Original research

Analysis of Rotterdam Study cohorts confirms a previously identified *RIPOR2* in-frame deletion as a prevalent genetic factor in phenotypically variable adult-onset hearing loss (DFNA21) in the Netherlands

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ABSTRACT

Background A 12-nucleotide *RIPOR2* in-frame deletion was recently identified as a relatively common and highly penetrant cause of autosomal dominant non-syndromic sensorineural hearing loss, type DFNA21, in the Netherlands. The associated hearing phenotype is variable. The allele frequency (AF) of 0.039% of this variant was determined in a local cohort, and the reported phenotype may be biased because studied families were identified based on index patients with hearing loss (HL). In this study, we determine the AF in a cohort from a different geographical region of the Netherlands. Additionally, we examine the hearing phenotype in individuals with the variant but not selected for HL.

Methods The AF was determined in participants of the Rotterdam Study (RS), a large cohort study. The phenotype was characterised using individual clinical hearing data, including audiograms.

Results The observed AF in the RS cohort was 0.072% and not statistically significantly different from the previously observed 0.039%. The AF in the two cohorts combined was 0.052%. Consistent with previous findings, we found a highly variable audiometric phenotype with non-penetrance of HL in 40% of subjects aged 55–81, which is higher than the 10% at age 50 previously observed.

Conclusion We found an overall higher AF and lower penetrance than previously reported, confirming that DFNA21 is relatively common in the Netherlands. This supports its potential suitability as a target for therapeutic development. Studying possible modifying factors is essential to explain the phenotypical variability and to identify patients eligible for such a therapy.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ In the Netherlands, a 12-nucleotide *RIPOR2* in-frame deletion is a relatively common cause of autosomal dominant non-syndromic sensorineural hearing loss (SNHL) with a variable phenotype (DFNA21).

WHAT THIS STUDY ADDS

⇒ This study confirms that this recently identified *RIPOR2* variant is a penetrant genetic factor in adult-onset SNHL in the Netherlands. Clinical evaluation of individuals with the variant from an unbiased cohort reinforces the previously described phenotypical variability.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The relatively high number of individuals at risk of hearing loss (HL) due to this variant reaffirms this type of hereditary HL to be an attractive target for developing a (genetic) therapy. Identifying genetic and environmental modifiers remains necessary to explain this high degree of variability and identify patients eligible for such a therapy.

INTRODUCTION

Sensorineural hearing loss (SNHL) is the most common sensory impairment. Although it can occur from birth, the prevalence increases with age. Estimates in the USA range from an incidence of one to two per 1000 newborns (congenital or early-onset hearing loss (HL)) to a prevalence of over 50% in individuals aged 70 or 75 years and older (adult-onset HL or age-related HL).[1–3] A genetic diagnosis is more likely in congenital or early-onset HL than in adult-onset or age-related HL. Adult-onset and age-related HL are highly heterogeneous, clinically as well as molecularly, and caused by (a combination of) genetic and environmental aetiologies.[4–6] The attribution of genetic factors to adult-onset HL is estimated at 36%–70% and may concern either rare Mendelian variants with large effect sizes or multiple variants with an accumulation of small contributions.[7] To date, over 120 genes have been associated with hereditary HL, both with early and adult onset.[8] Only a few single pathogenic variants have been associated with HL in multiple families.

Recently, a 12-nucleotide *RIPOR2* (OMIM 611410) in-frame deletion (Chr6(GRCh37):g.24843306_24843317del NM_014722.5:c.1696_1707del) was identified as a highly penetrant cause of autosomal dominant non-syndromic SNHL (DFNA21, OMIM 607017) in 12 Dutch families. A functional effect of the variant was studied in mice, demonstrating an aberrant localisation of mutant RIPOR2 in stereocilia of cochlear hair cells and failure of repair of morphological defects in RIPOR2-deficient hair cells.[9] Additional analysis revealed an allele frequency (AF) of this variant of 0.039% in Southeast Netherlands (SE-NL).[9] If that AF is comparable throughout the Netherlands, more than 13 000 Dutch individuals could be at risk of HL due to this particular variant. The phenotype of DFNA21

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is variable; the age of onset in the 12 studied families ranged from congenital to 70 years of age, and four distinctive audiometric patterns were observed.[9] However, the phenotypical characterisation in that study might be biased since families were identified based on index cases with a clear family history and early onset of HL.

