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Genotype-phenotype Correlation Study in a Large Series of Patients Carrying the p.Pro51Ser (p.P51S) Variant in *COCH* (DFNA9): Part I—A Cross-sectional Study of Hearing Function in 111 Carriers

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Introduction: DFNA9 is characterized by adult-onset progressive sensorineural hearing loss (SNHL) and vestibular impairment. More than 15 years ago, genotype-phenotype correlation studies estimated the initial age of hearing deterioration in the fourth to fifth decade (ranging from 32 to 43 years). However, these analyses were based on relatively limited numbers of mainly symptomatic carriers using markedly different methodologies. The starting point for the hearing deterioration is more correctly determined with larger numbers of carriers and with a more clearly defined starting point of the hearing deterioration.

Aim: The aim of this study was to determine milestone ages (start and maximal hearing deterioration, potential eligibility for hearing aids and cochlear implants based on pure-tone average [PTA]) in a large series of p.Pro51Ser *COCH* variant carriers. The degree of individual interaural asymmetry and the degree of variability (interquartile range) with which the hearing deterioration progresses across ages were also studied, and age-related typical audiograms (ARTA) were constructed.

Material and methods: One hundred eleven Belgian and Dutch p.P51S variant carriers were identified and recruited for audiological investigation. Their hearing thresholds were compared with p50th, p95th, and p97.5th percentile values of presbyacusis (ISO 7029 standards). The onset and degree of hearing deterioration were defined and assessed for each frequency and with three PTAs ($PTA^{0.5–4}$ [0.5, 1, 2, and 4 kHz]; $PTA^{4–8}$ [4 and 8 kHz]; and $PTA^{6–8}$ [6 and 8 kHz]). The milestones ages were derived from nonlinear regression model of hearing thresholds against age, for male and female carriers separately, because of different age-referenced limits. Interaural right-left asymmetry was assessed, and variability of hearing thresholds were calculated using interquartile range. ARTAs were built with both observed data and a prediction model.

Results: Hearing dysfunction in p.P51S carriers begins at about 38 years of age (ranging from 28 to 43 years) on average in female and 46 years (ranging from 42 to 49 years) in male carriers (third decade: female, fifth decade: male carriers), depending on the hearing frequency and with differences in deterioration sequence between both genders. These differences, however, were mainly due to more stringent age-referenced limits for men. In contrast, predictions (ARTA) did not show any difference of phenotypic expression between genders. At about 48 to 50 years of age on average, the majority of DFNA9 patients may need conventional hearing aids (PTA ≥ 40 dB HL), whereas this is about 56 to 59 years for cochlear implants (PTA ≥ 70 dB HL). There is a high degree of individual interaural asymmetry and interindividual variability throughout all ages.

Conclusion: This study demonstrates that the onset of sensorineural hearing deterioration starts in the third decade and probably even earlier. Regardless of differences in estimates, DFNA9 expresses similarly in male and female carriers, but male carriers are much more difficult to identify in early stages of the disease. Comprehensive assessment of the natural course of DFNA9 is of particular interest to predict the age of onset or critical period of most significant function deterioration in individual carriers of the pathogenic variant. This will help to design studies in the search for disease-modifying therapies.

Key words: SNHL, Cochlear hereditary hearing loss, DFNA9, COCH, Age-related typical audiograms.

Abbreviations: WHO = World Health Organization; SNHL = Sensorineural hearing loss; COCH = Coagulation Factor C Homology; p.P51S = c.151C>T, p.Pro51Ser (p.P51S) missense mutation in *COCH*; AC = air conduction; BC = bone conduction; dB HL = decibel hearing level; PTA = pure tone average; ATD = Annual Threshold Deterioration; CI = cochlear implantation; ICC = IntraClass Correlation; IQR = interquartile range; ARTA = Age-Related Typical Audiograms; ML = Machine Learning; ED = effective dose.

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INTRODUCTION

Hearing loss is a common neurosensory disease, and it was proclaimed a priority concern by the World Health Organization, partly because it causes communication disabilities resulting in dramatic social isolation and also because it yields an increasing impact on national social security costs (Hinchcliffe 1997).

Adult-onset progressive sensorineural hearing loss (SNHL) is often detected later in life, although it can be the result of an

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inborn DNA error. First signs may not be recognized in time or may remain untreated for a prolonged period of time until the auditory dysfunction becomes more apparent. This is absolutely also true for DFNA9, which is an autosomal dominantly inherited disease caused by pathogenic variants in the *COCH* (coagulation factor C Homology) gene (Robertson et al. 1994, 1997).

DFNA9 is the ninth locus that was identified for autosomal dominant hearing deterioration, and it is characterized by adult-onset SNHL and vestibular deterioration (Manolis et al. 1996). It was first described by Verhagen et al. (1988), and it was linked to chromosome 14q12-13 in 1992 and located in the *COCH* gene in 1996 (Verhagen et al. 1988; Manolis et al. 1996; Robertson et al. 1998, 2001; Fransen and Van Camp 1999). The *COCH* gene encodes for cochlin that represents 80% of all cochlear proteins (Robertson et al. 1997; Ikezono et al. 2001; Li et al. 2010). Cochlin plays important, however, not yet entirely elucidated, functions in maintaining inner ear architecture and local peri- and endolymphatic immunity (Py et al. 2013; Jung et al. 2019). To date, 31 different pathogenic variants have been described in *COCH* worldwide, all associated with late-onset SNHL with asymmetric interaural audiological thresholds and variability as well as variable degree of vestibular impairment, depending on their molecular location. All show autosomal dominant heredity, except for several autosomal recessive deterioration-of-function mutations that cause singular congenital hearing loss without apparent vestibular loss (Bae et al. 2014; JanssensdeVarebeke et al. 2018; Downie et al. 2020; Booth et al. 2020).

In 2019, a systematic review of the auditory and vestibular signs expressed by the c.151C>T, p.Pro51Ser (p.P51S), demonstrated that the current knowledge of the natural course of the disease is mainly based on data derived from studies that have used significantly different strategies and methodologies to phenotype symptomatic carriers (JanssensdeVarebeke et al. 2019). The alleged ages of onset for the hearing deterioration should therefore be confirmed with a larger number of participants and with a better-defined starting point for the beginning of the hearing deterioration. Comprehensive assessment of the natural course of DFNA9 is of particular interest to determine or predict the age of symptom onset or critical period of most significant function deterioration of inner ear function in individual carriers of the pathogenic variant. This will help to design studies in the search for disease-modifying therapies.

The aim of this study was to comprehensively evaluate hearing thresholds in a large series of 111 Belgian and Dutch p.P51S carriers. By doing this, we aimed (1) to define hearing deterioration in relation to age and gender using the ISO 7209 statistical distribution; (2) to determine ages of deterioration (age at onset of hearing deterioration, age at potential hearing aid eligibility, and age at potential cochlear implant candidacy); (3) to determine the deterioration rate (decibels per year (dB/y); and (4) to investigate the degree of intrasubject right/left asymmetry as well as the interindividual variability or interquartile range (IQR) of hearing thresholds across ages.

