4063-4071.
58. Isken O, Maquat LE. Quality control of eukaryotic mRNA: safeguarding cells from abnormal mRNA function. Genes Dev 2007;21(15):1833-1856.
59. Pagon RA. Retinitis pigmentosa. Surv Ophthalmol 1988;33(3):137-177.
60. Bressers I, Tham G. Extremely soft, yet incredibely close. Exploring hearing aid fitting in patients with Usher syndrome type II In; 2012.
61. Blauert J. Spatial hearing : the psychophysics of human sound localization. Rev. ed. ed. Cambridge, Mass. ; London: MIT Press; 1997.

Chapter 2

Genotype–phenotype correlations in patients with mutations in *USH2A*

Chapter 2.1

A combination of two truncating mutations in *USH2A* causes more severe and progressive hearing impairment in Usher syndrome type IIa

ABSTRACT

Objectives: Usher syndrome is an inherited disorder that is characterized by hearing impairment (HI), retinitis pigmentosa, and in some cases vestibular dysfunction. Usher syndrome type IIa is caused by mutations in *USH2A*. HI in these patients is highly heterogeneous and the present study evaluates the effects of different types of *USH2A* mutations on the audiometric phenotype. Data from two large centres of expertise on Usher syndrome in the Netherlands and Sweden were combined in order to create a large combined sample of patients to identify possible genotype-phenotype correlations.

Design and methods: A retrospective study on HI in 110 patients (65 Dutch and 45 Swedish) genetically diagnosed with Usher syndrome type IIa. We used methods especially designed for characterizing and testing differences in audiological phenotype between patient subgroups. These methods included Age Related Typical Audiograms (ARTA) and a method to evaluate the difference in the degree of HI developed throughout life between subgroups.

Results: Cross-sectional linear regression analysis of last-visit audiograms for the best hearing ear demonstrated a gradual decline of hearing over decades. The congenital level of HI was in the range of 16 - 33 dB at 0.25 - 0.5 kHz, and in the range of 51 - 60 dB at 1 - 8 kHz. The annual threshold deterioration was in the range of 0.4 - 0.5 dB/year at 0.25 - 2 kHz and in the range of 0.7 - 0.8 dB/year at 4 - 8 kHz. Patients with two truncating mutations, including homozygotes for the common c.2299delG mutation, developed significantly more severe HI throughout life than patients with one truncating mutation combined with one nontruncating mutation, and patients with two nontruncating mutations.

Conclusions: The results have direct implications for patient counselling in terms of prognosis of hearing and may serve as baseline measures for future (genetic) therapeutic interventions.

Published as: Hartel, B.P., Lofgren, M., Huygen, P.L., Guchelaar, I., Lo-A-Njoe - Kort, N., Sadeghi, A.M., van Wijk, E., Tranebjaerg, L., Kremer, H., Kimberling, W.J., Cremers, C.W., Moller, C., & Pennings, R.J. (2016). A Combination of Two Truncating Mutations in *USH2A* causes more Severe and Progressive Hearing Impairment in Usher syndrome type IIa. *Hear Res*. doi: 10.1016/j.heares.2016.06.008

INTRODUCTION

Usher syndrome is an autosomal recessively inherited disorder that is characterized by sensorineural hearing impairment (HI), retinitis pigmentosa (RP), and in some cases vestibular dysfunction. RP is a progressive retinal degenerative disease that eventually leads to functional blindness. It first presents with nightblindness and, as the disease progresses, deterioration in both visual field size and visual acuity.[1] Usher syndrome is clinically and genetically heterogeneous and the leading cause of hereditary deaf-blindness. The estimated prevalence ranges from 4.4 to 6.2 per 100,000 inhabitants.[2-4]

Three types of Usher syndrome can be distinguished. These types are differentiated by degree and progression of HI, onset of RP, and the presence or absence of vestibular abnormalities. Usher syndrome type I (OMIM276900) is characterized by congenital, severe to profound HI, pre-pubertal onset of RP, and vestibular areflexia. Usher syndrome type II (OMIM276901) presents with congenital, moderate to severe HI, RP that first manifests in the second decade of life and in rare cases vestibular abnormalities. Finally, Usher syndrome type III (OMIM276903) has a variable onset and degree of HI, RP, and vestibular abnormalities.[5] In addition, HI in Usher syndrome type III has been reported to be progressive.[6, 7]

Further classification is based on genetic aetiology. To date, 14 loci and 11 genes have been identified to be associated with Usher syndrome.[8] Three genes have been associated with Usher syndrome type II: *USH2A*, GPR98, and DFNB31.[9-13]

Usher syndrome type II is the most common type of Usher syndrome accounting for more than half of the patients.[4] About 72-87% of Usher syndrome type II patients carry mutations in *USH2A* leading to Usher syndrome type IIa (USH2a).[14-16] This gene encodes a transmembrane protein called usherin (OMIM608400). In the cochlea, usherin is present in the developing hair bundles and the synapse of hair cells, as well as in the spiral ganglion cells.[13, 17, 18] In the retina, usherin has been localised in the periciliary membrane complex of mouse, frog, and primate photoreceptors.[17, 19-21] Apart from causing USH2a, mutations in *USH2A* can also lead to nonsyndromic autosomal recessively inherited RP.[22]

Mutations in *USH2A* can generally be distinguished into two types. The first group of mutations is collectively named protein truncating mutations. The second group of mutations is called protein nontruncating mutations, for example missense mutations.

The most common mutation in *USH2A* is c.2299delG, p.(Glu767Serfs*21) with a carrier allele frequency of 16-44%.[10, 23] This truncating mutation causes a frameshift at codon 767 resulting in a premature termination codon presumably leading to nonsense-mediated mRNA decay.[24] Recently, it was demonstrated that this frameshift mutation most probably results in the combined skipping of exon 12 and 13.[25]

Previous studies have evaluated the degree and progression of HI in relatively small groups of subjects with USH2a. The audiogram configuration in patients with USH2a is down-sloping with mild-to-moderate HI in the low-mid frequencies and moderate-to-severe HI in the higher frequencies. Several studies reported a progressive type of HI.[26-29] To the best of our knowledge, no previous studies have analysed the audiometric data of more than 100 patients carrying two pathogenic mutations in *USH2A*. Since the identification of *USH2A*, and the identification of the transcript encoding the long isoform of usherin, many mutations in *USH2A* have been identified.[10, 11, 30] As previously suggested, the observed variation in the USH2a audiometric phenotype could in part be the result of different mutations in *USH2A*.[31, 32] Previous studies have tried to explain the observed variation by comparing audiometric phenotypes of patients carrying specific mutations or mutation combinations.

This study presents the overall audiometric phenotype of 110 genotyped USH2a patients. A comparison was made between the phenotypes of four subgroups with different combinations of types of mutations: two truncating mutations, including a separate subgroup of patients that are homozygous for the common c.2299delG mutation, the combination of a truncating mutation and a nontruncating mutation, and two nontruncating mutations. Knowledge of these audiometric phenotypes is important for counselling and for the establishment of baseline characteristics for future genetic therapeutic approaches.

PATIENTS AND METHODS

Patients

All patients with USH2a were extracted from databases of the Swedish and Dutch expertise centres on Usher syndrome. A total number of 208 patients were identified (Swedish: 87 and Dutch: 121). All patients were clinically diagnosed with Usher syndrome type II based on medical history, ophthalmologic, and audiovestibular examinations. These examinations were performed in various clinical settings in Sweden and the Netherlands over the past decades. Patients without any mutation identified (n=23), only one mutation identified (n=50), more than two mutations identified (n=5), and missing audiological data (n=13) were excluded from the study. In revision, seven additional patients (six Swedish, two men and four women, and one Dutch woman) were excluded because it was questioned whether they had two pathogenic *USH2A* mutations. After exclusion, 110 USH2a patients with two pathogenic mutations in *USH2A* were evaluated. These patients, 54 men and 56 women, had a mean age of 44 years (range 14-70).

Audiometric evaluation

Pure tone air and bone conduction thresholds for sound frequencies ranging from 0.25 to 8 kHz were assessed according to common clinical standards. Bone conduction thresholds were only measured to exclude middle ear problems. The patients' demographic, clinical, audiological, and genetic data were obtained from the respective databases at the University Hospital Orebro in Sweden (n=45) and the Radboud university medical centre in Nijmegen, the Netherlands (n=65). Some of the presented patients have been included in previous reports from both centres.[27-29, 33-35]

Data analysis

The last audiogram for the best hearing ear, obtained at an age of between 10 and 70 years, was used for cross-sectional analyses. The lower age limit was used because data often showed inconsistencies in audiometric evaluations under the age of 10 years. An upper age limit of 70 years was used because many of the thresholds that were measured at the higher frequencies appeared to be out of scale above this age.

Cross-sectional linear regression analyses were performed, for each frequency separately, to evaluate the congenital HI level (threshold intercept), the progression, i.e. slope or Annual Threshold Deterioration, (ATD, in dB/year), as well as the degree of HI developed throughout life in these patients. Residuals around the regression lines were inspected and it was tested whether they showed a normal distribution in first approximation by applying the Kolmogorov-Smirnov (KS) test. The congenital level of HI was considered to be significant if the 95% confidence interval (CI) for the threshold intercept did not include 0 dB HL. Progression was considered to be significant if the 95% CI for the positive slope (ATD) did not include 0 dB/ year.

Patient subgroups for analysing genotype-phenotype correlations

To determine pathogenicity of the truncating mutations the Leiden Open Variant Database was consulted. If a truncating mutation was not previously described, the Exome Aggregation Consortium (ExAC) database was consulted for the percentage of alleles with the newly found mutation in normal hearing patients. For all missense mutations, in addition to the above described method, the Combined Annotation Dependent Depletion (CADD) score was used. All nontruncating variants exceeded a CADD score of 15, the score suggested by the authors to identify predicted pathogenic mutations.

The present 110 patients were divided into three subgroups by their combination of mutation types to evaluate genotype-phenotype correlations. The first subgroup, designated as 2T, comprised 58 patients with two truncating mutations. The second subgroup, 1T, consisted of 31 patients with one truncating and one nontruncating mutation. Finally, subgroup 2nT consisted of 21 patients with two nontruncating mutations. A separate subgroup of 15 patients with homozygous c.2299delG mutations was selected from subgroup 2T.

Age related typical audiograms (ARTA)

The results of the cross-sectional linear regression analyses on the whole group of patients and the subgroups 2T, 1T and 2nT, as well as the separate subgroup of homozygous c.2299delG patients were used to calculate the ATD values and to construct Age Related Typical Audiograms (ARTA) as described previously.[36]

Comparing HI between mutation types

Pairwise testing focused on the level of HI developed throughout life, while controlling for age. This test was applied to pairwise comparisons between the subgroups 2T, 1T and 2nT, as well as between the subgroup of patients with homozygous c.2299delG mutations, and either of the subgroups 1T and 2nT. The procedure is illustrated in Supplemental Figure SF.1. For each audio frequency, i.e. pair of regression lines, a mean regression line was constructed. The intercept and slope of this line represented the mean value of the intercepts and slopes of the regression lines pertaining to the respective subgroups. The calculated mean intercept and slope values of this line were used to generate forced residuals in a "nonlinear" fitting procedure, using the equation Y = slope. X + intercept, with threshold Y (dB HL) and age X (years). For the validity of this method, it was first checked for each separate subgroup that the forced residuals passed the KS test, that linear regression of the residuals on age did not show any significant correlation, and that one-way analysis of variance (ANOVA) between the residuals at each frequency did not disclose any significant differences. Then a paired Student's t-test with 6 frequency pairs was performed between the residuals in each subgroup. The outcome of this test included the across-frequencies mean difference in threshold between the subgroups, with its 95% CI.

Apart from testing the degree of HI developed throughout life between subgroups, the values obtained for the intercepts and slopes for each frequency were compared between the subgroups 2T, 1T and 2nT by applying one-way ANOVA, followed by Tukey's Multiple Comparison test. We wished to make pairwise comparisons also in a second set of subgroups with the subgroup 2T replaced by the subgroup of homozygous c.2299delG patients. Applying again one-way ANOVAs and Tukey's tests would have caused duplicate testing of the same pair of subgroups 1T and 2nT in the context of different data sets. We therefore applied Student's t-test (unpaired) to test on the difference in intercept or slope only in pairwise comparisons between the subgroup of c.2299delG homozygotes and either subgroup 1T or 2nT. Welch's correction was applied if Bartlett's test detected unequal variances.

The significant differences found were labelled as substantial, in the sense of being clinically relevant, if the 95% CI of the difference for intercept was sufficiently remote (>5 dB) from zero and/or the 95% CI of the difference for slope was sufficiently remote (>0.20 dB/year) from zero.

General and simultaneous testing

A general significance level of P=0.05 was applied in all separate tests. Binomial distribution statistics were generally used for simultaneous assessment of any pairwise test items over all audio frequencies. Simultaneous significance was accepted if such differences were significant at two or more frequencies (P<0.05 tail probability in the binomial distribution with N=6, p=0.05, q=0.95). All analyses in this study were performed using Prism 5.03 software (GraphPad, San Diego, CA, USA).

RESULTS

Audiometric evaluation

Cross-sectional analyses of the pure tone threshold data of all patients were performed (Figure 1). The panel for each frequency in this figure includes the linear regression line with the fitted equation. The residuals from the linear regression line passed the normality (KS) test. The threshold intercepts were significantly positive at all frequencies, which underlines the known congenital component of HI in patients with USH2a. The congenital level of HI was in the range of 16-33 dB at 0.25-0.5 kHz, and of 51-60 dB at 1-8 kHz. Progression was in the range of 0.4-0.5 dB/year at 0.25-2 kHz and of 0.7-0.8 dB/year at 4-8 kHz; it was significant at all frequencies.

ARTA

For each frequency, thresholds predicted by the linear regression line for the fixed ages of 10, 20, 30, 40, 50, 60, and 70 years were used to construct the ARTA shown in Figure 2. The ATD values are reflected in the ARTA by the distance between the consecutive thresholds at each frequency depicted per decade. This distance (in dB) equals the deterioration in 10 years' time, i.e. 10 times the ATD, which was approximately 4-5 dB at 0.25-2 kHz, and 7-8 dB at 4-8 kHz.

USH2A mutations

Table 1 shows all the different pathogenic *USH2A* mutations (n=48) that were identified in the present combined patient sample. Thirty-two of these mutations were predicted to be truncating (18 nonsense mutations, 12 deletions or insertions and two intronic mutations predicted to affect splicing), and 16 mutations were predicted to be nontruncating. A total number of 48 c.2299delG alleles were identified, which represent 21.8% of all alleles. A total of 15 patients (14%) were homozygous and 18 (16%) heterozygous for the c.2299delG mutation.

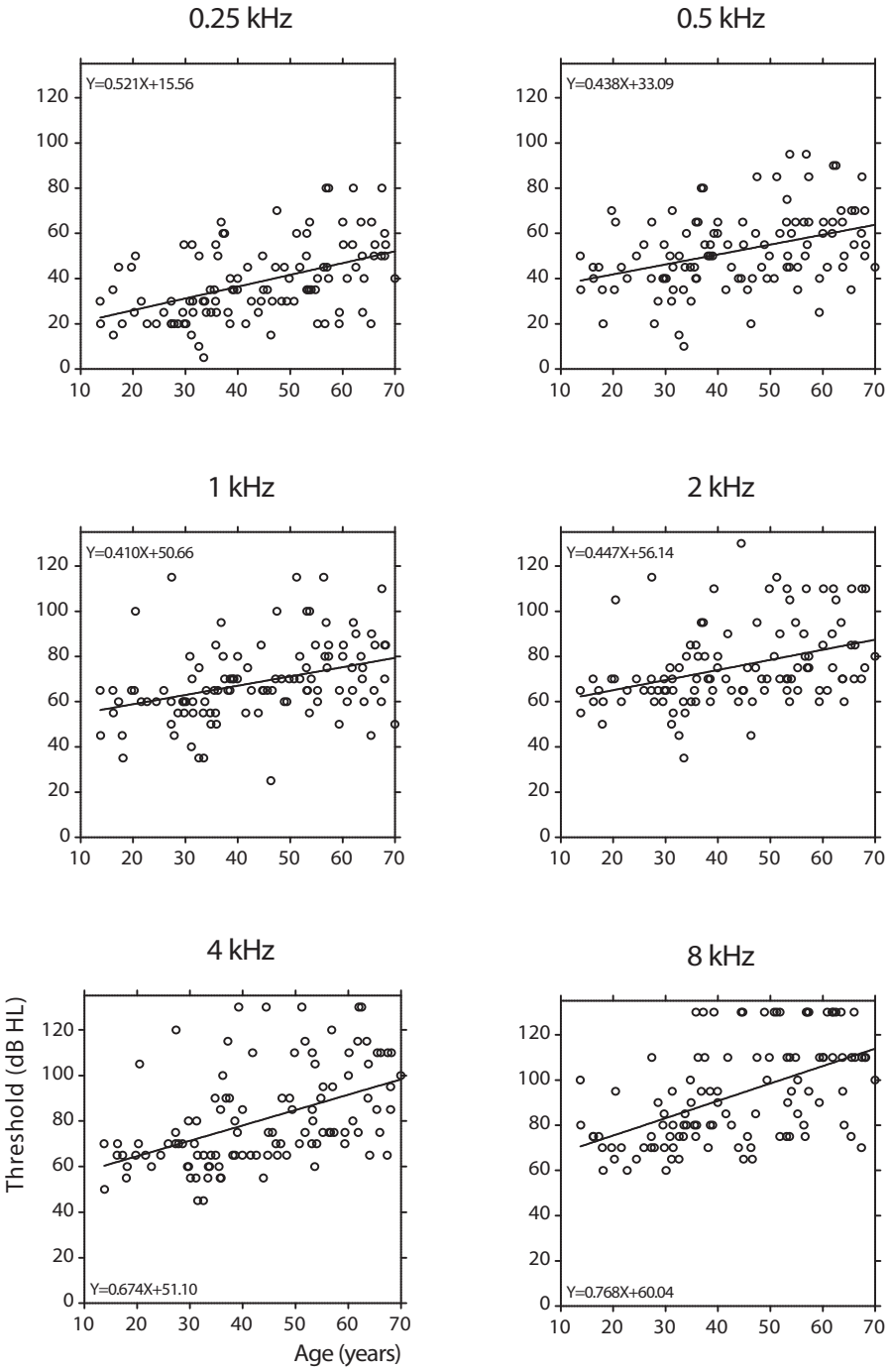


Figure 1. Cross-sectional analyses of best-ear air conduction thresholds (dB HL) in 110 patients with Usher syndrome type IIa. Each data point represents one patient's threshold. Panel inset: the linear regression equation Y = slope. X + intercept, with Y for threshold (dB HL) and X for age (years).

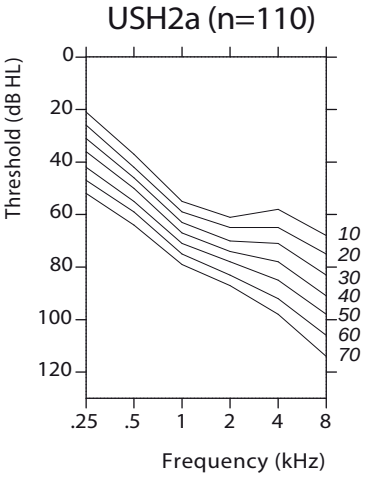


Figure 2. Age Related Typical Audiograms (ARTA) for the total Usher syndrome type IIa (USH2a) group (n=110). Italics indicate age (years).

Comparing HI between mutation types

Similar cross-sectional regression analyses as shown in Figure 1 were performed on the previously described subgroups of 2T, 1T, 2nT and homozygous c.2299delG patients; the obtained linear regression parameter values were used to construct four ARTA (Figure 3).

Inspection of the threshold lines in these ARTA gives the impression that the level of HI developed throughout life was systematically higher in either the 2T or the homozygous c.2299delG subgroup as compared with the 2nT or the 1T subgroup. The results of formal tests on the level of HI developed throughout life are listed in Table 2. The patients in either subgroup 2T or in the subgroup of homozygous c.2299delG developed a significantly higher level of HI throughout life compared to patients in either subgroup 1T or 2nT. All the corresponding significant across-frequencies mean differences between these subgroups were about 10-11 dB HL. There was no significant difference in the level of HI developed throughout life between the subgroups 1T and 2nT.

Pairwise comparisons focusing on progression and the level of congenital HI

We wished to find out whether the detected significant differences in the level of HI developed throughout life were associated primarily with a difference in the level of congenital HI (intercept of regression line) or in progression (slope of regression line). The ARTA depicted in Figure 3 seem to suggest more progression in HI in either the 2T or the homozygous c.2299delG subgroup as compared with the 2nT or the 1T subgroup at most frequencies. This appears from comparing the distances between the threshold lines in the separate ARTA for the subgroups involved. Table 3 lists the results of Tukey's

Table 1. Spectrum of *USH2A* mutations in this study

Nucleotide change	Predicted effect	No. (%) of alleles		LOVD	ExAC (%)
A. Truncating mutations					
Nonsense mutations					
c.187C>T	p.(Arg63*)	7	(3.2)	P	
c.779T>G	p.(Leu260*)	3	(1.4)	P	
c.949C>A	p.(Tyr318Cysfs*17)	3	(1.4)	UV4	
c.1227G>A	p.(Trp409*)	12	(5.5)	P	
c.1876C>T	p.(Arg626*)	4	(1.8)	P	
c.2242C>T	p.(Gln748*)	1	(0.5)	P	
c.2983C>T	p.(Gln995*)	4	(1.8)	P	
c.3932C>A	p.(Ser1311*)	3	(1.4)	P	
c.4405C>T	p.(Gln1469*)	1	(0.5)	P	
c.7931G>A	p.(Trp2644*)	2	(0.9)	P	
c.8079G>A	p.(Trp2693*)	2	(0.9)	New	0
c.8557A>T	p.(Arg2853*)	3	(1.4)	P	
c.10450C>T	p.(Arg3484*)	4	(1.8)	P	
c.10525A>T	p.(Lys3509*)	3	(1.4)	New	0
c.10684G>T	p.(Glu3562*)	1	(0.5)	P	
c.11864G>A	p.(Trp3955*)	9	(4.1)	P	
c.13822C>T	p.(Arg4608*)	2	(0.9)	P	
c.14131C>T	p.(Gln4711*)	1	(0.5)	P	
Deletions and insertions					
c.238_239insCGTA	p.(Thr80Argfs*29)	2	(0.9)	P	
c.545_546delAA	p.(Lys182Argfs*33)	1	(0.5)	P	
c.920_923dup	p.(His308Serfs*16)	3	(1.4)	P	
c.2299delG	p.(Glu767Serfs*21)	48	(21.8)	P	
c.3558delT	p.(Cys1186Trpfs*51)	1	(0.5)	P	
c.4628-30487_6325+8822del	p.(Gly1542_Leu2110delinsAsp)	6	(2.7)	P	
c.4773del	p.(Val1592*)	1	(0.5)	New	0
c.7121-8313_11048-962delins12	del Ex 38-56	10	(4.5)	New	0
c.8954delG	p.(Gly2985Alafs*3)	2	(0.9)	P	
c.9372-?_9570+?	del Ex 48	2	(0.9)	New	0
c.11875_11876delCA	p.(Gln3959Asnfs*53)	2	(0.9)	P	
c.13207-13208delGG	p.(Gly4403Profs*15)	1	(0.5)	P	

Nucleotide change	IVS position	No. (%) of alleles		LOVD	ExAC (%)
B. Intronic mutations					
c.8682-9A>G	IVS43-9A>G	2	(0.9)	UV2	0.009
c.15053-2A>T	p.(?)	1	(0.5)	New	0

Table 1. Spectrum of *USH2A* mutations in this study (continued)

Nucleotide change	Predicted effect	No. (%) of alleles		LOVD	ExAC (%)	CADD
C. Nontruncating mutations						
c.653T>A	p.(Val218Glu)	1	(0.5)	UV3	0.004	29.7
c.802G>A	p.(Gly268Arg)	1	(0.5)	UV3	0.001	34.0
c.1036A>C	p.(Ans346His)	16	(7.3)	P	0.010	26.6
c.1256G>T	p.(Cys419Phe)	25	(11.4)	P	0.005	34.0
c.1606T>C	p.(Cys536Arg)	11	(5.0)	P	0.001	25.3
c.2276G>T	p.(Cys759Phe)	3	(1.4)	P	0.078	33.0
c.4810G>C	p.(Asp1604His)	1	(0.5)	UV3	0	26.9
c.5018T>C	p.(Leu1673Pro)	2	(0.9)	New	0	31.0
c.6722C>T	p.(Pro2241Leu)	1	(0.5)	New	0	29.0
c.9815C>T	p.(Pro3272Leu)	2	(0.9)	UV3	0.003	35.0
c.10421A>G	p.(Tyr3474Cys)	1	(0.5)	New	0.001	27.5
c.10561T>C	p.(Trp3521Arg)	3	(1.4)	UV3	0.002	26.2
c.11819A>C	p.(Tyr3940Ser)	2	(0.9)	New	0	26.5
c.12695C>G	p.(Pro4232Arg)	1	(0.5)	UV3	0.001	27.2
c.13262T>C	p.(Leu4421Pro)	2	(0.9)	New	0	25.6
c.14408T>C	p.(Ile4808Thr)	1	(0.5)	New	0	24.0
Total number of alleles		**220**	**(100)**			

tests following one-way ANOVAs in pairwise comparisons focussing on subgroup 2T versus 1T.

As compared to subgroup 1T, subgroup 2T showed a significantly higher level of congenital HI at 4-8 kHz, which was only substantial at 4 kHz. The congenital level of HI in subgroup 2T was significantly lower than in subgroup 1T at 0.5 kHz, but the difference was not substantial (Table 3).

Subgroup 2T also showed significantly more progression than subgroup 1T at 0.25-2 kHz, which was substantial at 0.25-0.5 kHz (Table 3 and Supplemental Figure SF.2A, B). It seems that the higher level of HI developed throughout life by subgroup 2T as compared to subgroup 1T was predominantly associated with a higher level of congenital HI at the higher frequencies and more progression at the lower frequencies.

As compared to subgroup 2nT, subgroup 2T showed a significantly higher level of congenital HI at 0.25-4 kHz, which was substantial at 0.25-0.5, and 2 kHz (Supplemental Figure SF.3A, B and Supplemental Table ST1.A).

Subgroup 2T showed significantly more progression than subgroup 2nT only at 8 kHz, which was almost substantial (Supplemental Figure SF.3C, D and Supplemental Table ST1.A). Apart from that, subgroup 2T showed significantly less progression than

Table 2. Results (*P* value and across-frequencies mean difference with 95% CI) of the paired Student's t test for pairwise comparison of the level of HI developed throughout life between the subgroups as indicated. An example of such a test is shown in figure 1.

Subgroups	*P* value	Mean difference (dB)	95%CI (dB)	Frequency summary
2T vs. 1T	**0.00018**	10.39	7.67 to 13.10	**HI(2T) > HI(1T)**
2T vs. 2nT	**5.81×10^{-5}**	9.70	7.70 to 11.69	**HI(2T) > HI(2nT)**
1T vs. 2nT	0.71	0.55	-3.06 to 4.16	HI(1T) = HI(2nT)
c.2299delG.Hz vs. 1T	**0.0023**	10.71	8.01 to 13.42	**HI(c.2299delG.Hz) > HI(1T)**
c.2299delG.Hz vs.2nT	**0.00074**	9.51	6.17 to 12.84	**HI(c.2299delG.Hz) > HI(2nT)**

Abbreviations: 2T, two truncating mutations; 1T, one truncating mutation and one non-truncating mutation; 2nT, two nontruncating mutations; c.2299delG.Hz, subgroup of 2T patients with homozygous c.2299delG mutations, HI=Hearing Impairment, kHz=kilohertz; **bold**=significant ($P < 0.05$).

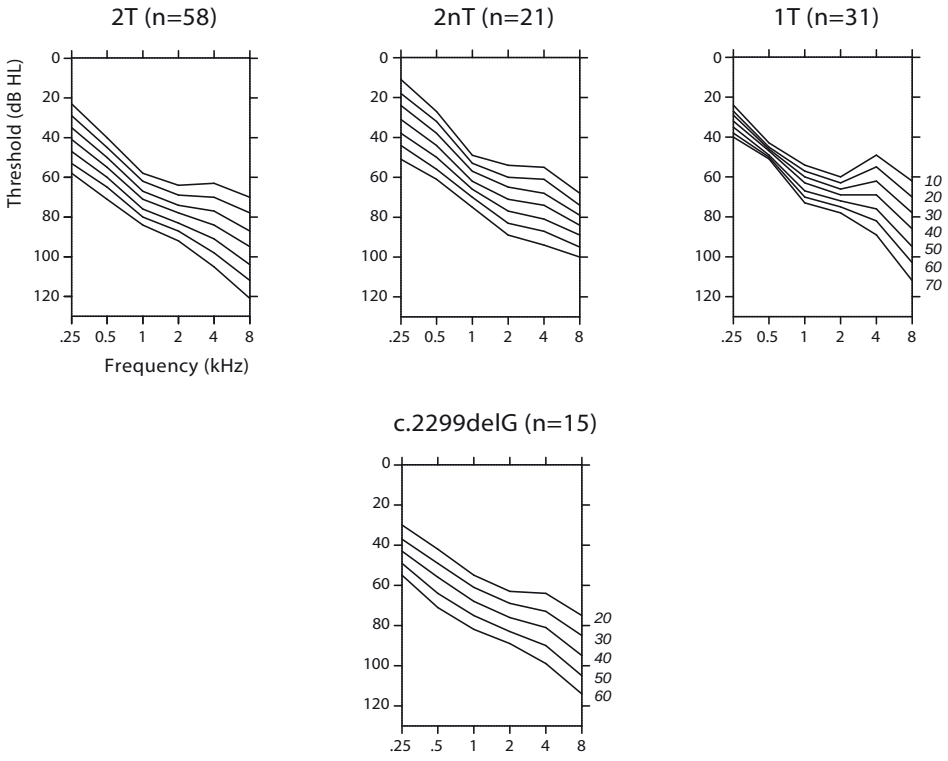


Figure 3. ARTA for the subgroups of patients with two truncating mutations (2T), two nontruncating mutations (2nT), one truncating mutation in combination with a missense mutation (1T), and for the subgroup of patients with homozygous c.2299delG mutations. Italics indicate age (years).

Table 3. 2T versus 1T (ANOVA with Tukey's test)

Results of Tukey's tests following one-way ANOVAs on intercept and slope for the pairwise comparison of subgroups 2T and 1T. Entries in column Summary significant were only made for significant results. **Bold print** in bottom rows indicates relative frequencies that are simultaneously significant (2/6 or higher) according to binomial distribution statistics.

Frequency (kHz)	Δ(Intercept) (dB)				Δ(Slope) (dB/year)			
	Δ	*P* value	95%CI	Summary	Δ	*P* value	95%CI	Summary
0.25	-3.78	$P > 0.05$	-7.64 to 0.08		0.31	$P < 0.001$	0.23 to 0.39	2T > 1T
0.5	-6.90	$P < 0.001$	-10.84 to -2.96	2T < 1T	0.38	$P < 0.001$	0.29 to 0.46	2T > 1T
1	2.81	$P > 0.05$	-1.42 to 7.04		0.12	$P < 0.01$	0.03 to 0.22	2T > 1T
2	3.22	$P > 0.05$	-0.97 to 7.41		0.16	$P < 0.001$	0.07 to 0.25	2T > 1T
4	13.94	$P < 0.001$	9.42 to 18.46	2T > 1T	0.03	$P > 0.05$	-0.06 to 0.13	
8	8.11	$P < 0.001$	3.19 to 13.03	2T > 1T	0.01	$P > 0.05$	-0.10 to 0.12	
Summary significant				2T < 1T in 1/6 0.5 kHz				**2T > 1T in 4/6 0.25-2 kHz**
				2T > 1T in 2/6 4-8 kHz				
Summary substantial				2T > 1T in 1/6 4 kHz				2T > 1T in 2/6 0.25-0.5 kHz

Abbreviations: 2T, two truncating mutations; 1T, one truncating mutation and one non-truncating mutation, Δ, difference in slope or intercept.

subgroup 2nT at 2 kHz. The difference was not substantial. Given the isolated finding of a significant difference (in progression) at a specific frequency (one out of six frequencies), this finding was not simultaneously significant (Methods).

It seems that the higher level of HI developed throughout life by subgroup 2T as compared to subgroup 2nT was primarily associated with a higher level of congenital HI at most frequencies.

As compared to subgroup 2nT, subgroup 1T showed a significantly higher level of congenital HI than subgroup 2nT at 0.25-2 kHz, which was also substantial at 0.25-0.5 kHz. It also showed a significantly, but not substantially, lower level of congenital HI at 4-8 kHz. Furthermore, subgroup 1T showed significantly less progression at 0.25, 0.5 and 2 kHz; the difference was substantial at 0.25-0.5 kHz. It also showed significantly more progression at 8 kHz, which was neither substantial nor simultaneously significant (Supplemental Table ST1.B).

There is no doubt that the subgroups 1T and 2nT developed fairly similar mean levels of HI throughout life (Table 2). However, the contributions to that level of HI made by the congenital level of HI and by postnatal progression were quite different, and counteractive, in different frequency regions. At all frequencies, except 4-8 kHz, the 1T

patients initially showed a higher level of HI than the 2nT patients, but later in life they also developed a lower degree of progression at most of these frequencies. At 8 kHz, the opposite was true: the 1T patients initially had a lower level of HI than the 2nT patients, but later in life they developed relatively more progression.

DISCUSSION

This study presents detailed audiometric analyses of 110 patients with a clinical and genetic diagnosis of USH2a. This is the most extensive audiological study of genotyped USH2a patients, so far. Cross-sectional linear regression analyses demonstrated a significant level of congenital HI, that ranged from ~15 dB at the lower frequencies to ~60 dB at the higher frequencies, as well as significantly progressive HI with an ATD that ranged from ~0.4 dB/year at the lower frequencies to ~7 dB/year at the higher frequencies (Figure 1). The HI developed throughout life in patients with two truncating mutations in *USH2A*, including patients with homozygous c.2299delG mutations, was significantly more severe compared to HI in patients with a combination of one truncating mutation and one nontruncating mutation, as well as compared to that of patients with two nontruncating mutations in this gene.

Smith et al. (1994) described a set of clinical criteria to be used for the diagnosis of Usher syndrome type I and Usher syndrome type II.[5] Usher syndrome type III had previously been proposed as a separate type by Davenport and Omenn (1977) but was not yet fully defined in 1994.[37] As a minimum diagnostic criterion for Usher syndrome type II, "no progression" of HI was proposed. Progression of HI was described to possibly differentiate between Usher syndrome type II and Usher syndrome type III. At that time the first loci were identified for Usher syndrome, but it was not until 1995 that the first Usher syndrome type I gene (MYO7A) was identified.[38] In 1998, Eudy et al. identified *USH2A* as the first gene for Usher syndrome type II and since then more genes associated with Usher syndrome have been discovered (see Introduction). With the current genotyping techniques, phenotypic features, such as progression of HI, became less important for establishing a diagnosis. An example of this was described by Pennings et al. (2003) who stated that Usher syndrome type III can mimic Usher syndrome type I, as well as type II.[39] However, knowledge of HI characteristics, including progression, is of utmost importance for patient counselling and for establishing baseline characteristics for the evaluation of future genetic therapeutics.