To determine whether the AF of the c.1696_1707del *RIPOR2* variant in other regions of the Netherlands is as high as in the SE-NL cohort, we examined the prevalence of this variant in a large Dutch cohort of the Rotterdam Study (RS).[10] The RS is a cohort study in the Ommoord district of Rotterdam. That district differs geographically from the SE-NL region. In addition, we evaluated clinical data, including audiograms, to study the phenotype and its variability in individuals from this unbiased cohort, that is, individuals who were not selected for HL, who carry the c.1696_1707del *RIPOR2* variant.

METHODS

For this study, the AF of the c.1696_1707del *RIPOR2* variant was determined in participants of the RS, a large prospective cohort study of individuals from the Ommoord district of Rotterdam aged 40 years or older.[10] The participation rate of inhabitants of this district was high, and there were no specific inclusion or exclusion criteria. In particular, participants were not selected or excluded based on HL. In addition, clinical data on HL, including audiograms, were obtained from a subgroup of individuals with the variant. These data were collected only in more recent subcohorts of the RS. The subgroup thus consisted of all individuals from RS subcohorts in which these data had been collected.

Subject identification

Genomic DNA of~15 000 participants of the RS was successfully genotyped for the presence of the c.1696_1707del *RIPOR2* variant (rs760676508). Genotyping was done in a 384-well PCR plate using a custom allelic discrimination TaqMan assay (Thermo Fisher Scientific, Waltham, USA). Each well contained 2 ng DNA, 1× Type-it Fast SNP PCR Mix (Qiagen, Hilden, Germany) and 1× TaqMan assay (Thermo Fisher Scientific) in a reaction volume of 2 μL. Each PCR plate contained five heterozygous and homozygous control samples and three blancs. Amplification was done in a PCR machine with recommended cycling conditions and end-point analysis was done in a QuantStudio V.7 Flex (Thermo Fisher Scientific). All samples with a heterozygous call were run a second time to validate the results.

AF of the c.1696_1707del *RIPOR2* variant

The AF of this variant was determined within RS participants in whom genotyping, as part of the RS, had been successfully performed. Kinship was examined up to six generations back, as kinship between subjects can influence the AF. The AF was compared with the previously determined AF of 0.039% in an SE-NL cohort of individuals not selected for HL.[9] Fisher's exact test was performed to determine the statistical significance of the difference in the observed AFs between the SE-NL and RS cohorts. The OR with 95% CI was determined to indicate the extent of the difference in the observed AFs. These calculations were performed with IBM SPSS Statistics for Windows V.27.

Clinical and audiological evaluation

We retrospectively collected the following self-reported audiovestibular data from RS questionnaires (online supplemental table 1): HL symptoms, use of hearing aids, problems following a conversation, tinnitus, symptoms of dizziness and balance function.

In addition, we evaluated the results of pure tone audiometry performed as part of the RS, as previously described.[11] In line with the previously studied DFNA21 families,[9] the prevalence of HL in this cohort was determined using the International Organisation for Standardisation (ISO) standard 7029:2017 (HL_{ISO}).[12] Individuals were considered to have HL_{ISO} when air conduction thresholds for at least three individual frequencies were below the age-specific and sex-specific 95th percentile for the best hearing ear. To determine whether subjects scored better or worse than the median for their age and sex, we compared their hearing thresholds (pure tone averages (PTAs) of 0.5–4.0 kHz ($PTA_{0.5–4kHz}$)) to the 50th percentile (P50).[12] If multiple audiograms were available, individual progression rates were determined, defined as the mean increase ($PTA_{0.5-4kHz}$) in decibel hearing level (dB HL) per year between the first and last audiometry measurement.

Additionally, we compared the hearing phenotype of the identified individuals with a subset of the full RS cohort from whom audiometry was available.[11] After exclusion of individuals with the *RIPOR2* variant from the latter, this cohort consisted of 4736 individuals. We assessed the association between the group (audiometrically studied RS cohort vs analysed subcohort with the *RIPOR2* variant) and three different outcome measures: $PTA_{0.5-4kHz}$ of the better ear and prevalence of HL based on two definitions previously used in analysis of the RS cohort[11] ($PTA_{0.5–4.0kHz}$ of ≥35 dB HL (HL_{35dB}) and ≥41 dB HL (HL_{41dB}) in the better ear). The association with the continuous outcome measure ($PTA_{0.5–4.0kHz}$ of the better ear) was assessed with linear regression analysis, the association with the dichotomous outcome measures (HL_{35dB} and HL_{41dB}) with logistic regression analyses. We corrected for age and sex. These analyses were performed with IBM SPSS Statistics for Windows V.27.