MATERIALS AND METHODS

Ethics Approval

The study was designed and conducted according to the Declaration of Helsinki (1996), and it was approved by the local ethics committees of the Antwerp University Hospital and the Hasselt Jessa Hospital (B300201630243) (Dale and Salo 1996). The study was registered in ClinicalTrials.gov (NCT03716908, updated August 21, 2019).

Enrollment, Inclusion, and Exclusion Criteria

Patient enrollment, including identification and audiological as well as vestibular investigations, started on January 1, 2019, and ended on January 31, 2020. All siblings of definite p.P51S variant carriers of at least 18 years of age were eligible for enrollment. The following exclusion criteria were used: all siblings younger than 18 years at the time of investigation, conductive hearing loss (difference of at least 15 dB HL between air [AC] and bone conduction [BC] measured on at least 3 contiguous frequencies), SNHL due to other concomitant disease, a history of significant occupational noise exposure, vestibular dysfunction due to causes other than DFNA9, previous middle ear surgery, known neurological disorders, known cerebral/cerebellar disorders, intracranial disease/tumors, unwillingness or inability to undergo thorough audiological and vestibular examination, and eardrum perforation.

Age Groups

The subject's age was allocated according to the age at the time of the investigation. All subjects were separated into six age groups (third decade: 18 to 29 years of age; fourth: 30 to 39 years; fifth: 40 to 49 years; sixth: 50 to 59 years; seventh: 60 to 69 years; and eighth decade: 70 to 80 years).

Audiological Testing and Outcome Measures

Pure-tone audiometry, including AC and BC (decibel hearing level [dB HL]), was performed by certified audiologists in a sound-attenuated room according to Hughson-West Lake methodology (Poling et al. 2016). An arbitrarily fixed value of 120 dB HL was assigned in cases where no measurable hearing was detected at equipment limits. Audiometry was conducted using an AC40 Clinical 2 channel audiometer (Interacoustics, Middlefart, Denmark) in the Antwerp center, whereas the Orbiter 922 2 channel audiometer (Madsen, Ballerup, Denmark) and the Affinity 2.0 digital 2 channel audiometer (Interacoustics, Middlefart, Denmark) were used in the Hasselt group. All audiometers were calibrated using ISO 389-I:2017 standards (https://www.iso.org/standard/69855.html?browse=tc). Air conduction hearing thresholds were collected from both ears for each subject and the following pure-tone averages (PTA) were calculated: $PTA^{0.5\text{-}4}$ (i.e., BIAP bureau international d'audiophonologie, https://www.biap.org [0.5, 1, 2, and 4 kHz]); $PTA^{4\text{-}8}$ (4 and 8 kHz); and $PTA^{6\text{-}8}$ (6 and 8 kHz).

Audiological data were used in the following ways: (1) calculating individual hearing thresholds of both left and right ears per subject, (2) to estimate a dose-response curve, (3) relationship between audiological data and individual interaural hearing thresholds, and (4) comparing thresholds by age and gender.

Statistical Analysis

For statistical analysis and graphic illustrations, R software Version 1.3.1056 (R: a language and environment for statistical computing, Vienna, Austria) was used. For the comparison of the hearing deterioration between male and female carriers and between the true mean of the samples with the theoretical median (age-referenced limits) for each frequency, gender, and decade, parametric tests (Welsh two-sample t test and one-sample t test, respectively) were used because sample sizes were large enough to tolerate possible slight deviations from normality. In addition, we repeated nonparametric methods (two-sample Wilcoxon test [Aka Mann Whitney *U* test] and Wilcoxon

signed rank test, respectively) and we obtained the same results and conclusions (data not shown).

Spearman's correlation, nonlinear drc-regression (CRAN drc-package in R) and simple linear regression were calculated for relationships between numeric variables. Statistical significance was fixed at $p < 0.05$, unless Bonferroni correction was warranted due to multiple comparisons being made.

Nonlinear dose-response regression models were used for the evaluation of the hearing decline progression across ages. A one-sample t test was used to endorse visual identification of onset ages.

In the following sections, the analyses of the audiological data are further developed, more specifically (1) to obtain Box & Whisker plots from individual hearing thresholds of both left and right ears for each subject for the assessment of the age of onset in decades using ISO7029 standard deviation, (2) to conduct nonlinear regression analysis (dose-response curve drc) to assess the age of onset in years, (3) to calculate the median and to perform interquartile range (IQR) analysis to investigate the degree of individual interaural difference of hearing thresholds between left and right ear and (4) to use Spearman's correlation of individual interaural hearing threshold difference, (5) to build age-related typical audiograms (ARTA) (Bom et al. 2003), both based on observed and predicted values (derived from drc-regression) to enable a clear visualization of the hearing decline at all frequencies across the decades, (6) to use one-sample t tests to compare means with age-referenced limits to check for the significance of hearing threshold deviation from age-referenced limits for all frequencies, PTAs, gender, and decades and (7) to use two-sample t tests to compare means of hearing threshold between both genders to analyze possible gender-effect of the p.P51S carriers status on the progression of the hearing decline across all decades.

Determination of Normative Age-referenced Limits for Male and Female Carriers

The ISO 7029 method models the median AC pure-tone threshold and the distribution around the median using upper halves of Gaussian distribution and corresponding standard deviation (s_u) (in decibels [dB]), for each age and gender separately (https://www.iso.org/standard/42916.html). Using the formula provided by ISO 7029, we calculated the p50th, p95th, and p97.5th percentile values (i.e., age-referenced limits) for the age and gender, respectively.

To check for possible hearing threshold differences between male and female p.P51S carriers, unpaired Welch two-sample t tests (significance level $p < 0.001$ [Bonferroni correction 12*6=72]) were conducted for each frequency as well as all three PTAs, for all ages together as well as for each decade separately to check for any possible hearing threshold differences between male and female p.P51S carriers.

In the next paragraphs, respective descriptive and inference statistical analyses for each research questions, as mentioned in previous audiological section, are explained in more detail.

Estimation of Age of Onset and Decline Rate

Age of onset of hearing deterioration was estimated by applying both descriptive and inferential statistics in line with previous publications (Verstreken et al. 2001; Bom et al. 2003; Lemaire et al. 2003; Bischoff et al. 2005). For the former, which is a categorical approach, we used age as categorical variable (age pooled in decades ranging from third to eighth) resulting in estimation of the age decade in which the hearing starts to deviate from age-referenced limits for a given frequency and gender. Onset was determined by visual comparison of the median with age-referenced limits and a one-sample t test. For the inferential analysis, the estimation of the age of onset was obtained using regression models and outcome was expressed in years (numeric approach).