In their report, Pennings et al. (2003) included ARTA based on 36 USH2a patients with at least one known mutation in *USH2A*.[40] They found a downsloping audiogram configuration with a mean threshold slope of -9 dB per octave and progression by 0.5 dB per

year. After comparison with the ISO 7029 norms, progression only persisted at 0.25-0.5 kHz.[27] In 2004, Sadeghi et al. reported on audiological thresholds of 80 USH2a patients with one or two mutations in *USH2A*.[28] They presented decade-specific progression rates with a peak progression in the fifth decade of almost 10 dB per 10 years covering the frequencies 0.5-2 kHz. Compared to Sadeghi et al. (2004), our data suggest a more linear progression, as shown in Figure 1. This difference might be explained by the difference in patient sample. In this study, all patients carried two pathogenic mutations whereas in the study of Sadeghi et al. (2004) patients with one mutation in *USH2A* or linkage analyses were also included. This might have created a phenotypically more heterogeneous sample. Another explanation might be the difference in analyses. Sadeghi et al. (2004) constructed decade audiograms using more than one audiogram from their patients, whereas this study only used one (the last) audio-gram from each patient to construct the ARTA. Several of the patients from the previously mentioned studies were included in this study.[16, 27, 28, 40, 41] In the present study, in contrast to these previous studies, all patients carry two mutations in *USH2A*.

To investigate genotype-phenotype correlations, the present study focussed on the predicted truncating or nontruncating effect of mutations on usherin, the *USH2A* protein product. Predicted truncating mutations lead to premature termination of translation and result in a truncated or even absent protein as a result of nonsense-mediated mRNA decay. The nearly complete absence of usherin might result in a more severe phenotype compared to an altered protein resulting from a nontruncating missense mutation. Such a difference is supported by previous findings in patients with mutations in *CDH23*, the gene coding for cadherin 23. Truncating mutations usually lead to Usher syndrome type Id, whereas two alleles with missense mutations lead to DFNB12, a nonsyndromic type of recessively inherited sensorineural hearing.[42, 43]

Using the method illustrated in Supplemental Figure SF.1, it appeared that patients carrying two truncating mutations (subgroup 2T or the subgroup of c.2299delG homozygotes) developed significantly more HI throughout life than patients with only one truncating mutation (1T) or patients with no truncating mutations (2nT). The across-frequencies mean difference was 10-11 dB (Table 2). This might seem to be a minor difference, however, focussing on separate frequencies disclosed incidental differences between subgroups as great as ~20 dB (data not shown), which are certainly clinically relevant. Such incidental differences were associated with pairwise comparisons between ARTA pertaining to the oldest patients at either the lowest frequencies, 0.25-0.5 kHz, (subgroups 2T and 1T, as well as subgroups c.2299delG.Hz and 2nT), or at the highest frequency, 8 kHz (subgroups 2T and 2nT, as well as subgroups c.2299delG.Hz and 2nT). In

addition, a similar significant, and substantial, difference in the congenital level of HI was found at 0.25-0.5 kHz between the subgroups 1T and 2nT (Supplemental Table ST1.B).

Almost all pairwise comparisons were associated with a significant across-frequency mean difference in the level of HI developed throughout life (Table 2). Fairly balanced contributions to that difference were made by two components, the difference in the level of congenital HI (intercept) and the difference in progression incorporated in the difference in threshold increase, i.e. Δ(ATD*age). In the comparison between 2T versus 1T and 2T versus 2nT, the major contributions by these two components were co-operative. However, in the two comparisons c.2299delG versus 1T, and c.2299delG versus 2nT, the two components made mainly counteractive contributions Supplemental Table ST.1. In the end, the predominant positive contribution associated with progression accounted for the significant difference in the level of HI developed throughout life (Table 2).

Although it seems clinically relevant to be able to conclude that the patients in subgroups 1T and 2nT showed similar features in terms of the level of HI developed throughout life, it is intriguing to see that there were significant differences in terms of the congenital level of HI and the degree of progression. We have no ready explanation in terms of genotypic differences. However, such an explanation might be found in the heterogeneous compositions of the patient subgroups in terms of mutation combinations (Supplemental Table ST.2). In a separate study, we are attempting to pinpoint significant phenotypic differences between different mutation combinations by analysing extensive, individual, longitudinal data.

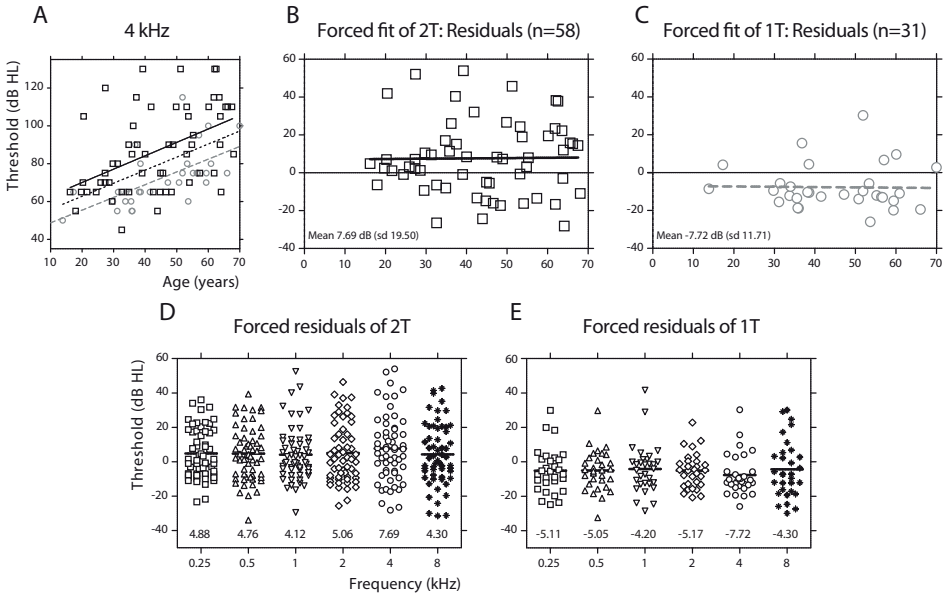
In conclusion, it can be stated that there is variability in the phenotypic presentation in USH2a. Overall, HI is congenital, progressive and typically presents with downsloping audiograms. Patients with two truncating mutations in *USH2A* show more severe and progressive sensorineural HI compared to USH2a patients without two truncating mutations. The exact mechanisms behind these phenotypic variations are not yet elucidated but we speculate that a complete lack of usherin due to two truncating mutations has a more negative effect on hearing physiology compared to the presence of an altered form of usherin in cases with one or two nontruncating mutations. This does not exclude possible effects of environmental factors, epigenetics or genetic modifiers. Further molecular studies are needed to clarify the function of usherin and to unfold the pathophysiology of hearing impairment caused by specific *USH2A* mutations.

REFERENCES

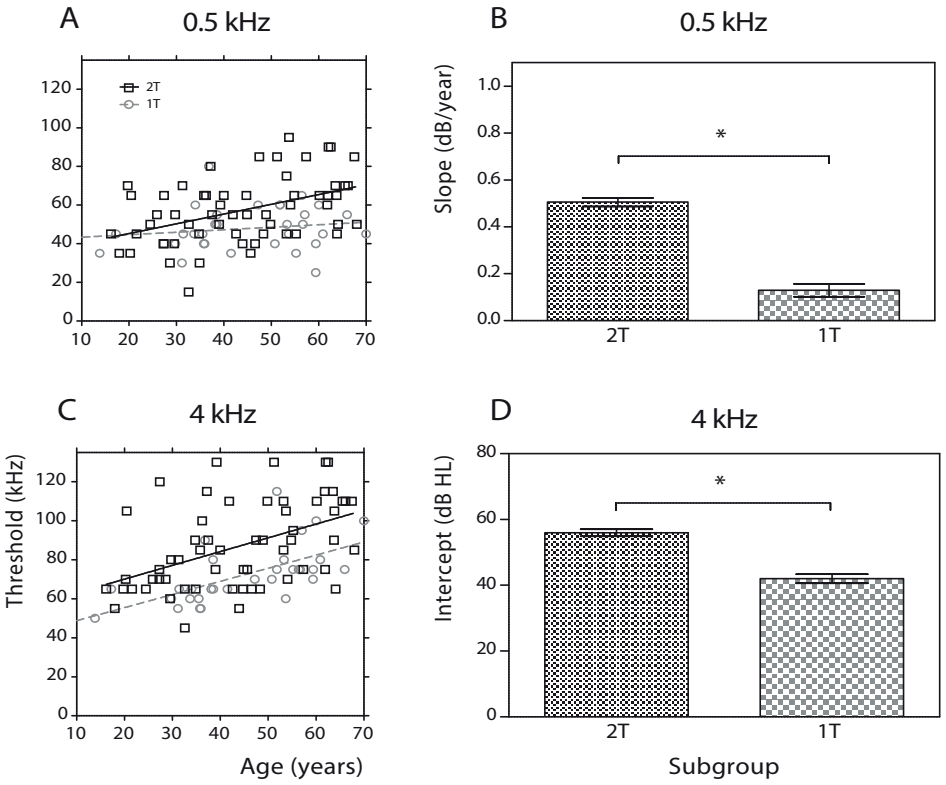
1. Pagon RA. Retinitis pigmentosa. Surv Ophthalmol 1988;33(3):137-177.
2. Boughman JA, Vernon M, Shaver KA. Usher syndrome: definition and estimate of prevalence from two high-risk populations. J Chronic Dis 1983;36(8):595-603.
3. Rosenberg T, Haim M, Hauch AM, et al. The prevalence of Usher syndrome and other retinal dystrophy-hearing impairment associations. Clin Genet 1997;51(5):314-321.
4. Spandau UH, Rohrschneider K. Prevalence and geographical distribution of Usher syndrome in Germany. Graefes Arch Clin Exp Ophthalmol 2002;240(6):495-498.
5. Smith RJ, Berlin CI, Hejtmancik JF, et al. Clinical diagnosis of the Usher syndromes. Usher Syndrome Consortium. Am J Med Genet 1994;50(1):32-38.
6. Cohen M, Bitner-Glindzicz M, Luxon L. The changing face of Usher syndrome: clinical implications. Int J Audiol 2007;46(2):82-93.
7. Plantinga RF, Kleemola L, Huygen PL, et al. Serial audiometry and speech recognition findings in Finnish Usher syndrome type III patients. Audiol Neurootol 2005;10(2):79-89.
8. Mathur P, Yang J. Usher syndrome: Hearing loss, retinal degeneration and associated abnormalities. Biochim Biophys Acta 2015;1852(3):406-420.
9. Ebermann I, Scholl HP, Charbel Issa P, et al. A novel gene for Usher syndrome type 2: mutations in the long isoform of whirlin are associated with retinitis pigmentosa and sensorineural hearing loss. Hum Genet 2007;121(2):203-211.
10. Eudy JD, Yao S, Weston MD, et al. Isolation of a gene encoding a novel member of the nuclear receptor superfamily from the critical region of Usher syndrome type IIa at 1q41. Genomics 1998;50(3):382-384.
11. van Wijk E, Pennings RJ, te Brinke H, et al. Identification of 51 novel exons of the Usher syndrome type 2A (*USH2A*) gene that encode multiple conserved functional domains and that are mutated in patients with Usher syndrome type II. Am J Hum Genet 2004;74(4):738-744.
12. Weston MD, Luijendijk MW, Humphrey KD, et al. Mutations in the VLGR1 gene implicate G-protein signaling in the pathogenesis of Usher syndrome type II. Am J Hum Genet 2004;74(2):357-366.
13. van Wijk E, van der Zwaag B, Peters T, et al. The DFNB31 gene product whirlin connects to the Usher protein network in the cochlea and retina by direct association with USH2A and VLGR1. Hum Mol Genet 2006;15(5):751-765.
14. Baux D, Larrieu L, Blanchet C, et al. Molecular and in silico analyses of the full-length isoform of usherin identify new pathogenic alleles in Usher type II patients. Hum Mutat 2007;28(8):781-789.
15. Dreyer B, Brox V, Tranebjaerg L, et al. Spectrum of *USH2A* mutations in Scandinavian patients with Usher syndrome type II. Hum Mutat 2008;29(3):451.
16. Pennings RJ, Te Brinke H, Weston MD, et al. *USH2A* mutation analysis in 70 Dutch families with Usher syndrome type II. Hum Mutat 2004;24(2):185.
17. Liu X, Bulgakov OV, Darrow KN, et al. Usherin is required for maintenance of retinal photoreceptors and normal development of cochlear hair cells. Proc Natl Acad Sci U S A 2007;104(11):4413-4418.
18. Michalski N, Michel V, Bahloul A, et al. Molecular characterization of the ankle-link complex in cochlear hair cells and its role in the hair bundle functioning. J Neurosci 2007;27(24):6478-6488.
19. Maerker T, van Wijk E, Overlack N, et al. A novel Usher protein network at the periciliary reloading point between molecular transport machineries in vertebrate photoreceptor cells. Hum Mol Genet 2008;17(1):71-86.
20. Sahly I, Dufour E, Schietroma C, et al. Localization of Usher 1 proteins to the photoreceptor calyceal processes, which are absent from mice. J Cell Biol 2012;199(2):381-399.

21. Zou J, Luo L, Shen Z, et al. Whirlin replacement restores the formation of the USH2 protein complex in whirlin knockout photoreceptors. Invest Ophthalmol Vis Sci 2011;52(5):2343-2351.
22. Rivolta C, Sweklo EA, Berson EL, et al. Missense mutation in the *USH2A* gene: association with recessive retinitis pigmentosa without hearing loss. Am J Hum Genet 2000;66(6):1975-1978.
23. Dreyer B, Tranebjaerg L, Brox V, et al. A common ancestral origin of the frequent and widespread 2299delG *USH2A* mutation. Am J Hum Genet 2001;69(1):228-234.
24. Isken O, Maquat LE. Quality control of eukaryotic mRNA: safeguarding cells from abnormal mRNA function. Genes Dev 2007;21(15):1833-1856.
25. Lenassi E, Robson AG, Luxon LM, et al. Clinical heterogeneity in a family with mutations in *USH2A*. JAMA Ophthalmol 2015;133(3):352-355.
26. Abadie C, Blanchet C, Baux D, et al. Audiological findings in 100 USH2 patients. Clin Genet 2012;82(5):433-438.
27. Pennings RJ, Huygen PL, Weston MD, et al. Pure tone hearing thresholds and speech recognition scores in Dutch patients carrying mutations in the *USH2A* gene. Otol Neurotol 2003;24(1):58-63.
28. Sadeghi M, Cohn ES, Kelly WJ, et al. Audiological findings in Usher syndrome types IIa and II (non-IIa). Int J Audiol 2004;43(3):136-143.
29. van Aarem A, Pinckers AJ, Kimberling WJ, et al. Stable and progressive hearing loss in type 2A Usher's syndrome. Ann Otol Rhinol Laryngol 1996;105(12):962-967.
30. Roux AF, Faugere V, Vache C, et al. Four-year follow-up of diagnostic service in USH1 patients. Invest Ophthalmol Vis Sci 2011;52(7):4063-4071.
31. Aller E, Najera C, Millan JM, et al. Genetic analysis of 2299delG and C759F mutations (*USH2A*) in patients with visual and/or auditory impairments. Eur J Hum Genet 2004;12(5):407-410.
32. Bernal S, Ayuso C, Antinolo G, et al. Mutations in *USH2A* in Spanish patients with autosomal recessive retinitis pigmentosa: high prevalence and phenotypic variation. J Med Genet 2003;40(1):e8.
33. Leijendeckers JM, Pennings RJ, Snik AF, et al. Audiometric characteristics of USH2a patients. Audiol Neurootol 2009;14(4):223-231.
34. Sadeghi AM, Cohn ES, Kimberling WJ, et al. Expressivity of hearing loss in cases with Usher syndrome type IIA. Int J Audiol 2013;52(12):832-837.
35. Wagenaar M, van Aarem A, Huygen P, et al. Hearing impairment related to age in Usher syndrome types 1B and 2A. Arch Otolaryngol Head Neck Surg 1999;125(4):441-445.
36. Huygen P, Pennings R, Cremers C. Characterizing and distinguishing progressive phenotypes in nonsyndromic autosomal dominant hearing impairment. Audiological Medicine 2003;1(1):37-46.
37. Davenport SLH, Omenn GS. The heterogeneity of Usher syndrome. In: Fifth International Conference on Birth Defects; 1977; Amsterdam: Excerpta Medica; 1977. p. 87-88.
38. Weil D, Blanchard S, Kaplan J, et al. Defective myosin VIIA gene responsible for Usher syndrome type 1B. Nature 1995;374(6517):60-61.
39. Pennings RJ, Fields RR, Huygen PL, et al. Usher syndrome type III can mimic other types of Usher syndrome. Ann Otol Rhinol Laryngol 2003;112(6):525-530.
40. Pennings RJ, Huygen PL, Cremers WR. Hearing impairment in Usher syndrome type II. Ann Otol Rhinol Laryngol 2003;112(9 Pt 1):825.
41. Pennings RJ, Huygen PL, Orten DJ, et al. Evaluation of visual impairment in Usher syndrome 1b and Usher syndrome 2a. Acta Ophthalmol Scand 2004;82(2):131-139.
42. Bork JM, Peters LM, Riazuddin S, et al. Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene CDH23. Am J Hum Genet 2001;68(1):26-37.
43. Pennings RJ, Topsakal V, Astuto L, et al. Variable clinical features in patients with CDH23 mutations (USH1D-DFNB12). Otol Neurotol 2004;25(5):699-706.

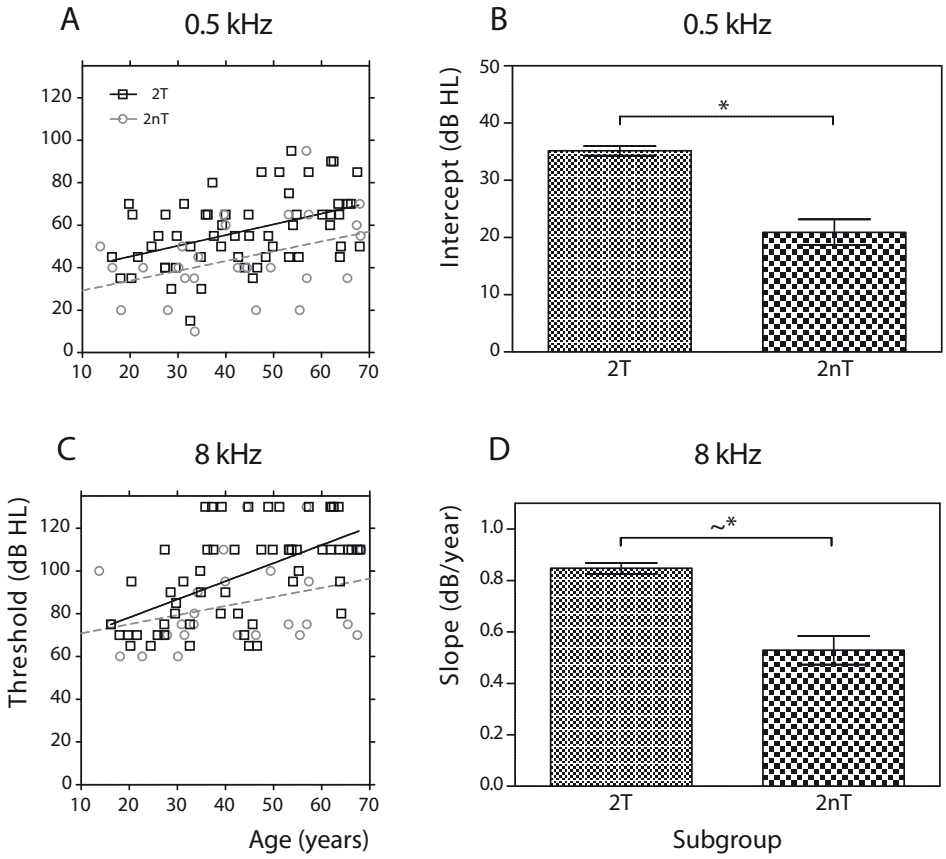
SUPPLEMENTAL MATERIALS



Supplemental Figure SF.1 (A-E). Plots explaining pairwise testing on the HI level developed throughout life between subgroups (2T and 1T). (A-C), Combined regression plots for both subgroups at 4 kHz, as an example, with a common dividing line (A), and plots of the residuals for both subgroups (B-C) forced around that line. (D-E), Outline of the procedure relating to all frequencies. (A), The (dotted) dividing line is the mean regression line, i.e., its slope and intercept each represent the mean value of the slopes and the intercepts of the regression lines pertaining to the respective subgroups 2T (black continuous line and open squares) and 1T (grey dashed line and open circles). (B-C), Residuals from forced linear regression of the thresholds at 4 kHz for subgroup 2T (B) and subgroup 1T (C) on age, using the obtained mean slope and intercept values as imposed regression parameter values. (D-E), Scatter plots showing the forced residuals at each frequency for subgroup 2T (D) and 1T (E). A paired t test (6 pairs) was applied to the forced residuals in both subgroups with a significant outcome ($P = 0.00018$).). The difference between the across-frequency means of the subgroups was 10.39 dB with a 95%CI of 7.67 to 13.10 dB. The conclusion is that the patients in subgroup 2T developed significantly more HI throughout life than the patients in subgroup 1T, with an overall mean, age-independent difference of ~10 dB.



Supplemental Figure SF.2 (A-D). Comparison between the subgroups 2T and 1T. (A), Plots with regression lines for the thresholds at 0.5 kHz. The dashed gray line represents the 1T subgroup; the continuous line represents the 2T subgroup. (B), Results of Tukey's test following ANOVA on the slopes for the subgroups at this frequency. Box and whisker plot showing mean and SD. Asterisk indicates a significant and substantial difference. (C), Regression plots for 4 kHz. (D), Results of Tukey's test following ANOVA on the intercepts for the subgroups at this frequency. The differences in slope and intercept were significant and substantial, as is shown in panels (B) and (D).



Supplemental Figure SF.3 (A-D). Comparison between the subgroups 2T and 2nT. Similar presentations as in figure 5, now for 0.5 kHz (A, B) and 8 kHz (C, D). The dashed gray line represents the 2nT subgroup, the continuous line represents the 2T subgroup. The differences in slope and intercept were significant. The difference in intercept (B) was also substantial (*), but the difference in slope (D) was marginally substantial (~*), i.e. close to but just under 0.20 dB/y.

Supplemental Table ST.1. Results of Tukey's tests following one-way ANOVAs on intercept and slope(A-B) for the pairwise comparison of subgroups 2T - 2nT (A),1T - 2nT (B), and results of Student's t test on intercept and slope (C-D) for the pairwise comparison of the subgroups c.2299delG homozygotes and 1T (C), as well as the subgroups c.2299delG homozygotes and 2nT (D). Further legend and abbreviations: see Table 3.

A. 2T versus 2nT (ANOVA with Tukey's test)

Frequency (kHz)	Δ(Intercept) (dB)				Δ(Slope) (dB/year)			
	Δ	***P* value**	**95%CI**	**Summary**	**Δ**	***P* value**	**95%CI**	**Summary**
0.25	12.64	*P* < 0.001	8.16 to 17.11	2T > 2nT	-0.07	*P* > 0.05	-0.17 to 0.02	
0.5	14.24	*P* < 0.001	9.70 to 18.78	2T > 2nT	-0.07	*P* > 0.05	-0.17 to 0.03	
1	9.15	*P* < 0.001	4.28 to 14.02	2T > 2nT	0.01	*P* > 0.05	-0.10 to 0.11	
2	12.05	*P* < 0.001	7.22 to 16.88	2T > 2nT	-0.13	*P* < 0.01	-0.24 to -0.03	2T < 2nT
4	7.64	*P* < 0.01	2.43 to 12.85	2T > 2nT	0.05	*P* > 0.05	-0.06 to 0.17	
8	-1.69	*P* > 0.05	-7.36 to 3.98		0.32	*P* < 0.001	0.20 to 0.44	2T > 2nT
Summary significant				**2T > 2nT in (5/6) 0.25-4 kHz**				2T > 2nT in (1/6) 8 kHz
Summary substantial				2T > 2nT in (3/6) 0.25-0.5, 2 kHz				

B. 1T versus 2nT (ANOVA with Tukey's test)

Frequency (kHz)	Δ(Intercept) (dB)				Δ(Slope) (dB/year)			
	Δ	***P* value**	**95%CI**	**Summary**	**Δ**	***P* value**	**95%CI**	**Summary**
0.25	16.42	*P* < 0.001	11.44 to 21.39	1T > 2nT	-0.39	*P* < 0.001	-0.49 to -0.28	1T < 2nT
0.5	21.14	*P* < 0.001	16.10 to 26.18	1T > 2nT	-0.45	*P* < 0.001	-0.56 to -0.34	1T < 2nT
1	6.34	*P* < 0.05	0.93 to 11.75	1T > 2nT	-0.12	*P* > 0.05	-0.23 to 0.001	
2	8.83	*P* < 0.001	3.49 to 14.19	1T > 2nT	-0.29	*P* < 0.001	-0.41 to -0.17	1T < 2nT
4	-6.30	*P* < 0.05	-12.09 to -0.51	1T < 2nT	0.02	*P* > 0.05	-0.11 to 0.15	
8	-9.80	*P* < 0.01	-16.08 to -3.52	1T < 2nT	0.31	*P* < 0.001	0.17 to 0.44	1T > 2nT
Summary significant				**1T > 2nT in 4/6 0.25-2 kHz**				**1T < 2nT in 3/6 0.25-0.5, 2 kHz**
				1T < 2nT in 2/6 4-8 kHz				1T > 2nT in 1/6 8 kHz
Summary substantial				1T > 2nT in 2/6 0.25-0.5 kHz				1T < 2nT in 2/6 0.25-0.5 kHz

C. c.2299delG homozygotes versus 1T (Student's t test)

Frequency (kHz)	Δ(Intercept) (dB)				Δ(Slope) (dB/year)			
	Δ	***P* value**	**95%CI**	**Summary**	**Δ**	***P* value**	**95%CI**	**Summary**
0.25	-3.11	0.48	-12.26 to 6.04		0.34	0.0014	0.15 to 0.53	delG > 1T
0.5	-13.59	0.0071	-22.89 to -4.29	delG < 1T	0.57	*P* < 0.001	0.38 to 0.76	delG > 1T
1	-9.85	0.0025	-16.03 to -3.67	delG < 1T	0.37	*P* < 0.001	0.24 to 0.50	delG > 1T
2	-7.54	0.057	-15.35 to 0.27		0.37	0.0001	0.21 to 0.53	delG > 1T
4	4.34	0.37	-5.68 to 14.36		0.20	0.054	-0.004 to 0.41	
8	1.62	0.71	-7.43 to 10.67		0.16	0.053	-0.002 to 0.32	
Summary significant				**delG < 1T in 2/6 0.5-1 kHz**				**delG > 1T in 4/6 0.25-2 kHz**
Summary substantial								delG > 1T in 3/6 0.5-2 kHz

D. c.2299delG homozygotes versus 2nT (Student's t test)

Frequency (kHz)	Δ(Intercept) (dB)				Δ(Slope) (dB/year)			
	Δ	***P* value**	**95%CI**	**Summary**	**Δ**	***P* value**	**95%CI**	**Summary**
0.25	13.31	0.0085	3.83 to 22.79	delG > 2nT	-0.04	0.65	-0.24 to 0.15	
0.5	7.55	0.13	-2.32 to 17.42		0.12	0.23	-0.08 to 0.33	
1	-3.51	0.34	-10.82 to 3.80		0.25	0.0025	0.10 to 0.41	delG > 2nT
2	1.29	0.76	-7.21 to 9.79		0.08	0.36	-0.10 to 0.26	
4	-1.96	0.68	-11.61 to 7.69		0.22	0.033	0.02 to 0.43	delG > 2nT
8	-8.18	0.073	-17.18 to 0.82		0.47	*P*< 0.001	0.27 to 0.66	delG > 2nT
Summary significant				delG > 2nT in 1/6 0.25 kHz				**delG > 2nT in 3/6 1, 4-8 kHz**
Summary substantial								delG > 2nT in 1/6 8 kHz

Supplemental Table ST.2. Spectrum of *USH2A* mutation combinations in this study.

Abbreviations: see Table 2.

2T		
Allele 1	**Allele 2**	**Patients (%)**
c.187C>T, p.(Arg63*)	c.187C>T, p.(Arg63*)	3 (2.7)
c.238_239insCGTA, p.(Thr80Argfs*29)	c.238_239insCGTA, p.(Thr80Argfs*29)	1 (0.9)
Tac.545_546delAA, p.(Lys182Argfs*33)	c.11875_11876delCA, p.(Gln3959Asnfs*53)	1 (0.9)
c.779T>G, p.(Leu260*)	c.2299delG, p.(Glu767Serfs*21)	1 (0.9)
c.779T>G, p.(Leu260*)	c.10450C>T, p.(Arg3484*)	1 (0.9)
c.779T>G, p.(Leu260*)	c.4628-30487_6325+8822del, p.(Gly1542_ Leu2110delinsAsp)	1 (0.9)
c.920_923dup, p.(His308Serfs*16)	c.920_923dup, p.(His308Serfs*16)	1 (0.9)
c.949C>A , p.(Tyr318Cysfs*17)	c.4773del, p.(Val1592*)	1 (0.9)
c.949C>A , p.(Tyr318Cysfs*17)	c.11864G>A, p.(Trp3955*)	1 (0.9)
c.949C>A , p.(Tyr318Cysfs*17)	c.2299delG, p.(Glu767Serfs*21)	1 (0.9)
c.1227G>A, p.(Trp409*)	c.1227G>A, p.(Trp409*)	3 (2.7)
c.1227G>A, p.(Trp409*)	c.2299delG, p.(Glu767Serfs*21)	2 (1.8)
c.1876C>T, p.(Arg626*)	c.1876C>T, p.(Arg626*)	1 (0.9)
c.1876C>T, p.(Arg626*)	c.2299delG, p.(Glu767Serfs*21)	1 (0.9)
c.2242C>T, p.(Gln748*)	c.4405C>T, p.(Gln1469*)	1 (0.9)
c.2299delG, p.(Glu767Serfs*21)	c.2299delG, p.(Glu767Serfs*21)	15 (13.6)
c.2299delG, p.(Glu767Serfs*21)	c.7121-8313_11048-962delins12, del Ex 38-56	1 (0.9)
c.2299delG, p.(Glu767Serfs*21)	c.11875_11876delCA, p.(Gln3959Asnfs*53)	1 (0.9)
c.2299delG, p.(Glu767Serfs*21)	c.15053-2A>T, p.(?)	1 (0.9)
c.2983C>T, p.(Gln995*)	c.2983C>T, p.(Gln995*)	2 (1.8)
c.3932C>A, p.(Ser1311*)	c.10450C>T, p.(Arg3484*)	2 (1.8)
c.3932C>A, p.(Ser1311*)	c.13207-13208delGG, p.(Gly4403Profs*15)	1 (0.9)
c.4628-30487_6325+8822del, p.(Gly1542_Leu2110delinsAsp)	c.4628-30487_6325+8822del, p.(Gly1542_ Leu2110delinsAsp)	1 (0.9)
c.7121-8313_11048-962delins12, del Ex 38-56	c.7121-8313_11048-962delins12, del Ex 38-56	2 (1.8)
c.7121-8313_11048-962delins12, del Ex 38-56	c.8954delG, p.(Gly2985Alafs*3)	2 (1.8)
c.8079G>A, p.(Trp2693*)	c.8079G>A, p.(Trp2693*)	1 (0.9)
c.8557A>T, p.(Arg2853*)	c.4628-30487_6325+8822del, p.(Gly1542_ Leu2110delinsAsp)	2 (1.8)
c.8682-9A>G, IVS43-9A>G	c.8682-9A>G, IVS43-9A>G	1 (0.9)
c.9372-?_9570+?, del Ex 48	c.9372-?_9570+?, del Ex 48	1 (0.9)
c.10450C>T, p.(Arg3484*)	c.10684G>T, p.(Glu3562*)	1 (0.9)
c.10525A>T, p.(Lys3509*)	c.2299delG, p.(Glu767Serfs*21)	1 (0.9)
c.11864G>A, p.(Trp3955*)	c.11864G>A, p.(Trp3955*)	3 (2.7)
Total		**58 (52.7)**

1T		
Allele 1	**Allele 2**	**Patients (%)**
c.187C>T, p.(Arg63*)	c.1036A>C, p.(Ans346His)	1 (0.9)
c.920_923dup, p.(His308Serfs*16)	c.1036A>C, p.(Ans346His)	1 (0.9)
c.1227G>A, p.(Trp409*)	c.1256G>T, p.(Cys419Phe)	2 (1.8)
c.1227G>A, p.(Trp409*)	c.13262T>C, p.(Leu4421Pro)	2 (1.8)
c.1876C>T, p.(Arg626*)	c.10561T>C, p.(Trp3521Arg)	1 (0.9)
c.2299delG, p.(Glu767Serfs*21)	c.1036A>C, p.(Ans346His)	2 (1.8)
c.2299delG, p.(Glu767Serfs*21)	c.1256G>T, p.(Cys419Phe)	3 (2.7)
c.2299delG, p.(Glu767Serfs*21)	c.1606T>C, p.(Cys536Arg)	3 (2.7)
c.2299delG, p.(Glu767Serfs*21)	c.9815C>T, p.(Pro3272Leu)	1 (0.9)
c.3558delT, p.(Cys1186Trpfs*51)	c.1256G>T, p.(Cys419Phe)	1 (0.9)
c.4628-30487_6325+8822del, p.(Gly1542_Leu2110delinsAsp)	c.12695C>G, p.(Pro4232Arg)	1 (0.9)
c.7121-8313_11048-962delins12, del Ex 38-56	c.1256G>T, p.(Cys419Phe)	2 (1.8)
c.7121-8313_11048-962delins12, del Ex 38-56	c.6722C>T, p.(Pro2241Leu)	1 (0.9)
c.7931G>A, p.(Trp2644*)	c.11819A>C, p.(Tyr3940Ser)	2 (1.8)
c.8557A>T, p.(Arg2853*)	c.802G>A, p.(Gly268Arg)	1 (0.9)
c.10525A>T, p.(Lys3509*)	c.1256G>T, p.(Cys419Phe)	2 (1.8)
c.11864G>A, p.(Trp3955*)	c.1256G>T, p.(Cys419Phe)	1 (0.9)
c.11864G>A, p.(Trp3955*)	c.2276G>T, p.(Cys759Phe)	1 (0.9)
c.13822C>T, p.(Arg4608*)	c.1036A>C, p.(Ans346His)	2 (1.8)
c.14131C>T, p.(Gln4711*)	c.1606T>C, p.(Cys536Arg)	1 (0.9)
Total		**31 (28.2)**

2nT		
Allele 1	**Allele 2**	**Patients (%)**
c.653T>A, p.(Val218Glu)	c.14408T>C, p.(Ile4808Thr)	1 (0.9)
c.1036A>C, p.(Ans346His)	c.1036A>C, p.(Ans346His)	4 (3.6)
c.1036A>C, p.(Ans346His)	c.2276G>T, p.(Csy759Phe)	1 (0.9)
c.1036A>C, p.(Ans346His)	c.4810G>C, p.(Asp1604His)	1 (0.9)
c.1256G>T, p.(Cys419Phe)	c.1256G>T, p.(Cys419Phe)	2 (1.8)
c.1256G>T, p.(Cys419Phe)	c.1606T>C, p.(Cys536Arg)	7 (6.4)
c.1256G>T, p.(Cys419Phe)	c.2276G>T, p.(Csy759Phe)	1 (0.9)
c.1256G>T, p.(Cys419Phe)	c.9815C>T, p.(Pro3272Leu)	1 (0.9)
c.1256G>T, p.(Cys419Phe)	c.10421A>G, p.(Tyr3474Cys)	1 (0.9)
c.5018T>C, p.(Leu1673Pro)	c.5018T>C, p.(Leu1673Pro)	1 (0.9)
c.10561T>C, p.(Trp3521Arg)	c.10561T>C, p.(Trp3521Arg)	1 (0.9)
Total		**21 (19.1)**

Chapter 2.2

Visual prognosis in *USH2A*-associated retinitis pigmentosa is worse for patients with Usher syndrome type IIa than for those with nonsyndromic retinitis pigmentosa

ABSTRACT

Objectives: *USH2A* mutations are an important cause of retinitis pigmentosa (RP) with or without congenital sensorineural hearing impairment. We studied genotype-phenotype correlations and compared visual prognosis in patients with Usher syndrome type IIa (USH2a) and *USH2A*-associated nonsyndromic RP (nsRP).