Age-related typical audiograms (ARTAs) were calculated for comparison with the ARTA obtained for individuals with DFNA21.[9 13] The k-means clustering analysis that previously yielded four audiometric patterns with different audiometric configurations[9] was updated by adding the audiometric data of RS subjects with the *RIPOR2* variant. The original DFNA21 audiometric patterns were classified as mild HL with an inverse U-shape audiogram, moderate HL with relatively worse hearing in the lower frequencies, moderate high-frequency HL with a gently down-sloping audiogram configuration and moderate mid-frequency HL with a U-shape audiogram.[9] For the ARTA and the k-means clustering analysis, only the most recent audiogram of each subject was used. These calculations and analyses were performed with RStudio V.1.4.1106 (PBC, Boston, Massachusetts, USA).

RESULTS

Subject identification and AF of the c.1696_1707del *RIPOR2* variant

The analysed RS cohort comprised 15 336 subjects who had been successfully genotyped for the c.1696_1707del *RIPOR2* variant (table 1). A total of 22 individuals carried this variant in the heterozygous state. We found no evidence of kinship between these individuals up to six generations back. The difference between the AFs observed in the RS (0.072%) and the previously reported SE-NL cohort[9] (0.039%) is not statistically significant (p=0.073; OR 1.83, 95% CI 0.98 to 3.41). The combined AF for both cohorts, consisting of 38 288 subjects, was 0.052%.

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Table 1 Allele frequency of the NM_014722.3:c.1696_1707del *RIPOR2* variant in the RS compared with the previously reported SE-NL cohort

Cohort	Total of individuals	Carriers of variant	Frequency (%)
RS	15336	22	0.072*
SE-NL[9]	22952	18	0.039*
Total	38288	40	0.052

*The difference between these allele frequencies is not statistically significant (p=0.073, OR 1.83, 95% CI 0.98 to 3.41).
RS, Rotterdam Study; SE-NL, Southeast Netherlands.

Clinical and audiological evaluation

In 10 of the 22 identified RS subjects with the c.1696_1707del *RIPOR2* variant, clinical and audiovestibular data, including audiograms, had been obtained as part of the RS (figure 1 and online supplemental table 1). There was no overlap between these and previously described subjects.[9] The age of these 10 subjects ranged from 53 to 83 years. Six individuals were female. In eight cases, a single audiogram was available and two audiograms were available from subjects RS05 and RS06 (online supplemental figure 1).

Nine out of these 10 subjects reported symptoms of HL, and four used conventional hearing aids (online supplemental table 1). In one case (subject RS05), hearing problems led to difficulties following conservations and avoiding social gatherings. Six subjects had tinnitus; this interfered with daily activities in one of them (subject RS05). Four subjects (RS04, RS05, RS08, RS10), aged 53 to 83, reported balance problems or frequent dizziness.

Based on the age- and sex-specific ISO norms, we identified six individuals (3 men, 3 women) with HL_{ISO}: RS01, RS02, RS04, RS05, RS07 and RS10 (figure 1). The penetrance of HL_{ISO} in this cohort aged 55 to 81 years was 60%. All HL was classified as purely sensorineural. The other four subjects (RS03, RS06, RS08, RS09) had worse hearing ($PTA_{0.5\text{-}4kHz}$) than could be expected according to the ISO median (P50) for their age and sex. Pronounced asymmetry was observed in subjects RS05 and RS09 (figure 1). In these subjects, the differences in $PTA_{0.5\text{-}4kHz}$ between the two ears were 56 dB HL (RS05, aged 83) in favour of the left ear and 20 dB HL (RS09, aged 54) in favour of the right ear (figure 1). Progression of HL was calculated over a period of 5 years for two subjects. The progression of subject RS05 was 1.25 dB HL per year ($PTA_{0.5\text{-}4kHz}$ of the best ear); there was no progression of HL in subject RS06.

Linear regression analysis showed that the cohort (audiometrically studied RS cohort versus analysed subcohort with the *RIPOR2* variant) was statistically significantly associated with hearing thresholds (p=0.004) (online supplemental table 2). The median and IQR of hearing thresholds ($PTA_{0.5\text{-}4kHz}$ of the better ear) in the ten subjects with the *RIPOR2* variant was 32 (IQR 21), compared with 21 (IQR 18) in the audiometrically studied RS cohort. Logistic regression analyses also showed a statistically significant association of the cohort and HL_{35dB} (p=0.049) and HL_{41dB} (p=0.047).

The ARTA demonstrated a down-sloping configuration with an onset of HL (WHO cut-off value of 25 dB HL, slight impairment[14]) in the low frequencies (0.25–2 kHz) at the age of 65 and in the high frequencies (4–8 kHz) at the age of 55 (figure 2). The ARTA of the 12 families previously studied show a similar audiogram configuration, with an earlier onset of HL at the age of 40 in the low and 30 in the high frequencies.[9] The updated k-means cluster analysis distinguished four audiometric patterns slightly different from those previously identified[9], the audiograms of the RS subjects were assigned to all four patterns (figure 3).