Age of Onset in Decade

Box & Whisker plots of the hearing thresholds (dB HL) were constructed and displayed per frequency (ranging from 0.125 to 8 kHz) for each decade and for both genders separately, with the corresponding p50th, p95th, and p97.5th percentile values superimposed. The same method was applied to three different PTA-types ($PTA^{0.5–4}$, $PTA^{4–8}$, $PTA^{6–8}$), superimposing corresponding averaged p50th, p95th, and p97.5th percentile values, again for both genders separately.

To determine the starting point (onset) of hearing deterioration, the following criteria were applied: (1) the median derived from all measured hearing thresholds of all subjects included in a given age group (decade), at a given frequency and of a given gender, had to exceed the corresponding p97.5th percentile value, (2) with an increasing deviation across the following decades, and (3) comparison of the observed mean of the sample (of a given frequency, decade, and gender) had to be significantly greater than corresponding age-referenced limits (one-sample t test). The same method was applied for all frequencies and for the three PTAs ($PTA^{0.5-4}$, PTA^{4-8}, PTA^{6-8}). For the comparison of the observed mean of the sample with age-referenced limits, the one-sample t test was applied for each frequency and for all decades, for both genders separately. With a $p < 0.001$ (Bonferroni correction; 9*6 = 54) as significance level, the observed mean of the sample was compared with the corresponding p97.5th percentile value for presbyacusis (mμ) to test the null hypothesis (the observed mean of the sample is greater than mμ). This was chronologically applied from the third to eighth decade, both for male and female carriers.

Age of Onset in Years and Annual Threshold Deterioration

Estimation of the age of onset and progression (decline rate) of hearing threshold was carried out using nonlinear dose-response curve regression analysis (drc), with hearing threshold as dependent and age as independent variable. Separate drc-models were fit for each frequency from 0.125 to 8 kHz (0.125, 0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz), as well as for the three PTA-types and for both genders separately.

The age of onset was estimated with the use of these drc-regression equations by applying corresponding age-referenced limits according to frequency and gender (Ritz et al. 2015).

The annual threshold deterioration (ATD) or slope was calculated based on the method described by Bom et al. (2003).

The model function of the generalized log-logistic equation c+(d-c/(1+exp(b(log(x)-log(e))))) was used to calculate the coordinates y1(lower), y2(upper) values, which were in turn derived by estimating effective dose (e), where "b" stands for slope (Hill coefficient), and "d" and "c" are saturation (maximal or upper level) and minimal (bottom level or offset threshold), respectively, where "e" or ED50 (i.e., effective dose 50%) referred to half-way progression of the hearing deterioration

TABLE 1. Demographics of study population, all carriers of the p.Pro51Ser COCH mutation (P51S) (n = 111)

Age (Decade)	Age (Mean)	Age (Range)	Number (Ears)	Number Male (Ears)	Number Female (Ears)	SD
Third	22.4	(18–25)	10	2	8	2.55
Fourth	35.19	(30–39)	32	18	14	3.10
Fifth	45.44	(40–49)	42	26	16	2.61
Sixth	54.96	(50–59)	52	16	36	2.70
Seventh	64.54	(60–69)	58	34	24	2.12
Eighth	75.07	(70–80)	28	12	16	3.18

from offset, while ED20 and ED80 represented 20% and 80% progression of the hearing deterioration from baseline threshold, respectively. The age of onset was estimated with the use of these drc-regression equations by applying corresponding age-referenced limits according to frequency and gender (Ritz et al. 2015).

To compute the slope (as a measure of progression, decline rate or ATD in dB/y), corresponding ×1 (lower) and ×2 (upper) values were obtained to complete the coordinates of the linear segment of the resulting S-shaped dose-response curve. Simple linear regression of this linear segment was then computed with corresponding 95% confidence intervals, according to the