Design and participants: Clinic-based, longitudinal, multicenter study. Consecutive patients with USH2a (n=152) and *USH2A*-associated nsRP (n=73) from ophthalmogenetic clinics in the Netherlands and Belgium.

Methods: Data on clinical characteristics, visual acuity, visual field measurements, retinal imaging, and electrophysiological features were extracted from medical charts over a mean follow-up of nine years. Cumulative lifetime risks of low vision and blindness were estimated using Kaplan-Meier survival analysis.

Results: Participant groups had similar distributions of gender (48% versus 45% males in USH2a versus *USH2A*-associated nsRP; P=0.8), ethnicity (97% versus 99% European; P=0.3), and median follow-up time (6.5 years versus 3 years; P=0.3). USH2a patients demonstrated symptoms at a younger age (median age, 15 years versus 25 years; P<0.001), were diagnosed earlier (median age, 26 years versus 36.5 years; P<0.001), and became visually impaired 13 years earlier (median age, 41 years versus 54 years; P<0.001) based on VF and 18 years earlier based on VA (median age, 54 years versus 72 years; P<0.001) than *USH2A*-associated nsRP patients. The presence of two truncating mutations in *USH2A* was associated mostly with the syndromic phenotype, whereas other combinations were present in both groups. We found novel variants in USH2a (25%) and *USH2A*-associated nsRP (19%): 29 missense mutations, 10 indels, 14 nonsense mutations, nine frameshift mutations, and five splice-site mutations.

Conclusions: Most patients with *USH2A*-associated nsRP have severe visual impairment by age 50. However, those with USH2a have an earlier decline of visual function and a higher cumulative risk of visual impairment than those with *USH2A*-associated nsRP. Complete loss of function of the *USH2A* protein predisposes to USH2a, but remnant protein function can lead to RP with or without hearing loss.

Published as: Pierrache, L.H., Hartel, B.P., van Wijk, E., Meester-Smoor, M.A., Cremers, F. P., de Baere, E., de Zaeytijd, J., van Schooneveld, M.J., Cremers, C.W., Dagnelie, G., Hoyng, C.B., Bergen, A.A., Leroy, B.P., Pennings, R.J., van den Born, L.I., & Klaver, C.C. (2016). Visual Prognosis in *USH2A*-Associated Retinitis Pigmentosa Is Worse for Patients with Usher Syndrome Type IIa Than for Those with Nonsyndromic Retinitis Pigmentosa. *Ophthalmology, 123*(5), 1151-1160. doi: 10.1016/j.ophtha.2016.01.021

INTRODUCTION

Retinitis pigmentosa (RP) is a genetic disorder marked by progressive retinal degeneration leading to severe visual impairment. Patients initially experience nightblindness and visual field (VF) constriction resulting from rod degeneration, followed by deterioration of central vision caused by loss of cone function. Mutations in *USH2A* form a substantial cause of RP and can give rise to two distinct phenotypes: Usher syndrome type IIa (USH2a) and nonsyndromic RP (*USH2A*-associated nsRP). Patients with USH2a are characterized by RP and congenital sensorineural hearing impairment, whereas patients with *USH2A*-associated nsRP do not experience extraocular symptoms.

The *USH2A* gene is located on chromosome 1q41 and codes for the usherin protein, which plays an important role in the development of cochlear hair cells and long-term maintenance of photoreceptors. There are two isoforms, a short isoform of 170 kDa, which is translated from 21 exons,[1] and a long isoform of 580 kDa, translated from an extra 51 exons.[2] Both isoforms are expressed in the photoreceptors and cochlear hair cells. Approximately 50% to 75% of Usher syndrome patients and 12% to 25% of nsRP patients carry mutations in *USH2A*, making it one of the most important mutated genes in these populations.[3] Generally, patients with USH2a can be identified early because of their congenital hearing impairment. At this young age, most photoreceptors are still viable and would be amenable targets for gene replacement therapy.[4] *USH2A* is a challenge for developers of gene therapy because the size of the gene largely exceeds the capacity of adeno-associated virus and lentivirus vectors. Other strategies such as antisense oligonucleotide-based therapy [5] and cell replacement therapy with induced pluripotent stem cells seem promising.[4] As treatment options for *USH2A* mutations become apparent, it is important to identify individuals who have the greatest chance to benefit from these therapies. Therefore, being able to predict the course of the disease early in the process is highly desirable.

Thus far, genotype-phenotype correlations have not been very distinct in patients with *USH2A* mutations. Certain mutations in *USH2A*, such as p.(Glu767Serfs*21), have been associated mainly with the syndromic phenotype.[6] Others seem to have a predilection for *USH2A*-associated nsRP, such as p.(Cys759Phe).[7] It remains unknown why some mutations in *USH2A* lead to USH2a and others to *USH2A*-associated nsRP. A great number of mutations are missense mutations and are private, which means that they are observed only in one family.[6, 8, 9] In addition, most patients are compound heterozygotes because they carry different mutations on the maternal and paternal allele. This makes it even more difficult to predict the effect of each of these mutations on the phenotype and complicates assessing a possible allelic hierarchy.[10] Affected siblings are prone to having the same phenotype, but differences in severity occur.[11] This suggests that each phenotype may be caused by a distinct set of genotypes.

The aim of this study was to investigate the visual prognosis in a large series of patients with retinal degenerations resulting from *USH2A* mutations. We compared the course of disease in patients with USH2a with that of patients with *USH2A*-associated nsRP and aimed to investigate whether their genetic constitutions can predict the progression of visual function loss.

PATIENTS AND METHODS

Study population

We ascertained 225 consecutive subjects with RP resulting from mutations in *USH2A* from five ophthalmogenetic clinics in the Netherlands and Belgium. Of these, 152 had a diagnosis of congenital hearing impairment based on audiologic test results and were identified as having USH2a (Figure 1). The remaining 73 did not have childhood-onset hearing impairment and were classified as having *USH2A*-associated nsRP. We included siblings and offspring of 22 probands in our cohort. There were 18 USH2a families and four families with *USH2A*-associated nsRP in total; the same phenotype was observed in all siblings. The study adhered to the tenets of the Declaration of Helsinki, and all procedures were reviewed by the Medical Ethics Committee of Erasmus Medical Centre and

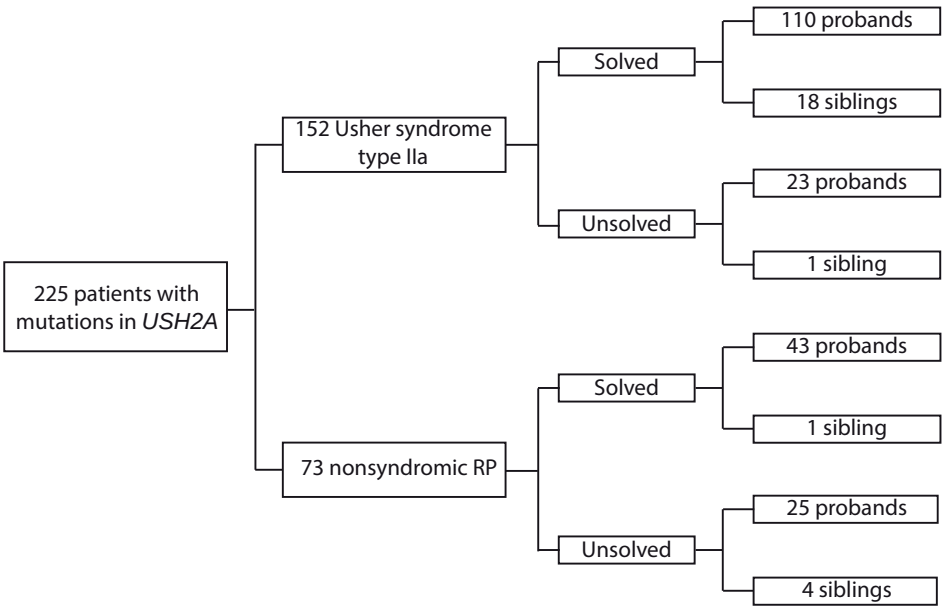


Figure 1. Flow chart of subjects included in the study.

Patients with biallelic pathogenic mutations were defined as solved, and patients with only 1 known pathogenic mutation were considered unsolved. RP = retinitis pigmentosa

the Medical Ethics Committee of the University Hospital of Ghent. Participants provided a written informed consent to retrieve data from medical records.

Clinical examination

We strived to establish a database with virtually complete longitudinal data. Participants were queried for all ophthalmologists and otolaryngologists they had consulted during their lifetimes, and medical records were retrieved. We gathered longitudinal data from 171 patients and examined 54 patients cross-sectionally. Eye examinations were performed in accordance with good clinical practice at regular intervals by a small number of ophthalmologists (n = 6) with expertise in ophthalmogenetics and included best-corrected Snellen visual acuity (VA), Goldmann VFs, electroretinography (full-field electroretinography according to the standards of the International Society for Clinical Electrophysiology of Vision; available at www.iscev.org), colour vision testing, slit-lamp examination, and ophthalmoscopy. Not all of these examinations had been performed at each visit, and not all participants had undergone all examinations. We digitized VF retinal area of the V4 target using a method described by Dagnelie.[12]

Genetic Analyses

To provide the molecular diagnosis, different molecular testing approaches were used over the course of 20 years (1996-2015). In the initial years, participants were analysed with polymerase chain reaction amplification and subsequent Sanger sequencing. From 2006 through 2013, participants were analysed with an Usher syndrome APEX (arrayed primer extension) genotyping micro-array or an autosomal recessive RP APEX genotyping microarray. Sanger direct sequencing was performed subsequently to confirm the identified mutation(s). When only 1 heterozygous mutation in *USH2A* was found, the entire *USH2A* gene was sequenced to screen for a second pathogenic mutation. From 2014 onward, ophthalmogenetic laboratories used targeted next-generation sequencing of 160 genes associated with hereditary blindness or whole-exome sequencing to identify mutations for RP.[13, 14] Pathogenicity of mutations was scored using Combined Annotation Dependent Depletion (CADD).[15]

Statistical Analyses

Differences in age at onset and age at diagnosis were compared using a Wilcoxon-Mann-Whitney test. To compare differences in gender ratio, ethnicity, and refractive error, we used a chi-square test. Outcome variables were low vision and blindness. These functional stages were based on VA and VF and were in accordance with World Health Organization standards. Visual impairment was defined as either low vision ($0.05 \leq VA < 0.3$, $10° \leq VF < 20°$ central VF diameter, or both) or blindness ($VA < 0.05$, central VF diameter $< 10°$, or both). Lifetime cumulative risk of the outcome variables was esti-

mated using Kaplan-Meier product-limit survival analysis. The log-rank test was used to determine the statistical significance of risk differences. Analyses were stratified for clinical diagnosis and the number of truncating variants present, because these variants are predicted to lead to nonsense-mediated decay, significant truncation of the protein, or both if translated. Progression of VF loss was evaluated with mixed- model analysis.

RESULTS

Clinical Characteristics

The average follow-up time did not differ significantly between the groups (Table 1) and was 9 years on average with a maximum of 43 years. Participants with USH2a in our cohort were younger than participants with *USH2A*-associated nsRP, but did not differ in gender. The vast majority of participants in both groups were of European descent. All participants had at least two RP hallmarks on fundus examination, that is, the presence of a waxy optic disc, narrow blood vessels, midperipheral and peripheral bone spicules,

Table 1. Distribution of clinical characteristics in patients with *USH2A* mutations.

Characteristics	*USH2a* (n=152)	*USH2A*-associated nsRP (n=73)	*P* value
Mean age ± SD, yrs	48 (±2.5)	55 (±3.3)	<0.001[a]
Male gender, no. (%)	73 (48)	33 (45)	0.8[b]
Median follow-up (range) (y)	6.5 (0-43)	3 (0-37)	0.3[c]
European ethnicity, no. (%)	148 (97)	72 (99)	0.3[b]
Median refractive error (SE; n=189), D	-1.50 (-16 to +5)	-0.75 (-14 to +4)	0.2[c]
High myopia (SE < - 6 D), no. (%)	9 (7)	6 (9)	0.3[b]
Mild myopia (-2D > SE > - 6D) no. (%)	51 (39)	16 (25)	
Emmetropia (2 D > SE > -2 D), no. (%)	66 (50)	38 (60)	
Hyperopia (2 D > SE), no. (%)	6 (5)	4 (6)	
Cataract extraction			
Right Eye, no.	30	21	
Median age (range), yrs. (n = 46)	45 (27-64)	56 (31-73)	0.002[c]
Left eye, no.	32	19	
Median age (range), yrs. (n=46)	43 (28-64)	57 (32-78)	0.003[c]
Electroretinography results (n=142), no.(%)			<0.001[b]
Reduced rods and cones	1 (1)	18 (35)	
Rods extinguished, reduced cones	9 (9)	9 (17)	
Rods and cones extinguished	86 (90)	26 (48)	

[a] Student t-test, [b] Chi Square test, [c] Wilcoxon–Mann–Whitney test, SE = spherical equivalent, USH2a = Usher syndrome type IIa, D = Diopter, SD = standard deviation

and atrophy of the retinal pigment epithelium. There were no differences in refractive error between the patient groups (Table 1). Subcapsular cataract was common (overall median age, 48 years), and 46 patients (21%) had undergone cataract extraction. Participants with USH2a underwent cataract extraction approximately 10 years earlier than participants with *USH2A*-associated nsRP (Table 1).

Visual Function

Subjects with USH2a were younger at age of onset of symptoms, that is, nightblindness and VF constriction, and were diagnosed with RP at a younger age (Table 2). The VF constriction preceded VA loss in all but 1 patient who had irreversible central visual loss resulting from longstanding cystoid macular edema. Participants with USH2a became visually impaired approximately 13 years earlier based on VF constriction criteria (VF < 20°; $P<0.001$) and 18 years earlier based on VA criteria (VA < 0.3; $P<0.001$) than subjects with *USH2A*-associated nsRP (Table 2; Figures 2 and 3). At 50 years of age, 83% of participants with USH2a had VF constriction versus only 40% of participants with *USH2A*-associated nsRP. These numbers were 46% versus 6% for VA. Exclusion of relatives did not alter these risk estimates significantly (data not shown). To validate our findings, we repeated the analyses and excluded subjects with a single *USH2A* mutation. This exclusion did not alter our estimates (Table 3, available as supplemental table at *http://www.aaojournal.org*). Progression of VF loss was evaluated with mixed-model analysis. At baseline (intercept), there was no difference between subjects with USH2a and *USH2A*-associated nsRP ($P=0.3$), but with aging, the difference in progression became statistically significant ($P<0.01$; Figure 4).

Table 2. Comparison of age difference at onset of disease, at diagnosis, and at onset of low vision and legal blindness

Median Age Time Point (yrs)	USH2a (n=152)	*USH2A*-associated nsRP (n=73)	*P* value
Onset of symptoms	15 (0-46)	25 (0-68)	<0.001[a,c]
Diagnosis	26 (8-56)	36.5 (12-74)	<0.001[a,c]
Low vision based on VF (n=176)	41 (15-67)	54 (32-78)	<0.001[b,c]
Low vision based on VA (n=225)	54 (20-74)	72 (15-72)	<0.001[b,c]
Legal blindness based on VF (n=176)	54 (19-58)	80 (34-80)	0.01[b,c]
Legal blindness based on VA (n=225)	74 (32-74)	77 (49-77)	0.01[b,c]

[a] Wilcoxon–Mann–Whitney test, [b] Log-rank test, [c] $P>0.008$, after Bonferroni correction. USH2a = Usher syndrome type IIa, nsRP = nonsyndromic retinitis pigmentosa, VA = visual acuity, VF = visual field. Data are median (range).

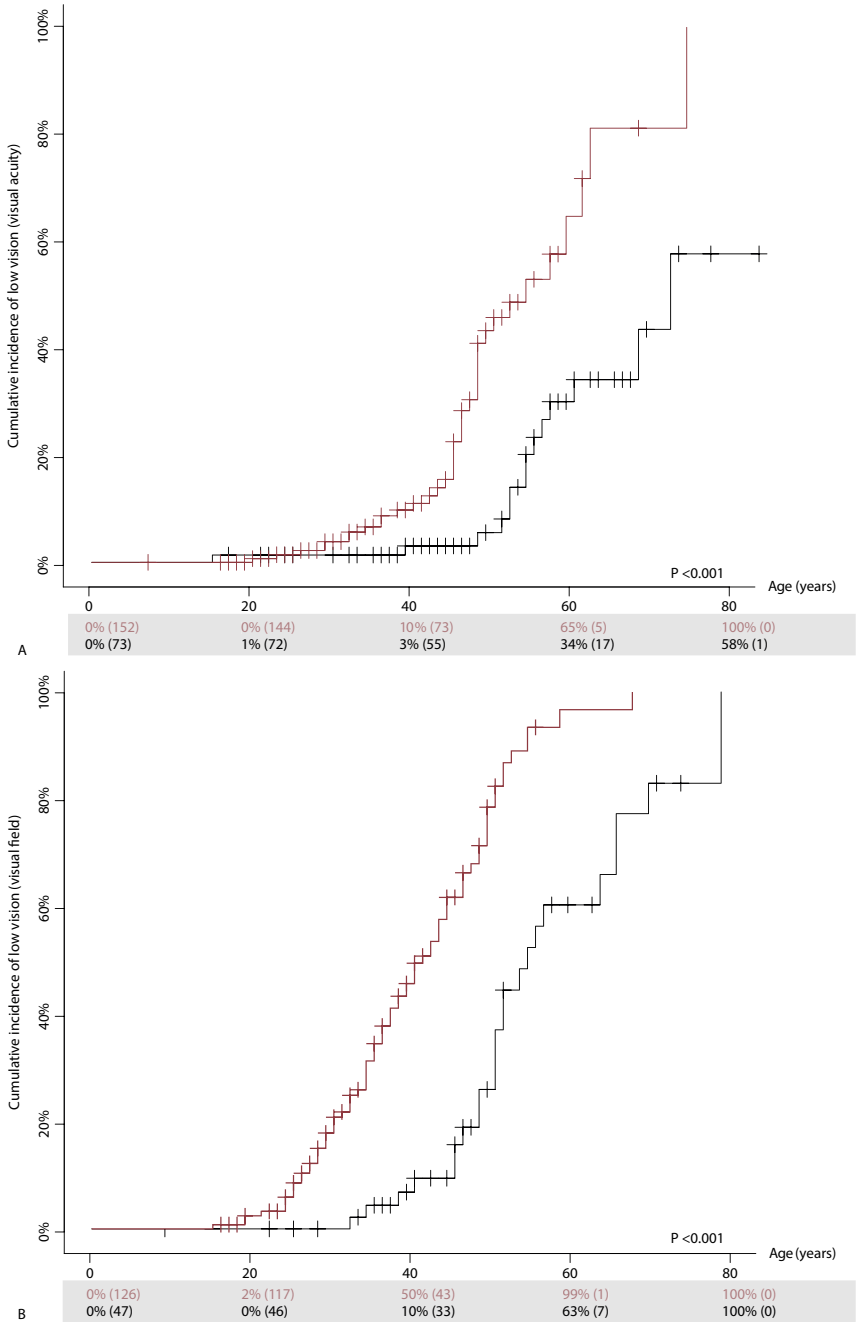


Figure 2. Graphs showing cumulative incidence (%) of low vision based on (A) visual acuity of less than 0.3 and (B) GVF of less than 20 as a function of age stratified for Usher syndrome type IIa and *USH2A*-associated nonsyndromic retinitis pigmentosa (nsRP). The cumulative incidence of low vision at 0, 20, 40, 60, and 80 years of age is indicated in the grey box below the graph. Red = Usher syndrome type IIa; Black =*USH2A*-associated nsRP

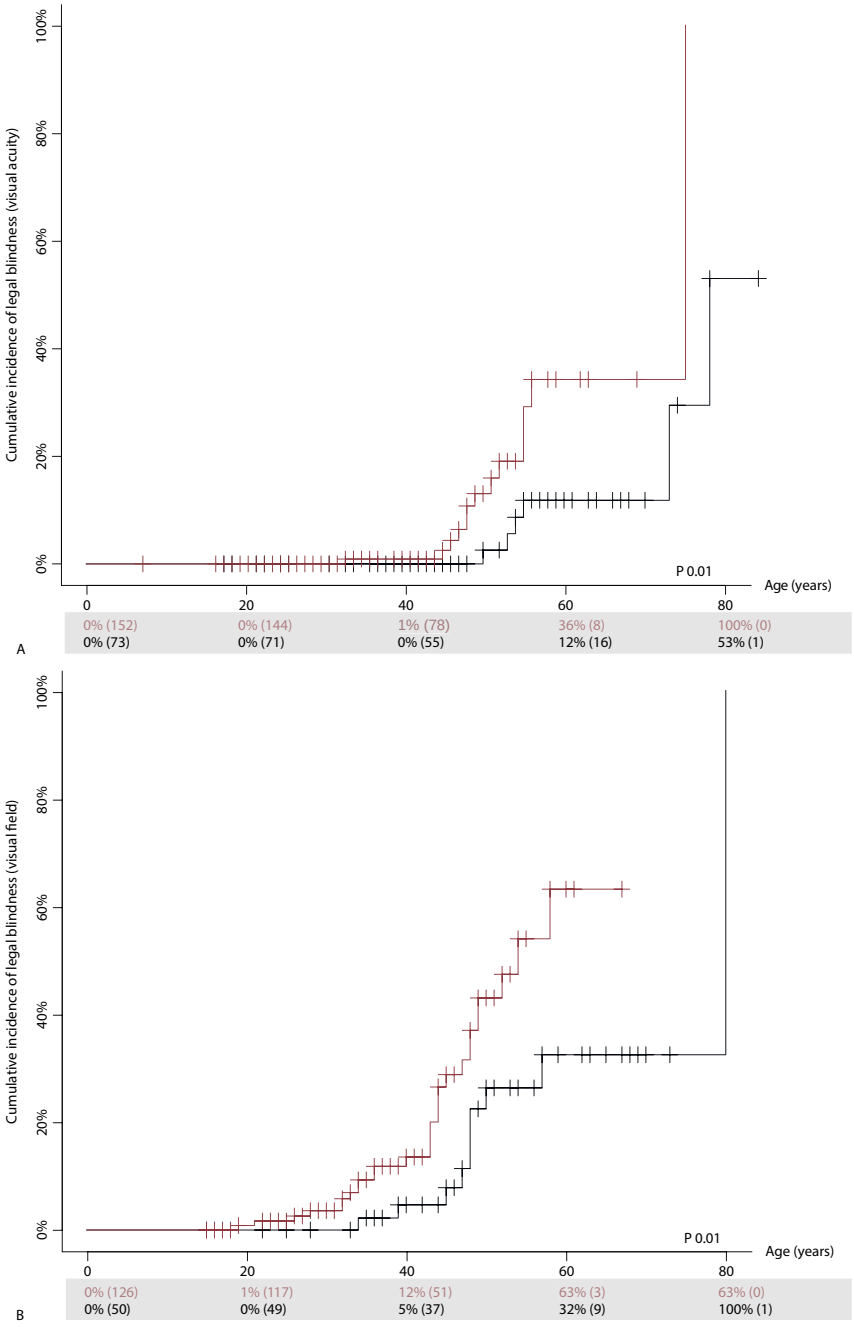


Figure 3. Graphs showing cumulative incidence (%) of legal blindness based on (A) visual acuity of less than 0.05 and (B) GVF of less than 10 as a function of age stratified for Usher syndrome type IIa and *USH2A*-associated nonsyndromic retinitis pigmentosa (nsRP). The cumulative incidence of legal blindness at 0, 20, 40, 60, and 80 years of age is indicated in the grey box below the graph. Red = Usher syndrome type IIa; Black = *USH2A*-associated nsRP

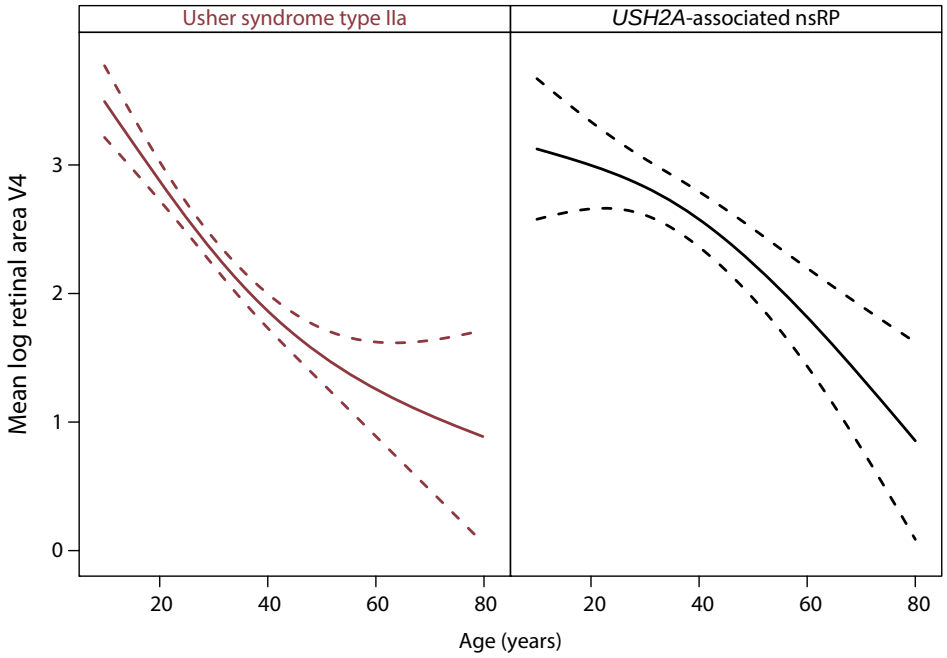


Figure 4. Graphs showing the relationship between mean log retinal area and age. Solid line = mixed model estimation; dashed line = confidence interval. RP = retinitis pigmentosa

Genotype

Pathogenic mutations on both alleles were found in 160 participants (71%), among whom 31 (19%) were homozygous and 129 (81%) were compound heterozygous. In 53 patients (24%), only one pathogenic mutation was detected, and in 12 participants (5%), multiple mutations (≥3) in *USH2A* were found (Figure 1). One hundred twenty-eight different mutations were recorded, of which 65 were missense mutations, 10 were insertions or deletions, 30 were nonsense mutations, 16 were frame-shift mutations, and seven were splice-site mutations. Sixty-eight of these variants were newly identified, because they were not present in the Leiden Open Variant Database (accessed July 17, 2015): 29 missense mutations, 10 insertions or deletions, 14 nonsense mutations, nine frame-shift mutations, and five splice-site mutations (Table 4, available as supplemental table at *http://www.aaojournal.org*). The most common mutations in syndromic participants were p.(Glu767Serfs*21) (65 of 250 observed *USH2A* variants), p.(Cys419Phe) (29 of 250 observed *USH2A* variants), and p.(Cys536Arg) (15 of 250 observed *USH2A* variants). The most frequent mutations in *USH2A*-associated nsRP participants were p.(Cys759Phe) (33 of 130 observed *USH2A* variants), p.(Glu767Serfs*21) (13 of 130 observed *USH2A* variants), and p.(Arg4115Cys) (8 of 130 observed *USH2A* variants; Table 4, available as supplemental table at *http://www.aaojournal.org*). Twenty-one of the 225 participants

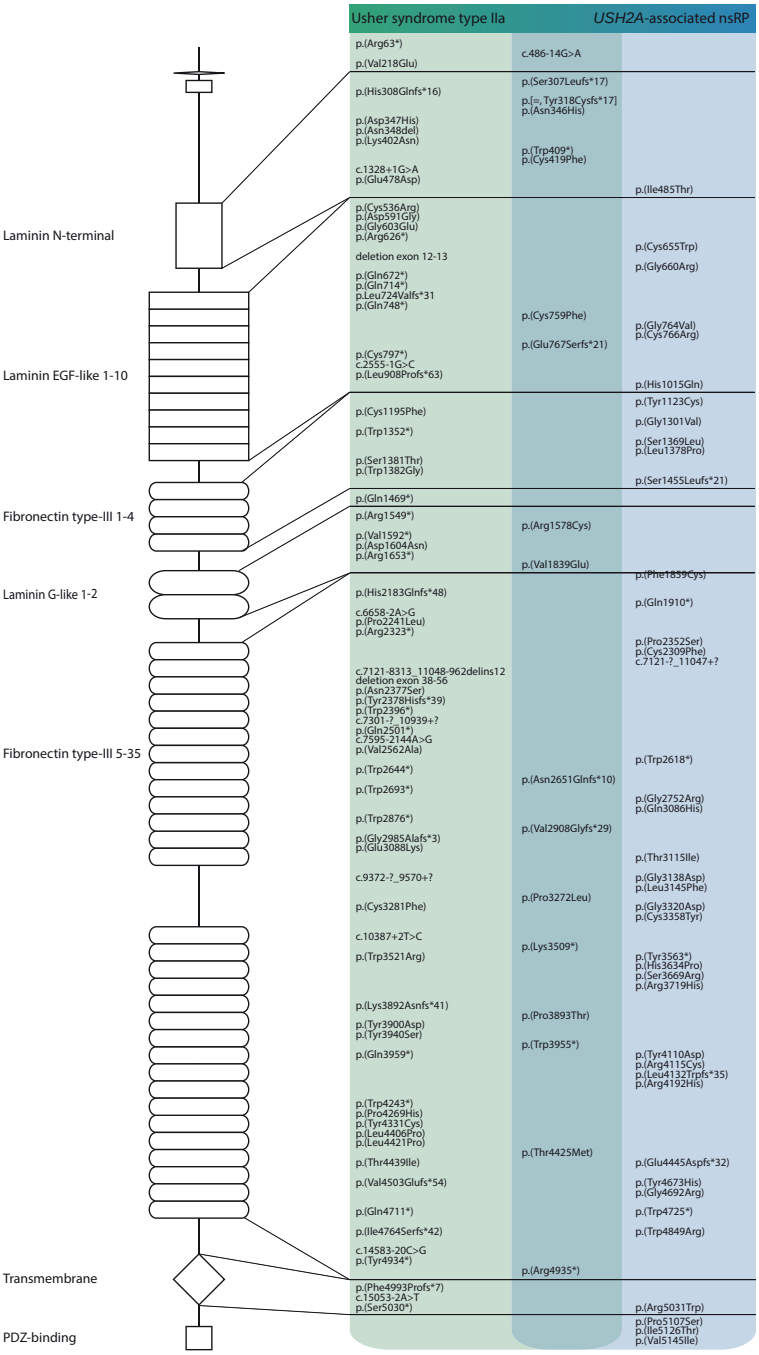


Figure 5. Schematic representation of the usherin protein and localisation of mutations. Mutations in the first column were found only in participants with Usher syndrome type IIa. Mutations in the last column were found only in the *USH2A*-associated nonsyndromic retinitis pigmentosa participants. Mutations in the middle column were present in both phenotype groups.

(9%) resulted from a consanguineous marriage, and in only 10 of them did we find a homozygous mutation. In the remaining participants carrying homozygous mutations, p.(Glu767Serfs*21) was the most frequently observed mutation (n = 12). Figure 5 shows the protein structure of usherin and the locations of mutations in the protein per phenotype. Strikingly, participants with two mutations in the N-terminal laminin domain always had the USH2a phenotype at presentation, independent of the effect on the protein (Table 5, available as supplemental table at *http://www.aaojournal.org*).

We stratified all variants into two groups: truncating variants (also referred to as 'inactivating' or 'null variants') and nontruncating variants. Participants carrying two nontruncating variants or one nontruncating and one truncating variant had both phenotypes at presentation. All but one participant carrying truncating variants on both alleles had USH2a. The number of truncating variants present seems to be associated with an earlier decline in visual function (Table 6). As the number of truncating variants increases, participants become visually impaired earlier in life. More detailed analysis of pathogenicity was performed using CADD scores. Most variants exceeded a CADD score of 15, the cut-off score suggested by the authors to identify potentially pathogenic variants. No significant correlations between the score and age at diagnosis or age at of visual impairment were found (data not shown). The CADD score can be used to predict whether a variant is deleterious in diagnostics, but we could not use it as a proxy for remaining protein function.

Table 6. Mutations in Usher syndrome type IIa and *USH2A*-associated nsRP

	Nontruncating Nontruncating	Nontruncating Truncating	Truncating Truncating	*P* value
USH2a, no. (%)	19 (16)	48 (40)	54 (44)	< 0.001[a]
USH2A-associated nsRP, no. (%)	14 (36)	24 (62)	1 (3)	
Median age low vision by VA, yrs (n=160)	59	57	48	0.4[b]
Median age low vision by GVF, yrs (n=124)	45	48	38	0.06[b]

[a] Chi square test, [b] Log-rank test, USH2a = Usher syndrome type IIa, nsRP = nonsyndromic retinitis pigmentosa, GVF = Goldmann visual field, VA = visual acuity

DISCUSSION

In this study, we compared the visual course between subjects with USH2a and *USH2A*-associated nsRP. We found that USH2a patients demonstrated visual symptoms at an earlier age, had an earlier onset of disease, and became visually impaired at a younger age than participants with *USH2A*-associated nsRP. We observed several genotype-phenotype correlations: the presence of two truncating mutations was restricted to

the USH2a phenotype, as was the presence of two missense variants in the N-terminal laminin of the gene. The presence of at least one truncating mutation was associated with an earlier visual decline, regardless of the phenotype.

Our study has benefits and drawbacks. Among the benefits is our establishment of the largest cohort of *USH2A* patients with genetic and longitudinal follow-up data to date. The 225 Dutch and Belgian participants had been examined by a small number of ophthalmologists and had been followed up for a relatively long period. Finally, our analysis focused on the lifetime course of disease and used end points, which are considered gold standards for functional vision. The drawbacks include the lack of power for detailed subgroup analysis and the incomplete genotyping in a proportion of the patients. There may have been selection bias because patients with hearing loss are more sensitive to visual decline than nsRP patients because they rely more on visual cues in daily life. These patients may have been referred for ophthalmologic screening at a younger age, and thereby may have received an earlier diagnosis of RP. We do not think that the raised alertness explains the difference in visual decline, because this was registered by severe outcome measures using standardized criteria. We believe these differences to be genuine.

Others studied disease progression of USH2a or *USH2A*-associated nsRP in smaller groups of patients. Fishman et al examined the course of visual function in 58 subjects with USH2a by examining change in VF over 3 years.[16] They found that the progression rate did not depend on initial localisation of the VF defect. Blanco- Kelly et al. compared 276 subjects with USH2a with 93 subjects with Usher syndrome type I cross-sectionally using historical data and found an earlier onset for Usher syndrome type I.[17] For subjects with USH2a in this study, the age at onset of symptoms (18.1 years versus 15 years) and diagnosis (26.8 years versus 26 years) was similar to our findings. Another large study by Sandberg et al. investigated 125 RP patients with mutations in *USH2A* with and without hearing loss and calculated rates of decline in electroretinography results, VA, and VF.[18] Annual decline of cone electroretinography amplitudes was 13.2%, which was a rate faster than that of patients with mutations in retinitis pigmentosa GTPase regulator (*RPGR*) or rhodopsin (*RHO*). The estimated decline of VA and VF in this study occurred earlier than in our study. At 65 years of age, 50% were legally blind based on VA or VF in the study of Sandberg et al, whereas these proportions were 22% and 49% at 65 years of age in our study for both phenotypes combined. Their risk estimates were derived from a fitted model based on only a small number of observations in the higher age range, whereas our estimates were based on actual data and included a larger number of data points. Therefore, we believe our estimates may be more realistic. Note that the rate of VF loss, expressed as remaining retinal area capable of detecting the target, follows the same time course in both subgroups, with a plateau before onset in the nonsyndromic

group and a slowing at older age in the syndromic group. The mixed-model analysis we used does not test for the concept of a critical age as postulated by Massof.[19]

We studied the distribution of *USH2A* genotypes and found that less than 19% of mutations were homozygous and that most mutations were private (69%). This indicates that the heterogeneity is very large and that de novo mutations may occur frequently. We found two pathogenic *USH2A* mutations in 72%, three mutations in 5%, and only one pathogenic mutation in 23% of our study population. The most frequently occurring mutation in the USH2a group was p.(Glu767Serfs*21), but this was also the second most common variant in the nonsyndromic group. This mutation was found in seven of 16 homozygous subjects. Carrier frequency of this allele is 0.08% in the general population according to the Exome Aggregation Consortium data set (accessed July 17, 2015). This mutation was found in control populations of European, African, and Latin descent, but seems to be of European ancestral origin.[20] In the 25% (n=52) of patients in whom we detected only one *USH2A* mutation using APEX microarray we did not find a second mutation with Sanger sequencing. This may be the result of deep intronic variants that affect splicing, intronic or intergenic variants that affect transcription or deletions, or duplications that escaped detection. The disease may also be the result of mutations in other genes that mimic this phenotype.