DISCUSSION

We determined an AF of the c.1696_1707del *RIPOR2* variant of 0.072% in the RS cohort and an overall AF of 0.052% for the combined RS and SE-NL cohorts. In the RS cohort, penetrance of age-corrected HL_{ISO} in subjects identified with the variant was 60%. Affected individuals had mild to moderate SNHL.

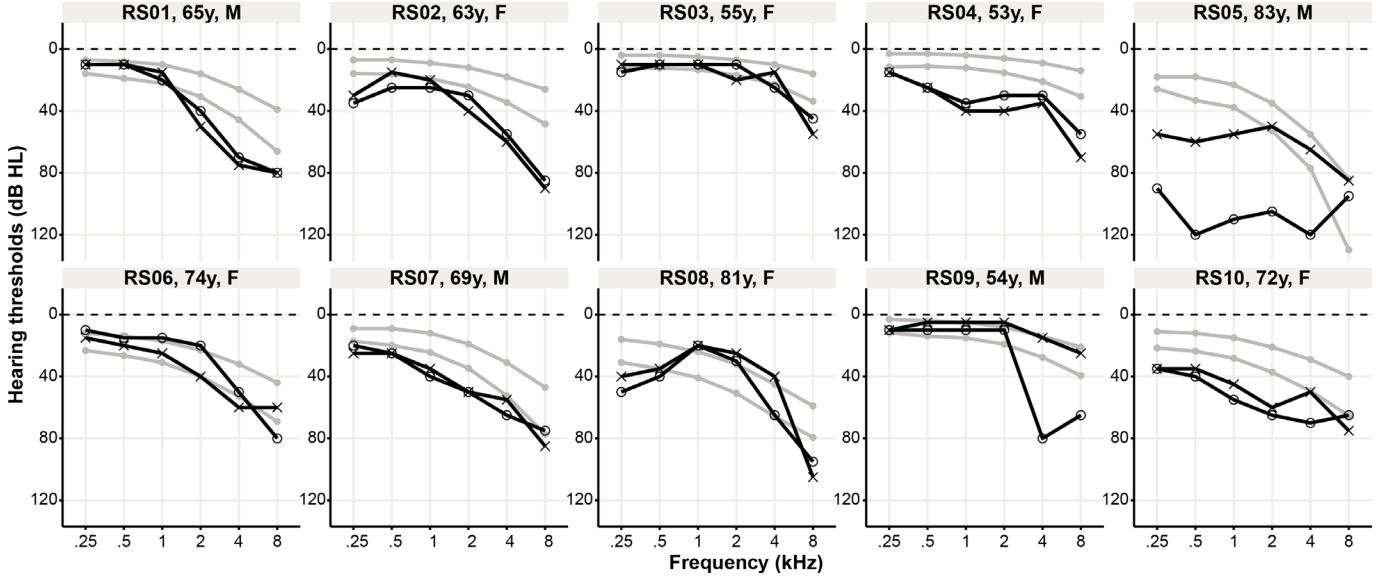

Figure 1 Individual audiograms of 10 individuals identified with the NM_014722.3:c.1696_1707del *RIPOR2* variant from the RS cohort. Black lines with crosses represent thresholds of the left ear, black lines with circles of the right ear, grey lines represent the age- and gender-specific 50th (upper lines) and 95th (lower lines) percentiles. The audiograms generally show mild to moderate HL with a down-sloping audiogram configuration. HL is obviously asymmetric in subjects RS05 and RS09. dB HL, decibel hearing level; F, female; kHz, kilohertz; M, male; y, years.

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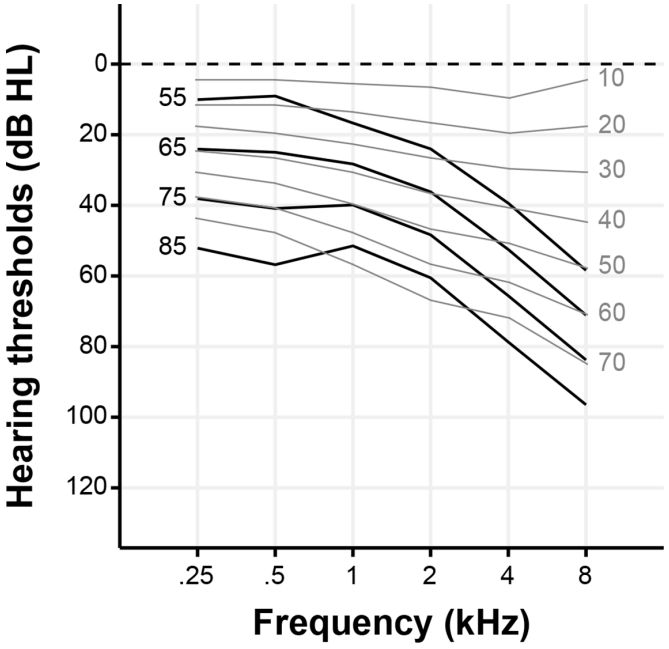