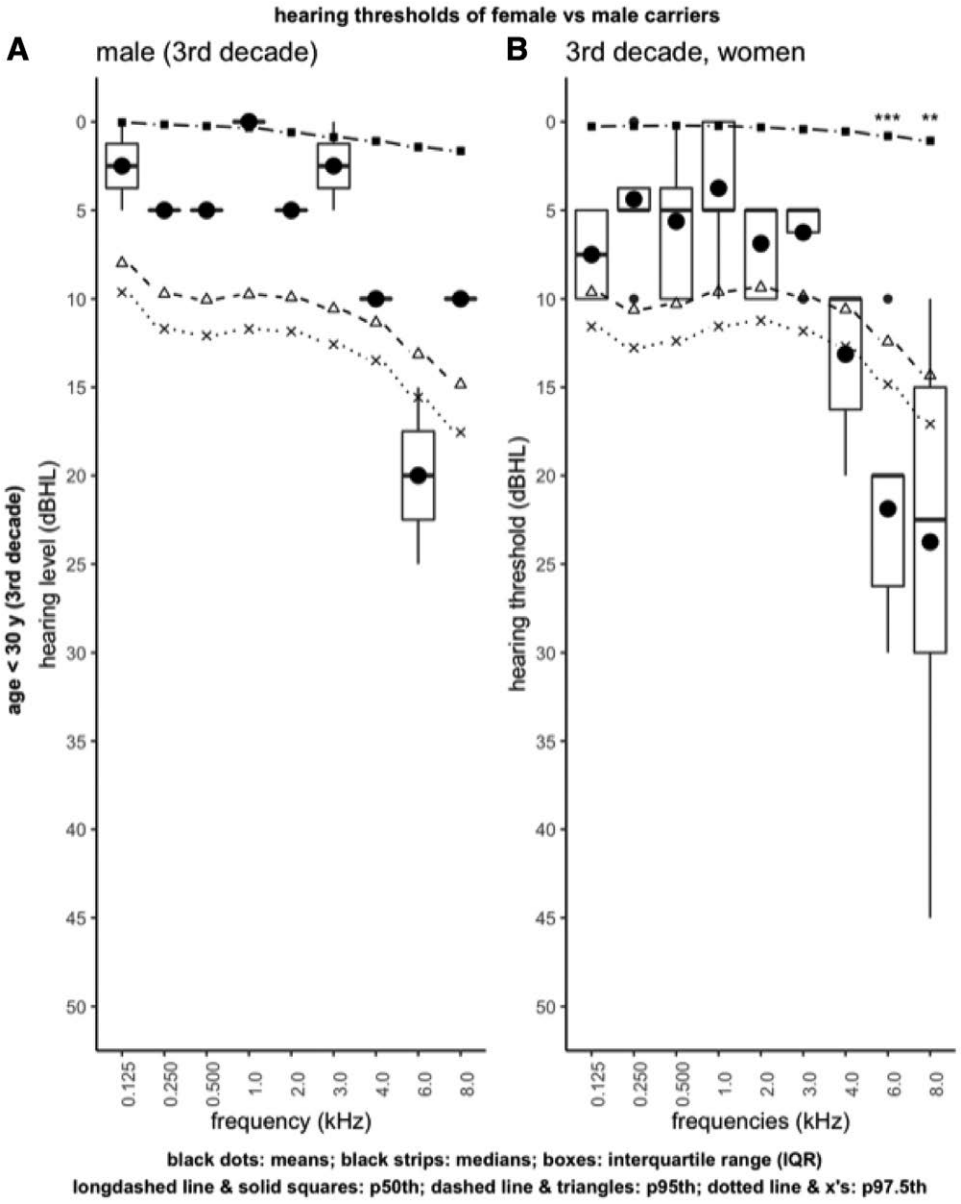

Fig. 1. A, B, Hearing thresholds of 5 (10 ears) p.P51S variant carriers aged in the third decade in relation to the corresponding p50th, p95th, and p97.5th percentile values for presbyacusis, for male (A) and female carriers (B). Note that (A) only represents data from one subject. The upper and lower borders of the boxes represent 25th and 75th quartiles, respectively. The boxes contain 50% of the values and represent the IQR. The bold horizontal lines in the boxes are the medians (asterix [*]: the mean value of the hearing thresholds of corresponding decade is significantly greater than corresponding age-referenced limit [ISO 7029]). IQR, interquartile range.

methodology described by Bom et al (2003), with the exception that a linear segment between ED20 and ED80 was chosen over ED10 and ED90, that audiological data were separated according to gender and that individual right and left hearing thresholds were used instead of binaural averaged thresholds (Bom et al. 2003).

This was applied to all frequencies (ranging from 0.125 to 8 kHz) as well as for the three PTAs, for both genders separately. For all models, a 95% confidence interval of all 5 parameters of the drc-regression fit ("b," "c," "d," "e," "f") were calculated. The age at maximal hearing decline for each frequency was estimated using the same method, with the y-variable fixed at 120 dB HL.

Estimation of Potential Treatment Eligibility

The methodology, described in the previous section, was also applied for the calculation of the age of theoretical eligibility for treatment with hearing aids and cochlear implantation (CI). For this purpose, The $PTA^{0.5\text{-}4}$ was used, since this frequency range was in agreement with the one that is used for determining the current reimbursement PTA criteria for hearing aids (≥40 dBHL) and CI (≥70 dBHL) as imposed by the Belgian National Health Insurance, even though PTA levels are only part of these criteria, which also includes impaired speech perception tests (https://kce.fgov.be). To adjust progression range (0% to 100%) to the hearing threshold range (0 to 120 dBHL), the ED of the drc-regression

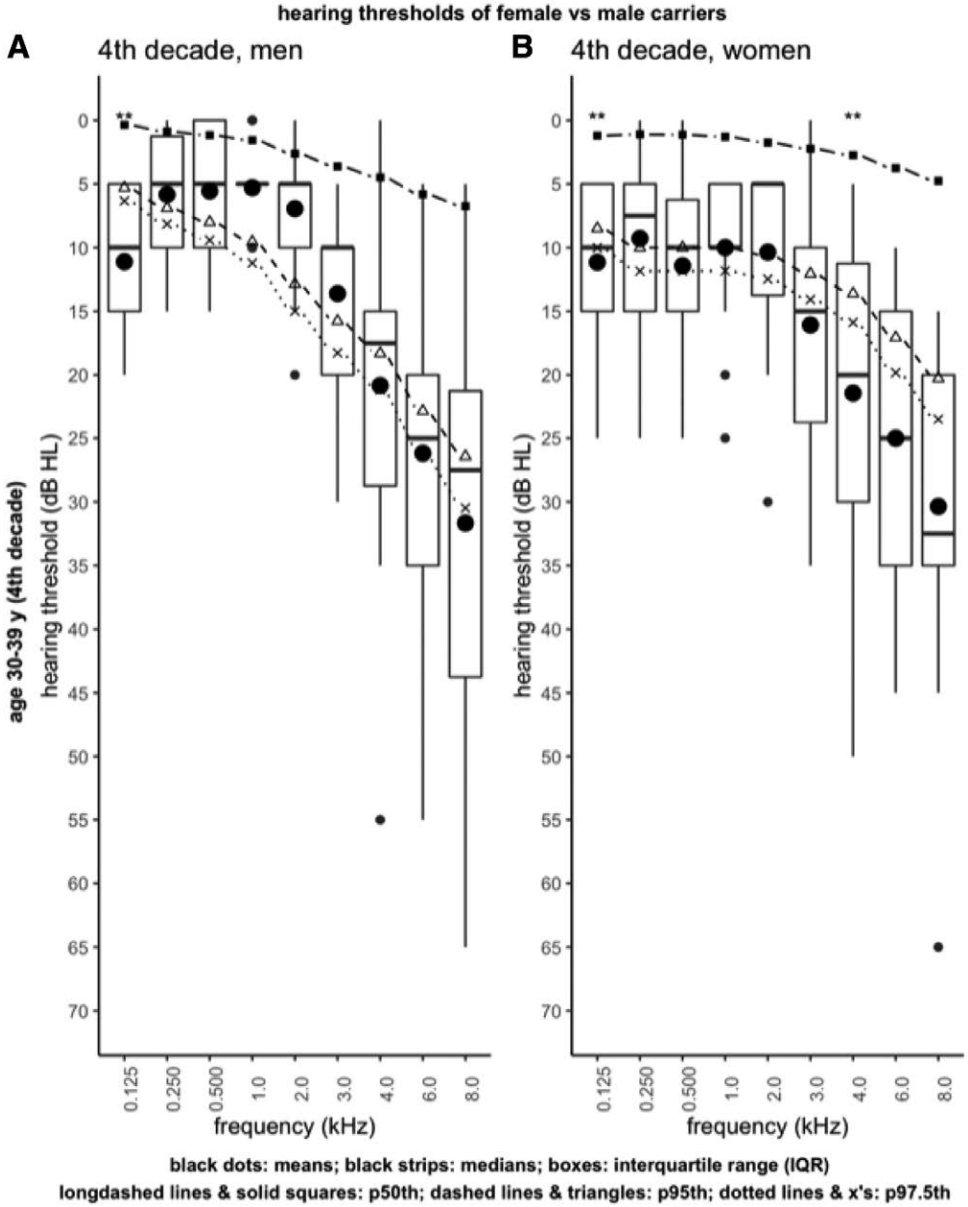

Fig. 2. A, B, Hearing thresholds of 16 (32 ears) p.P51S variant carriers aged in the fourth decade in relation to the corresponding p50th, p95th, and p97.5th percentile values for presbyacusis, for male (A) and female carriers (B). The upper and lower borders of the boxes represent 25th and 75th quartiles, respectively. The boxes contain 50% of the values and represent the IQR. The bold horizontal lines in the boxes are the medians (asterix [*]: the mean value of the hearing thresholds of corresponding decade is significantly greater than corresponding age-referenced limit [ISO 7029]). IQR, interquartile range.