Variants were distributed all over the gene for both phenotypes (Figure 5). We observed only one distinct hotspot; this was located in the N-terminal laminin domain, which was associated with the USH2a phenotype. Subjects in our cohort with biallelic variants in this domain always had impaired hearing, implying that this protein domain is essential for normal cochlear development (Table 5, available as supplemental table at *http://www.aaojournal.org*). Although Baux et al. found a high density of pathogenic variants in this domain, they did not report a relationship with function.[21] There were no other apparent genotype-phenotype relationships; however, all 21 sibling pairs with *USH2A* mutations shared the same phenotype. Most of the unrelated patients with identical genotypes (13/15) also shared the same phenotype. Therefore, the combination of mutations seems to predispose individuals to a certain phenotype.

Approximately half (49.2%) of the mutations were truncating, leading to a shortened or absent protein resulting from nonsense-mediated mRNA decay. These were twice as frequent in USH2a (60.33% versus 25.00%), and we confirmed that two truncating variants always caused congenital hearing loss, but not congenital blindness.[3] However, we observed that biallelic missense mutations also caused congenital hearing loss in 19 patients (Table 5, available as supplemental table at *http://www.aaojournal.org*; and Table 6). Patients with two truncating mutations have an earlier onset of RP and an earlier progression to visual impairment than those with residual protein function (Table 6). It seems that at least one functional allele is needed for normal cochlear development, but not for retinal development. In the retina, the function of the *USH2A* protein usherin

is maintenance of photoreceptor cells.[22] Zebrafish studies have shown that the long isoform of the protein is located at the connecting cilium and photoreceptor synapse and that knockdown of the gene caused photoreceptor degeneration.[23] In the cochlea, both isoforms are present transiently during development, and functional studies in mice have shown that knockout of the gene leads to nonprogressive sensory hearing loss.[22] The protein in the ear seems to function in the ankle link complex that organizes stereocilia in a V-shaped pattern in the developmental phase. It does not seem to have a role in maintenance of the cochlea. The dissimilar roles of the protein in the eye and the ear clarify the difference in age of onset and clinical course.

Our data do not support the allelic hierarchy theory proposed by Lenassi et al., which stratifies disease-causing variants in retinal-disease specific alleles and USH2a-specific alleles.[10] Retinal disease-specific alleles had to be present in more than one patient with *USH2A*-associated nsRP and did not occur in USH2a. The authors proposed that one or more retinal disease-specific alleles were associated with preserved hearing. In our cohort, six patients with USH2a were heterozygous for the c.2276G>T allele, which they attributed to the retinal disease-specific group.[21] In the Leiden Open Variant Database (accessed July 17, 2015), 31 patients with USH2a also carried the c.2276G>T variant.[5, 7, 8, 18, 21, 24-31] We suggest that the effect of this variant on usherin is not confined to the retina, but also can affect cochlear development. The c.2276G>T variant causes a change in amino acid residue from cysteine to phenylalanine. Cysteine is crucial for the formation of 1 of the 4 disulphide bonds required for proper protein folding in the epidermal growth factor (EGF) domain. Another variant, c.11156G>A, which Lenassi et al. proposed to be retina specific, also was present in the Leiden Open Variant Database in an USH2a patient.[10, 32] Therefore, we do not support this allelic hierarchy theory, but suggest that normal cochlear development depends on the presence of at least one functional copy of the *USH2A* protein.

In conclusion, we studied the progression of visual function in a large cohort (n=225) of patients with RP resulting from mutations in *USH2A*. Participants with USH2a had a worse visual prognosis than patients with *USH2A*-associated nsRP. Hence, variants causing USH2a seem to have a more deleterious effect on the protein in the retina. Our data aid in patient counselling by clinicians and geneticists and can provide valuable information for researchers developing therapy for this debilitating disease.

REFERENCES

1. Weston MD, Eudy JD, Fujita S, et al. Genomic structure and identification of novel mutations in usherin, the gene responsible for Usher syndrome type IIa. Am J Hum Genet 2000;66(4):1199-1210.
2. van Wijk E, Pennings RJ, te Brinke H, et al. Identification of 51 novel exons of the Usher syndrome type 2A (*USH2A*) gene that encode multiple conserved functional domains and that are mutated in patients with Usher syndrome type II. Am J Hum Genet 2004;74(4):738-744.
3. McGee TL, Seyedahmadi BJ, Sweeney MO, et al. Novel mutations in the long isoform of the *USH2A* gene in patients with Usher syndrome type II or non-syndromic retinitis pigmentosa. J Med Genet 2010;47(7):499-506.
4. Tucker BA, Mullins RF, Streb LM, et al. Patient-specific iPSC-derived photoreceptor precursor cells as a means to investigate retinitis pigmentosa. Elife 2013;2:e00824.
5. Vache C, Besnard T, le Berre P, et al. Usher syndrome type 2 caused by activation of an *USH2A* pseudoexon: implications for diagnosis and therapy. Hum Mutat 2012;33(1):104-108.
6. Le Quesne Stabej P, Saihan Z, Rangesh N, et al. Comprehensive sequence analysis of nine Usher syndrome genes in the UK National Collaborative Usher Study. J Med Genet 2012;49(1):27-36.
7. Seyedahmadi BJ, Rivolta C, Keene JA, et al. Comprehensive screening of the *USH2A* gene in Usher syndrome type II and non-syndromic recessive retinitis pigmentosa. Exp Eye Res 2004;79(2):167-173.
8. Baux D, Larrieu L, Blanchet C, et al. Molecular and in silico analyses of the full-length isoform of usherin identify new pathogenic alleles in Usher type II patients. Hum Mutat 2007;28(8):781-789.
9. Dreyer B, Brox V, Tranebjaerg L, et al. Spectrum of *USH2A* mutations in Scandinavian patients with Usher syndrome type II. Hum Mutat 2008;29(3):451.
10. Lenassi E, Vincent A, Li Z, et al. A detailed clinical and molecular survey of subjects with nonsyndromic USH2A retinopathy reveals an allelic hierarchy of disease-causing variants. Eur J Hum Genet 2015.
11. Lenassi E, Robson AG, Luxon LM, et al. Clinical heterogeneity in a family with mutations in *USH2A*. JAMA Ophthalmol 2015;133(3):352-355.
12. Dagnelie G. Conversion of planimetric visual field data into solid angles and retinal areas. Clin Vision Sciences 1990(5):95-100.
13. Neveling K, Collin RW, Gilissen C, et al. Next-generation genetic testing for retinitis pigmentosa. Hum Mutat 2012;33(6):963-972.
14. van Huet RA, Pierrache LH, Meester-Smoor MA, et al. The efficacy of microarray screening for autosomal recessive retinitis pigmentosa in routine clinical practice. Mol Vis 2015;21:461-476.
15. Kircher M, Witten DM, Jain P, et al. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet 2014;46(3):310-315.
16. Fishman GA, Bozbeyoglu S, Massof RW, et al. Natural course of visual field loss in patients with Type 2 Usher syndrome. Retina 2007;27(5):601-608.
17. Blanco-Kelly F, Jaijo T, Aller E, et al. Clinical aspects of Usher syndrome and the *USH2A* gene in a cohort of 433 patients. JAMA Ophthalmol 2015;133(2):157-164.
18. Sandberg MA, Rosner B, Weigel-DiFranco C, et al. Disease course in patients with autosomal recessive retinitis pigmentosa due to the *USH2A* gene. Invest Ophthalmol Vis Sci 2008;49(12):5532-5539.
19. Massof RW. First order dynamics of visual field loss in retinitis pigmentosa. Clin Vision Sciences 1990;5(1):1-26.
20. Aller E, Larrieu L, Jaijo T, et al. The *USH2A* c.2299delG mutation: dating its common origin in a Southern European population. Eur J Hum Genet 2010;18(7):788-793.

21. Baux D, Blanchet C, Hamel C, et al. Enrichment of LOVD-USHbases with 152 USH2A genotypes defines an extensive mutational spectrum and highlights missense hotspots. Hum Mutat 2014;35(10):1179-1186.
22. Liu X, Bulgakov OV, Darrow KN, et al. Usherin is required for maintenance of retinal photoreceptors and normal development of cochlear hair cells. Proc Natl Acad Sci U S A 2007;104(11):4413-4418.
23. Ebermann I, Phillips JB, Liebau MC, et al. PDZD7 is a modifier of retinal disease and a contributor to digenic Usher syndrome. J Clin Invest 2010;120(6):1812-1823.
24. Aller E, Jaijo T, Beneyto M, et al. Identification of 14 novel mutations in the long isoform of *USH2A* in Spanish patients with Usher syndrome type II. J Med Genet 2006;43(11):e55.
25. Bernal S, Meda C, Solans T, et al. Clinical and genetic studies in Spanish patients with Usher syndrome type II: description of new mutations and evidence for a lack of genotype--phenotype correlation. Clin Genet 2005;68(3):204-214.
26. de Castro-Miro M, Pomares E, Lores-Motta L, et al. Combined genetic and high-throughput strategies for molecular diagnosis of inherited retinal dystrophies. PLoS One 2014;9(2):e88410.
27. Garcia-Garcia G, Aparisi MJ, Jaijo T, et al. Mutational screening of the *USH2A* gene in Spanish USH patients reveals 23 novel pathogenic mutations. Orphanet J Rare Dis 2011;6:65.
28. Jaijo T, Aller E, Garcia-Garcia G, et al. Microarray-based mutation analysis of 183 Spanish families with Usher syndrome. Invest Ophthalmol Vis Sci 2010;51(3):1311-1317.
29. Najera C, Beneyto M, Blanca J, et al. Mutations in myosin VIIA (MYO7A) and usherin (*USH2A*) in Spanish patients with Usher syndrome types I and II, respectively. Hum Mutat 2002;20(1):76-77.
30. Sodi A, Mariottini A, Passerini I, et al. MYO7A and *USH2A* gene sequence variants in Italian patients with Usher syndrome. Mol Vis 2014;20:1717-1731.
31. Vona B, Muller T, Nanda I, et al. Targeted next-generation sequencing of deafness genes in hearing-impaired individuals uncovers informative mutations. Genet Med 2014;16(12):945-953.
32. Chen X, Sheng X, Liu X, et al. Targeted next-generation sequencing reveals novel *USH2A* mutations associated with diverse disease phenotypes: implications for clinical and molecular diagnosis. PLoS One 2014;9(8):e105439.

Chapter 2.3

Evaluation of hearing in patients with *USH2A*-associated nonsyndromic retinitis pigmentosa

ABSTRACT

Objectives: Mutations in *USH2A* are associated with nonsyndromic retinitis pigmentosa (nsRP), which is an inherited progressive retinal degenerative disease. Mutations in this gene are also associated with Usher syndrome type IIa (USH2a), which is characterized by the combination of retinitis pigmentosa and congenital hearing impairment. The aim of this study was to examine hearing in patients with *USH2A*-associated nsRP, in order to draw clear conclusions on their hearing phenotype.

Design: In this cross-sectional study, 21 patients with confirmed *USH2A*-associated nsRP were subjected to audiological tests and a questionnaire to properly examine their hearing phenotype. Test results were compared to those previously reported for specific groups of normal hearing subjects, patients with USH2a and norms published for presbyacusis.

Results: Patients had a mean pure-tone average (0.5 - 4 kHz) of 20.2 dB HL (sd 13.3). A significant high number of patients (8/18) showed hearing impairment beyond presbyacusis. There was no subjective or statistical congenital hearing impairment. There was some progression of hearing impairment at 4 kHz, but this was not significant after correcting for presbyacusis and significantly less than observed in patients with USH2a. The outcomes on phoneme perception tests in both silence and noise were comparable to those of normal hearing subjects, and significantly better than data from patients with USH2a. The scores obtained from the questionnaire placed the *USH2A*-associated nsRP patients close to older normal hearing subjects.

Conclusions: The studied patients, clinically and genetically diagnosed with *USH2A*-associated nsRP, presented with mild hearing impairment that was not significantly progressive, but attained higher levels than could be expected for normal presbyacusis in 8 out of 18 patients. Phoneme and sentence perception were normal. Hearing impairment was significantly milder than usual for patients with USH2a. Following the clinical criteria, *USH2A*-associated nsRP represents a different entity compared to USH2a. However, in this study, almost half of the *USH2A*-associated nsRP patients present with an adult onset mild hearing impairment.

Submitted as: Hartel, B.P., Pierrache, L.H.M., Huygen, P.L., Homans, N.C., Goedegebure, A., van Wijk, E., Snik, A.F., Klaver, C.C., van den Born, L.I., & Pennings,. R.J. Evaluation of hearing in patients with *USH2A*-associated nonsyndromic retinitis pigmentosa

INTRODUCTION

Nonsyndromic retinitis pigmentosa (nsRP, OMIM 613809) is a progressive inherited retinal degenerative disease. It first presents with nightblindness and, as the disease progresses, a deterioration in both visual field size and visual acuity as a consequence of rod and later cone degeneration.[1] NsRP has an overall prevalence of 1:4,000 individuals and is inherited in an autosomal dominant, autosomal recessive, or X-linked manner. In 5-20% of the cases, nsRP inherits autosomal recessively. Mutations in *USH2A* represent the most common cause of autosomal recessive retinitis pigmentosa (RP), accounting for 7-23% of cases.[2-5]

In addition to causing nsRP, mutations in *USH2A* are also the leading cause of Usher syndrome (USH). USH is an autosomal recessively inherited disorder that is characterized by congenital sensorineural hearing impairment (HI), progressive RP and occasionally vestibular dysfunction. USH is an orphan disease, with a reported prevalence of 4.4 to 6.2 per 100,000 inhabitants [6-8]. The most common genetic type is Usher syndrome type IIa (USH2a), accounting for more than half of all USH patients. Patients with USH2a present with congenital sensorineural HI, RP diagnosed at a post-pubertal age, and intact vestibular function. USH2a is caused by genomic deletions and multiple missense, nonsense, frameshift, and splice-modulating mutations in *USH2A*. This gene encodes a transmembrane protein called usherin (OMIM 608400), which has an important role in the developing hair bundles, the synapses of hair cells, as well as in the spiral ganglion cells in the cochlea.[9-13] In the retina, usherin has been localised to the periciliary region of photoreceptors.[9, 14-16]

The association between *USH2A* and nsRP was first described by Rivolta et al. in 2000.[4] Patients with nsRP present without extraocular symptoms like hearing loss, hence the term nonsyndromic. Audiometric testing is, however, often not performed due to the absence of subjective HI. Most studies performed on patients with *USH2A*-associated nsRP focused on the visual phenotype and only marginally described the audiological phenotype. However, in their original study Rivolta et al. already suggested that *USH2A*-associated nsRP patients might exhibit adult onset HI, and therefore develop a syndromic phenotype at later age.

The aim of this study was to properly examine hearing function in patients with *USH2A*-associated nsRP to draw clear conclusions on their hearing status. The results may have an impact on disease classification and therefore on patient counselling.

PATIENTS AND METHODS

Patients

All patients, clinically and genetically diagnosed with *USH2A*-associated nsRP, were extracted from the databases of three Dutch ophthalmogenetic clinics (Radboud university medical centre, Nijmegen, the Netherlands; the Erasmus Medical centre, Rotterdam, the Netherlands; the Rotterdam Eye Hospital, the Netherlands). The most important criteria in the clinical diagnosis of nsRP was the absence of extraocular symptoms such as congenital HI. A total of 28 patients was identified. Five patients refused participation and two patients were excluded because of a positive otologic history or ear surgery. Finally, 21 patients were included in this study. Some of the presented patients have been included in a previous report from one of the participating centres.[17] All patients agreed to participate in this study by informed consent. This study was approved by the local ethics committee (nr. 2015/2030).

Audiometric evaluation

Pure tone air and bone conduction thresholds for sound frequencies ranging from 0.25 to 8 kHz were assessed according to common clinical standards. Bone conduction thresholds and tympanometry were evaluated to exclude middle ear problems. Speech perception was evaluated using Dutch consonant-vowel-consonant monosyllables according to Bosman and Smoorenburg [18] in silence and using standardised Dutch sentences according to Plomp et al. in silence and in noise.[19] Speech perception of monosyllables was measured monaurally and perception of sentences was evaluated in sound field. The patients' demographic, clinical, audiological, and genetic data were obtained from databases of participating centres.

Questionnaire

The Dutch translation* of the Speech, Spatial and Qualities of Hearing Scale (SSQ) questionnaire was used to evaluate subjective hearing complaints over a wide range of hearing situations.[20] This questionnaire consists of 45 items divided into three subscales. Participants responded using a scale from 0 to 10, in which a higher score represents better self-reported ability. The results were compared to the outcomes of a young normal hearing cohort (n=48, mean age 18.6 years (range 18-22)) and an old normal hearing cohort (n=48, mean age 70 years (range 67-80)).[21]

* The SSQ was translated to Dutch by ExpORL (Department of Neurosciences, Leuven, Belgium) in collaboration with VUMC, Amsterdam; AMC, Amsterdam; Erasmus MC, Rotterdam and AZ St.-. Jan, Brugge

Data analysis

The audiogram (air conduction threshold) of the best-hearing ear (based on the pure-tone average for the frequencies 0.5-4 kHz; $PTA_{0,5\text{-}4kHz}$) was used for further analyses. For each patient aged below 70 years (n=18), the threshold at a given audio frequency was compared to the $P95_{ISO7029}$ threshold, i.e. the 95th percentile for normal hearing subjects, according to the patient's gender and age.[22] HI beyond presbyacusis was accepted if the patient's threshold was higher than the $P95_{ISO7029}$ threshold at 2 or more frequencies (simultaneous significance, see below).

Cross-sectional linear regression analysis was performed for each frequency, separately. From these analyses, the onset level of HI (threshold intercept, in dB HL at age 0 years) and progression of HI, i.e. slope (in dB/year) could be obtained. The residuals around the regression line were inspected, and it was tested whether they showed a normal distribution around the regression line in first approximation by applying the D'Agostino and Spearman (D & S) test. Congenital HI was considered to be significant if the 95% confidence interval (95% CI) of the threshold intercept did not include 0 dB HL. Progression was considered to be significant, if there was a significant correlation and the slope was positive, in which case the 95% CI for slope should not include the value of 0 dB/year. If, at any frequency, significant progression was found, it was tested whether this represented progression beyond (normal) presbyacusis. This was done by subtracting the median ($P50_{ISO7029}$) threshold for normal presbyacusis according to the patient's gender and age from the raw thresholds of the patients aged below 70 years (n=18). The linear regression analysis was then repeated and it was tested whether the slope differed significantly from zero. If it did not, it was accepted that the patients had progression in line with normal presbyacusis.

The results of the cross-sectional linear regression analysis were used to derive Age Related Typical Audiograms (ARTA) as described previously.[23] For comparison, similar ARTA were constructed for the $P50_{ISO7029}$ thresholds.

The 50% speech reception thresholds (SRT) of the participant's ear that performed best on monosyllables and sentences (without noise) were compared to the results of normal hearing individuals (n=20, [18]). For the perception of sentences in noise, the Speech in Noise Ratio (SNR) at 50% speech perception was determined and compared to normal hearing individuals (n=20), as well as to previously published results for USH2a patients (n=11) by Leijendeckers et al.[24]

All analyses were performed using Prism 4.02 software (GraphPad, San Diego, CA, USA). For the comparison between 2 subgroups (SRT) Student's t test (unpaired) was used, with Welch's correction if Bartlett's test had detected unequal variances. For the comparison between 3 subgroups (SNR and SSQ) one-way analyses of variance (ANOVA)

was used, followed by Tukey's Multiple Comparison test. A general significance level of P=0.05 was applied in all separate tests. Binomial distribution statistics were invoked for the simultaneous assessment of significant results in repeated tests, applied on separate audio frequencies, or on separate subjects within a subgroup. Simultaneous significance was accepted for across-frequencies test if 2 or more frequencies tested significantly (tail probability P<0.05 in the binomial distribution with N=6, P=0.05 and q=0.95).

RESULTS

Patient characteristics

Twenty-one patients with a mean age of 55 years (range 28-79), clinically and genetically diagnosed with *USH2A*-associated nsRP, were included in this study. Twelve of these patients reported their hearing to be stable ("unaffected"), six patients reported a "decline" of their hearing, and three patients reported their hearing to be "affected". Three of the six patients with hearing that subjectively was getting worse, used bilateral hearing aids. None of the other patients used hearing aids. There were no patients with a history of noise exposure. The mean age at diagnosis of RP was 39.2 years (sd 13.0). The identified pathogenic mutations in *USH2A* are shown in Table 1.

Audiometric evaluation

There were no signs of conductive hearing loss according to the measurements of bone conduction thresholds and tympanometry. The first screening of the thresholds indicated the presence of mild HI. The across-subjects $PTA_{0,5\text{-}4kHz}$ of air conduction thresholds for all patients was 20.2 dB HL (sd 13.3). A significantly high proportion of patients aged below 70 years (8 out of 18) had thresholds beyond presbyacusis at 2-6 out of the 6 frequencies. Six of these 8 patients had worse thresholds mainly at the higher frequencies, i.e. at 2-8 or 1-8 kHz. One patient had such thresholds at 1 and 8 kHz, and another at all frequencies (black squares in Figure 1).

Cross-sectional analyses of the threshold data are presented in Figure 1. Each frequency panel shows the measured thresholds as a function of age. The fitted linear regression line and its equation, with 95% CI for slope and intercept, are also presented. The residuals around the regression line passed the normality (D & S) test at all frequencies. The threshold intercept did not differ significantly from 0 at any frequency; in other words, the onset of hearing loss is probably not congenital. The slope was only significantly different from 0 dB/year at 4 kHz. To compare this progression to the progression caused by presbyacusis, the threshold was corrected for the $P50_{ISO7029}$ norm value. Linear regression analysis for (Threshold - $P50_{ISO7029}$) on age did not disclose any slope that differed significantly from zero. The analysis is shown only for 4 kHz in supplemental figure SF.1.

Table 1. Spectrum of *USH2A* mutations in this study

A. Truncating mutations

Nucleotide change	Predicted effect	No. of alleles	LOVD	ExAC (%)
Nonsense mutations				
c.1227G>A	p.(Trp409*)	1	P	
c.4957C>T	p.(Arg1653*)	1	P	
c.5728C>T	p.(Gln1910*)	1	New	0
c.10525A>T	p.(Lys3509*)	2	New	0
c.14174G>A	p.(Trp4725*)	1	P	
Deletions and insertions				
c.2299delG	p.(Glu767Serfs*21)	4	P	
c.7121-?_11047-?	del Ex 38-56	1	New	
c.8723_8724del	p.(Val2908fs)	1	P	

B. Intronic mutations

Nucleotide change	IVS position	No. of alleles	LOVD	ExAC (%)
c.486-14G>A	p.(Met162Ile,Met162_ Cys163insCysPheLeuArg)	2	UV4	0.047

C. Nontruncating mutations

Nucleotide change	Predicted effect	No. of alleles	LOVD	ExAC (%)	CADD
c.1256G>T	p.(Cys419Phe)	2	P	0.005	34.0
c.1965T>G	p.(Cys655Trp)	1	New	0	23.4
c.2276G>T	p.(Cys759Phe)	12	P	0.078	33.0
c.2296T>C	p.(Cys766Arg)	1	UV3	0.001	25.8
c.3902G>T	p.(Gly1301Val)	2	UV2	0.095	32
c.4106C>T	p.(Ser1369Leu)	1	New	0.013	7.59
c.4732C>T	p.(Arg1578Cys)	1	UV3	0.003	34
c.5516T>A	p.(Val1839Glu)	1	New	0	28
c.6926G>T	p.(Cys2309Phe)	1	UV3	0.002	33
c.10073G>A	p.(Cys3358Tyr)	1	UV3	0.029	28.8
c.12343C>T	p.(Arg4115Cys)	4[a]	UV1	0.031	24.2
c.12575G>A	p.(Arg4192His)	1	UV2	0.047	24
c.13274C>T	p.(Thr4425Met)	3[a]	UV3	0.002	32
Total number of alleles		**42**			

Abbreviations: 2T=two truncating mutations, 1T=one truncating mutation and one non-truncating mutation, 2nT=two nontruncating mutations, LOVD=Leiden Open Variant Database, P=pathogenic, UV2=likely neutral, UV4=certainly pathogenic, ExAC=Exome Aggregation Consortium, CADD=Combined Annotation Dependent Depletion score. [a] three patients presented with both these mutations on one allele.

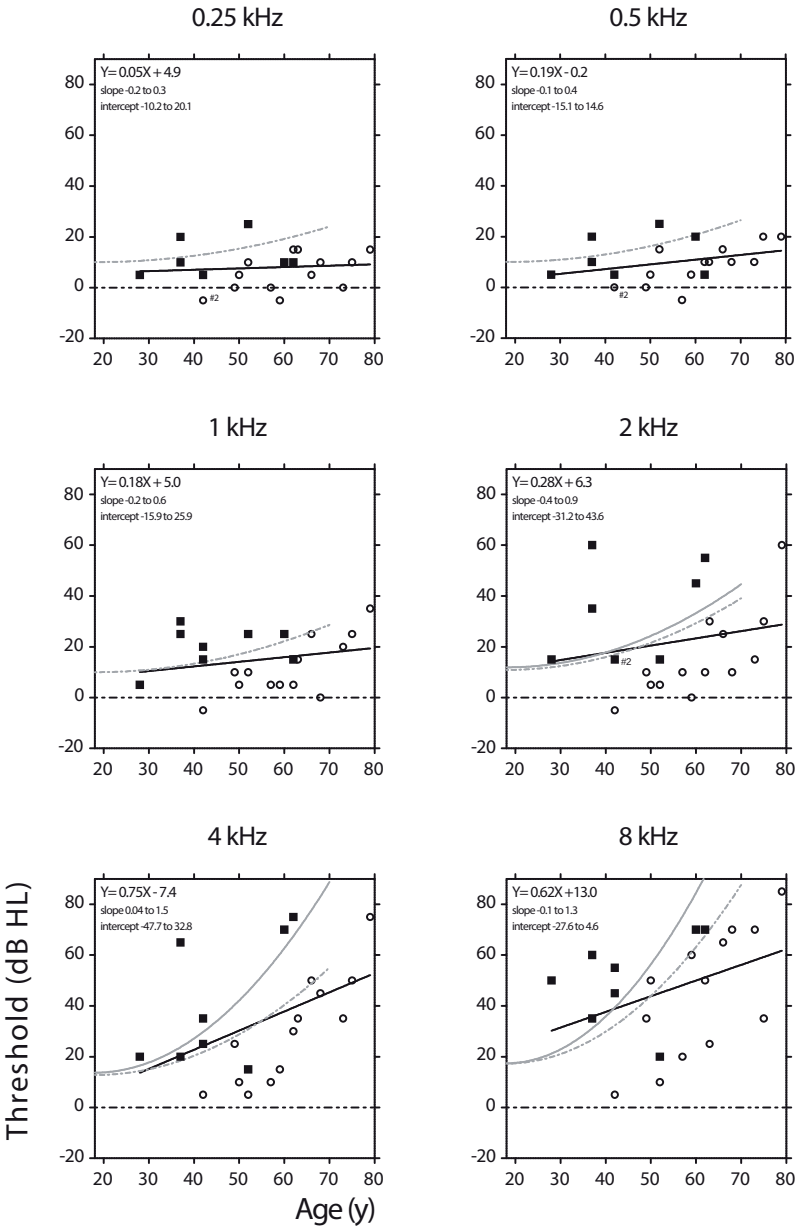


Figure 1. Cross-sectional analyses of best-hearing ear air conduction thresholds (dB HL) in 18 patients with *USH2A*-associated nonsyndromic retinitis pigmentosa.

Each data point represents one patient's threshold; [#2] indicates two patients with the same age and threshold. The regression lines are shown as continuous lines. The grey lines represent the $P95_{ISO7029}$ thresholds for men (continues line) and women (dotted line). At 0.25, 5 and 1 kHz the grey lines are superimposed. The black squares represent the patients with thresholds above the $P95_{ISO7029}$ threshold at 2 or more frequencies (simultaneous significance). Panel insets: the linear regression equation Y = slope.X + intercept, with Y for threshold (dB HL) and X for age (years), and the 95% CIs for slope and intercept.

The HI characteristics of the *USH2A*-associated nsRP patients were compared to $P50_{ISO7029}$ and USH2a prediction in ARTA (Figure 2). At each frequency, the vertical equidistant differences between adjacent threshold lines reflect the progression per decade. The hearing level at 2-8 kHz is generally lower in the $P50_{ISO7029}$ prediction than in the *USH2A*-associated nsRP patients during the first 5 decades of life. However, the $P50_{ISO7029}$ prediction shows more progression in later decades at these frequencies, consequently diminishing the difference in hearing levels. Compared to the USH2a ARTA (adapted from Figure 3 in Hartel et al. 2016) *USH2A*-associated nsRP patients have milder HI than patients with USH2a, already from early childhood onwards have. In addition, they show less progression at the lower frequencies (0.25-1 kHz), however, progression at the higher frequencies is almost similar (0.7-0.8 dB/year at 4-8 kHz in patients with USH2a versus 0.6-0.8 dB/year in patients with *USH2A*-associated nsRP).

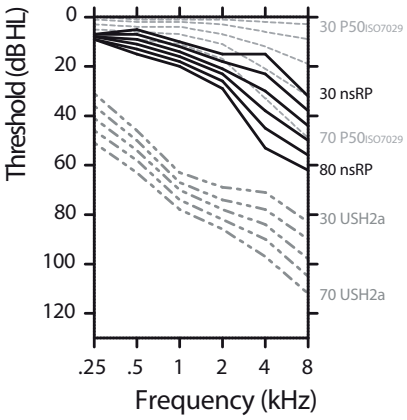


Figure 2. ARTA of the 21 *USH2A*-associated nonsyndromic retinitis pigmentosa patients (nsRP). Derived from the regression lines shown in Figure 1 (black continuous lines). For comparison ARTA for the $P50_{ISO7029}$ presbyacusis are included in for 10-year intervals between 30-70 years (light grey dashed lines), based on weighted mean P50 thresholds for 8 men and 10 women. The ARTA for patients with Usher syndrome type IIa (USH2a), adapted from Figure 3 in Hartel el al. (2016), are added in dark grey dashed lines. Age (years) in italics.

The three panels in Figure 3 represent the phoneme SRT, sentence SRT and sentence SNR of all patients. All but two patients attained an unaided maximum phoneme score of 100%. One patient attained 98%, and another patient 90%. This last patient was the oldest participant aged 79 years and used hearing aids in daily life.

Phoneme SRT values of the *USH2A*-associated nsRP patients did not differ from the normal hearing group (15.4 dB HL versus 15.5 dB HL). Also, the sentence SNR results were comparable between the *USH2A*-associated nsRP and normal hearing group (-4.9 dB versus -5.5 dB HL, difference of means 0.6 dB HL, Tukey's test not significant), and

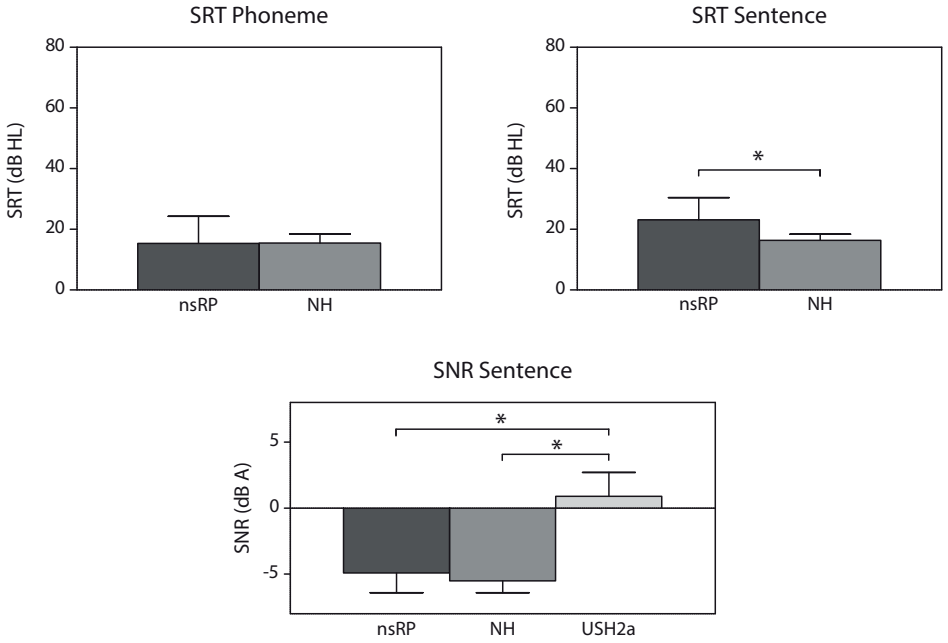


Figure 3. Three panels representing the Speech Reception Thresholds (SRTs) for phonemes and sentences in silence (A and B) and noise (C) for the studied population of *USH2A*-associated nonsyndromic retinitis pigmentosa patients, normal hearing patients and USH2a patients.[24]

Abbreviations: SRT = Speech Reception Threshold, SNR = Speech in Noise Ratio, NH = Normal Hearing. * indicates significant difference.

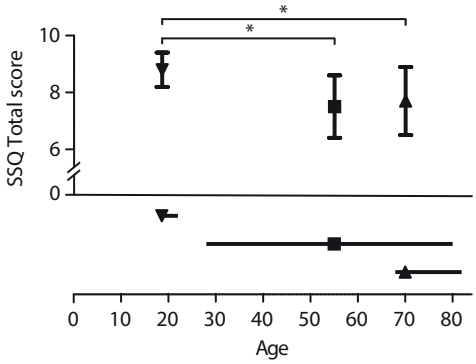


Figure 4. Total scores on the Speech, Spatial and Qualities of hearing (SSQ) questionnaire. The total scores of the *USH2A*-associated nonsyndromic retinitis pigmentosa patients (black square) were compared to those of the normal hearing young (upside-down black triangles) and old (upright black triangles) groups described by Banh et al. in 2012. The mean age and age range (horizontal lines) are shown in the lower part of the figure. * indicates significant difference.

these results were significantly better compared to patients with USH2a (0.9 dB, difference of means 5.8 dB HL, Tukey's test significant, $P<0.001$, 95% CI of difference 4.5 to 7.1 dB HL). However, sentence SRT values of the *USH2A*-associated nsRP patients were significantly worse compared to the normal hearing group (23.1 dB HL versus 16.4 dB HL, difference of means 6.7 dB; Student's t test with Welch's correction, $P=0.0007$, 95% CI of difference 3.2 to 10.2 dB HL).

Questionnaire

The total scores for the SSQ, and the Speech, Spatial and Qualities subscale scores for the group of *USH2A*-associated nsRP patients were 7.5 (sd 1.1), 6.8 (sd 1.7), 7.6 (sd 1.3) and 8.1 (sd 1.0), respectively. The total score, as well as each of the subscale scores appeared to be comparable to the corresponding scores for the older normal hearing patient cohort. ANOVA followed by Tukey's test showed that each of these scores was significantly lower than the corresponding one for the cohort of younger normal hearing subjects in the study of Banh et al. (2012). The mean total scores were 7.5 versus 8.8 ($P<0.001$, difference of means 1.3, 95% CI 0.7 to 1.9). Finally, there was also a similar significant difference in score between the reported young and old normal hearing: 8.8 versus 7.7 ($P<0.001$, difference of means 1.1, 95% CI 0.7 to 1.5; Figure 4).