Figure 2 Age-related typical audiograms (ARTA) of RS subjects with the NM_014722.3:c.1696_1707del *RIPOR2* variant compared with the previously calculated ARTA.[9] ARTA (black lines) determined by cross-sectional linear regression analysis of 10 audiograms obtained from the RS for subjects with the c.1696_1707del *RIPOR2* variant compared with ARTA (grey lines) as previously calculated and published.[9] The ARTA start at the age of 55 years as the youngest subject in the RS cohort was 53 years. Age (in years) is indicated at the right end of all lines. dB HL, decibel hearing level; kHz, kilohertz. loss.

The degree of HL, corrected for age and sex, and the audiogram configurations were variable. Phenotypical analysis could only be performed in a subset of 10 of the 22 individuals who were identified with the *RIPOR2* variant because clinical data on HL and audiograms were only obtained in the most recent RS subcohorts. Unlike the families studied previously,[9] these individuals were not selected based on HL. Therefore, their phenotype is likely to be more representative of individuals with this variant and, thus, an important addition to the understanding of DFNA21.

AF of the c.1696_1707del *RIPOR2* variant

The AF that we observed is higher than the previously observed AF of 0.039% in the SE-NL cohort,[9] while not statistically significant, and confirms that this variant is a frequent cause of HL throughout the Netherlands. Based on the previously established AF, approximately 13 000 individuals were estimated to be at risk of developing HL due to this variant.[9] Based on the updated AF of 0.052% of both cohorts combined, this number rises to 18 000. Taking into account the lower penetrance found in this study, the prevalence of this variant as a genetic factor of HL in the Netherlands is likely to be similar to what was estimated based on the AF and penetrance rate determined in the SE-NL cohort. The *RIPOR2* variant is indicated to be inherited from a common ancestor.[9] The high AF in another Dutch region supports the possibility of this ancestor being of Dutch origin.

Based on the updated AF, there are approximately five individuals in the Netherlands expected to be homozygous for the variant. To the best of our knowledge, these have not been identified so far. A homozygous loss-of-function variant in *RIPOR2* has previously been associated with autosomal recessively inherited HL (DFNB104, OMIM 616515).[15] In the heterozygous state, the loss-of-function variant was not associated with HL[15 16] and therefore, we previously concluded that the c.1696_1707del *RIPOR2* variant has a toxic gain-of-function or dominant negative effect.[9] One could argue that individuals homozygous for this variant have a more severe HL phenotype, similar to DFNB104, than individuals who are heterozygous. Also, the HL might be syndromic as *RIPOR2* is expressed in many cell types and tissues.

Clinical and audiological evaluation

We observed a milder hearing phenotype in terms of degree of HL and ARTA-determined age of onset of HL in this cohort than in the previously studied families, which were identified based on index patients with a clear family history and childhood to adulthood onset age of HL.[9] The variability of the audiogram configuration was comparably high to what had been previously observed. The degree of HL, corrected for the ISO age-specific and sex-specific median, showed a range of almost 30 dB HL. The difference between men and women was remarkable, though not statistically significant; women scored 9 dB HL worse than the population P50 and men 24 dB HL. Presumably, the degree of HL is at least partially correlated with the age of onset, which was not addressed in the RS cohort. Therefore, we can only conclude that there was HL at the age at which the RS audiogram was obtained. These ages range from 53 years to 78 years (RS05 was 78 and 83 years old at the first and second audiometric measurements, respectively). Given the high prevalence of HL in the elderly and its aetiological heterogeneity, multiple factors could have played a role in developing HL. The *RIPOR2* variant might be one component of a multifactorial cause of HL.