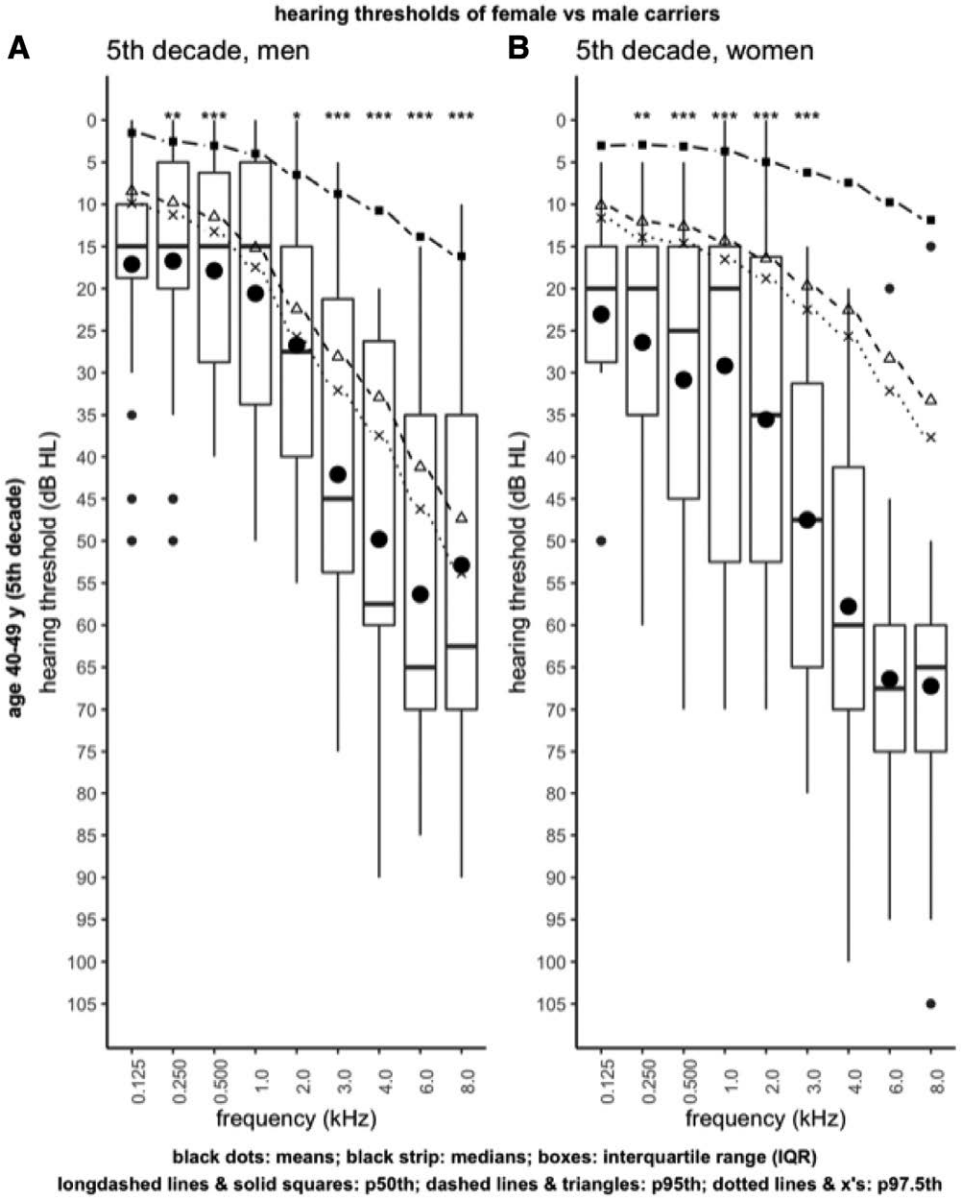

Fig. 3. A, B, Hearing thresholds of 21 (42 ears) p.P51S variant carriers aged in the fifth decade in relation to the corresponding p50th, p95th, and p97.5th percentile values for presbyacusis, for male (A) and female carriers (B). The upper and lower borders of the boxes represent 25th and 75th quartiles, respectively. The boxes contain 50% of the values and represent the IQR. The bold horizontal lines in the boxes are the medians (asterix [*]: the mean value of the hearing thresholds of corresponding decade is significantly greater than corresponding age-referenced limit [ISO 7029]). IQR, interquartile range.

TABLE 2. Age of onset of hearing loss according to age for male and female carriers of the P51S COCH mutation (n = 111)

Frequency (kHz)	Male Carriers (n = 54)	Female Carriers (n = 57)
0.125	Fourth	Fourth
0.250	Fifth	Fifth
0.500	Fifth	Fifth
1	Sixth	Fifth
2	Fifth	Fifth
3	Fifth	Fifth
4	Fifth	Fourth
6	Fifth	Third
8	Fifth	Third
PTA 0.5–4	Fifth	Fifth
PTA 4–8	Fifth	Third
PTA 6–8	Fifth	Third

PTA, pure-tone average.

model corresponding with 40 dB HL and 70 dB HL were set at 33.33% and 58.33% progression, respectively (ED33.33 = 40 dB HL, ED58.33 =70 dB HL).

Defining Individual Asymmetry (Interaural Difference) and Interindividual Variability Of Hearing Thresholds

To visualize the degree of individual asymmetry of hearing thresholds (right-left interaural threshold difference per subject) according to the frequencies and age, individual difference of hearing thresholds between right and left ear were calculated and pooled in three different age groups (18 to 39 years; 40 to 59 years; 60 to 80 years). To quantify the interindividual variability of audiological data across the three different age groups, the interquartile range (IQR) was calculated for all frequencies (also represented as boxes in Box & Whisker plots). Spearman's

TABLE 3. Significance level of one-sample t test per frequency and PTAs of auditory data of male (A) and female (B) p.P51S carriers when compared with respective p95th percentile values (age-referenced ISO 7029 limits): (–: mμ = not greater than; +: mμ = greater than the p95th age-referenced value)

(A)												
Male Carriers (n = 54)	0.125 kHz	0.250 kHz	0.500 kHz	1 kHz	2 kHz	3 kHz	4 kHz	6 kHz	8 kHz	PTA 0.5–4	PTA 4–8	PTA 6–8
Third decade	–	–	–	–	–	–	–	–	–	–	–	–
Fourth decade	+	–	–	–	–	–	–	–	–	–	–	–
Fifth decade	+	+	+	–	+	+	+	+	+	+	+	+
Sixth decade	+	+	+	+	+	+	+	+	+	+	+	+
(B)												
Female Carriers (N = 57)	0.125 kHz	0.250 kHz	0.500 kHz	1 kHz	2 kHz	3 kHz	4 kHz	6 kHz	8 kHz	PTA 0.5–4	PTA 4–8	PTA 6–8
Third decade	–	–	–	–	–	–	–	+	+	–	+	+
Fourth decade	+	–	–	–	–	–	+	+	+	–	+	+
Fifth decadE	+	+	+	+	+	+	+	+	+	+	+	+
Sixth decade	+	+	+	+	+	+	+	+	+	+	+	+

PTA, pure-tone average.