DISCUSSION

This study shows that a proportion of *USH2A*-associated nsRP patients presented with a level of HI that was higher compared to presbyacusis (8/18 patients). However, the overall patient sample did not report or statistically show a congenital onset of HI. Neither did the HI show progression beyond presbyacusis. Finally, patients with *USH2A*-associated nsRP show normal speech perception in both silence and noise.

From the initial association of nsRP with mutations in *USH2A* (Rivolta et al., 2000), there was uncertainty about its clinical classification. The absence of HI at birth was a clear criterion, but Rivolta suggested that *"many patients with presumed nonsyndromic RP who report mild, subjective hearing impairment actually have Usher syndrome type II"*.[4] This instigated us to speculate about the spectrum of clinical presentations associated with pathogenic mutations in *USH2A*. In the present study, congenital HI did not occur in medical history, nor could congenital HI be substantiated from the data analyses. Following the clinical criteria, *USH2A*-associated nsRP is considered as a different entity from USH2a. However, in this study, almost half of the *USH2A*-associated nsRP patients present with a mild adult onset mild hearing impairment.

With the improved methods for genetic testing, many large cohorts of RP patients have been screened for mutations in *USH2A*. In most of these studies, no or only subjective absence of HI was reported.[3, 5, 25-30] In a few studies, the authors mentioned audiograms, and some presented audiogram data. Only five studies reported possible adult-onset HI [4, 31-34] of which two corrected for presbyacusis.[4, 33] They could not confirm any substantial HI in patients with *USH2A*-associated nsRP.

Most of the mutations found in patients with *USH2A*-associated nsRP are also identified in USH2a patients. There is no clear evidence of specific mutations being solely associated with either *USH2A*-associated nsRP or USH2a. However, none of the combinations of *USH2A* mutations identified in the present cohort was found in a previously described international cohort of 110 patients with USH2a.[35] Possibly, there are unique combinations of mutations that affect hearing more mildly. See Supplemental Table ST.1 for all combinations of mutations in the present study.

In two previous studies, differences in auditory and ocular phenotypes between *USH2A*-associated nsRP and USH2a were shown to be associated with different types of mutations in *USH2A*.[17, 35] Mutations can be predicted to result in a truncated protein. Protein truncating mutations will most often result in nonsense-mediated mRNA decay [36], whereas nontruncating mutations will presumably lead to altered protein folding, localisation and function. As Pierrache et al. (2016) described, none of the patients with *USH2A*-associated nsRP presented with two predicted protein truncating mutations. In the present study, thirteen patients (62%) carried one allele with a protein truncating mutation and one allele with a protein nontruncating mutation. The other 8 patients carried two protein nontruncating alleles. No obvious differences between the above-mentioned subgroups with different types of mutation combinations were observed in PTA (0.5 - 4 kHz), SRT, SNR or SSQ outcomes. This suggests that there is no additional negative effect on hearing of a protein truncating mutation in *USH2A* in patients with *USH2A*-associated nsRP. In contrast, Pierrache et al. (2016) demonstrated a positive correlation between the number of truncating mutations in *USH2A* and an earlier onset of RP and more progressive visual deterioration in patients with USH2a. Similarly, it was shown that patients with USH2a and two truncating mutations in *USH2A* presented with more severe and progressive HI as compared to patients with one or no truncating mutations.[35] Still, not all variations found in HI in patients with USH2a could be explained by the presence of absence of truncating mutations. It seems possible that patients with *USH2A*-associated nsRP have mutations, or combinations of mutations with a milder effect on the phenotype compared to patients with USH2a. Currently, a large cohort of USH2a patients is being analysed in an attempt to correlate the observed variability to individual mutations or mutation combinations. This might result in the identification

of mutations or combinations of mutations with a milder or more severe effect on the phenotype.

When comparing outcomes of the present study to those of previously reported studies, there was no age matching between the different data sources. The patients with *USH2A*-associated nsRP in the present study had a mean age of 55 years, range 28-79. The normal hearing subjects as studied by Bosman et al. and used for the comparison of SRT values had a mean age of 25 years, range 21 - 29.[18] The difference in age might explain the difference found in sentence SRT. The cohort of patients with USH2a as described by Leijendeckers et al., that was used for comparing the SNR, had a mean age of 41 years, range 28 - 59.[24] Banh et al. presented the SSQ results of two normal hearing cohorts, a young cohort with a mean age of 18.6 years, range 18-22 and an old cohort with a mean age of 70, range 67-80 (Figure 4).[21] The patients with *USH2A*-associated nsRP reported the same scores as the old normal hearing cohort presented by Banh et al., but the scores of both groups were lower than those reported for young normal hearing subjects. As illustrated in Figure 4, the *USH2A*-associated nsRP patient group had a large overlap with the old normal hearing cohort, possibly explaining the apparent similarity of their scores.

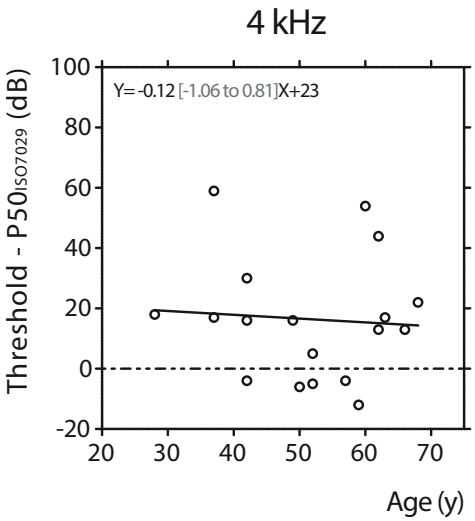
In conclusion, the patients in this study that were clinically and genetically diagnosed with *USH2A*-associated nsRP presented with a level of HI higher than in presbyacusis in 8/18 patients. There was, however, no reported or statistically extrapolated congenital hearing impairment. In addition, there was no progression beyond presbyacusis, and the HI appeared to be milder than the HI typical for patients with USH2a. Hence, USH2a and *USH2A*-associated nsRP are considered as different entities. The difference might be explained by differences in the effects of specific mutations or mutation combinations, or perhaps other factors, such as epigenetics or strong environmental factors, important points of interest for future research.

REFERENCES

1. Pagon RA. Retinitis pigmentosa. Surv Ophthalmol 1988;33(3):137-177.
2. Bernal S, Ayuso C, Antinolo G, et al. Mutations in *USH2A* in Spanish patients with autosomal recessive retinitis pigmentosa: high prevalence and phenotypic variation. J Med Genet 2003;40(1):e8.
3. McGee TL, Seyedahmadi BJ, Sweeney MO, et al. Novel mutations in the long isoform of the *USH2A* gene in patients with Usher syndrome type II or non-syndromic retinitis pigmentosa. J Med Genet 2010;47(7):499-506.
4. Rivolta C, Sweklo EA, Berson EL, et al. Missense mutation in the *USH2A* gene: association with recessive retinitis pigmentosa without hearing loss. Am J Hum Genet 2000;66(6):1975-1978.
5. Seyedahmadi BJ, Rivolta C, Keene JA, et al. Comprehensive screening of the *USH2A* gene in Usher syndrome type II and non-syndromic recessive retinitis pigmentosa. Exp Eye Res 2004;79(2):167-173.
6. Boughman JA, Vernon M, Shaver KA. Usher syndrome: definition and estimate of prevalence from two high-risk populations. J Chronic Dis 1983;36(8):595-603.
7. Rosenberg T, Haim M, Hauch AM, et al. The prevalence of Usher syndrome and other retinal dystrophy-hearing impairment associations. Clin Genet 1997;51(5):314-321.
8. Spandau UH, Rohrschneider K. Prevalence and geographical distribution of Usher syndrome in Germany. Graefes Arch Clin Exp Ophthalmol 2002;240(6):495-498.
9. Liu X, Bulgakov OV, Darrow KN, et al. Usherin is required for maintenance of retinal photoreceptors and normal development of cochlear hair cells. Proc Natl Acad Sci U S A 2007;104(11):4413-4418.
10. Michalski N, Michel V, Bahloul A, et al. Molecular characterization of the ankle-link complex in cochlear hair cells and its role in the hair bundle functioning. J Neurosci 2007;27(24):6478-6488.
11. van Wijk E, van der Zwaag B, Peters T, et al. The DFNB31 gene product whirlin connects to the Usher protein network in the cochlea and retina by direct association with USH2A and VLGR1. Hum Mol Genet 2006;15(5):751-765.
12. Eudy JD, Yao S, Weston MD, et al. Isolation of a gene encoding a novel member of the nuclear receptor superfamily from the critical region of Usher syndrome type IIa at 1q41. Genomics 1998;50(3):382-384.
13. van Wijk E, Pennings RJ, te Brinke H, et al. Identification of 51 novel exons of the Usher syndrome type 2A (*USH2A*) gene that encode multiple conserved functional domains and that are mutated in patients with Usher syndrome type II. Am J Hum Genet 2004;74(4):738-744.
14. Maerker T, van Wijk E, Overlack N, et al. A novel Usher protein network at the periciliary reloading point between molecular transport machineries in vertebrate photoreceptor cells. Hum Mol Genet 2008;17(1):71-86.
15. Sahly I, Dufour E, Schietroma C, et al. Localization of Usher 1 proteins to the photoreceptor calyceal processes, which are absent from mice. J Cell Biol 2012;199(2):381-399.
16. Zou J, Luo L, Shen Z, et al. Whirlin replacement restores the formation of the USH2 protein complex in whirlin knockout photoreceptors. Invest Ophthalmol Vis Sci 2011;52(5):2343-2351.
17. Pierrache LH, Hartel BP, van Wijk E, et al. Visual Prognosis in *USH2A*-Associated Retinitis Pigmentosa Is Worse for Patients with Usher Syndrome Type IIa Than for Those with Nonsyndromic Retinitis Pigmentosa. Ophthalmology 2016;123(5):1151-1160.
18. Bosman AJ, Smoorenburg GF. Intelligibility of Dutch CVC syllables and sentences for listeners with normal hearing and with three types of hearing impairment. Audiology 1995;34(5):260-284.
19. Plomp R, Mimpen AM. Speech-reception threshold for sentences as a function of age and noise level. J Acoust Soc Am 1979;66(5):1333-1342.

20. Gatehouse S, Noble W. The Speech, Spatial and Qualities of Hearing Scale (SSQ). Int J Audiol 2004;43(2):85-99.
21. Banh J, Singh G, Pichora-Fuller MK. Age affects responses on the Speech, Spatial, and Qualities of Hearing Scale (SSQ) by adults with minimal audiometric loss. J Am Acad Audiol 2012;23(2):81-91; quiz 139-140.
22. ISO 7029: Acoustics -- Statistical distribution of hearing thresholds as a function of age. Geneva, 2000.
23. Huygen P, Pennings R, Cremers C. Characterizing and distinguishing progressive phenotypes in nonsyndromic autosomal dominant hearing impairment. Audiological Medicine 2003;1(1):37-46.
24. Leijendeckers JM, Pennings RJ, Snik AF, et al. Audiometric characteristics of USH2a patients. Audiol Neurootol 2009;14(4):223-231.
25. Tucker BA, Mullins RF, Streb LM, et al. Patient-specific iPSC-derived photoreceptor precursor cells as a means to investigate retinitis pigmentosa. Elife 2013;2:e00824.
26. Sandberg MA, Rosner B, Weigel-DiFranco C, et al. Disease course in patients with autosomal recessive retinitis pigmentosa due to the *USH2A* gene. Invest Ophthalmol Vis Sci 2008;49(12):5532-5539.
27. Katagiri S, Akahori M, Sergeev Y, et al. Whole exome analysis identifies frequent CNGA1 mutations in Japanese population with autosomal recessive retinitis pigmentosa. PLoS One 2014;9(9):e108721.
28. Jinda W, Taylor TD, Suzuki Y, et al. Whole exome sequencing in Thai patients with retinitis pigmentosa reveals novel mutations in six genes. Invest Ophthalmol Vis Sci 2014;55(4):2259-2268.
29. de Castro-Miro M, Pomares E, Lores-Motta L, et al. Combined genetic and high-throughput strategies for molecular diagnosis of inherited retinal dystrophies. PLoS One 2014;9(2):e88410.
30. Avila-Fernandez A, Cantalapiedra D, Aller E, et al. Mutation analysis of 272 Spanish families affected by autosomal recessive retinitis pigmentosa using a genotyping microarray. Mol Vis 2010;16:2550-2558.
31. Aller E, Najera C, Millan JM, et al. Genetic analysis of 2299delG and C759F mutations (*USH2A*) in patients with visual and/or auditory impairments. Eur J Hum Genet 2004;12(5):407-410.
32. Bernal S, Meda C, Solans T, et al. Clinical and genetic studies in Spanish patients with Usher syndrome type II: description of new mutations and evidence for a lack of genotype--phenotype correlation. Clin Genet 2005;68(3):204-214.
33. Lenassi E, Vincent A, Li Z, et al. A detailed clinical and molecular survey of subjects with nonsyndromic USH2A retinopathy reveals an allelic hierarchy of disease-causing variants. Eur J Hum Genet 2015;23(10):1318-1327.
34. Perez-Carro R, Corton M, Sanchez-Navarro I, et al. Corrigendum: Panel-based NGS Reveals Novel Pathogenic Mutations in Autosomal Recessive Retinitis Pigmentosa. Sci Rep 2016;6:24843.
35. Hartel BP, Lofgren M, Huygen PL, et al. A Combination of Two Truncating Mutations in *USH2A* causes more Severe and Progressive Hearing Impairment in Usher syndrome type IIa. Hear Res 2016.
36. Isken O, Maquat LE. Quality control of eukaryotic mRNA: safeguarding cells from abnormal mRNA function. Genes Dev 2007;21(15):1833-1856.

SUPPLEMENTAL MATERIALS



Supplemental Figure SF.1. Cross-sectional analysis of 'threshold - $P50_{ISO7029}$' on age. Each data point represents one patient's threshold. The regression line is shown as continuous line. Panel inset: the linear regression equation Y = slope.X + intercept, with Y for threshold (dB HL) and X for age (years). In grey the 95% confidence interval values for the slope.

Supplemental Table ST.1. Spectrum of *USH2A* mutation combinations in this study.

Abbreviations: see Table 1.

1T		
Allele 1	**Allele 2**	**Patients (%)**
c.1227G>A, p.(Trp409*)	c.12575G>A, p.(Arg4192His)	1 (4.8)
c.2276G>T, p.(Cys759Phe)	c.486-14G>A, p.(Met162Ile,Met162_Cys163insCysPheLeuArg)	2 (9.5)
c.2276G>T, p.(Cys759Phe)	c.8723_8724del, p.(Val2908fs)	1 (4.8)
c.2276G>T, p.(Cys759Phe)	c.10525A>T, p.(Lys3509*)	1 (4.8)
c.2276G>T, p.(Cys759Phe)	c.14174G>A, p.(Trp4725*)	1 (4.8)
c.2296T>C, p.(Cys766Arg)	c.4732C>T, p.(Arg1578Cys)	1 (4.8)
c.2299del, p.(Glu767fs)	c.2276G>t, p.(Cys759Phe)	4 (19.0)
c.7121-?_11047+?, del Ex 38-56	c.2276G>T, p.(Cys759Phe)	1 (4.8)
c.12343C>T, p.(Arg4115Cys) c.13274C>T, p.(Thr4425Met)	c.5728C>T, p.(Gln1910*)	1 (4.8)
c.12343C>T, p.(Arg4115Cys) c.13274C>T, p.(Thr4425Met)	c.10525A>T, p.(Lys3509*)	1 (4.8)
2nT		
Allele 1	**Allele 2**	**Patients (%)**
c.1256G>T, p.(Cys419Phe)	c.4106C>T, p.(Ser1369Leu)	1 (4.8)
c.1965T>G, p.(Cys655Tr)	c.6926G>T, p.(Cys2309Phe)	1 (4.8)
c.2276G>T, p.(Cys759Phe)	c.5516T>A, p.(Val1839Glu)	1 (4.8)
c.2276G>T, p.(Cys759Phe)	c.12343C>T, p.(Arg4115Cys)	1 (4.8)
c.3902G>T, p.(Gly1301Val)	c.3902G>T, p.(Gly1301Val)	1 (4.8)
c.4957C>T, p.(Arg1653X) ; c.7379G>A, p.(Arg2460His)	c.10073G>A, p.(Cys3358Tyr)	1 (4.8)
c.12343C>T, p.(Arg4115Cys) ; c.13274C>T, p.(Thr4425Met)	c.1256G>T, p.(Cys419Phe)	1 (4.8)
Total		**21 (100)**

Chapter 3

Rehabilitation of patients with Usher syndrome type IIa

Chapter 3.1

Hearing aid fitting for visual and hearing impaired patients with Usher Syndrome type IIa

ABSTRACT

Objectives: Usher syndrome is the leading cause of hereditary deafblindness. Most patients with Usher syndrome type IIa start using hearing aids from a young age. A serious complaint refers to interference between sound localisation abilities and adaptive sound processing (compression), as present in today's hearing aids. The aim of this study was to investigate the effect of advanced signal processing on binaural hearing, including sound localisation.

Methods: In this prospective study, patients were fitted with hearing aids with a nonlinear (compression) and linear amplification programs. Data logging was used to objectively evaluate the use of either program. Performance was evaluated with a speech-in-noise test, a sound localisation test and two questionnaires focusing on self-reported benefit.

Results: Data logging confirmed that the reported use of hearing aids was high. The linear program was used significantly more often (average use: 77%) than the nonlinear program (average use: 17%). The results for speech intelligibility in noise and sound localisation did not show a significant difference between types of amplification. However, the self-reported outcomes showed higher scores on 'ease of communication' and overall benefit, and significant lower scores on disability for the new hearing aids when compared to their previous hearing aids with compression amplification.

Conclusions: Patients with Usher syndrome type IIa prefer a linear amplification over nonlinear amplification when fitted with novel hearing aids. Apart from a significantly higher logged use, no difference in speech in noise and sound localisation was observed between linear and nonlinear amplification with the currently used tests. Further research is needed to evaluate the reasons behind the preference for the linear settings.

Published as: Hartel, B.P., Agterberg, M.J., Snik, A.F., Kunst, H.P., van Opstal, A.J., Bosman, A.J., & Pennings, R.J. (2016). Hearing aid fitting for visual and hearing impaired patients with Usher Syndrome type IIa. *Clin Otolaryngol*. doi: 10.1111/coa.12775

INTRODUCTION

Usher syndrome (USH) is the leading cause of hereditary deafblindness. This autosomal recessively inherited disorder is characterised by sensorineural hearing impairment, retinitis pigmentosa (RP) and in part of the cases vestibular dysfunction. USH is clinically and genetically heterogeneous and has a prevalence of 4.4–6.2 per 100.000 inhabitants.[1-3] Usher syndrome type II is one of the three clinical types of USH, and Usher syndrome type IIa (OMIM276901) is the most common genetic type, accounting for more than half of the USH patients.[4-6] Pathogenic mutations of this type are identified in the *USH2A* gene located on chromosome 1q41.[7, 8] Patients with Usher syndrome type IIa (USH2a) have a congenital moderate to severe high-frequency hearing impairment, intact vestibular function and RP, a progressive retinal degenerative disease that usually first becomes manifest in the second decade of life and eventually leads to blindness.

Most patients with USH2a use hearing aids from a young age.[9] During their lives, these patients will therefore face multiple hearing aid fitting procedures. Owing to the multiple complex settings and programs of today's sophisticated hearing aids, fitting periods will be prolonged to find the best settings for these double sensory-impaired patients as described by patients with this specific syndrome.[10] Their report pointed out the difficulties experienced during hearing aid fitting and hearing aid use, which is the motivation for this study. The additional onset of visual impairment in young adulthood is thought to play a major role in the lengthy fitting procedures. The impact of the hearing disability is probably more severe because of the additional effects of RP, like the loss of visual feedback.

Nowadays, hearing aids are equipped with advanced algorithms focusing on the largest consumer group: the elderly. Many algorithms have been developed to optimise speech intelligibility and to provide comfortable hearing. However, less attention is given to the preservation of natural cues used for localisation, such as interaural time difference (ITD) and interaural level difference (ILD); especially in the horizontal (azimuth) plane, the difference in sound arrival at both ears (ITD) for the lower frequencies (<1.5 kHz) and the difference in sound level between both ears (ILD) for the higher frequencies (>3 kHz) are important for sound localisation.[11]

The aim of this study was to investigate whether advanced signal processing of sound by hearing aids has a positive or negative effect on speech intelligibility in noise (with spatially separated speech and noise sources) and on sound localisation. Both tasks require binaural hearing abilities. The patients were fitted with novel hearing aids with a nonlinear (compressive) and linear amplification program. It was hypothesised that the linear program affects sound localisation minimally because the ITD and ILD-cues are potentially less perturbed.[12] This may lead to better sound localisation performance

when compared to the nonlinear program. In contrast, the nonlinear program may provide better audibility and speech recognition in noisy environments, when compared to a linear fitting.[13] To test these hypotheses, details of hearing aid use were retrieved from data logging and speech reception in noise, and sound localisation was tested in an elaborate set-up.[14] Furthermore, two questionnaires and a diary were used to evaluate the subjective benefit and reported use in daily life of the newly fitted hearing aids.

PATIENTS AND METHODS

Patients

The patients with USH2a were extracted from the Nijmegen Usher syndrome database. Patients were included if they were clinically diagnosed with Usher syndrome type II, had two identified pathogenic mutations in *USH2A*, had a pure-tone average (0.5–8 kHz) better than 80 dB HL and were above 18 years of age. Twenty-four adults were selected for participation and contacted. Finally, eighteen of them decided to participate in this study. Six patients refused without specified reasons. Some of the patients participated in former studies on hearing in USH2a from our centre.[15, 16] This study was approved by the local ethics committee (nr. 2012/520).

Audiometric evaluation and hearing aids

At the first visit, pure-tone air and bone-conduction thresholds were assessed for frequencies ranging from 0.25 to 8 kHz according to the ISO 8253-1 standard.[17] All patients were bilaterally fitted with Phonak Naida Q SP hearing aids (Phonak AG, Stäfa, Switzerland), hereafter referred to as 'new hearing aids'. The fitting was performed using the Target system 3.1. In six patients', ear-moulds were replaced with the standard occluding ear-moulds. These patients could adapt at least 2 weeks with the Phonak hearing aids and a nonlinear program to get used to the ear-moulds before the tests started. The vent diameter varied according to hearing impairment, but all 18 patients were fitted with vents smaller than 2 mm.

Two listening programs were activated, and for 13 patients, a telecoil program was added. The first program consisted of a nonlinear fitting on the basis of the NAL-NL2 rule.[18] Minor adjustments in gain were made according to remarks from the patients. The nonlinear program used syllabic compression (SC) with an attack time of 10 ms and a release time of 80 ms. In this program, microphone directionality was set as a static, input independent, beam former ('Real-Ear sound'; to compensate to some extent for pinna function) and the default adaptive features were activated. The linear program was adapted to the nonlinear program by setting the same gain for a 65 dB SPL input. A compression ratio of one was aimed for as allowed by the patient's dynamic range, and

all adaptive features were deactivated, apart from feedback suppression, which was set to medium. Microphone directionality was fixed to omnidirectional.

Figure 1 shows that the CR in the linear program (mean 1.2, standard deviation (SD) 0.2) was significantly lower at all frequencies compared to the CR in the nonlinear program (mean 1.8, SD 0.4) (panel a). The mean CR for the linear program was not 1.0 because of the reduced dynamic range in the higher frequencies. This is illustrated in panel b, in which a slight increase in CR can be seen in the higher frequencies at input levels between 65 and 80 dB SPL.

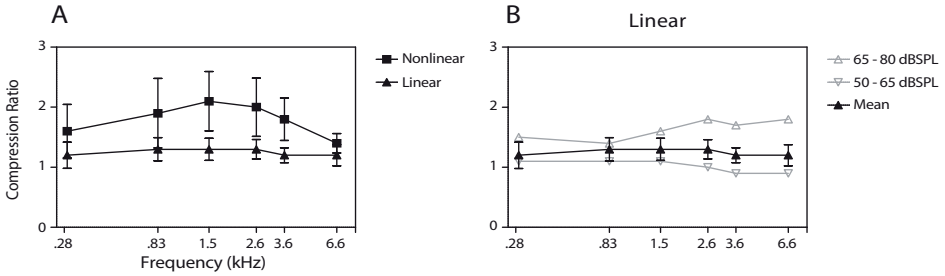


Figure 1. Compression ratios for the linear and nonlinear program.

(A) The mean compression ratios in the nonlinear program are represented by squares and continuous line with the standard deviation on either side of the squares. The mean compression ratios in the linear program are represented by the triangles and continuous line with the standard deviation on either side of the triangles. (B) Compression ratios for the linear program between 50–65 dB SPL and 65–80 dB SPL. The mean compression ratios in the linear program are represented by black triangles and continuous black line with the standard deviation on either side of the triangles. The average compression ratios in the linear program between 50 and 65 dB SPL are represented by the grey downward triangles and continuous grey line. The average compression ratios in the linear program between 65 and 80 dB SPL are represented by grey upward triangles and continuous grey line.

When the hearing aids were switched on, half of the patients started with the linear program, whereas the other half started with the nonlinear program. Wireless communication between both hearing aids was activated (Quick-Sync) to enable simultaneous change of sound level and program across ears. Volume control was available but at follow-up, none of the participants reported to have daily changed the volume. All patients were instructed to try both programs in all situations. Between the two test sessions, all patients were contacted and five patients were subsequently referred to their own hearing aid dispenser for minor (documented) adjustments of gain, only for the nonlinear program. At follow-up, the overall use, individual program use and changes in volume were retrieved from the new hearing aids. Moreover, data logging was activated to retrieve the number of days, hours and used programs.

Speech intelligibility

Speech intelligibility was measured in silence and in noise with the patient's own hearing aids before fitting and with their new hearing aids with either program (nonlinear and linear) directly after fitting and at follow-up. The speech test used was the Dutch matrix test[19] in an open-set response format, in which listeners had to repeat the words they understood from target sentences spoken by a female. These sentences were always presented from the front (at 1 m), and noise was either presented from the front or randomly from +90 or -90 degrees (at 1 m, S_0/N_{s90} and S_0/N_{f90}). The stationary noise had an average power spectrum equal to that of the sentences. Additionally, a single-talker male babble noise was used based on the International Speech Test Signal.[20, 21] The level of noise was adaptively varied according to the Brand and Kollmeier procedure,[22] with a minimum step size of ±1 dB. In the measurement of speech intelligibility in silence, the same set-up was used without noise and the first word was presented at 65 dB(A). Two lists of ten sentences were presented per noise configuration and listening condition and the outcomes averaged to limit the intra-individual variation. Two training lists were used to familiarise subjects with the task, the first starting with an easy speech level of 65 dB(A) without noise. The second training list was presented with a 15 dB SNR (target speech at 65 dB(A), noise at 50 dB(A)). Noise started 2 s before and ended 2 s after the target sentence presentation. The speech level was always held constant at 65 dB(A). The 50% speech reception threshold (SRT) was determined over the last 7 reversals.

Sound localisation

Apparatus

The patients were seated in a comfortable chair in the centre of a completely dark and sound-attenuated room. The ambient background noise level in the room was 30 dB(A). Horizontal head movements were recorded using the magnetic search coil induction technique. Patients wore a lightweight spectacle frame on which a small coil was mounted.[14] They were asked to turn their head as quickly and as accurately as possible in the perceived stimulus direction. The patient controlled stimulus onset by pressing a hand-held button while facing straight ahead.

Stimuli

The stimuli were digitally generated in Matlab (R2012a, The Mathworks, Inc., Natick, MA, USA). The sound was presented by a broad-range loudspeaker (MSP-30; Monacor International GmbH, Bremen, Germany). Fifty-eight loudspeakers were mounted on a vertical hoop at 100 cm of the patient that could turn to every position in azimuth. Three different acoustic stimuli were presented; a broadband stimulus (0.5–20 kHz; BB), a low-pass filtered noise stimulus (LP; high-frequency cut-off at 1.5kHz) and a high-pass filtered noise stimulus (HP; low-frequency cut-off at 3 kHz). For the BB and HP stimuli,

sound intensities were roved from 55 to 75 dB(A) in steps of 10 dB. This was carried out to prevent the use of perceived loudness as a cue for localisation.[23] For a more detailed description of set-up and stimuli, see reference[14]

Experiments

A visual calibration experiment was first run to map the head-position data to known spatial locations and to demonstrate that patients had no motoric problems to direct their head to the stimulus positions. After this calibration run, 10 practice trials were presented to become accustomed to the sounds and the open-loop head-movement response procedure.

For the localisation experiment, the patient was asked to orient towards 12 LP stimuli and 36 BB and 36 HP stimuli in each condition (own hearing aids, new hearing aids nonlinear, new hearing aids linear). The stimuli were presented at randomised locations between -75 and 75 degrees in azimuth with a minimum of 20 degrees between consecutive stimuli and at zero degrees' elevation. Regular breaks were introduced to prevent fatigue and to motivate the patients.

Self-reported outcomes

In addition to the objective outcome measures, two questionnaires and a diary were used to assess each patient's reported benefit, satisfaction and use of the new hearing aids.

The Abbreviated Profile of Hearing Aid Benefit (APHAB) is a hearing related benefit questionnaire.[24] It contains 24 items and is a disability-based inventory to document the outcome of hearing aid fitting and to evaluate fitting over time. The questionnaire yields scores on four subscales: ease of communication, listening under reverberant conditions, listening in background noise and aversiveness of sounds. The APHAB was used to qualify the disability and the differences in disability between hearing aids. The maximum disability was represented by a 100% of the time that certain situation occurred, the patient felt disabled, and the minimum (best score) was 0% (never disabled).

The Glasgow Hearing Aid Difference Profile (GHADP) was designed to evaluate patient reported hearing disability, handicap, hearing aid use, benefit, residual disability and satisfaction.[25] This inventory provided eight possible environments. Four of them were predetermined, and four others could be added by the patient. This questionnaire was used to address the differences between the patient's own and new hearing aids.

A nonvalidated diary was provided to the patient for the first week after fitting and the last week before the follow-up visit. Questions about the number of changes between programs, use of programs and satisfaction on a 1–10 Likert scale were included. Finally, patients were also asked to describe situations in which they used a specific program.

Data analysis

Patient characteristics were compared using Student's t-test or their nonparametric counterpart if the data deviated from a normal distribution. All analyses were performed using Prism 5.03 software (GraphPad, San Diego, CA, USA). A paired t-test was performed for comparison of compression ratios between programs. Binomial distribution statistics were used for simultaneous assessment of the CR over all six audio frequencies. Simultaneous significance was accepted if such differences were significant at two of more frequencies (P<0.05 tail probability in the binomial distribution with n=6, P=0.05, q=0.95). Furthermore, one-way analysis of variance (ANOVA) with post hoc Bonferroni correction was used to compare speech intelligibility and self-reported outcomes between the two fittings and conditions. A general significance level of P=0.05 was applied in all separate tests.

Sound localisation

All responses were analysed with Matlab for each patient and condition (R2012a, The Mathworks, and Inc.). The best linear fit (least squares criterion) of the stimulus response relationship on the azimuth data was determined with the following equation:

$$\alpha_r = b + G\alpha_S$$

in which α_r is the response azimuth in degrees, b the response bias in degrees, G the response gain (dimensionless) and α_S the presented stimulus azimuth in degrees.[26] From the regression, we calculated the coefficient of determination (r^2) of the fit, as well as the mean absolute error (MAE; in degrees). A good performer should produce a gain and r^2 close to 1.0 and a bias and MAE close to 0.0 degrees. Results for each condition were averaged across patients, and the gain changes were normalised to enable a direct and unbiased comparison between conditions as described by Zwiers et al.[27] The normalisation of the gain change was obtained with the following equation:

$$G_E = |G/G_C - 1|$$

In which G is the measured gain for a particular condition in the patient and G_C the gain for the control condition in the patient. A value of $G_E = 0$ indicates that the measured gain was equal to the control value, that is no change in gain. The absolute value ensures that systematic overshoots and undershoots yielded similar measures.

RESULTS

Patients

A total of 18 patients (nine male and nine female) with a mean age of 38.8 years (range 20–55) were included, and Table 1 shows their general characteristics. Most patients reported a childhood onset of hearing impairment and an almost lifetime use of hearing aids. All patients but one used their own hearing aids during at least 1 year. The median follow-up period was 6.3 weeks (range 5.3– 16.9).

Table 1. Patient characteristics

Number of patients, n (m/f)	18 (9/9)
Mean age, y (range)	38.8 (20-55)
Median age start hearing impairment, y (range)	0 (0-5)
Median age first hearing aid, y (range)	4 (2-36)
Mean use own hearing aids, y (sd)	3.8 (1.7)
Median follow-up, weeks (range)	6.3 (5.3-16.9)

Audiometric evaluation

A symmetrical, high-frequency, sensorineural hearing impairment was observed in all patients. The mean audiogram, obtained from thresholds of the best hearing ear, is shown in Figure 2. The Loudness Discomfort Levels (LDL), relative to the mean thresholds, clearly demonstrates a reduction in dynamic range of hearing, most pronounced at the high frequencies. This influences the calculated compression ratios, as shown in Figure 1.

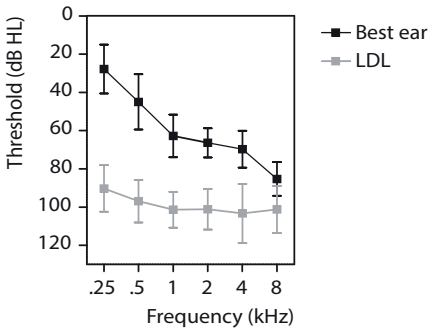


Figure 2. Average thresholds and Loudness Discomfort Levels of the best ear.

Average thresholds are represented by black squares and continuous line. Per frequency, the standard deviations are represented by thick black lines on either side of the squares. The loudness discomfort levels are represented by the grey squares and continuous line. Per frequency, the standard deviations are represented by thick grey lines on either side of the squares. Abbreviations: LDL, Loudness Discomfort Levels; HL, hearing level.

Hearing aids

All patients used their new hearing aids on a daily basis as shown in Table 2 with a mean overall use of 11.6 h/day. All but one patient kept using the new hearing aids after the study ended. That one patient preferred his old hearing aids. All patients reported to have tried the two programs in different situations. Overall, patients used the linear program on average 77% of the time (range 56%–99%), whereas this was only 17% (range 1%–44%) for the nonlinear program (two-sided; $P>0.001$; 95% Confidence Interval (CI) 46.0–75.2). This difference was statistically significant (bold values in Table 2). No difference was noted between subgroups based on program at starting up.

Table 2. Data logging from the new hearing aids

Mean data logging, days (sd)	53.7 (18.0)
Mean use new hearing aids/day, hours (sd)	11.6 (4.7)
Mean use linear program, % (range)	**77 (56-99)**
Mean use nonlinear program, % (range)	**17 (1-44)**
Mean use telecoil program, % (range)	6 (0-28)

In bold the values which differed significantly between the linear and nonlinear program.

Speech in noise

Table 3a shows no significant differences in mean SRT between the patient's own and new hearing aids with either the linear or nonlinear program. At the second session, a decrease in SRT was seen in patients with new hearing aids in both programs compared to the first measurement directly after the fitting, but this difference was not significant.

Likewise, no significant differences were found in mean SRT in noise between both programs at either visit. However, a significant improvement was seen with the own and new hearing aids (in either program) when the noise (stationary or babbled noise) was presented at -90 or +90 degrees (Table 3b).

Sound localisation

For four patients, we could not obtain localisation performance with their own hearing aids: one patient did not use his hearing aids, for one patient the hearing aids failed and for two patients the set-up failed. Figure 3 shows two typical results of sound localisation for broadband stimuli for two of the patients (#6 and #16). Patient #16 localised well with his/her own hearing aids, as well as with the two programs for the new hearing aids. Note that the bias decreased from -13 deg (with own hearing aids) to nearly 0 deg (new hearing aids). Patient #6 localised much better with the new hearing aids for either program, when compared to the own hearing aids.