Four subjects reported vestibular symptoms. Specification of these symptoms or functional vestibular tests was not available since this was not part of the RS. Vestibular tests in a subset of subjects from the 12 families previously studied showed no vestibular dysfunction.[9]

Non-penetrance, the extreme of phenotypical variability, was found in four subjects (RS03, RS06, RS08 and RS09; aged 55–81) based on ISO age-specific and sex-specific thresholds.[12] Also using the WHO definition of slight hearing impairment ($PTA_{0.5–2.0kHz}$ >25 dB HL for the best ear[14]), non-penetrance was seen in four cases. However, according to the WHO definition, RS08 is affected, and RS01 is not. Compared with the RS age-specific and sex-specific thresholds,[11] only one individual (RS02) scored worse than the 95th percentile and thus would be considered affected. In conclusion, it is crucial to consider the used definition of HL to interpret values such as penetrance.

The milder phenotype in these additional individuals with the variant from the RS cohort confirms a degree of expected bias in the previously studied families identified based on index cases with HL.[9] The high degree of interfamilial and intrafamilial phenotypical variability of *RIPOR2*-associated HL in those families was hypothesised to result from an interplay between environmental and genetic modifying factors. Our findings provide further indication of modifying factors that led to a higher penetrance and a more severe phenotype within those families. Differences in wild-type and mutant *RIPOR2* transcript levels in peripheral blood cells have been addressed as possible modifiers, but no correlation with the age of onset of HL could be demonstrated.[9] Studying potential modifying factors was beyond the scope and possibilities of the present study due to limitations in genetic analyses and the collection of clinical data. Only the

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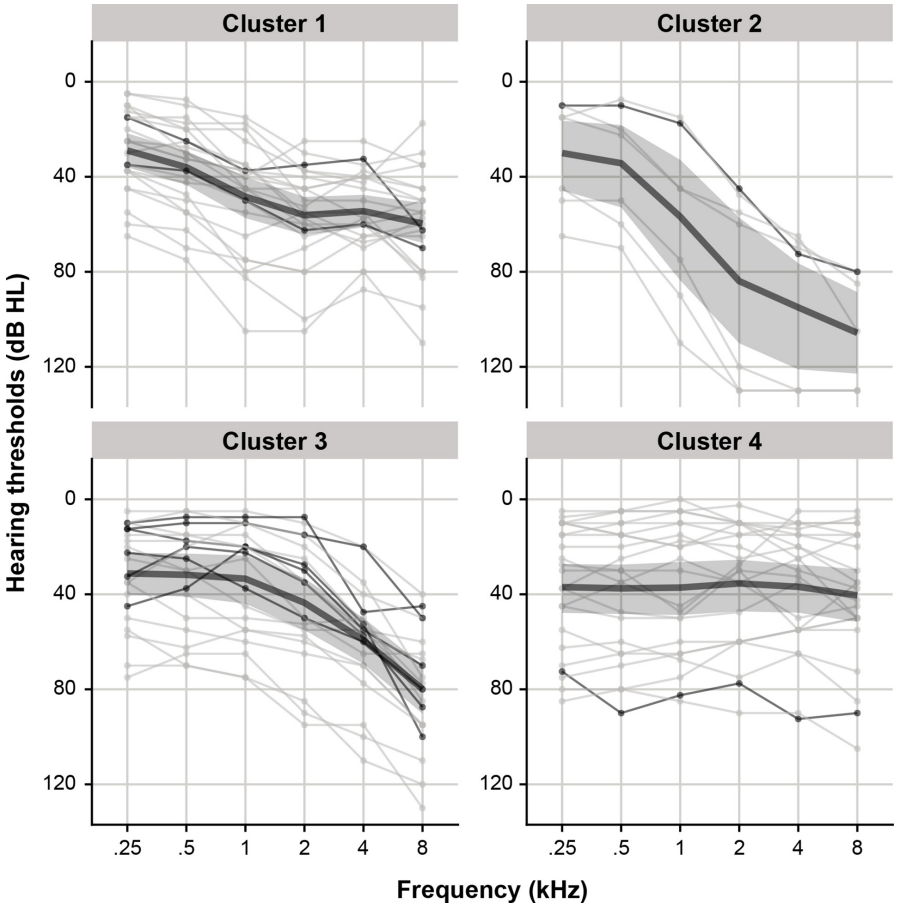

Figure 3 Updated audiometric patterns of *RIPOR2*-associated hearing loss**.** A k-means clustering analysis was performed on the air conduction thresholds (average of right and left ear) of all subjects as described previously.[9] The grey lines represent the audiograms that were included in the original k-means analysis[9] while the black lines represent the additional audiograms obtained in this study. The bold black line represents the mean thresholds per cluster, including the audiograms obtained in this study, and the grey highlighting represents the 95% confidence interval around the mean threshold. Cluster 1: moderate hearing loss (average $PTA_{0.5\text{-}4kHz}$ of 49 dB HL) with a U-shape audiogram. Cluster 2: moderate-to-severe hearing loss (average 68 dB HL) of predominantly the mid and high frequencies. Cluster 3: mild-to-severe hearing loss (average 42 dB HL) of mainly the high frequencies. Cluster 4: mild-to-severe hearing loss (average 37 dB HL) with a flat audiogram configuration. dB HL, decibel hearing level; kHz, kilohertz.

presence of this specific variant was determined in genetic analyses, and clinical data were collected retrospectively within a cohort study not specifically addressing HL.