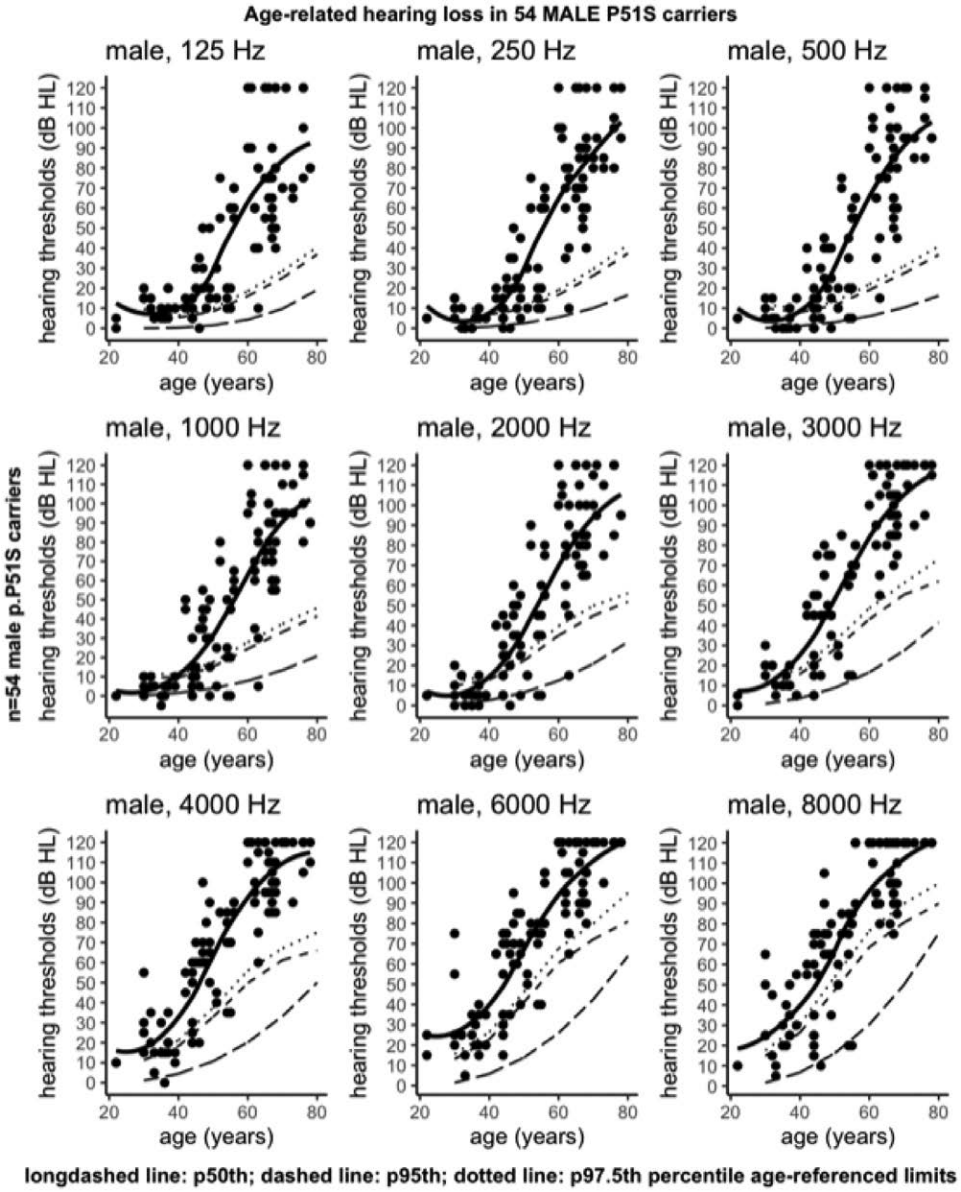

Fig. 4. Frequency-specific progression of hearing thresholds in 54 male p.P51S variant carriers in function of their age (108 ears). Note that hearing loss starts earlier with higher frequencies, whereas there is a constant offset hearing threshold at lower frequencies which lies within range of normative values for 0.125 to 4 kHz.