Table 3.

(a) Mean SRT values for the own and new hearing aids without noise

	Own	New nonlinear		New linear	
		At fitting	Follow-up	At fitting	Follow-up
SRT, mean (sd)	39,4 (4,6)	38,9 (4,6)	37,4 (4,7)	42,3 (4,2)	40,6 (4,9)

(b) Mean signal-to-noise ratio values for the own and new hearing aids

	Own	New nonlinear		New linear	
		At fitting	Follow-up	At fitting	Follow-up
SNR S_0/N_{s0}, mean (sd)	-4.1 (1.6)	-3.2 (1.5)	-4.2 (1.6)	-3.8 (1.2)	-4.8 (1.8)
SNR S_0/N_{s90}, mean (sd)	-7.4 (4.3)*	-8.1 (3.3)*	-9.9 (3.9)*	-7.9 (3.5)*	-9.6 (3.7)*
SNR S_0/N_{f0}, mean (sd)	n.p.	n.p.	-6.9 (3.5)	n.p.	-6.3 (3.6)
SNR S_0/N_{f90}, mean (sd)	n.p.	n.p.	-11.0 (4.6)*	n.p.	-12.2 (4.4)*

a) Abbreviations: SRT, speech reception threshold. b) The signal-to-noise ratio is the ratio at which the SRT is 50%. Abbreviations: SNR, signal-to-noise ratio; S, Signal; $N_{s,}$ Speech noise; $N_{f,}$ male babble noise; $N_{0,}$ 0 degrees; N_{90}, 90 degrees. *SNR S_0/N_s (or $_f$) $_{90}$ significantly better compared to SNR S_0/N_s (or $_f$) $_0$.

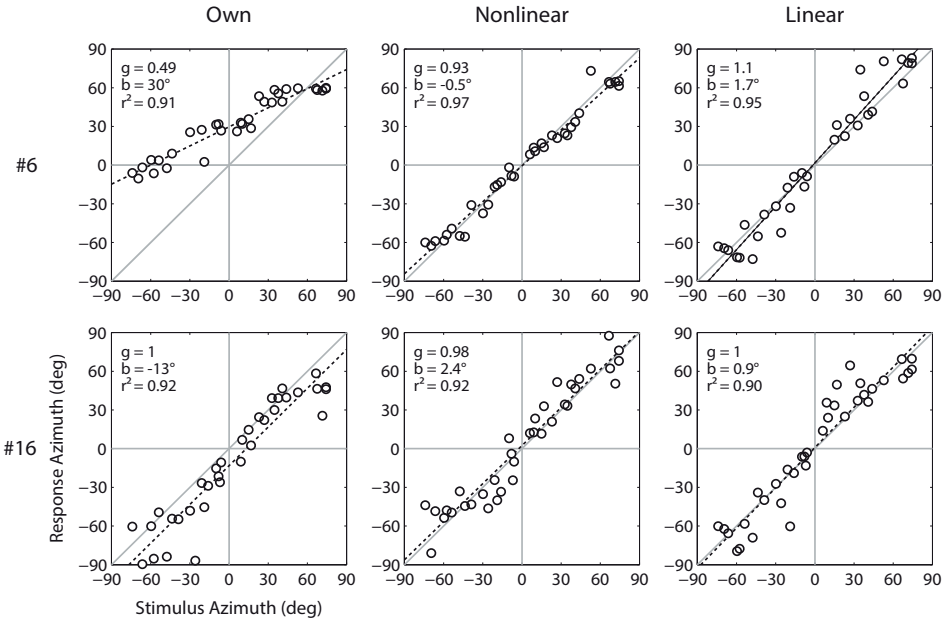


Figure 3. Sound localisation in azimuth in three conditions for two patients. Graph representing the results of two individual patients (#6 and #16) for sound localisation in azimuth (horizontal plane) in three conditions: with their own hearing aids, with the new hearing aids with the nonlinear amplification program and with the new hearing aids with the linear amplification program. Each dot represents one of the 36 broadband stimuli. The dotted line represents the best linear fit (least squares criterion) of the stimulus–response relationship. The parameters of the fit are shown in the panel: g = response gain, b = response bias and r^2 = coefficient of determination (see 'Patients and methods'). Abbreviations: deg, degrees.

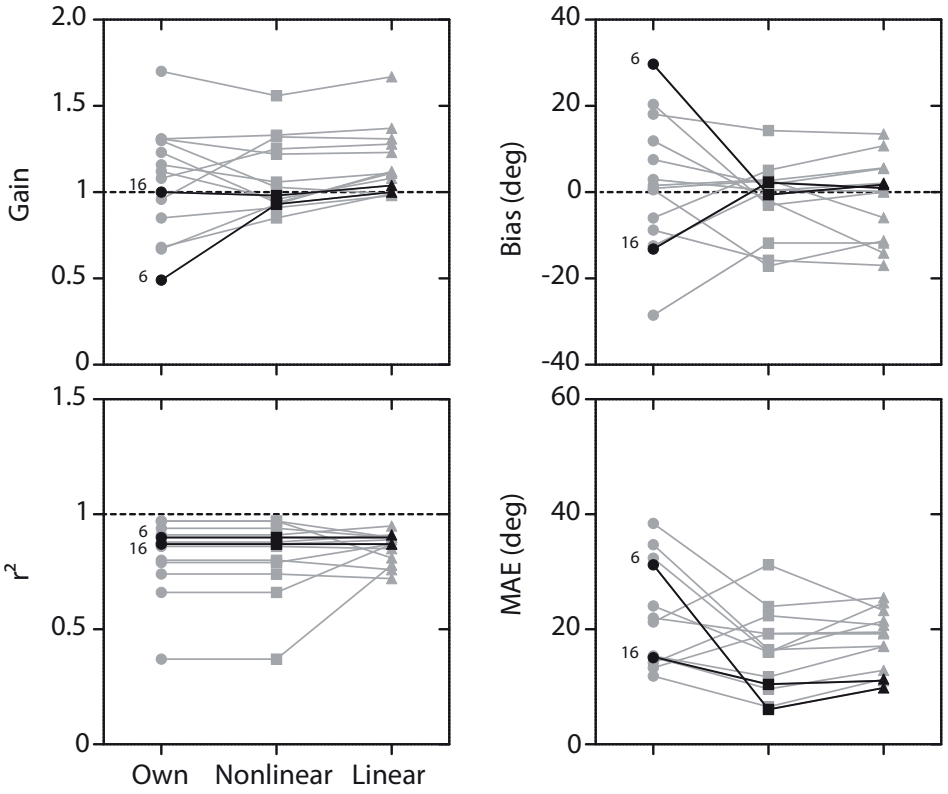


Figure 4. Individual sound localisation parameters. Graphs representing individual gain, bias, coefficient of determination and Mean Absolute Error (MAE) values for broadband stimuli in three conditions: with the patient's own (dots) and new hearing aids in nonlinear (squares) and linear (triangles) program. The values for patients #6 and #16 are highlighted for they were represented in Figure 3.

Figure 4 shows all the measured values for G, r^2, b and MAE for the three conditions with broadband sounds. The results for patients #6 and #16 are highlighted. On an individual level, the good performers with their own hearing aids seem to perform equally well in all three conditions. However, the poor performers with their own hearing aids seem to perform better with the new hearing aids, and equally well for both programs.

The normalised gain (G_E) was used to compare the average gains between the three conditions. Note that $G_E = 0$ when the gains for two conditions are the same (see 'Patients and methods'). Figure 5 demonstrates that the overall mean values did not differ significantly.

Overall, on all parameters, in all conditions of the sound localisation test, no significant differences in sound localisation were found between the own and new hearing aids

nor between the two programs in the new hearing aids. For the average values of these parameters, see Supplemental Table ST.1 (A and B).

Questionnaires

Figure 6 shows the results of the AHPAB per subscale compared to the norm percentiles obtained in 2010 by Johnson et al.[28] for successful hearing aid users. When comparing the AHPAB concerning the previous hearing aids and that concerning the new hearing aids, on each subscale a decline in disability was seen, which was statistically significant on the subscale ease of communication (*P*=0.018; 95% CI 2.47–22.69). All items in the subscale ease of communication concerned situations in quiet, in small groups without background noise. Further-more, the overall score on the APHAB improved significantly

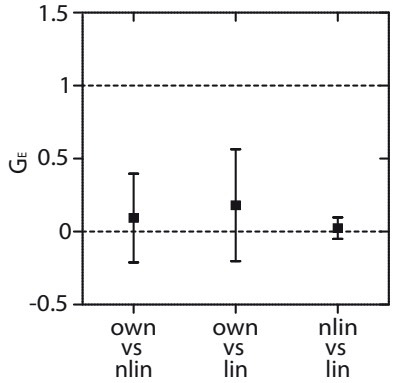


Figure 5. Comparison of normalised average gains. Graph representing the mean gain-error (G_E) for the differences between the own and new hearing aids in the nonlinear amplification program (nlin), between the own and new hearing aids in the linear amplification program (lin) and between the nonlinear amplification program of the new hearing aids. Per mean G_E, the standard deviations are represented by black lines on either side of the squares.

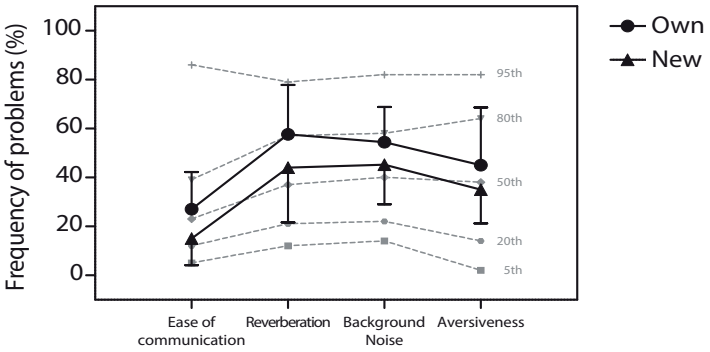


Figure 6. Abbreviated Profile of Hearing Aid Benefit (APHAB). Mean scores for the AHPAB subscales for the own (black squares _SD) and new (black triangles +SD) hearing aids. For comparison, the norm percentiles as defined by Johnson et al. in 2010 for successful hearing aid users were added.[28]

(from 46.0, SD 12.4 to 34.8, SD 12.5, *P*=0.018; 95% CI 2.02–18.46), representing an overall benefit of the new hearing aids compared to their own hearing aids. Concerning listening in background noise or in reverberant places, no significant changes were found.

On the GHADP questionnaire, a high score on device use is reported, with median scores above 90% for their own as well as new hearing aids. After using the new hearing aids, the mean reported disability, in pre-determined and personal relevant situations, showed a significant drop of 16.3% on a baseline of 58.6% (*P*=0.002).

Finally, using their diary, the patients reported a major decrease in change of programs between the first and last week of the study, from 6.6 (SD 4.6) to 2.4 (SD 1.7) changes per day (*P*=0.001). More important, the satisfaction with the linear program, 7.7 (range 1.0-9.6), was significantly higher than the satisfaction with the nonlinear program, which was 5.9 (range 1.1-8.5) (*P*=0.02).

DISCUSSION

The present study showed that the included patients with USH2a demonstrate a significant preference for the linear amplification program with omnidirectional microphone over the nonlinear program with pinna imitating directionality, measured objectively (mean logged use of 77% versus 17%) as well as subjectively (satisfaction of 7.7 versus 5.9 out of 10). In addition, the self-reported outcomes showed significantly higher scores on ease of communication and overall benefit and lower scores on disability for the new hearing aids compared to their own hearing aids. These results are complemented with good results for speech intelligibility in noise and sound localisation. The latter tests, however, did neither show a difference between hearing aids nor programs. It should be noted that the two domains of the APHAB that deal with spatial hearing (hearing in noisy places and in reverberant surroundings) did not show a significant improvement, which seems to agree with the objective measurements. Therefore, the hypothesis that a linear amplification program of hearing aids in patients with USH2a would lead to improved sound localisation when compared to a nonlinear amplification setting could so far not be confirmed.

On an individual level, differences in localisation were found between the patient's own hearing aids and their new hearing aids at follow-up for BB (Figure 5) and LP sounds. Patients who performed poorly with their own hearing aids performed better with the new hearing aids in either program. Patients who already performed quite well with their own hearing aids, performed equally well with the new hearing aids in both programs.

The good sound localisation performance of the patients with USH2a is not in agreement with their reported subjective difficulties.[10] A possible explanation for these differences might lie in the validity of the used method. The laboratory set-up and test protocol used in this study may not appropriately assess the reported difficulties. Further research is needed to evaluate the experienced difficulties by these patients. Possible interfering difficulties might be distance estimation, moving sound stimuli, localisation of sound stimuli in noise or reverberation.

In 2006, Keidser et al. performed localisation tests on hearing impaired patients with different compression techniques and directional microphone settings. They could not detect any difference in left–right localisation between a linear program with omnidirectional microphone and a program with syllabic compression and omnidirectional microphone. These results corroborate the findings of the present study. However, the nonlinear program (with syllabic compression) in our study was complemented by a moderate static directional microphone. A possible explanation for the absence of a difference may lie in the localisation environment. In our study, no noise was presented during the localisation task. A more complex localisation task, with noise, might accentuate any differences in program or microphone setting.

In conclusion, the examined patients with USH2a prefer the linear program over the nonlinear program. However, apart from a significantly higher logged use, no difference in speech in noise and sound localisation was observed. Further research is needed to address the preference of a linear over a nonlinear amplification program and to replicate the present results in hearing impaired patients without additional visual impairment.

REFERENCES

1. Boughman JA, Vernon M, Shaver KA. Usher syndrome: definition and estimate of prevalence from two high-risk populations. J Chronic Dis 1983;36(8):595-603.
2. Rosenberg T, Haim M, Hauch AM, et al. The prevalence of Usher syndrome and other retinal dystrophy-hearing impairment associations. Clin Genet 1997;51(5):314-321.
3. Spandau UH, Rohrschneider K. Prevalence and geographical distribution of Usher syndrome in Germany. Graefes Arch Clin Exp Ophthalmol 2002;240(6):495-498.
4. Baux D, Larrieu L, Blanchet C, et al. Molecular and in silico analyses of the full-length isoform of usherin identify new pathogenic alleles in Usher type II patients. Hum Mutat 2007;28(8):781-789.
5. Dreyer B, Tranebjaerg L, Rosenberg T, et al. Identification of novel *USH2A* mutations: implications for the structure of USH2A protein. Eur J Hum Genet 2000;8(7):500-506.
6. Pennings RJ, Te Brinke H, Weston MD, et al. *USH2A* mutation analysis in 70 Dutch families with Usher syndrome type II. Hum Mutat 2004;24(2):185.
7. van Wijk E, Pennings RJ, te Brinke H, et al. Identification of 51 novel exons of the Usher syndrome type 2A (*USH2A*) gene that encode multiple conserved functional domains and that are mutated in patients with Usher syndrome type II. Am J Hum Genet 2004;74(4):738-744.
8. Weston MD, Eudy JD, Fujita S, et al. Genomic structure and identification of novel mutations in usherin, the gene responsible for Usher syndrome type IIa. Am J Hum Genet 2000;66(4):1199-1210.
9. Lentz J, Keats B. Usher Syndrome Type II. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews(R). Seattle (WA); 1993.
10. Bressers I, Tham G. Extremely soft, yet incredibely close. Exploring hearing aid fitting in patients with Usher syndrome type II In; 2012, unpublished manuscript.
11. Blauert J. Spatial hearing : the psychophysics of human sound localization. Rev. ed. ed. Cambridge, Mass. ; London: MIT Press; 1997.
12. Keidser G, Rohrseitz K, Dillon H, et al. The effect of multi-channel wide dynamic range compression, noise reduction, and the directional microphone on horizontal localization performance in hearing aid wearers. Int J Audiol 2006;45(10):563-579.
13. Dillon H. Compression? Yes, but for low or high frequencies, for low or high intensities, and with what response times? Ear Hear 1996;17(4):287-307.
14. Agterberg MJ, Snik AF, Hol MK, et al. Contribution of monaural and binaural cues to sound localization in listeners with acquired unilateral conductive hearing loss: improved directional hearing with a bone-conduction device. Hear Res 2012;286(1-2):9-18.
15. Leijendeckers JM, Pennings RJ, Snik AF, et al. Audiometric characteristics of USH2a patients. Audiol Neurootol 2009;14(4):223-231.
16. Pennings RJ, Huygen PL, Weston MD, et al. Pure tone hearing thresholds and speech recognition scores in Dutch patients carrying mutations in the *USH2A* gene. Otol Neurotol 2003;24(1):58-63.
17. ISO 8253-1: Acoustics -- Audiometric test methods -- Part 1: Pure-tone air and bone conduction audiometry. Geneva; 2010.
18. Keidser G, Dillon H, Carter L, et al. NAL-NL2 empirical adjustments. Trends Amplif 2012;16(4):211-223.
19. Houben R, Koopman J, Luts H, et al. Development of a Dutch matrix sentence test to assess speech intelligibility in noise. Int J Audiol 2014;53(10):760-763.
20. Holube I, Fredelake S, Vlaming M, et al. Development and analysis of an International Speech Test Signal (ISTS). Int J Audiol 2010;49(12):891-903.

21. Veugen LC, Chalupper J, Snik AF, et al. Matching Automatic Gain Control Across Devices in Bimodal Cochlear Implant Users. Ear Hear 2016;37(3):260-270.
22. Brand T, Kollmeier B. Efficient adaptive procedures for threshold and concurrent slope estimates for psychophysics and speech intelligibility tests. J Acoust Soc Am 2002;111(6):2801-2810.
23. Van Wanrooij MM, Van Opstal AJ. Contribution of head shadow and pinna cues to chronic monaural sound localization. J Neurosci 2004;24(17):4163-4171.
24. Cox RM, Alexander GC. The abbreviated profile of hearing aid benefit. Ear Hear 1995;16(2):176-186.
25. Gatehouse S. A self-report outcome measure for the evaluation of hearing aid fittings and services. Health Bull (Edinb) 1999;57(6):424-436.
26. Van Wanrooij MM, Van Opstal AJ. Sound localization under perturbed binaural hearing. J Neurophysiol 2007;97(1):715-726.
27. Zwiers MP, Van Opstal AJ, Cruysberg JR. A spatial hearing deficit in early-blind humans. J Neurosci 2001;21(9):RC142: 141-145.
28. Johnson JA, Cox RM, Alexander GC. Development of APHAB norms for WDRC hearing aids and comparisons with original norms. Ear Hear 2010;31(1):47-55.

SUPPLEMENTAL MATERIALS

Supplemental Table ST.1. (A) Mean values for *Gain*, *bias*, r^2 and MAE for BB, LP and HP stimuli for the own and new hearing aids in linear program. (B) Mean values for *Gain*, *bias*, r^2 and MAE for BB, LP and HP stimuli for new hearing aids in nonlinear versus linear program at follow up

A

Stimulus	Own hearing aids				New hearing aids, linear program			
	Gain	Bias	r^2	MAE	Gain	Bias	r^2	MAE
Broadband, Mean (sd)	1.1 (0.3)	1.8 (15.3)	0.8 (0.2)	22.2 (9.1)	1.2 (0.2)	-1.4 (9.3)	0.9 (0.1)	18.0 (5.3)
Low Pass, Mean (sd)	1.0 (0.3)	-1.4 (14.8)	0.8 (0.3)	24.2 (13.1)	1.2 (0.2)	-3.3 (10.0)	0.9 (0.1)	19.7 (9.9)
High Pass, Mean (sd)	0.8 (0.2)	2.2 (17.8)	0.7 (0.3)	27.0 (12.1)	1.1 (0.3)	-2.0 (9.1)	0.8 (0.1)	21.4 (9.9)

Abbreviations: HA = Hearing Aids, MAE = Mean Absolute Error, r^2 = coefficient of determination, sd = Standard Deviation

B

Stimulus	New hearing aids, nonlinear program				New hearing aids, linear program			
	Gain	Bias	r^2	MAE	Gain	Bias	r^2	MAE
Broadband, Mean (sd)	1.1 (0.2)	-0.7 (8.0)	0.9 (0.1)	19.3 (9.4)	1.2 (0.2)	-0.2 (8.5)	0.9 (0.1)	16.9 (8.7)
Low Pass, Mean (sd)	1.1 (0.2)	-0.3 (10.1)	0.9 (0.1)	18.5 (10.8)	1.2 (0.2)	-2.8 (10.4)	0.9 (0.1)	18.7 (9.6)
High Pass, Mean (sd)	1.0 (0.3)	0.1 (9.0)	0.9 (0.1)	20.2 (10.4)	1.1 (0.3)	0.1 (9.1)	0.8 (0.1)	21.4 (9.8)

Abbreviations: HA = Hearing Aids, MAE = Mean Absolute Error, r^2 = coefficient of determination, sd = Standard Deviation

Chapter 3.2

Cochlear implantation in patients with Usher syndrome type IIa increases performance and quality of life

ABSTRACT

Objectives: Usher syndrome type IIa (USH2a) is characterized by congenital moderate to severe hearing impairment and retinitis pigmentosa. Hearing rehabilitation starts in early childhood with the application of hearing aids. In a select group of USH2a patients, severe progression of hearing impairment leads to insufficient speech intelligibility with hearing aids and issues with adequate communication and safety. Cochlear implantation (CI) is the next step in rehabilitation of these patients. This study evaluates the performance and benefit of CI in patients with USH2a.

Methods: Retrospective case-control study to evaluate performance and benefit after CI in 16 postlingually deaf adults (8 patients with USH2a and 8 matched controls). Performance and benefit were evaluated with a speech intelligibility test and three quality of life questionnaires.

Results: Patients with USH2a and a mean age at implantation of 59 years, show good performance after cochlear implantation. The phoneme scores improved significantly from 41 to 87% in the USH2a patients (P=0.02), and from 30 to 86% in the control group (P=0.001). Evaluation of the questionnaires demonstrated a clear benefit from CI. There were no differences in performance or benefit between USH2a and control patients before and after implantation.

Conclusions: Cochlear implantation in patients with USH2a increases speech intelligibility and improves quality of life.

Submitted as: Hartel, B.P., van Nierop, J.W., Huinck, W.J., Rotteveel, L.J., Mylanus, E.A., Snik, A.F., Kunst, H.P., & Pennings R.J. Cochlear implantation in patients with Usher syndrome type IIa increases performance and quality of life

INTRODUCTION

Usher syndrome is an autosomal recessively inherited disorder that is characterized by sensorineural hearing impairment (HI), retinitis pigmentosa (RP) and in some cases vestibular dysfunction. Usher syndrome is the leading cause of hereditary deafblindness with a prevalence of ~ 1:20,000.[1-3] RP is a slowly progressive retinal degenerative disease that leads to severe visual impairment over decades.

Usher syndrome type II (OMIM276901) is one of the three clinical types of Usher syndrome. Usher syndrome type IIa (USH2a) is the most common genetic type and accounts for more than half of all patients with USH.[4, 5] It is clinically characterized by congenital moderate to severe progressive HI, RP and intact vestibular function. Hearing rehabilitation is usually started with the application of hearing aids in early childhood. Adequate long-term hearing rehabilitation is critical as the RP progresses and eventually leads to legal blindness based on visual field size at an average age of 54 years.[6] Therefore, with increasing age, these patients depend more on hearing for communication and safety.

Previous studies have demonstrated that HI in USH2a is variable.[7-10] In the first classification of the different types of USH, Usher syndrome type II was characterized by stable HI.[11, 12] The current concept is, however, that progression of HI is seen in most patients with the genetic type USH2a.[13] In a select group of patients, severe progression of HI leads to insufficient speech intelligibility with hearing aids and issues with adequate communication and safety. In these cases, cochlear implantation (CI) is the designated choice of rehabilitation. So far, to the best of our knowledge, no results on CI in USH2a have been reported in literature.

This study presents the results on performance and an evaluation of quality of life after CI in patients with USH2a. The presented results are useful for counselling of USH2a patients with progressive sensorineural HI. In addition, the results on performance and benefit may serve as baseline data for comparison in future studies on bilateral cochlear implantation in these deafblind adults.

PATIENTS AND METHODS

Patients and controls

From 292 known Usher syndrome patients in the Nijmegen Usher syndrome database (June 2016), 186 (64%) have a clinical diagnosis of Usher syndrome type II. In 113 of them (61%), an USH2a diagnosis was confirmed by the identification of two pathogenic mutations in *USH2A*. Eight (7%) of these fully genotyped patients received a cochlear implant and were included in the present study. Three more USH2a patients are booked for CI the coming year. All patients agreed to participate in this study by informed consent.

Implantation and rehabilitation, performed according to current standard procedures, took place at the Radboud university medical centre for seven patients and for one patient at the Leiden University Medical Centre.

For comparison, a matched control group was selected from the Nijmegen Cochlear Implantation database. This group consisted of eight adults implanted with a multichannel cochlear implant. The matched criteria were in order of importance: audiogram before implantation (pure tone average; PTA 0.5-4 kHz and the PTA 0.25-2 kHz), age at implantation (the maximal deviation was nine years in one patient), gender and availability of outcome scores. This study was approved by the local ethics committee (nr. 2015/1911).

Audiometric evaluation

The demographic, clinical, audiologic, and genetic data were retrieved from the Nijmegen Usher database and available medical records at the Radboud university medical centre (n=15) and Leiden University Medical Centre (n=1). Pre- and postoperative PTA air conduction thresholds for sound frequencies ranging from 0.25 to 2 kHz (PTA Low 0.25-2) and 0.5 to 4 kHz (PTA 0.5-4) were assessed according to the ISO 8253-1.[14] Furthermore, a Dutch standardised speech perception test was used to obtain phoneme scores: the NVA test (an open speech recognition test that consists of monosyllabic wordlists).[15] All words were presented in free field at 65 dB SPL and the phoneme scores were recorded preoperatively and at 12 months postoperatively in the cochlear implant only and best aided condition.

Quality of life

Three questionnaires were used to evaluate the perceived quality of life after CI. The Glasgow Benefit Inventory (GBI) was used to assess the patient's benefit after CI. This questionnaire consists of 18 questions about changes in general, social and physical health benefits. Possible scores for each question were based on a five-point Likert scale and the numerical data were converted into a GBI score. This is an index score of -100 to +100 representing the worst to best outcome.[16]

The Nijmegen Cochlear Implant Questionnaire (NCIQ) is a hearing impairment/CI specific questionnaire. It contains three general domains: physical, psychological and social functioning. Each domain is further divided in subdomains, consisting of 10 items. The items are formulated as a statement with 5 possible answers. Final scores for the subdomains ranged from 0 to 100.[17] The GBI and NCIQ questionnaires are obtained from all implanted patients in the Radboud university medical centre.

The Usher lifestyle survey is a descriptive questionnaire. In 1980, the Nordic countries agreed to a common definition of domains of independence that are adversely affected in deafblindness. These domains are access to information, the ability to give and re-

ceive information (as in communication), and mobility. In the first domain (access to information), the recoded answers could be 1 = independently, 2 = with equipment, 3 = with others, and 4 = not aware. In the communication domain, the recoded answers could be 0 = no use to 8 = eight different ways of using modes of communication. At last, the last two answers of the communication domain and the two mobility domain answers could be 0 = independently and 1 = with others. These results were afterwards recoded in percentages of patients needing help from others.[18]

Data analysis

Analyses were performed using Prism 5.03 software (GraphPad, San Diego, CA, USA) and IBM SPSS Statistics 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). Descriptives were computed for the main characteristics (gender, age, age at implantation and follow-up period) of the patients. The results of the phoneme scores were compared pre- and postoperatively by the paired nonparametric Wilcoxon matched-pairs signed rank test. For comparison between groups, the unpaired Student's t-test and the nonparametric independent sample Mann-Witney U test were used.

The questionnaire scores were computed following the guidelines. Results were tested if they indicated significant benefit with a one sample t-test. Correlation analysis was done calculating Spearman's Rho. A general significance level of P=0.05 was applied in all separate tests.

RESULTS

Patients and Controls

The USH2a and control patients' mean characteristics are shown in Table 1. All patients were raised with oral communication and were postlingually hearing deprived. All but two patients were using bilateral hearing aids preoperatively. One USH2a patient (USH-3) had a unilateral active middle ear implant (vibrant soundbridge, Med-El Corporation, Austria) prior to CI in the contralateral ear and one control patient (CON-15) used a unilateral hearing aid in the to be implanted ear. Six USH2a patients received a multichannel cochlear implant from Cochlear (Cochlear Corporation, Australia) and two patients from Advanced Bionics (Advanced Bionics Corporation, USA). All controls received a multichannel cochlear implant from Cochlear. All patients had a follow-up of at least 12 months. For individual patient characteristics see Table 2.

Table 1. Distribution of clinical characteristics

	USH2a (n=8)	Control (n=8)
Gender, n (%) of females	7 (87)	7 (87)
Mean age, y (sd)	64 (10)	64 (9)
Mean HA use pre implantation, y (sd)	38 (18)	32 (20)
Mean age at implantation, y (sd)	59 (8)	60 (9)
PTA Low (0.25 - 2 kHz), dB HL (sd)	84 (16)	85 (15)
PTA (0.5 - 4 kHz), dB HL (sd)	98 (17)	101 (11)
Follow-up period, y (sd)	4 (6)	4 (1)

Abbreviations: USH2a = Usher syndrome type IIa, y = years, sd = standard deviation, HA = hearing aid, PTA = pure tone average, kHz = kilohertz, dB HL = decibel hearing level.

Audiometric evaluation

The mean preoperative PTA for frequencies ranging from 0.25 to 2 kHz (PTA Low) in the best ear was 84 ± 16 dB HL in the USH2a patients. This did not differ from the mean pre-implanted PTA Low in the control group (85 ± 15 dB HL). The mean preoperative PTA for the frequencies ranging from 0.5 to 4 kHz did not differ between the two groups (98 ± 17 dB HL in the USH2a patients versus 101 ± 11 dB HL in the control group). Pre-implantation, both the USH2a and the control group used hearing aids for a similar amount of time (38 years ± 18 versus 32 years ± 20). After implantation, some residual hearing in the implanted ear was preserved in four USH2a patients (USH-2, USH-3, USH-4 and USH-7). In USH-7, residual hearing declined over 12 months after surgery. In the remaining four USH2a patients, no residual hearing was seen postoperatively. In the control group, some residual hearing was observed in seven patients (CON-1, CON-2, CON-3, CON-4, CON-6, CON-7 and CON-8). The individual audiograms of patients and controls are shown in Figure 1. The mean postoperative aided hearing threshold in the implanted ear was 34 ± 18 dB HL in the USH2a group versus 28 ± 5 dB HL in the control group. This difference was not significant.

Figure 2 shows the best aided phoneme scores one year after implantation at 65 dB SPL for both groups. The phoneme score at 12 months of one USH2a patient (USH-5) could not be retrieved due to a logistical problem. None of the patients with USH2a, nor the controls, underwent bilateral CI due to the fact that in the Netherlands bilateral implantation is (still) not reimbursed for adults. All USH2a patients are using a hearing aid in the contralateral ear in the best aided condition after implantation. Two USH2a patients use an additional hearing aid in the implanted ear (USH-7 and USH-2) and use it for electro-acoustic stimulation (EAS). Five controls are using a hearing aid in the contralateral ear in the best aided condition, none of them use EAS. In the USH2a patients, phoneme scores improved significantly from 41 to 70% in the cochlear implant only condition

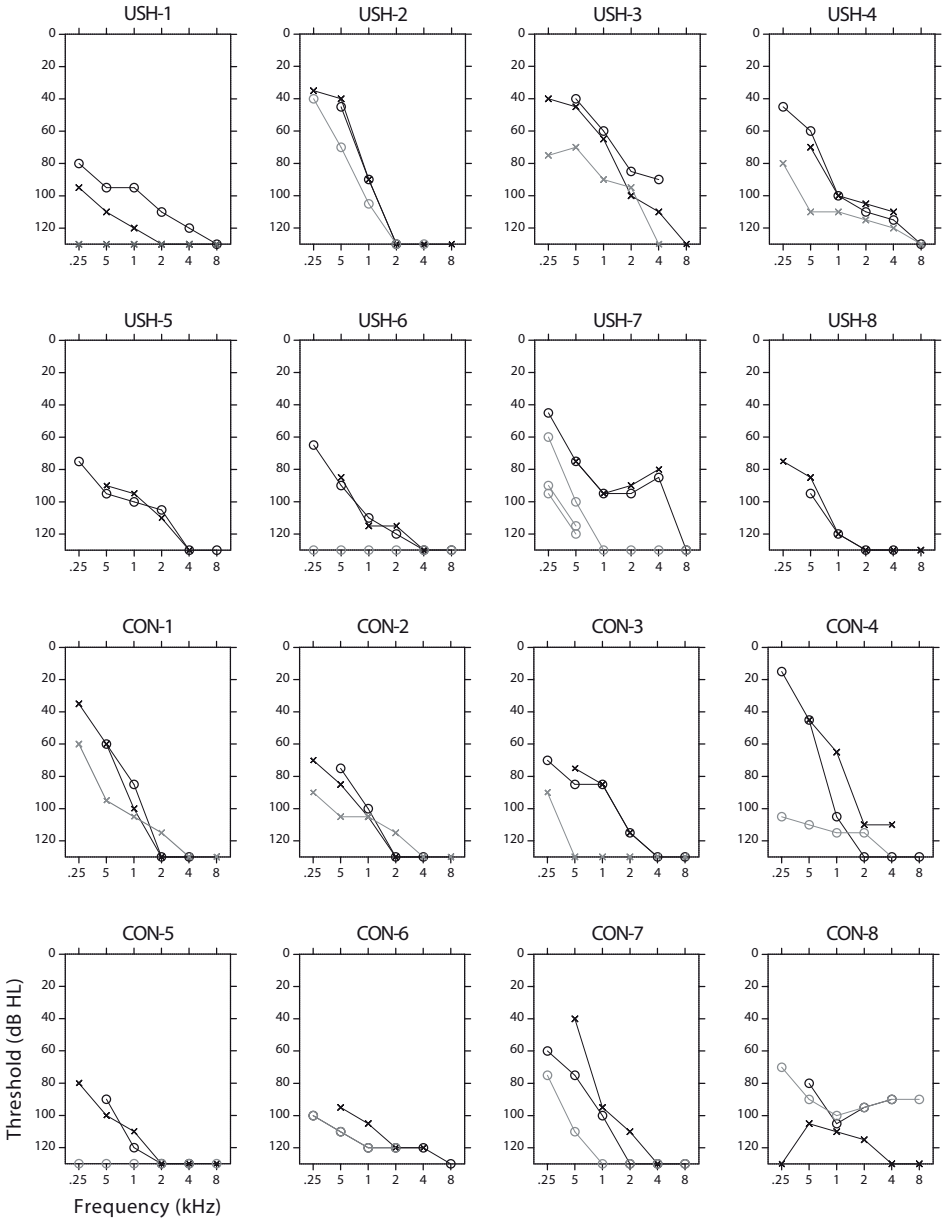


Figure 1. Pre- and postoperative audiograms of patients with Usher syndrome type IIa and controls. Circles represent the right ear and crosses represent the left ear. The grey line represents the threshold in the implanted ear post-implantation.

(*P*=0.02) and from 41 to 87% in the best aided condition (*P*=0.02). In the control patients, phoneme scores improved from 30 to 78% (*P*=0.008) and from 30 to 86% (*P*=0.008),

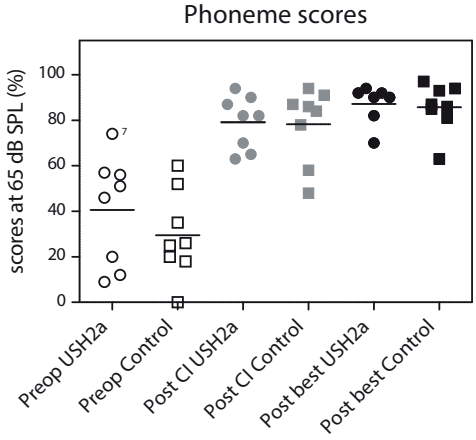


Figure 2. Individual phoneme scores pre and post implantation in the CI and best aided condition for USH2a (open, grey and black circles) and control patients (open, grey and black squares). The mean phoneme score is represented by the black line in each condition. Abbreviations: dB SPL = decibel sound pressure level, USH2a = Usher syndrome type IIa, Pre = before cochlear implantation, Post = after cochlear implantation, CI = cochlear implant, best = best aided condition.