DFNA21 may be an interesting candidate target for the development of gene therapy because it is relatively common and the onset of HL is generally late, providing a time window for intervention. However, its phenotypical variability and incomplete penetrance may hinder identifying patients eligible for such a therapy. First, individuals with the *RIPOR2* variant need to be identified, which can be particularly challenging for those with milder phenotypes outside known DFNA21 families. Second, assuming therapy should be initiated before a certain degree of HL is reached, reliable prediction of the expected progression and eventual severity of HL at the individual level is essential.

CONCLUSION

By determining the AF of the previously identified c.1696_1707del *RIPOR2* variant in a geographically distinct cohort, we confirm this variant as a prevalent genetic factor of adult-onset HL in the Netherlands. The associated phenotype varies from disabling to mild HL and even non-penetrance. Factors underlying intrafamilial and interfamilial phenotypical variability are unknown so far. Given the large number of individuals at risk of HL due to this *RIPOR2* variant, this type of HL may be an interesting target for the development of gene therapy. Research into possible environmental and genetic modifiers is essential for identifying eligible patients.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the medical ethics committee of the Erasmus MC (registration number MEC 02.1015) and The Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272–159,521-PG). The participants gave informed consent to participate in the study before taking part.

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Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. All data relevant to the study are included in the article or uploaded as supplemental information. Data not included in the manuscript or supplementary data file are available upon reasonable request (Hannie.Kremer@radboudumc.nl).

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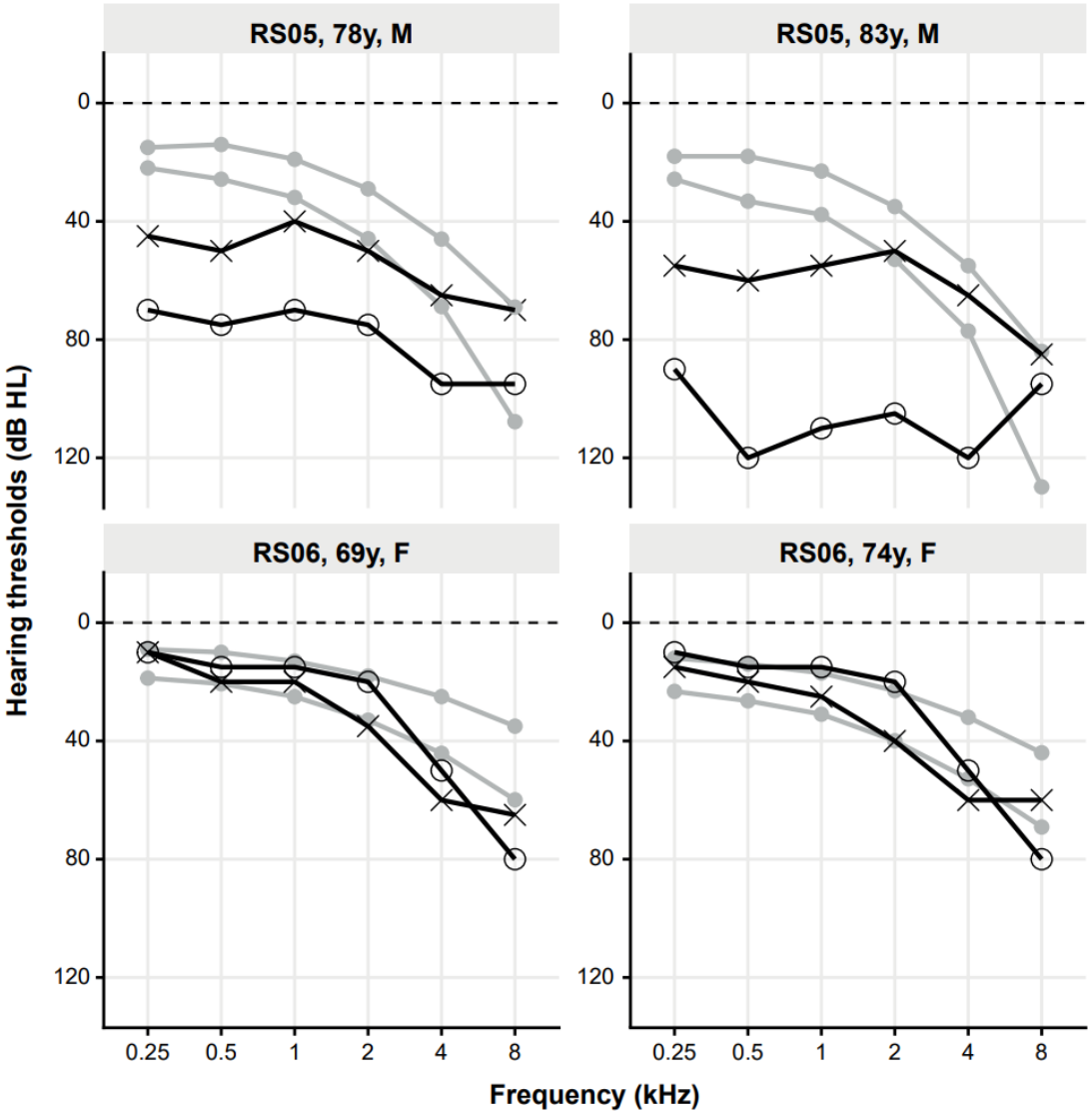