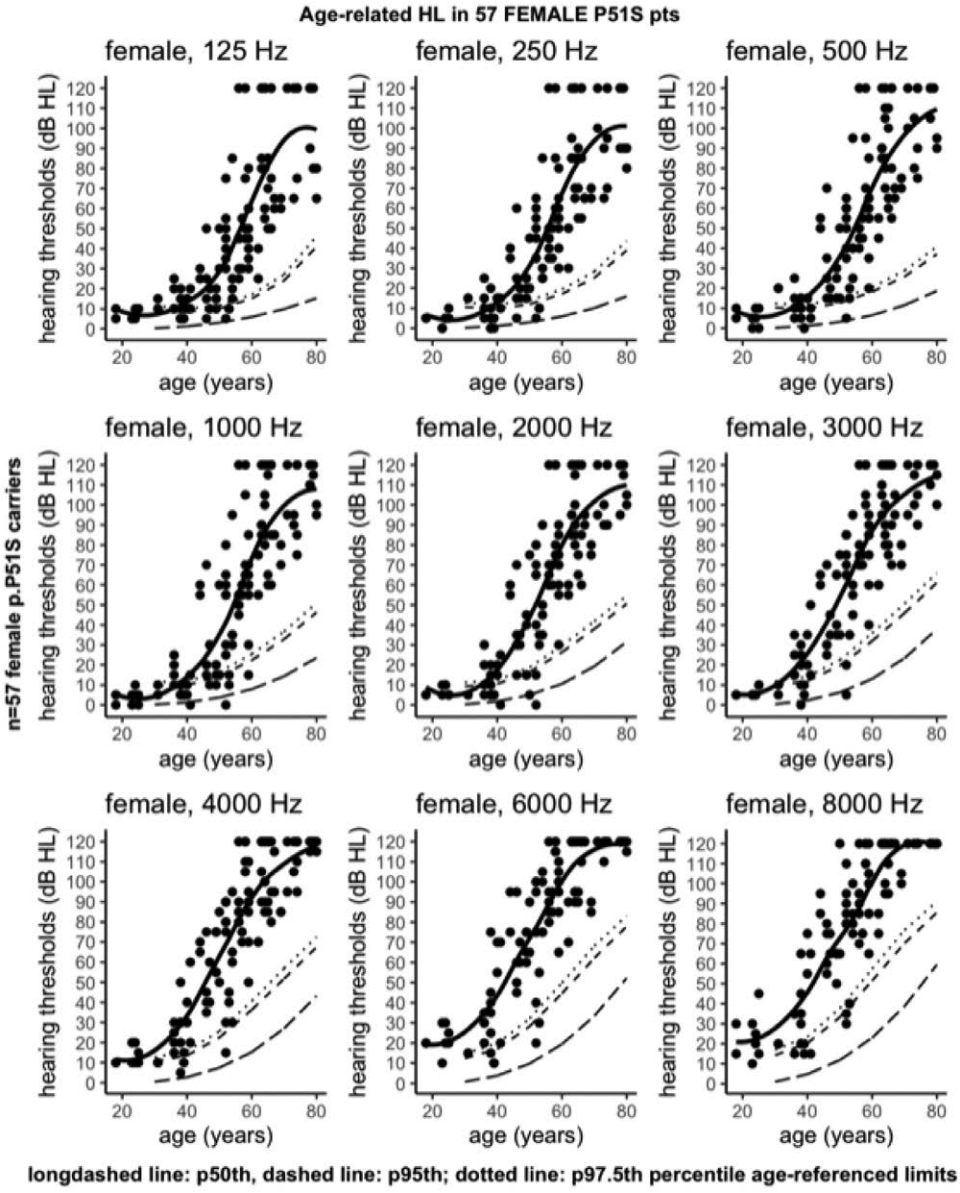

Fig. 5. Frequency-specific progression of hearing thresholds in 57 female p.P51S variant carriers in function of their age (114 ears). Note that hearing loss starts earlier with higher frequencies, whereas there is a constant offset hearing threshold at lower frequencies which lies within range of normative values for 0.125 to 3 kHz.

correlation between individual right and left auditory data was also computed for each age group separately.

Age-related Typical Audiograms

Progression of the hearing decline across the decades was visualized using "Age-related Typical Audiograms" (ARTA), as outlined elsewhere, with this difference that we also produced ARTAs with observed measurements (observed ARTA) besides the prediction-based ARTAs (predicted ARTA) and that both ARTAs were built for male and female carriers separately (Huygen et al. 2003). In brief, nonlinear drc-regression models were fit, modeling thresholds versus age at each frequency, to obtain a prediction of hearing threshold values for each decade, for both gender separately, which were plotted against frequencies 0.125 to 8 kHz. Because of the alleged high degree of asymmetry between the auditory data of both ears within the same individual, binaurally averaged values, as they were used in literature, were avoided. Finally, means and 95% confidence intervals of all measured hearing thresholds (observed ARTAs) were plotted against age for each decade to compare with the predicted ARTAs.

RESULTS

Age and Gender Distribution of the Study Population

One hundred eleven confirmed p.P51S variant carriers in the *COCH* gene were enrolled and had audiological tests as part of the study, giving 222 individual thresholds of the two ears per subject at all frequencies. Due to the aging demographic distribution in most of the studied family pedigrees, the age groups fifth, sixth, and seventh decade were better represented than the younger ones (Figure 1 in Supplemental

TABLE 4. Summary of all estimated ages at which the hearing deterioration starts, reaches 33.33% (=40 dB HL), 50%, 58.33% (=70 dB HL), and maximal progression, as well as estimates of the ATD, with 95% confidence intervals in 54 male P51S carriers (all estimated were computed based on nonlinear drc-regression models)

Male Carriers (n = 54) (kHz)	Onset (Decade)	Age of Onset (years)+ 95% CI	ED 33.33* (years) +95% CI	ED 50† (years) +95% CI	ED 58.33‡ (years) + 95% CI	ED max§ (years) +95% CI	Slope¶ (dB/y)
0.125	Fifth	44.983 (38.130; 51.837)	52.953 (48.011; 57.896)	57.158 (48.196; 65.621)	59.512 (48.695; 70.827)	87.583 (19.730; 155.436)	3.285 (1.178; 5.410)
0.250	Fifth	44.660 (40.400; 48.921)	54.197 (47.360; 61.014)	60.163 (48.476; 71.851)	63.667 (48.682; 78.652)	111.758 (34.405; 189.111)	2.095 (1.708; 3.502)
0.500	Fifth	44.620 (36.383; 52.857)	53.417 (46.209; 60.626)	58.711 (46.524; 70.898)	61.657 (45.500; 77.815)	97.167 (–1.923; 196.257)	2.729 (1.845; 3.609)
1	Sixth	46.455 (35.046; 57.863)	51.923 (43.737; 60.109)	57.523 (49.299; 65.757)	60.184 (50.505; 69.863)	82.360 (32.370; 132.349)	3.180 (2.229 – 4.136)
2	Fifth	45.596 (29.382; 61.811)	51.561 (37.244; 65.878)	57.566 (37.581; 77.551)	60.740 (35.080; 86.399)	95.169 (–61.108; 251.446)	2.778 (2.019; 3.540)
3	Fifth	41.929 (18.989; 64.869)	46.463 (28.559; 64.367)	53.083 (37.200; 68.967)	56.382 (38.709; 74.054)	87.570 (–9.634; 184.774)	2.609 (2.190; 3.029)
4	Fifth	45.672 (43.074; 48.271)	47.287 (44.321; 50.253)	52.099 (47.196; 57.001)	54.916 (48.565; 61.266)	93.045 (59.344; 126.746)	2.112 (1.534; 2.690)
6	Fifth	48.552 (44.006; 53.098)	48.217 (43.809; 52.624)	53.997 (46.525; 61.469)	57.402 (47.681; 67.123)	105.282 (49.674; 160.890)	1.991 (1.497; 2.486)
8	Fifth	48.595 (38.988; 58.202)	46.618 (36.412; 56.824)	51.972 (41.650; 62.295)	54.769 (42.369; 67.170)	84.272 (7.111; 161.433)	3.324 (2.460; 4.188)
PTA 0.5–4	Fifth	45.846 (37.263; 54.429)	52.073 (41.904; 62.242)	58.279 (41.020; 75.539)	61.790 (38.764; 84.834)	107.005 (–48.720; 262.730)	2.672 (1.994; 3.350)
PTA 4–8	Fifth	47.721 (44.182; 51.261)	47.697 (44.164; 51.229)	52.851 (47.023; 58.680)	55.855 (48.220; 63.490)	96.439 (51.646; 141.241)	2.159 (1.611; 2.707)
PTA 6–8	Fifth	49.423 (44.293; 54.554)	48.253 (43.662; 52.843)	54.020 (46.092; 61.948)	57.409 (46.975; 67.843)	104.760 (42.439; 167.081)	2.003 (1.534; 2.472)

ED or effective dose refers to the sigmoid-shaped dose-response relationship between the hearing threshold (dependent variable) with age (independent variable), which is expressed as the percentage by which the hearing theshold progresses with increasing age from the start to end. For example: ED50 refers to the value of the hearing threshold at half-way progression, ED100 refers to the end of the progression.