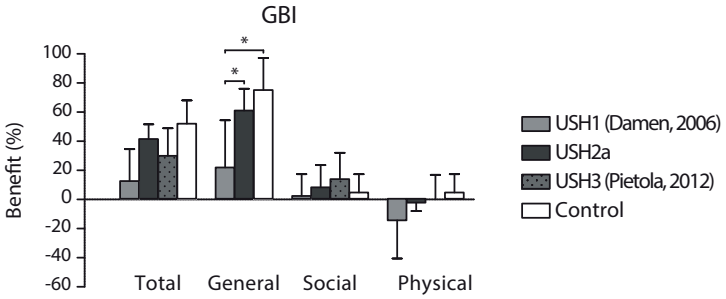


Figure 3. Mean benefit scores measured by the Glasgow Benefit Inventory (GBI) for USH1 patients (light grey), USH2a patients (dark grey), USH3 patients (grey) and control patients (white). Error bars represent one standard deviation. Abbreviations: GBI = Glasgow benefit inventory USH1 = Usher syndrome type I, USH2a = Usher syndrome type IIa, USH3 = Usher syndrome type 3.

respectively. No differences were seen between USH2a and control patients before and after implantation. Furthermore, no differences were observed between the cochlear implant only and best aided condition.

Questionnaires

Figure 3 shows the GBI scores for the USH2a patients and the control group. In one control patient (CON-13) the scores of the GBI and NCIQ could not be obtained after implantation. In both groups, the total GBI score and the general subdomain score demonstrated a significant benefit of CI. The mean scores of the total GBI were 41.6 ±

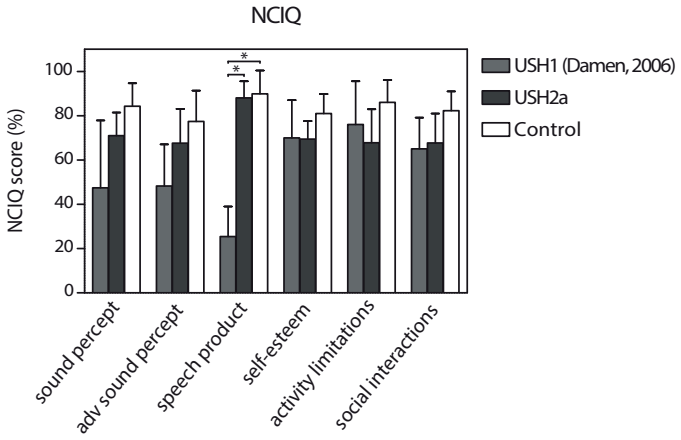


Figure 4. Mean scores measured by the Nijmegen Cochlear Implant Questionnaire (NCIQ) for the patients with USH1 (light grey), USH2a (dark grey) and the control patients (white). Error bars represent one standard deviation. Each pair of bars represents the scores for a subdomain. * = significant difference Abbreviations: NCIQ= Nijmegen cochlear implant questionnaire, adv = advances, percept = perception, product = production, USH1 = Usher syndrome type 1, USH2a = Usher syndrome type IIa

10.1 (P< 0.001; 95% CI 33.2 to 50.1) in the USH2a patients and 61.1 ± 15.0 (P<0.001; 95% CI 48.6 to 73.6) in the control group. The mean scores of the general GBI subdomain were 52.0 ± 15.6 (P>0.001; 95% CI 37.2 to 66.8) in the USH2a patients and 75.2 ± 22.1 (P>0.001; 95% CI 54.9 to 95.6) in the control group. The differences between both groups were not significant.

Table 3 demonstrates the mean scores of the NCIQ for the USH2a and control groups after CI. In five of the six subdomains, the control group scored higher, however, these differences were not significant.

Table 4 represents the results of the Usher Lifestyle survey in the USH2a group. Patients with USH2a mostly use additional equipment to wake up. Furthermore, half of the patients report to use some form of equipment to hear someone at the front door. Furthermore, to fill out a form, six out of eight USH2a patients report the use of help from others. Half of the patients use equipment to receive information on an emergency. For communication, half of the patients use the telephone without help and five out of eight patients use some form of equipment to help them write or read. To buy food or communicate with a doctor, 75% of the USH2a patients report the use of help from relatives or friends. Patients also indicate to need help from others to travel to the shop or doctor, in 87.5 and 62.5% of the patients, respectively.

Table 2. Individual patient characteristics

Patient No.	Gender	Age (y)	Age at implantation (y)	Period CI Use (y)	Implant	Electrode	Aetiology HI	Mutation 1 in *USH2A*	Mutation 2 in *USH2A*
USH-1	F	60	57	3	Cochlear, N	CI42RE	USH2a	c.1256G>T , p.(Cys419Phe)	c.1606T>C , p.(Cys536Arg)
USH-2	F	50	47	2	Cochlear, N	CI422	USH2a	c.5018T>C , p.(Leu1673Pro)	c.5018T>C , p.(Leu1673Pro)
USH-3	M	75	73	1	Cochlear, N	CI422	USH2a	c.2276G>T , p.(Cys759Phe)	c.11864G>A , p.(Trp3955*)
USH-4	F	67	65	1	AB, HIRes 90K	Hifocus MS	USH2a	c.2299delG, p.(Glu767Serfs*21)	c.14583-20C>T, p.(?)
USH-5	F	82	63	19	Cochlear, N	N22	USH2a	c.2299delG, p.(Glu767Serfs*21)	c.2299delG , p.(Glu767Serfs*21)
USH-6	F	56	54	4	Cochlear, N	CI42RE	USH2a	c.8079G>A, p.(Trp2693*)	c.8079G>A, p.(Trp2693*)
USH-7	F	63	62	1	Cochlear, N	CI422	USH2a	c.1256G>T, p.(Cys419Phe)	c.1256G>T , p.(Cys419Phe)
USH-8	F	59	55	4	AB, HIRes 90K	Hifocus 1J	USH2a	c.8954delG, p.(Gly2985Alafs*3)	c.7121-8313_11048-962delins12, del ex 38-56
CON-9	F	59	56	2	Cochlear, N	CI422	Hereditary		
CON-10	F	52	48	4	Cochlear, N	CI422	Hereditary		
CON-11	M	76	72	4	Cochlear, N	CI42RE	Hereditary		
CON-12	F	69	64	4	Cochlear, N	CI42RE	Hereditary		
CON-13	F	68	62	5	Cochlear, N	CI42RE	Hereditary		
CON-14	F	59	55	4	Cochlear, N	CI42RE	Hereditary		
CON-15	F	75	71	4	Cochlear, N	CI42RE	Hereditary		
CON-16	F	53	49	4	Cochlear, N	CI42RE	Hereditary		

Abbreviations: y = years, HI = hearing impairment, F = female, M = male, N = Nucleus, AB= Advanced Bionics, USH2a = Usher syndrome type IIa

Table 3. Mean Nijmegen Cochlear Implant Questionnaire (NCIQ) scores after cochlear implantation in the USH2a and control groups

	USH2a (n=8)			Control (n=8)		
	Mean (%)	**sd**	**Range**	**Mean (%)**	**sd**	**Range**
Sound perception basic	71.0	10.4	50.0 - 82.0	84.3	10.3	64.0 - 94.0
Sound perception advanced	67.6	15.5	50.0 - 86.7	77.4	13.9	58.0 - 96.0
Speech production	88.0	7.6	78.0 - 100.0	89.9	10.5	72.7 - 100.0
Self-esteem	69.4	15.1	35.6 - 84.0	80.9	8.9	64.0 - 90.0
Activity limitations	67.8	13.3	54.0 - 84.0	86.0	10.1	72.0 - 98.0
Social interactions	67.7	13.3	54.0 - 88.0	82.3	8.7	68.0 - 93.4

Abbreviations: USH2a = Usher syndrome type IIa

Correlations

Correlation analyses showed a significant correlation in the USH2a group between the phoneme scores at 12 months and total GBI score and two subdomains of the NCIQ. When the phoneme scores increase, the total GBI score increases (r=0.77, *P*=0.048, n=7), the NCIQ *self-esteem* subdomain increases (r=0.85, *P*=0.024, n=7) and the NCIQ *social interactions* subdomain increases (r=0.84, *P*=0.024, n=7).

Furthermore, significant correlations were seen between the phoneme score benefit ('post-implantation phoneme score' minus 'pre-implantation phoneme score') at 12 months and subdomains of the NCIQ in the control group. When the phoneme benefit increases, the NCIQ *speech perception* subdomain increases (r=0.92, *P*=0.006, n=7), the NCIQ *speech production* subdomain increases (r=0.85, *P*=0.024, n=7) and the NCIQ *activities* subdomain increases (r=0.85, *P*=0.024, n=7).

Table 4. Scores of the USH2a group on the Usher Lifestyle survey

		Independently	**equipment**	**others**	**Unaware**
Access to information	Wake up (n)	1	**6**	1	0
	Front door (n)	**4**	**4**	0	0
	Access form (n)	0	2	**6**	0
	Emergency (n)	3	**4**	1	0
Communication	Telephone use (n)	**4**	1	3	0
	Written communication (n)	2	**5**	0	1
	Buy food (% help from others)		75		
	Communicate doctor (% help from others)		75		
Mobility	Visit shop (% help from others)		87.5		
	Visit doctor (% help from others)		62.5		

DISCUSSION

About 10% of the patients (11/113) with USH2a known in our database have or will receive a cochlear implant in the near future. The present study shows that patients with USH2a have good performance after CI and that it is beneficial to them. All outcomes showed similar results when compared to a control group of postlingually deaf implanted adults.

Six patients (four with USH2a and two controls) had a pre-implantation phoneme score of more than 50% in best-aided conditions and speech presented at 65 dB SPL. Especially USH-7 (highlighted in Figure 2) showed an exceptionally high pre-implantation phoneme score of 74%. Although the hearing phenotype in USH2a patients is variable, progression is found in most patients.[13] Such was the case in USH-7, who experienced a progressive deterioration of hearing and visual function over the previous 10 years. This patient obtained good postoperative scores with a phoneme score of 90% and a total GBI score of 27.8. The double-sensory progression was fundamental for the decision to perform CI and as demonstrated in this study, individuals with exceptional progression might benefit from early CI.

In a previous study from our group, HI was evaluated in patients with different types of *USH2A* mutations.[13] Patients with two truncating mutations demonstrate a significantly more severe and progressive HI compared to patients with two nontruncating (missense) mutations or one truncating and one nontruncating mutation in *USH2A*. Of the eight USH2a patients included in this study, four patients carried two truncating mutations (USH-4, USH-5, USH-6 and USH-8), three patients carried two nontruncating mutations (USH-1, USH-2 and USH-7), and one patient carried one truncating and one nontruncating mutation (USH-3). This indicates that half of the patients belong to the two groups that are considered to have less severe and less progressive HI. A possible explanation for this might be that these patients have nontruncating mutations that do lead to more progression of HI, they might have genetic modifiers that influence HI or may have been exposed to negative environmental factors. Future individual longitudinal analyses could reveal specific nontruncating mutations that are linked to more severe and progressive HI. In addition, epidemiological studies might identify other negative environmental factors as well.

Previous studies demonstrated that CI in deafblind adults is as successful as in other postlingually deaf adults.[19-21] Furthermore, CI improves quality of life in adults with either Usher syndrome type 1 (USH1) [22, 23] or Usher syndrome type 3 (USH3).[21] Unlike USH2a, USH1 (OMIM276900) is characterized by congenital, severe to profound HI,

pre-pubertal onset of RP, and vestibular areflexia. In 2006, Damen et al. and Pennings et al. reported the performance and quality of life in 7 adults with USH1 who received a cochlear implant. In Figures 3 and 4, the current results of the USH2a patients were compared to those reported for USH1 patients (Damen et al. and Pennings et al.). Figure 3 shows the GBI scores. In the "general" domain, the USH2a patients (dark grey bar), experience significantly more benefit after CI compared to USH1. The "general" benefit domain is composed of 12 of the 18 items and represents all items about benefit itself. The questions in the other domains "social" and "physical health" benefits inform us about the needed help from others and physical problems. Therefore, the mean score on the most extensive domain of the GBI is significantly better in the USH2a subgroup compared to the USH1 subgroup. The factor influencing this outcome is most probably the onset of profound deafness. Patients with USH1 are prelingually deaf and the patients in Damen's study were implanted in early adulthood. This difference in onset also explains the significant difference in the NCIQ subdomain *speech production* (USH2a patients: 88 ± 7,6 versus USH1 patients 25,4 ±13,6 (P<0,001; 95% CI 34,44 - 90,76) as demonstrated in Figure 4. On the other domains of the NCIQ no significant differences were found (Figure 4).

Usher syndrome type 3 (USH3, OMIM276903) has a variable onset and degree of HI, RP, and vestibular abnormalities.[12] In addition, HI in USH3 is progressive, especially in the first three decades of life.[24, 25] However, this progression is not reported to be significantly more compared to USH2a patients.[24] In 2012, Pietola et al. studied the results of CI in 19 patients with USH3. With a mean age of 41 (sd 17) years, the studied patients were implanted at a significantly younger age compared to the USH2a patients in this study. (P=0.009). None of the available results for the GBI subdomains differed from the present results (Figure 3). The speech recognition scores could unfortunately not be compared due to the fact that only word scores and no phoneme scores were published.

Due to the visual decline in RP, especially the peripheral vision, USH2a patients are dependent on auditory input for proper sound localisation. Adequate sound localisation is of paramount importance to patients with deafblindness to locate for example cars or cyclists, providing a safe situation in traffic. Adults with one cochlear implant are less capable of sound localisation compared to adults with bilateral cochlear implants.[26] For this reason, patients with deafblindness, such as USH2a patients, may especially benefit from bilateral CI.

In conclusion, CI in patients with USH2a and severe progression of HI increases speech intelligibility and improves quality of life, leading to improved communication.

REFERENCES

1. Boughman JA, Vernon M, Shaver KA. Usher syndrome: definition and estimate of prevalence from two high-risk populations. J Chronic Dis 1983;36(8):595-603.
2. Rosenberg T, Haim M, Hauch AM, et al. The prevalence of Usher syndrome and other retinal dystrophy-hearing impairment associations. Clin Genet 1997;51(5):314-321.
3. Spandau UH, Rohrschneider K. Prevalence and geographical distribution of Usher syndrome in Germany. Graefes Arch Clin Exp Ophthalmol 2002;240(6):495-498.
4. Baux D, Faugere V, Larrieu L, et al. UMD-USHbases: a comprehensive set of databases to record and analyse pathogenic mutations and unclassified variants in seven Usher syndrome causing genes. Hum Mutat 2008;29(8):E76-87.
5. Dreyer B, Tranebjaerg L, Rosenberg T, et al. Identification of novel *USH2A* mutations: implications for the structure of USH2A protein. Eur J Hum Genet 2000;8(7):500-506.
6. Pierrache LH, Hartel BP, van Wijk E, et al. Visual Prognosis in *USH2A*-Associated Retinitis Pigmentosa Is Worse for Patients with Usher Syndrome Type IIa Than for Those with Nonsyndromic Retinitis Pigmentosa. Ophthalmology 2016.
7. Abadie C, Blanchet C, Baux D, et al. Audiological findings in 100 USH2 patients. Clin Genet 2012;82(5):433-438.
8. Blanco-Kelly F, Jaijo T, Aller E, et al. Clinical aspects of Usher syndrome and the *USH2A* gene in a cohort of 433 patients. JAMA Ophthalmol 2015;133(2):157-164.
9. Leijendeckers JM, Pennings RJ, Snik AF, et al. Audiometric characteristics of USH2a patients. Audiol Neurootol 2009;14(4):223-231.
10. Pennings RJ, Huygen PL, Weston MD, et al. Pure tone hearing thresholds and speech recognition scores in Dutch patients carrying mutations in the *USH2A* gene. Otol Neurotol 2003;24(1):58-63.
11. Davenport S, Omenn GS. The heterogeneity of Usher syndrome [abstract]. In: Littlefield JW, Ebbing FJG, Henderson JW, editors. Fifth International Conference on Birth Defects. Amsterdam; 1977. p. 87–88.
12. Smith RJ, Berlin CI, Hejtmancik JF, et al. Clinical diagnosis of the Usher syndromes. Usher Syndrome Consortium. Am J Med Genet 1994;50(1):32-38.
13. Hartel BP, Lofgren M, Huygen PL, et al. A Combination of Two Truncating Mutations in *USH2A* causes more Severe and Progressive Hearing Impairment in Usher syndrome type IIa. Hear Res 2016.
14. ISO 8253 Acoustics -- Audiometric test methods. Part 1: Pure-tone air and bone conduction audiometry. In: International Organizationfor Standardization, Geneva 2010.
15. Bosman AJ, Smoorenburg GF. Intelligibility of Dutch CVC syllables and sentences for listeners with normal hearing and with three types of hearing impairment. Audiology 1995;34(5):260-284.
16. Robinson K, Gatehouse S, Browning GG. Measuring patient benefit from otorhinolaryngological surgery and therapy. Ann Otol Rhinol Laryngol 1996;105(6):415-422.
17. Hinderink JB, Krabbe PF, Van Den Broek P. Development and application of a health-related quality-of-life instrument for adults with cochlear implants: the Nijmegen cochlear implant questionnaire. Otolaryngol Head Neck Surg 2000;123(6):756-765.
18. Damen GW, Krabbe PF, Kilsby M, et al. The Usher lifestyle survey: maintaining independence: a multi-centre study. Int J Rehabil Res 2005;28(4):309-320.
19. El-Kashlan HK, Boerst A, Telian SA. Multichannel cochlear implantation in visually impaired patients. Otol Neurotol 2001;22(1):53-56.
20. Hinderink JB, Mens LH, Brokx JP, et al. Performance of prelingually and postlingually deaf patients using single-channel or multichannel cochlear implants. Laryngoscope 1995;105(6):618-622.

21. Pietola L, Aarnisalo AA, Abdel-Rahman A, et al. Speech recognition and communication outcomes with cochlear implantation in Usher syndrome type 3. Otol Neurotol 2012;33(1):38-41.
22. Damen GW, Pennings RJ, Snik AF, et al. Quality of life and cochlear implantation in Usher syndrome type I. Laryngoscope 2006;116(5):723-728.
23. Pennings RJ, Damen GW, Snik AF, et al. Audiologic performance and benefit of cochlear implantation in Usher syndrome type I. Laryngoscope 2006;116(5):717-722.
24. Plantinga RF, Kleemola L, Huygen PL, et al. Serial audiometry and speech recognition findings in Finnish Usher syndrome type III patients. Audiol Neurootol 2005;10(2):79-89.
25. Cohen M, Bitner-Glindzicz M, Luxon L. The changing face of Usher syndrome: clinical implications. Int J Audiol 2007;46(2):82-93.
26. Smulders YE, van Zon A, Stegeman I, et al. Comparison of Bilateral and Unilateral Cochlear Implantation in Adults: A Randomized Clinical Trial. JAMA Otolaryngol Head Neck Surg 2016;142(3):249-256.

Chapter 4

Discussion

The aims of this thesis were to examine patients with pathogenic mutations in *USH2A* in order to elucidate the phenotype, to identify putative genotype-phenotype correlations and to evaluate hearing rehabilitation options. In the following discussion, the results of this thesis will be discussed according to their position in a care program. Furthermore, the effect of the results on this care program, future research fields and the importance of patient participation will be discussed.

GENETIC DIAGNOSIS

During the first appointment of a hearing impaired patient at an ENT outpatient clinic, the physician will differentiate between an acquired cause, such as infection, ototoxic drugs or loud sound exposure, or an inherited cause of hearing impairment. Generally, once the acquired causes are unlikely or ruled out, genetic testing may be performed to identify the potential cause of the phenotype. Recent developments in genome diagnostics permit us to detect the genetic defects underlying hereditary disease more efficiently and at an earlier age. Where only a few years ago, it was common to order sequencing of a single gene, nowadays, this is largely replaced by whole exome sequencing (WES). Subsequently, targeted analysis of WES data is performed using disease-specific gene panels. In this way, only variants in genes that were previously identified to be associated with a certain condition are selected and analysed. In our institute, the deafness gene panel currently consists of 141 genes associated with syndromic and nonsyndromic forms of hearing impairment.[1] Hearing impaired children as well as adults are eligible to undergo screening using this diagnostic tool. Since implementation of WES for diagnostic purposes significantly increased the percentage of genetically solved patients as compared to single gene testing (33.5% versus 16% genetically solved patients), we try to reduce single gene testing to a minimum.[2]

The use of WES as a diagnostic tool presents both new challenges and advantages.[3] The former order of events in a care program to diagnose patients with Usher syndrome has changed. Usher syndrome was previously always clinically diagnosed based on the combination of hearing impairment, retinitis pigmentosa and the presence or absence of vestibular dysfunction. The order of clinical diagnosis of Usher syndrome > genetic diagnosis of Usher syndrome > rehabilitation is labelled as 'forward genetics' in literature.[4] However, nowadays the diagnosis Usher syndrome is more often made based on the outcome of genetic screening and given way before the first clinical presentation of ocular symptoms. The order thus changes to genetic diagnosis of Usher syndrome > clinical symptoms of Usher syndrome > rehabilitation, also known as 'reverse phenotyping'.[5]

A possible disadvantage from this change is that early identified mutations in a hearing impairment-associated gene may not always provide a definite clinical diagnosis. This is due to the fact that different phenotypes might result from mutations in one single gene. For example, infants with pathogenic mutations in *MYO7A* (OMIM 276903), may develop a syndromic (Usher syndrome type Ib (OMIM 276900) or nonsyndromic phenotype (autosomal recessive deafness type 2 (DFNB2, OMIM 600060)).[6, 7] This also highlights the importance of re-evaluating available WES data as new clinical features emerge in a patient over time.

Another disadvantage of a genetics-based diagnosis of Usher syndrome before the onset of visual deterioration may be that it will have a considerable psychosocial effect on the parents. To our knowledge, no studies have been performed on the parental impact of receiving an early genetic diagnosis of a child with Usher syndrome. Oonk et al. studied the psychosocial impact of a genetic diagnosis of nonsyndromic hereditary hearing impairment in adults (unpublished work).[8] They found no differences in psychosocial outcomes between patients receiving a genetic diagnosis for their hearing impairment as compared to patients for whom no genetic diagnosis could be obtained after testing. However, the impact of a genetic diagnosis of syndromic hearing impairment in patients and parents remains to be studied.

Finally, an early genetic diagnosis of Usher syndrome raises ethical questions. To answer these, a care professional has to integrate a new ethical part in the parental counselling. This consists of discussing whether the child must be informed before or after developing initial visual symptoms.

A genetic diagnosis for Usher syndrome early in life also presents advantages for patients, parents and scientists. As a result, patients will receive a treatment already at an early stage and will be informed about prognosis. Furthermore, it may lead to anticipatory guidance for rehabilitation and the existence of support groups. Parents can be informed about the recurrence risks and may become aware of possible Usher syndrome-related problems that might arise as a consequence of the defects in hearing, balance and vision. Parents of patients with Usher syndrome type I, for example, can expect delayed motor milestones of their child as a result of the presented vestibular dysfunction. In addition, they may seek support from the patient and parent community at an earlier stage. For scientists, a strict follow-up of genetically diagnosed patients with specific evaluations is useful to obtain a detailed natural course of the disease, which may serve as a basis for further genotype-phenotype studies. Natural history studies in patients that are eligible to receive future genetic treatments are of utmost importance in order to determine the therapeutic efficacy.

To identify all the challenges and advantages of early genetic testing in the current era, it is essential to study the effect of an early genetic diagnosis of Usher syndrome on patients and parents. The involvement of different care professionals, like an ENT doctor, an audiologist, an ophthalmologist, a clinical geneticist, a psychologist and a social worker, may add to the completeness of such a study.

COUNSELLING AFTER A GENETIC DIAGNOSIS

Counselling is the action of providing information and advice to an individual patient. To do so, we not only need the patient's information, like (medical) background, family and social status, symptoms and genetic information, but also knowledge about the (course of) disease. Natural history studies are the preferred choice to examine the course of a disease in order to obtain information useful for counselling. These studies can be approached in a cross-sectional or longitudinal way. Chapter 2.1 is an example of a cross-sectional study and chapter 2.2 of a longitudinal study. Both studies were performed on a retrospective basis. Differences in used equipment, experimental setup and care professionals that conducted the measurements in both studies may have introduced a bias.

In literature, there is extensive knowledge about the type of hearing impairment but less about progression of hearing impairment in patients with USH2a. A congenital moderate to severe hearing impairment, more pronounced in the higher frequencies is generally observed.[9-13] The studied populations were, however, small and only in part genotyped. In addition, a large variability in hearing impairment was identified. In chapter 2.1, we studied severity and progression of hearing impairment in patients with USH2a. This study demonstrated that the hearing impairment in these patients is progressive. This result has a direct impact on the clinical characterization of USH2a as described by Davenport et al. and Smith et al.[14, 15] Therefore, we propose a refinement of the criteria for clinical characterization of USH2a, including progressive hearing impairment in most patients (Table 1).

Table 1. Proposed refinement of the criteria for clinical characterization of Usher syndrome

	Hearing impairmentt	Visual impairment	Vestibular function
Usher syndrome type I	Congenital, severe to profound	Diagnosis before puberty, progressive	Absent
Usher syndrome type II	Congenital, moderate to severe **mildly progressive in most patients**	Diagnosis around puberty, progressive	Intact
Usher syndrome type III	Variable onset progressive	Variable age of diagnosis, progressive	Variable

In chapter 2.2, a similar genotype-phenotype correlation was observed in patients with *USH2A*-associated nonsyndromic retinitis pigmentosa (nsRP) and USH2a. In this study, the presence of two truncating mutations in *USH2A* was always associated with hearing impairment (thus USH2a), and the number of truncating mutations predicted the severity of the visual phenotype. The observed association between truncating mutations and the course of both the hearing and visual phenotypes has greatly improved our knowledge of USH2a and *USH2A*-associated nsRP. This insight is essential for proper counselling of these patients.

Even after taking into account the above described genotype-phenotype correlations, the large variability of hearing impairment in USH2a patients remains unexplained. This suggests that other genetic and/or environmental factors are important in the development of hearing impairment as already suggested by Sadeghi et al. in 2013.[16] Possibly, individual or combinations of certain mutations may have a more severe effect on the phenotypic outcome than others. This theory is supported by the results of chapter 2.3. In this study, the hearing phenotype of patients with *USH2A*-associated nsRP was compared to presbyacusis and hearing impairment in patients with USH2a. The observed (combinations of) *USH2A* mutations in patients with *USH2A*-associated nsRP proved to be unique as compared to a large sample of 110 patients with USH2a. It was demonstrated that a proportion of patients (8/18), initially diagnosed with *USH2A*-associated nsRP, developed a mild, adult onset hearing impairment. However, the differences in the level of hearing impairment and the age of onset at which the hearing impairment manifests clearly differentiates patients with *USH2A*-associated nsRP from patients with USH2a.

Furthermore, mutations in other genes known to be associated with (non)syndromic forms of hearing impairment may influence the severity and/or progression of hearing impairment in patients with USH2a and *USH2A*-associated nsRP. To identify these mutations, large cohorts of patients should undergo extensive genetic screening by WES.[17]

This could result in the discovery of mutations or specific Single Nucleotide Polymorphisms (SNPs), which might modify the observed phenotype.

Finally, nongenetic factors may influence the hearing and visual phenotype. These might be environmental factors, like loud sound exposure or sunlight exposition, but also early rehabilitation, education and support. To identify and study these factors, a large database with detailed information of a large USH2a and *USH2A*-associated nsRP cohort could be a useful tool. To be able to aggregate this information, standardized evaluation protocols will be necessary on a national and international level.

Further awareness of genetic and environmental factors might improve individual counselling and prognosis. The knowledge of factors influencing hearing impairment, like the type of mutation in *USH2A*, might affect the counselling on hearing rehabilitation options in adulthood. The different hearing rehabilitation options will be discussed in the next part of the discussion.

HEARING REHABILITATION AFTER THE DIAGNOSIS OF USH2A

Chapters 3.1 and 3.2 present the results of the commonly used hearing rehabilitation options used in patients with USH2a: hearing aids and cochlear implants.

Due to the congenital aspect of hearing impairment, most patients use hearing aids from young age.[18] However, some patients start using hearing aids at later age because of the existing variability in hearing impairment. Modern hearing aids are primarily developed for sighted individuals with hearing impairment. The possible limitations in spatial hearing are considered negligible because these users will have good visual abilities to compensate for distorted sound localisation cues presented by the hearing aids. Instead, patients with low or no vision, such as patients with USH2a, cannot rely on visual cues and, therefore, auditory cues become critical.[19] Prolonged hearing aid fitting is often necessary to optimize both spatial hearing and communication. In chapter 3.1 of this thesis, we evaluated the effects of two hearing aid settings on speech perception (as an outcome for communication) and sound localisation (as an outcome for spatial hearing). The first setting consisted of a linear amplification setting. Hypothetically, this setting would preserve auditory cues necessary for sound localisation. The second setting applied a nonlinear amplification. This was thought to offer better performances in speech perception. We could, however, not determine any differences in speech perception and sound localisation between a linear and a nonlinear program. The results, with the tests we used, were equally good. Nonetheless, subjectively, patients preferred by

far the linear over the nonlinear program. The similar objective outcomes might be due to the compression ratios of the two settings. The compression ratio of the nonlinear setting was mild. A higher compression ratio might have had a more detrimental effect on the auditory cues for sound localisation.[20] Other tests to evaluate spatial hearing or to compare the effects of different hearing aid settings might include distance perception and moving sounds. In addition, more "real world' stimuli might be used. In other words, these suggested tests might be more ecological valid. Ecological validity refers to the extent to which results represent the range of experiences in daily life.[21] Tests with a high ecological validity can be generalised to real-life situations.

The study presented in chapter 3.2 evaluated the performance of and subjective outcomes after cochlear implantation, a more invasive rehabilitation option, in patients with USH2a with profound hearing impairment. In eight USH2a patients using a cochlear implant, speech perception and subjective outcomes were compared to matched adults with nonsyndromic congenital hearing impairment. Good performances, similar to the control group, were observed. In our institute, ten percent of all USH2a patients (all ages included) have or are eligible for a cochlear implant in the near future and above the age of 50 years, this percentage increases up to 15%. Furthermore, the raised awareness, changing implantation criteria and improving implant outcomes may possibly lead to a higher prevalence of cochlear implant recipients in patients with USH2a in the future.

Sound localisation with unilateral cochlear implantation, with or without hearing aid in the contralateral ear, is poor. Bilateral cochlear implantation has been proven to be of benefit for sound localisation. Concerning speech perception in noise, an absolute benefit of bilateral cochlear implantation over a bimodal situation (a cochlear implant in one ear and a hearing aid in the contra-lateral ear) is less evident.[22, 23] In the Netherlands, bilateral cochlear implantation is from 2016 onwards fully reimbursement for deafblind adults, including Usher syndrome. To complement chapter 3.2, future research should now focus on the additional benefits of a second cochlear implant in deafblind adults and the additional value compared to a bimodal situation with regard to speech perception in noise.

FUTURE TREATMENT OPTIONS

Besides improvements in diagnostics, counselling and rehabilitation, the most promising research in the coming decades will focus on the development of therapeutic treatments. When we talk about treatment we often think of curing deafness and/or blindness. In the case of treatment for USH2a, the current primary goal is to stop the progression of visual deterioration. This might be achieved by different forms of therapy.

An important concept is genetic therapy. An example of genetic therapy is gene augmentation therapy. This consists of supplying "healthy" copies of the gene (e.g. *USH2A*) to the retina using adeno-associated or lentiviral vectors. However, for USH2a this is very difficult due to the size of the coding sequence of *USH2A* (15.606 base pairs), which largely exceeds the capacity of the currently available vehicles for delivery. This problem of length may be bypassed by the use of minigenes. Minigenes are edited genes consisting of only the regions of the gene that encode the most important domains of the protein. Consequently, these shortened *USH2A* minigenes might be packaged into the available viral vectors. Another possible approach is the development of a genome-editing strategy by using CRISPR/Cas9-based RNA-guided DNA endonucleases.[24] By adopting this approach, specific genomic aberrations could be modified or repaired. Finally, another therapeutic option is the use of antisense oligonucleotides (AONs) to redirect aberrant splicing caused by (deep-)intronic mutations or to "skip" certain protein-encoding regions (exons) that contain deleterious mutations. [25, 26]

A cellular based therapy, such as cell replacement therapy with induced pluripotent stem cells also seems promising.[27] Stem cell therapy aims to introduce stem cells into the target organ (e.g. retina or cochlea) and allow them to differentiate into specific cells. These cells may turn into properly functioning photoreceptor cells in the retina or hair cells in the cochlea when transplanted.[28, 29]

Many mutations in *USH2A* are private and evenly distributed over the gene. They include nonsense, frame-shift, splice-modulating (= truncating mutations), and missense variants (= nontruncating mutations). It is generally believed that USH2a, due to mutations in this gene, is caused by a loss-of-function mechanism. However, it was recently demonstrated that an *USH2A* missense (nontruncating) mutation could result in the accumulation of non-functional usherin in the endoplasmatic reticulum of patient-derived retinal organoids, leading to cellular stress and consequently apoptosis.[27] This observed additional pathogenic effect of nontruncating mutations over truncating mutations might influence the choice of future therapeutic approaches for different types of mutations.

For now, therapeutic studies concentrate on the development of treatment options that enable to stop the progression of retinal degeneration in Usher syndrome patients in the future. An important reason for this is that good hearing rehabilitation options are available for hearing impairment, which is not the case for the visual impairment. Natural history studies have demonstrated that patients with USH2a are born with apparently normal vision and develop the first clinical symptoms shortly after puberty. This leaves a time frame for therapeutic intervention before the onset of the first clinical symptoms.

Moreover, the concept of delivering genetic material to the retina by injection into the subretinal space has been proven adequate.[30] In contrast, hearing impairment in USH2a has a congenital onset. This is probably caused by a defective cochlear hair bundle development as a result of defects in or absence of usherin. In humans, this development is completed *in utero*. Therefore, ideally, treatment should be applied *in utero* which is, both technically and ethically, challenging. Consequently, post-natal treatment options should be further explored in the future.[31]

PATIENT-PARTICIPATORY RESEARCH

To conclude the discussion of this thesis, it is wise to go back to where it all started: patient reported problems. The concept of patient involvement in science is spreading across the scientific community. Patients have always been involved in research, as object of interest or medium on which innovations were tested. Nowadays their engagement may, however, be different. Patients are nowadays encouraged to participate from the start of new research projects by formulating research questions and participate by attracting and including other patients. In the world of well-known medical journals, involvement of patients in journal review boards and strategy planning is a trend and is often called "patient partnership". For example, the British Medical Journal (BMJ) has adopted this strategy in 2014 and explains: "*it sees partnering with patients, their families, carers and support communities, and the public as an ethical imperative, which is essential to improving the quality, safety, value and sustainability of health systems*" (http://www/bmj/com/campaign/patiënt-parnership).