Supplemental Figure 1. Audiograms of RS05 and RS06 of whom two audiograms were available.

Black lines with crosses represent thresholds of the left ear, black lines with circles represent thresholds of the right ear, grey lines represent the age- and gender-specific 50th (upper lines) and 95th (lower lines) percentiles. dB HL, decibel hearing level; F, female; kHz, kilohertz; M, male; y, years.

Supplemental Table 1. Audiovestibular data from Rotterdam Study questionnaires.

Question	Yes	No
Hearing		
Hearing impaired without hearing aids	9 RS01, 02, 03, 04, 05, 07, 08, 09, 10	1 RS06
Use of hearing aids	4 RS05, 07, 08, 10	6 RS01, 02, 03, 04, 06, 09
Hearing impaired with hearing aids*	3 RS05, 07, 10	1 RS08
Difficulties in conversations with >3 people	1 RS05	9 RS01, 02, 03, 04, 06, 07, 08, 09, 10
Avoidance of gatherings because of hearing	1 RS05	9 RS01, 02, 03, 04, 06, 07, 08, 09, 10
Tinnitus	6 RS03, 04, 05, 06, 07, 08	4 RS01, 02, 09, 10
Interfering of tinnitus with daily activities*	2 RS05, 07	4 RS03, 04, 06, 08)
Balance		
Frequent dizziness	3 RS04, 05, 08	7 RS01, 02, 03, 06, 07, 09, 10
Difficulties if looking back while walking	4 RS04, 05, 08, 10	6 RS01, 02, 03, 06, 07, 09
Difficulties if looking back while riding a bike[$]	1 RS04	7 RS01, 02, 03, 06, 07, 08, 09
Difficulties in estimating uneven road surfaces or the location of obstacles	1 RS04	9 RS01, 02, 03, 05, 06, 07, 08, 09, 10

* Not applicable in six and four cases because the subjects did not use hearing aids or had no tinnitus, respectively; [$] Not applicable in two cases because the subjects could not ride a bike.

Supplemental Table 2. Comparison of hearing outcome measures.

Hearing outcome measure (better ear)	**Audiometrically studied RS cohort (n = 4,736)**	**Audiometrically studied RS subcohort with the *RIPOR2* variant (n = 10)**	***P***
$PTA_{0.5\text{-}4kHz}$	Median 21 (IQR 18)	Median 32 (IQR 21)	0.004
HL_{35dB}	26% with HL	67% with HL	0.049
HL_{41dB}	15% with HL	43% with HL	0.047

Comparison of three hearing outcome measures of the audiometrically studies RS cohort versus the analysed subcohort with the *RIPOR2* variant: $PTA_{0.5\text{-}4kHz}$ of the better ear and prevalence of HL based on two definitions previously used in analysis of the RS cohort[11] ($PTA_{0.5\text{-}4kHz}$ of ≥35 dB HL (HL_{35dB}) and ≥41 dB HL (HL_{41dB}) in the better ear). The association between the continuous outcome measure was assessed with linear regression analysis, the association with the dichotomous outcome measures with logistic regression analysis. We corrected for age and sex. HL, hearing loss; IQR, interquartile range; $PTA_{0.5\text{-}4kHz}$, pure tone averages of 0.5 to 4 kHz.