The idea of patient involvement in research is part of a bigger philosophy, the concept of "patient centered care". This idea of patient involvement in care and science is highly promoted by the Radboudumc. In this institute, "patient centered care" is one of the four core values. Our Usher-expert team (part of Hearing and Genes) has adopted this core value and promotes patient participation in care, education and research programs. Finally, this thesis has adopted and integrated this philosophy by studying research questions formulated by patients with USH2a and tried to provide directly applicable answers on their care program.[32]

REFERENCES

1. www.radboudumc.nl/Informatievoorverwijzers/Genoomdiagnostiek/en/Pages/Hearingimpairment. In; Accessed 28 november 2016.
2. Yntema H, Seco CZ, Wesdorp M, et al. The diagnostic yield of whole exome sequencing targeting a gene panel for hearing impairment in the Netherlands. Accepted for publication in the European Journal of Human Genetics, 2016.
3. Kimberling WJ, Hildebrand MS, Shearer AE, et al. Frequency of Usher syndrome in two pediatric populations: Implications for genetic screening of deaf and hard of hearing children. Genet Med 2010;12(8):512-516.
4. Takahashi JS, Pinto LH, Vitaterna MH. Forward and reverse genetic approaches to behavior in the mouse. Science 1994;264(5166):1724-1733.
5. Schulze TG, McMahon FJ. Defining the phenotype in human genetic studies: forward genetics and reverse phenotyping. Hum Hered 2004;58(3-4):131-138.
6. Liu XZ, Walsh J, Mburu P, et al. Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. Nat Genet 1997;16(2):188-190.
7. Weil D, Kussel P, Blanchard S, et al. The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene. Nat Genet 1997;16(2):191-193.
8. Oonk A, Ariens S, Kunst HP, et al. Psychological impact of a genetic diagnosis on hearing impairment, an exploratory study. In: Radboud university medical center.
9. Pennings RJ, Huygen PL, Orten DJ, et al. Evaluation of visual impairment in Usher syndrome 1b and Usher syndrome 2a. Acta Ophthalmol Scand 2004;82(2):131-139.
10. Hunter DG, Fishman GA, Mehta RS, et al. Abnormal sperm and photoreceptor axonemes in Usher's syndrome. Arch Ophthalmol 1986;104(3):385-389.
11. Pennings RJ, Huygen PL, Weston MD, et al. Pure tone hearing thresholds and speech recognition scores in Dutch patients carrying mutations in the *USH2A* gene. Otol Neurotol 2003;24(1):58-63.
12. van Aarem A, Pinckers AJ, Kimberling WJ, et al. Stable and progressive hearing loss in type 2A Usher's syndrome. Ann Otol Rhinol Laryngol 1996;105(12):962-967.
13. Sadeghi M, Cohn ES, Kelly WJ, et al. Audiological findings in Usher syndrome types IIa and II (non-IIa). Int J Audiol 2004;43(3):136-143.
14. Davenport SLH, Omenn GS. The heterogeneity of Usher syndrome. In: Fifth International Conference on Birth Defects; 1977; Amsterdam: Excerpta Medica; 1977. p. 87-88.
15. Smith RJ, Berlin CI, Hejtmancik JF, et al. Clinical diagnosis of the Usher syndromes. Usher Syndrome Consortium. Am J Med Genet 1994;50(1):32-38.
16. Sadeghi AM, Cohn ES, Kimberling WJ, et al. Expressivity of hearing loss in cases with Usher syndrome type IIA. Int J Audiol 2013;52(12):832-837.
17. Ebermann I, Phillips JB, Liebau MC, et al. PDZD7 is a modifier of retinal disease and a contributor to digenic Usher syndrome. J Clin Invest 2010;120(6):1812-1823.
18. Lentz J, Keats B. Usher Syndrome Type II. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews(R). Seattle (WA); 1993.
19. Simon HJ, Levitt H. Effect of dual sensory loss on auditory localization: implications for intervention. Trends Amplif 2007;11(4):259-272.
20. Bakke MH. The contribution of interaural intensity differences to the horizontal auditory localization of narrow bands of noise. New York: The City University of New York Graduate Center; 1999.
21. Stone A, Shiffman S, Atienza A, et al. The Science of Real-Time Data Capture: Oxford University Press; 2007. 416 p.

22. van Schoonhoven J, Sparreboom M, van Zanten BG, et al. The effectiveness of bilateral cochlear implants for severe-to-profound deafness in adults: a systematic review. Otol Neurotol 2013;34(2):190-198.
23. Smulders YE, van Zon A, Stegeman I, et al. Comparison of Bilateral and Unilateral Cochlear Implantation in Adults: A Randomized Clinical Trial. JAMA Otolaryngol Head Neck Surg 2016;142(3):249-256.
24. Savic N, Schwank G. Advances in therapeutic CRISPR/Cas9 genome editing. Transl Res 2016;168:15-21.
25. Slijkerman RW, Vache C, Dona M, et al. Antisense Oligonucleotide-based Splice Correction for *USH2A*-associated Retinal Degeneration Caused by a Frequent Deep-intronic Mutation. Mol Ther Nucleic Acids 2016;5(10):e381.
26. Vache C, Besnard T, le Berre P, et al. Usher syndrome type 2 caused by activation of an *USH2A* pseudoexon: implications for diagnosis and therapy. Hum Mutat 2012;33(1):104-108.
27. Tucker BA, Mullins RF, Streb LM, et al. Patient-specific iPSC-derived photoreceptor precursor cells as a means to investigate retinitis pigmentosa. Elife 2013;2:e00824.
28. Di Foggia V, Makwana P, Ali RR, et al. Induced Pluripotent Stem Cell Therapies for Degenerative Disease of the Outer Retina: Disease Modeling and Cell Replacement. J Ocul Pharmacol Ther 2016;32(5):240-252.
29. Chen JR, Tang ZH, Zheng J, et al. Effects of genetic correction on the differentiation of hair cell-like cells from iPSCs with MYO15A mutation. Cell Death Differ 2016;23(8):1347-1357.
30. Maguire AM, High KA, Auricchio A, et al. Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial. Lancet 2009;374(9701):1597-1605.
31. Kohrman DC, Raphael Y. Gene therapy for deafness. Gene Ther 2013;20(12):1119-1123.
32. Bressers I, Tham G. Extremely soft, yet incredibely close. Exploring hearing aid fitting in patients with Usher syndrome type II In; 2012.

Chapter 5

Summary and Conclusions

Samenvatting en Conclusies

SUMMARY AND CONCLUSIONS

This thesis is part of an ongoing research project in Nijmegen on Usher syndrome started by Cor Cremers in the nineties of the previous century. Unlike previous theses on this topic, the patients studied in this thesis were specifically patients with either Usher syndrome type IIa (USH2a) or *USH2A*-associated nonsyndromic retinitis pigmentosa (nsRP).

Chapter 1 is a general introduction. The introduction and following chapters are written following the most common steps in the care program of a patient with Usher syndrome. For the patient, this often starts shortly after birth with the detection of hearing impairment during the neonatal hearing screening. Afterwards, the patient visits a health care professional for a definite clinical and genetic diagnosis. The health care professional will try to provide individual information and advice concerning the disease (= counselling). Finally, together with the patient and parents, optimal hearing rehabilitation will be determined. Usher syndrome is a rare disease with a prevalence of about 4.4 - 6.3 per 100,000 inhabitants. Three clinical types can be distinguished based on the presence, severity, progression and age of onset of hearing impairment, retinitis pigmentosa (RP) and vestibular dysfunction. To date, mutations in 11 different genes have been identified that result in Usher syndrome. USH2a is the most common type of Usher syndrome (> 50% of all patients), and is caused by mutations in *USH2A*. Patients with USH2a present with congenital hearing impairment, a progressive deterioration of visual function as a consequence of retinitis pigmentosa (RP) and intact vestibular function. Mutations in *USH2A* are also associated with nonsyndromic retinitis pigmentosa (nsRP). Little is known about the cause of the large clinical variations seen in and between both groups. Some patients with USH2a develop severe progressive hearing impairment, whereas other patients suffer from a mild and stable hearing impairment. Detailed information about the expected course of the disease is, for patients diagnosed with mutations in *USH2A*, essential to his or her future societal perspective. This knowledge also has an influence on the determination of the hearing rehabilitation options.

Chapter 2 focuses on the identification of possible genotype-phenotype correlations in patients with mutations in *USH2A*, in order to explain (a part of) the observed variability of the audiological en visual phenotype. In **chapter 2.1**, hearing impairment of a sample of 110 patients with USH2a was studied. Based on this study we can conclude that the observed hearing impairment is generally congenital, moderate to severe and progressive. Also, the hearing impairment is more pronounced in the higher frequencies. Furthermore, patients with two protein-truncating mutations showed a more severe and progressive hearing impairment as compared to patients with only one or no protein-

truncating mutation. Our explanation is that, in this case, protein-truncating mutations must be regarded as mutations with a severe clinical effect, due to the fact that as a result of these mutations, no protein or a non-functional shortened protein is formed. The effect of nontruncating mutations on the phenotype is milder as still a protein is formed of which, however, the function is significantly reduced. In **chapter 2.2**, the visual phenotype was compared between patients with USH2a and *USH2A*-associated nsRP. This study demonstrated that RP is diagnosed at a younger age in patients with USH2a than in patients with *USH2A*-associated nsRP. In addition, the visual phenotype is more progressive in USH2a patients than in *USH2A*-associated nsRP patients. Consequently, patients with USH2a reach the international criteria for blindness at a younger age (average of 54 years in patients with USH2a versus an average of 80 years in patients with *USH2A*-associated nsRP). The effect of protein-truncating and nontruncating mutations on the visual phenotype was also studied. A combination of two truncating mutations was found only in patients with USH2a. Furthermore, independent of the audiological phenotype, a positive correlation was found between the number of protein-truncating mutations (0, 1 or 2), and the severity of the visual phenotype. **Chapter 2.3** of this thesis studied the hearing in patients with *USH2A*-associated nsRP. This was necessary to determine whether *USH2A*-associated nsRP could be considered as a different clinical entity from USH2a. Previously, the lack of subjective hearing loss was considered to be a criterion for nsRP. However, no comprehensive audiological data were available for *USH2A*-associated nsRP patients. After several hearing tests and a questionnaire, it could be concluded that 8 of the 18 patients developed a mild, adult-onset hearing impairment. Despite the variability, hearing impairment in patients with USH2a is generally present at birth in a moderate to severe form. As the hearing impairment in *USH2A*-associated nsRP patients is mild and starts at a later age in only a subset of patients, *USH2A*-associated nsRP can indeed be considered as a separate entity from USH2a.

Chapter 3 of this thesis focuses on the options for auditory rehabilitation in patients with USH2a. Fitting hearing aids in these patients is problematic. This was reported by two patients with Usher syndrome, Gracia Tham and Ivonne Bressers. In 2012, they interviewed both patients with Usher syndrome and healthcare professionals about the difficulties in fitting hearing aids. In **Chapter 3.1** we studied whether the adaptation of hearing aids in patients with USH2a could be improved. In this chapter, the results of sound localisation, speech perception in noise and quality of life were compared between two hearing aid programs. These programs were developed based on two hypotheses:

1. The first, experimental, **linear** program would interfere as little as possible with the incoming sound and should provide better sound localisation.

2. The second, regular, **compression** program would be considered more comfortable and offer better performance in speech perception in noise.

The results of this study showed that USH2a patients have a clear subjective preference for the linear program. However, we found no objective difference between the two programs in terms of sound localisation and speech perception. In **Chapter 3.2,** the objective (speech perception) and subjective (questionnaires) results after cochlear implantation in patients with USH2a were examined and compared with a control group. Cochlear implantation is an important option for rehabilitation in patients with USH2a that present with severe to profound hearing impairment. Approximately 10% of all known USH2a patients in our institute received a cochlear implant. Both subjective and objective results did not differ from the results in the control group.

In conclusion, the results of this thesis have a direct impact on the different stages of the care program for patients with USH2a and *USH2A*-associated nsRP. Patients, who receive this clinical and/or genetic diagnosis, may now be better informed about the prognosis of the visual and audiological phenotype and the rehabilitation of hearing impairment. This thesis is a new step in the understanding of factors affecting the severity and progression of both the audiological and visual symptoms in patients with USH2a and *USH2A*-associated nsRP.

SAMENVATTING EN CONCLUSIES

Dit proefschrift maakt deel uit van een groter onderzoek naar het Usher syndroom in Nijmegen, gestart door Cor Cremers in de jaren negentig van de vorige eeuw. De patiënten die in dit proefschrift bestudeerd zijn, hebben in tegenstelling tot in de voorgaande proefschriften over Usher syndroom, enkel Usher syndroom type IIa (USH2a) of niet-syndromale retinitis pigmentosa (nsRP) veroorzaakt door mutaties in *USH2A*.

Hoofdstuk 1 bestaat uit een algemene inleiding. Deze inleiding en de overige hoofdstukken zijn geschreven aan de hand van de meest voorkomende stappen in het zorgprogramma van een patiënt met USH2a. Voor de patiënt begint dit vaak al kort na de geboorte na het vaststellen van gehoorverlies tijdens de neonatale gehoorscreening. Vervolgens bezoekt de patiënt een zorgverlener voor een klinische en genetische diagnose. Hierna zal de zorgverlener proberen om op individueel niveau een advies te geven over de aandoening (counseling). Samen met de patiënt zal vervolgens naar een optimale vorm van revalidatie gezocht worden. Het Usher syndroom is een zeldzame aandoening met een prevalentie van ongeveer 4,4 - 6,3 per 100.000 inwoners. Drie klinische typen kunnen van elkaar onderscheiden worden op basis van de aanwezigheid, de ernst en het moment van optreden van slechthorendheid, retinitis pigmentosa (RP) en vestibulaire afwijkingen. Tot op heden zijn mutaties in 11 verschillende genen gevonden die allen resulteren in het ontstaan van Usher syndroom. USH2a is de meest voorkomende vorm van Usher syndroom (>50% van alle patiënten) en wordt veroorzaakt door mutaties in *USH2A*. Patiënten met USH2a worden slechthorend geboren maar ervaren pas de eerste klachten met hun visus rond de puberteit. Daarbij hebben ze een intacte vestibulaire functie. Mutaties in *USH2A* kunnen ook leiden tot niet-syndromale RP (nsRP). Er is nog maar weinig bekend over de oorzaak van de grote klinische variabiliteit die gezien wordt in en tussen deze beide groepen. Sommige patiënten met USH2a ontwikkelen een ernstige progressieve vorm van gehoorverlies, terwijl bij andere patiënten een mild, stabiel gehoorverlies wordt geconstateerd. Nauwkeurige informatie over het verwachte verloop van het ziektebeeld is, voor een patiënt die gediagnosticeerd is met mutaties in *USH2A*, van essentieel belang voor zijn of haar sociaal-maatschappelijk toekomstperspectief. Deze kennis heeft bovendien invloed op het bepalen van de opties voor gehoorrevalidatie.

Hoofdstuk 2 is gericht op de identificatie van mogelijke genotype-fenotype correlaties bij patiënten met mutaties in *USH2A*, met als doel om (een deel van) de waargenomen variabiliteit van het audiologische en visuele fenotype te kunnen verklaren. In **hoofdstuk 2.1** is het gehoorverlies onderzocht van 110 patiënten met USH2a. Aan de hand van deze studie kan geconcludeerd worden dat het gehoorverlies over het algemeen congenitaal,

matig tot ernstig en progressief is. Ook is het gehoorverlies meer uitgesproken in de hogere frequenties. Verder lieten patiënten met twee eiwit-truncerende mutaties een ernstiger en progressiever gehoorverlies zien in vergelijking met patiënten met maar één of geen eiwit-truncerende mutatie. Onze verklaring hiervoor is dat truncerende mutaties in dit geval moeten worden beschouwd als mutaties met een ernstig effect omdat er ten gevolge van deze mutaties geen of slechts een niet-functioneel, verkort eiwit wordt gevormd. Het effect van niet-truncerende mutaties is waarschijnlijk milder omdat er nog wel een eiwit gevormd wordt waarvan echter de functie sterk verminderd is. In **hoofdstuk 2.2** werd het visuele fenotype vergeleken tussen patiënten met USH2a en *USH2A*-geassocieerde nsRP. Hieruit bleek dat RP op jongere leeftijd gediagnosticeerd wordt bij patiënten met USH2a dan bij patiënten met *USH2A*-geassocieerde nsRP. Daarnaast gaat het zicht sneller achteruit bij USH2a patiënten dan bij *USH2A*-geassocieerde nsRP patiënten. Hierdoor voldeden patiënten met USH2a, in vergelijking met patiënten met *USH2A*-geassocieerde nsRP, op jongere leeftijd aan de internationale criteria voor blindheid (gemiddeld 54 jaar in patiënten met USH2a versus gemiddeld 80 jaar in patiënten met *USH2A*-geassocieerde nsRP). Ook werd in dit onderzoek gekeken naar de truncerende en niet-truncerende mutaties. Een combinatie van twee truncerende mutaties werd alleen gevonden bij patiënten met USH2a. Verder, onafhankelijk van het audiologische fenotype, werd er een positieve correlatie gevonden tussen het aantal eiwit-truncerende mutaties (0, 1 of 2) en de ernst van het visuele fenotype. **Hoofdstuk 2.3** van dit proefschrift is gewijd aan het bestuderen van het gehoor in patiënten met *USH2A*-geassocieerde nsRP. Dit was nodig om definitief vast te kunnen stellen of *USH2A*-geassocieerde nsRP daadwerkelijk als een andere klinische entiteit dan USH2a beschouwd kan worden. Voorheen werd de afwezigheid van subjectief gehoorverlies beschouwd als een criterium voor nsRP. Er was echter nog geen uitgebreid audiologisch onderzoek verricht naar het gehoor van *USH2A*-geassocieerde nsRP patiënten. Na diverse gehoortesten en aan de hand van een vragenlijst kon geconcludeerd worden dat 8 van de 18 onderzochte patiënten een mild gehoorverlies ontwikkelen op volwassen leeftijd. Ondanks de variabiliteit, is het gehoorverlies bij patiënten met USH2a bijna altijd bij de geboorte aanwezig in een matige tot ernstige vorm. Omdat het gehoorverlies bij *USH2A*-geassocieerde nsRP patiënten mild van aard is, slechts bij een deel van de patiënten en daarbij pas op latere leeftijd optreedt, wordt *USH2A*-geassocieerde nsRP daadwerkelijk beschouwd als een andere entiteit dan USH2a.

Hoofdstuk 3 van dit proefschrift richt zich op de opties voor gehoorrevalidatie bij patiënten met USH2a. In **Hoofdstuk 3.1** is onderzoek verricht ter verbetering van het aanpassen van hoortoestellen bij patiënten met USH2a. Het aanpassen van hoortoestellen bij deze patiënten is problematisch. Dit werd beschreven door twee patiënten met het syndroom van Usher, Gracia Tham en Ivonne Bressers. In 2012 interviewden zij zowel

patiënten met Usher syndroom als zorgprofessionals over de moeilijkheden bij het aanpassen van hoortoestellen. In dit hoofdstuk zijn de uitkomsten van geluidslokalisatie, spraakverstaan in ruis en de kwaliteit van leven vergeleken tussen twee hoortoestelprogramma's. Deze programma's werden ontwikkeld aan de hand van twee hypotheses:

1. Het eerste, experimentele, **lineaire** programma zou zo min mogelijk interfereren met het inkomende geluid en daardoor een betere geluidlokalisatie opleveren.
2. Het tweede, reguliere, **compressie** programma zou als comfortabeler worden beschouwd en beter functioneren met betrekking tot spraakverstaan in rumoer.

De resultaten van ons onderzoek lieten zien dat USH2a patiënten een duidelijke subjectieve voorkeur hebben voor het lineaire programma. Echter vonden we in de verrichte testen geen objectief verschil tussen de twee programma's wat betreft geluidlokalisatie en spraakverstaan. In **hoofdstuk 3.2** zijn objectieve (spraakverstaan) en subjectieve (vragenlijsten) resultaten na cochleaire implantatie bij patiënten met USH2a onderzocht en vergeleken met een controlegroep. Cochleaire implantatie is een zeer belangrijke optie voor revalidatie bij patiënten met USH2a en ernstig gehoorverlies. Ongeveer 10% van alle bij ons bekende USH2a patiënten heeft inmiddels een cochleair implantaat. Zowel de subjectieve als objectieve resultaten verschilden niet van de resultaten in de controlegroep.

Concluderend hebben de resultaten van dit proefschrift directe gevolgen voor de verschillende stadia van het zorgprogramma voor patiënten met USH2a en *USH2A*-geassocieerde nsRP. Patiënten die deze klinische en/of genetische diagnose ontvangen, kunnen nu beter worden geïnformeerd over de prognose van het audiovisuele fenotype en de revalidatie van het gehoorverlies. Dit proefschrift is een nieuwe stap in het onderzoek naar factoren die van invloed zijn op de ernst en progressie van zowel de auditieve als de visuele symptomen in patiënten met USH2a en *USH2A*-geassocieerde nsRP.

Chapter 6

Dankwoord

De publicatie van dit proefschrift is het resultaat van een gezamenlijke inspanning waar niet alleen ik, maar direct of indirect ook vele anderen aan hebben deelgenomen. De onderstaande personen hebben me enorm geholpen, aangemoedigd en gesteund.

Prof. dr. ir Snik. Beste Ad, ik kon altijd bij jou terecht voor vragen over de audiologie en de audiologische onderzoeken maar ook voor vragen over onderzoek in het algemeen. Je nam rustig de tijd voor de uitleg en ik ging vaak met nieuwe inzichten de deur uit. Ook lag er altijd wel een ietwat vergeeld manuscript op je bureau dat ik absoluut moest lezen. Dit past perfect bij het beeld van een zeer ervaren en bevlogen professor, waaraan jij zeker voldoet. Veel dank voor je begeleiding en ik wens je veel succes bij het afronden van de laatste promoties.

Dr. Pennings. Beste Ronald, we hebben elkaar veel gesproken en gezien in de afgelopen jaren. Hiervoor ben ik je enorm dankbaar. Jouw betrokkenheid en gestructureerde manier van werken, met deadlines en wekelijkse afspraken, werkte voor mij zeer motiverend. Door het intensieve contact zijn we er ook achter gekomen in welke opzichten we verschillen. Ik heb je open houding hierin zeer gewaardeerd en ik denk dat het uiteindelijk tot een nog sterkere samenwerking en wederzijds respect heeft geleid. Als copromotor hield je bovendien het overzicht over alle onderzoeken en kon je me op zowel audiologisch als genetisch gebied inhoudelijk adviseren. Tot slot heb ik veel geleerd van de professionele en geëngageerde manier waarop je samenwerkt met ouders en patiënten. Veel dank!

Dr. van Wijk. Beste Erwin, hoe verder ik in de promotie kwam, hoe meer we hebben samengewerkt. De genetisch georiënteerde stukken hebben veel meer diepgang gekregen door je toevoegingen en onze inhoudelijke conversaties. Deze gesprekken waren een soort privécolleges waarin je met veel enthousiasme zowel de basale genetica als de toekomstige therapeutische mogelijkheden uitlegde. Ik heb niet eerder iemand ontmoet die met zoveel energie kan vertellen over ingewikkelde basale wetenschap. De patiënten waarvoor je een mogelijke therapie aan het ontwikkelen bent, hebben geluk met een wetenschapper zoals jij. Erg veel dank voor je bijdrage aan de onderzoeken en dit proefschrift.

Dr. Huygen. Beste Patrick, je hebt een erg groot aandeel gehad in dit proefschrift. En dat terwijl je eigenlijk al een tijdje niet meer in dienst bent van het Radboudumc. Dit vind ik fantastisch en bewonderenswaardig want dat laat zien dat jouw interesse in het ontcijferen van het gehoor bij erfelijke slechthorendheid meer is dan alleen werk. Ik heb in de afgelopen jaren regelmatig hele pakketten met documenten ontvangen die zo groot waren dat ze per e-mail slechts gecomprimeerd verstuurd konden worden. Je

hebt vele uren besteed aan het nalopen van alle gegevens en elk cijfertje werd meermaals gecontroleerd. Ik heb veel geleerd van jouw aanpak bij een statistische analyse en de zorgvuldigheid met cijfers, dank daarvoor.

Beste leden van de werkgroep Otogenetica, door de maandelijks geplande vergaderingen werd mijn otogenetische blikveld opengehouden voor andere oorzaken van erfelijke slechthorendheid zodat ik geen kokervisie kreeg op enkel het Usher syndroom. Tevens heeft de feedback tijdens een presentatie ervoor gezorgd dat we de verschillen in gehoor tussen twee verschillende patiëntengroepen in acht hebben genomen zoals beschreven in hoofdstuk 2.1. In het bijzonder dank voor deze bijdrage en commentaar, Prof. dr. Hannie Kremer.

Medewerkers van het Donders instituut. Beste Martijn Agterberg, Beste Prof. dr. John van Opstal, veel dank voor jullie uitleg, expertise en begeleiding op het gebied van het richtinghoren. De geluidslokalisatietesten, verricht in het lab van de afdeling Biofysica, waren een zeer interessante afwisseling. Ik heb veel bewondering voor de onderzoeken die jullie uitvoeren bij zowel normaalhorende mensen als bij patiënten.

Beste Loes, veel dank voor de administratieve ondersteuning. Ik kon altijd bij je terecht voor uiteenlopende zaken zoals het plannen van afspraken, het opvragen van adreslijsten en voor het bemachtigen van onmogelijk verkrijgbare manuscripten.

De manuscriptcommissie. Hooggeleerde heren en dame, bedankt voor uw tijd ter beoordeling van dit manuscript.

Beste staf van de afdeling keel-, neus- en oorheelkunde van het Radboudumc, veel dank voor de prettige ambiance op deze afdeling. De goede sfeer heeft me zeer gemotiveerd om, naast het promoveren, ook bij alle andere sociale activiteiten aanwezig te zijn. In het bijzonder dank aan prof. dr. Marres en dr. van den Hoogen. Het vooruitzicht dat ik na dit promotietraject aan de opleiding tot KNO-arts mocht beginnen, was enorm motiverend.

Alle arts-assistenten KNO, dank voor de positieve en goede sfeer in en buiten het ziekenhuis. Ik heb enorm veel zin gekregen om jullie collega te worden en kijk uit naar alle toekomstige sociale aangelegenheden maar ook naar de aanstaande professionele samenwerking.

Lieve medeonderzoekers, heel veel dank voor alle gezellige koffiepauzes, leuke gesprekken, wetenschappelijke discussies, etentjes en voorbereidingen voor sociale activiteiten

van de afdeling. De hechte club op de researchgang heeft ervoor gezorgd dat ik met plezier naar het ziekenhuis kwam en vaak met energie weer naar huis ging.

Lieve Mieke, als kamergenoot en mede promovendus Otogenetica heb ik een ontzettend gezellige tijd met je doorgebracht. Bovendien heb je me veel inhoudelijke tips en waardevolle adviezen gegeven. Wat me altijd bij zal blijven is jouw tip om dingen niet uit te stellen. Of in jouw woorden: "Dit klusje kan ook nú in plaats van morgen, overmorgen of pas na de vakantie". Je bent een harde werker en jouw serieuze aanpak en no-nonsense-attitude heeft een positieve invloed op me gehad. Daarnaast ben je een lief en leuk mens en waarschijnlijk een topprofessional.

Lieve Luuk, drie jaar geleden zijn we tegelijk begonnen als promovendus op deze afdeling. Met heel veel plezier denk ik terug aan het samen reizen in de trein, onze gemeenschappelijke toewijding en inzet en de eindeloze gesprekken over onderzoek en de verdere indeling van het leven. Zonder enige twijfel is dit proefschrift deels te danken aan deze momenten. En nu op naar een nieuw soort professionele relatie, maar bovenal vriendschap!

Lieve vrienden, lieve Robin, Sigrid, Nina, en Harmen, veel dank voor alle gezellige momenten in Amsterdam, waar af en toe de wetenschap werd besproken maar veelal ook niet.

Lieve familie Hartel en familie de Jong, in de afgelopen jaren ben ik familie meer gaan zien als een essentieel onderdeel van een gelukkig leven. De hechte banden, het wonen in elkaars nabijheid en de frequente etentjes en kopjes koffie hebben gezorgd voor een fijne afwisseling van het onderzoeksleven. Tijd doorbrengen met jullie is echt thuiskomen. Veel dank daarvoor.

Lieve Eva, ik zou je bij deze gelegenheid kunnen bedanken voor ontelbaar veel dingen. Ik houd het echter bij de twee belangrijkste, dank voor het volle vertrouwen en de onvoorwaardelijke steun die ik van je krijg. Dit is liefde zoals ik hem ieder mens zou gunnen. Ik geniet van elk moment met je.

Chapter 7

Curriculum Vitae

Bas Pieter Hartel werd op 2 juli 1987 geboren te Gouda als oudste zoon van een gezin met drie kinderen. Al kort na zijn geboorte volgde een aaneenschakeling van verhuizingen naar onder andere Engeland, Almere, Delden en uiteindelijk eind 1998 naar Argentan, een klein dorp in het Franse Normandië. In Argentan voltooide hij de middelbare school en hij behaalde in 2006 het Franse Baccalauréat Scientifique (equivalent van het vwo-diploma in Nederland). Hoewel het leven in Frankrijk hem prima beviel, wilde hij toch het liefst studeren in Nederland. In datzelfde jaar verhuisde hij daarom naar Amsterdam om geneeskunde te studeren aan de Universiteit van Amsterdam (in het Academisch Medisch Centrum). Hoewel Bas zich als een vis in het water voelde in Amsterdam, kreeg hij al gauw de drang om meer van de wereld te zien. In het kader van zijn opleiding liep hij daarom onder andere stage in Zwitserland, India en Zuid-Afrika. Ook vond hij de tijd om zes maanden met zijn vriendin door Zuidoost-Azië te fietsen. Eenmaal terug in Amsterdam begon hij met z'n coschappen en ontdekte hij dat het coschap Keel-, Neus- en Oorheelkunde in het Sint Lucas Andreas Ziekenhuis er voor hem met kop en schouders bovenuit stak. Het is dan ook geen verrassing dat hij, na het behalen van zijn artsenbul in 2014, is gaan solliciteren voor een felbegeerde plek als arts-onderzoeker op de afdeling KNO van het Radboudumc te Nijmegen. De sollicitatie had een positief vervolg. Vanaf april 2014 is Bas op de bovengenoemde afdeling werkzaam als promovendus en hij zal, na een nieuwe fietsreis (dit keer door Zuid-Amerika), op 1 augustus 2017 beginnen aan de opleiding tot Keel-, Neus- en Oorarts in het Radboudumc.

Chapter 8

Abbreviations

ANOVA	analysis of variance
ARTA	age-related typical audiograms
ATD	annual threshold deterioration
BB	broadband
CADD	combined annotation dependent depletion score
CI	cochlear implantation
CR	compression ratio
DFNB	autosomal recessive inherited sensorineural HI
dB HL	decibel hearing level
ExAC	exome aggregation consortium
GBI	Glasgow benefit inventory
GVF	Goldmann visual field
HA	hearing aid
HI	hearing impairment
HP	high-pass
ILD	interaural level differences
ITD	interaural time differences
kHz	kilo hertz
KS	Kolmogorov-Smirnov
LDL	loudness discomfort levels
LOVD	Leiden open variant database
LP	low-pass
MAE	mean absolute error
n	number
NCIQ	Nijmegen cochlear implant questionnaire
NH	normal hearing
nsRP	nonsyndromic retinitis pigmentosa
nT	nontruncating
OMIM	online mendelian inheritance in man
P	pathogenic
PTA	pure tone average
RP	retinitis pigmentosa
SC	syllabic compression
SD	standard deviation
SNR	signal-to-noise ratio
SPL	sound pressure level
SPSS	statistical package for the social sciences
SRT	speech reception threshold
SSQ	speech, spatial and qualities of hearing scale

T	truncating
USH	Usher syndrome
USH2a	Usher syndrome type IIa (genetic type)
USH2A	gene of USH2a
VA	visual acuity
VF	visual field

Chapter 9

List of publications

PUBLISHED MANUSCRIPTS

Hartel, B.P., Alta, T.D., Sewnath, M.E., & Willems, W.J. (2015). Difference in clinical outcome between total shoulder arthroplasty and reverse shoulder arthroplasty used in hemiarthroplasty revision surgery. *Int J Shoulder Surg, 9*(3), 69-73. doi: 10.4103/0973-6042.161426

Hartel, B.P., Agterberg, M.J., Snik, A.F., Kunst, H.P., van Opstal, J., Bosman, A.J., & Pennings, R.J. (2016). Hearing aid fitting for visual and hearing impaired patients with Usher Syndrome type IIa. *Clin Otolaryngol*. doi: 10.1111/coa.12775

Hartel, B.P., Lofgren, M., Huygen, P.L., Guchelaar, I., Lo-A-Njoe - Kort, N., Sadeghi, A.M., van Wijk, E., Tranebjaerg, L., Kremer, H., Kimberling, W.J., Cremers, C.W., Moller, C., & Pennings, R.J. (2016). A Combination of Two Truncating Mutations in *USH2A* causes more Severe and Progressive Hearing Impairment in Usher syndrome type IIa. *Hear Res*. doi: 10.1016/j.heares.2016.06.008

Kooistra, B. W., Willems, W. J., Lemmens, E., Hartel, B.P., van den Bekerom, M.P., & van Deurzen, D.F. (2016). Comparative study of total shoulder arthroplasty versus total shoulder surface replacement for glenohumeral osteoarthritis with minimum 2-year follow-up. *J Shoulder Elbow Surg*. doi: 10.1016/j.jse.2016.07.029

Pierrache, L.H., Hartel, B.P., van Wijk, E., Meester-Smoor, M.A., Cremers, F.P., de Baere, E., de Zaeytijd, J., van Schooneveld, M.J., Cremers, C.W., Dagnelie, G., Hoyng, C.B., Bergen, A.A., Leroy, B.P., Pennings, R.J., van den Born, L I., & Klaver, C.C. (2016). Visual Prognosis in *USH2A*-Associated Retinitis Pigmentosa Is Worse for Patients with Usher Syndrome Type IIa Than for Those with Nonsyndromic Retinitis Pigmentosa. *Ophthalmology, 123*(5), 1151-1160. doi: 10.1016/j.ophtha.2016.01.021

Slijkerman, R.W., Vache, C., Dona, M., Garcia-Garcia, G., Claustres, M., Hetterschijt, L., Peters, T.A., Hartel, B.P., Pennings, R.J., Millan, J.M., Aller, E., Garanto, A., Collin, R.W., Kremer, H., Roux, A.F., & Van Wijk, E. (2016). Antisense Oligonucleotide-based Splice Correction for *USH2A*-associated Retinal Degeneration Caused by a Frequent Deep-intronic Mutation. *Mol Ther Nucleic Acids, 5*(10), e381. doi: 10.1038/mtna.2016.89

SCIENTIFIC COMMUNICATIONS

Hartel, B.P., Pennings, R.J., & van Wijk, E. (2016). Comment on "Usher's Syndrome: Evaluation of the Vestibular System with Cervical and Ocular Vestibular Evoked Myogenic Potentials and the Video Head Impulse Test". *Otol Neurotol, 37*(5), 608. doi: 10.1097/MAO.0000000000001031

SUBMITTED MANUSCRIPTS

Hartel, B.P., Pierrache, L.H.M., Huygen, P.L., Homans, N.C., Goedegebure, A., van Wijk, E., Snik, A.F., Klaver, C.C., van den Born, L.I., & Pennings,. R.J. Evaluation of hearing in patients with *USH2A*-associated nonsyndromic retinitis pigmentosa

Hartel, B.P., van Nierop, J.W., Huinck, W.J., Rotteveel, L.J., Mylanus, E.A., Snik, A.F., Kunst, H.P., & Pennings R.J. Cochlear implantation in patients with Usher syndrome type IIa increases performance and quality of life

Usher syndrome is the most common cause of hereditary deaf-blindness in man. This condition is characterized by a combination of hearing impairment, vision loss, and in some cases vestibular dysfunction. Usher syndrome type IIa is the most common type of this syndrome explaining up to 50% of all cases. In October 2012, two Usher syndrome patients published a report on problematic hearing aid fitting in patients with Usher syndrome, based on interviews with care professionals and patients. This report, together with ongoing questions about the variability in presentation of clinical symptoms in Usher syndrome type IIa patients, laid the foundation for this thesis.

The aims of this thesis were to clinically examine patients with mutations in *USH2A*, the gene causing Usher syndrome type IIa, in order to find explanations for the variability in clinical presentation and to evaluate hearing rehabilitation options.