*Estimated age at which the hearing deterioration has reached 33.33 % (effective dose) of its progression, which corresponds to 40 dB HL

†Estimated age at which the hearing deterioration has reached 50% (effective dose) of its progression

‡Estimated age at which the hearing deterioration has reached 58.33% (effective dose) of its progression, corresponding to 70 dB HL.

§Estimated age at which the hearing deterioration has reached its maximal progression

¶Slope is a measure of ATD in dB/y.

ATD, annual threshold deterioration; CI, confidence intervals; PTA, pure-tone average.

TABLE 5. Summary of all estimated ages at which the hearing deterioration starts, reaches 33.33% (=40 dB HL), 50%, 58.33% (=70 dB HL) and maximal progression, as well as estimates of the ATD, with 95% confidence intervals in 57 female P51S carriers (all estimated were computed based on nonlinear drc-regression models)

Female Carriers (n = 57) (kHz)	Onset (Decade)	Age of Onset (years) + 95% CI	ED 33.33* (years) + 95% CI	ED 50† (years) + 95% CI	ED 58.33‡ (years) + 95% CI	EDmax§ (years) + 95% CI	Slope¶ (dB/y) + 95% CI
0.125	Fifth	42.860 (30.798; 54.922)	53.891 (49.694; 58.088)	57.988 (54.923; 61.053)	59.818 (56.606; 63.031)	72.843 (56.498; 89.188)	2.396 (0.345; 5.574)
0.250	Fifth	38.622 (25.562; 51.681)	50.711 (44.805; 56.618)	55.765 (52.032; 59.497)	57.955 (54.420; 61.491)	71.938 (55.475; 88.401)	3.513 (2.195; 4.831)
0.500	Fifth	41.800 (29.016; 54.585)	52.149 (46.451; 57.846)	57.304 (51.210; 63.397)	59.830 (52.287; 67.372)	82.562 (38.090; 127.034)	3.433 (2.467; 4.433)
1	Fifth	42.132 (31.949; 52.316)	51.262 (46.823; 55.702)	55.815 (52.497; 59.133)	57.897 (54.298; 61.500)	73.635 (53.324; 93.946)	4.557 (3.135; 5.978)
2	Fifth	41.663 (32.868; 50.458)	49.371 (45.031; 53.711)	54.164 (50.129; 58.200)	56.514 (51.656; 61.372)	77.666 (46.581; 108.751)	3.583 (2.629; 4.536)
3	Fourth	34.443 (21.188; 47.698)	46.170 (40.290; 52.050)	51.851 (46.713; 56.988)	54.620 (48.644; 60.596)	79.339 (40.599; 118.059)	2.644 (1.921; 3.367)
4	Fourth	37.694 (30.431; 44.957)	46.561 (40.722; 52.400)	53.008 (42.728; 63.287)	56.754 (42.385; 71.123)	108.793 (–16.184; 233.771)	1.994 (1.573; 2.414)
6	Third	34.917 (–17.566; 87.400)	44.525 (25.778; 63.273)	50.172 (25.495; 74.849)	53.218 (18.276; 88.160)	88.167 (–261.760; 438.094)	2.319 (1.732; 2.906)
8	Third	27.851 (14.123; 41.579)	41.45 (32.57; 50.34)	48.52 (43.04; 54.00)	51.54 (47.30; 55.78)	66.87 (53.21; 80.53)	2.319 (1.732; 2.919)
PTA 0.5–4	Fifth	40.849 (31.235; 50.463)	49.432 (44.532; 54.332)	54.682 (50.180; 59.185)	57.213 (51.865; 62.562)	79.121 (46.596; 111.647)	3.392 (2.546; 4.237)
PTA 4–8	Third	35.603 (15.446 55.761)	45.513 (36.447; 54.579)	51.469 (38.755; 64.183)	54.714 (37.147; 72.281)	92.972 (–64.442; 250.369)	1.899 (1.516; 2.282)
PTA 6–8	Third	31.795 (–14.002; 77.591)	43.495 (25.713; 61.277)	49.398 (38.012; 60.784)	52.173 (39.927; 64.420)	74.546 (–37.884; 186.977)	2.799 (1.989; 3.609)

ED or effective dose refers to the sigmoid-shaped dose-response relationship between the hearing threshold (dependent variable) with age (independent variable), which is expressed as the percentage by which the hearing theshold progresses with increasing age from the start to end. For example: ED50 refers to the value of the hearing threshold at half-way progression, ED100 refers to the end of the progression.

*Estimated age at which the hearing deterioration has reached 33.33 % (effective dose) of its progression, which corresponds to 40 dB HL.

†Estimated age at which the hearing deterioration has reached 50% (effective dose) of its progression.

‡Estimated age at which the hearing deterioration has reached 58.33 % (effective dose) of its progression, corresponding to 70 dB HL.

§Estimated age at which the hearing deterioration has reached its maximal progression.

¶Slope is a measure of ATD in dB/y.

ATD, annual threshold deterioration; CI, confidence intervals; PTA, pure-tone average.

Digital Content 1, http://links.lww.com/EANDH/A857). Twenty-two asymptomatic carriers aged under 40 years were also included. The male/female ratio was 54/57. There were no statistically significant differences in mean hearing thresholds between both genders at any frequency nor PTA-types (Welsh two-sample t test), neither when analyzing all ages together, nor for each decade separately (Table 1 in Supplemental Digital Content 1, http://links.lww.com/EANDH/A857). The demographics of male and female carrier population are summarized in Table 1.

Estimation of Age of Onset and Decline Rate

Age of Onset in Decades • Box & Whisker plots of each frequency (ranging from 0.125 to 8 kHz) and the three PTAs ($PTA^{0.5\text{-}4}$, $PTA^{4\text{-}8}$, $PTA^{6\text{-}8}$) were drawn for each decade and for both genders separately (Figs. 1–3; Figures 2 to 6 in Supplemental Digital Content 2 to 6, http://links.lww.com/EANDH/A857).

The age decade of onset for each frequency and the three PTAs were summarized in Table 2, for male and female carriers separately. Hearing deterioration started as early as in the third decade at the highest frequency in female carriers, whereas this was observed in the fifth decade for male carriers. The starting point of all other frequencies (i.e., below 4 kHz) was observed in the fifth decade, regardless of gender.

Table 3 summarizes the chronology of onset of hearing deterioration, based on the one-sample t test (A: male; B: female carriers). Hearing deterioration started in the fifth decade at almost all frequencies in male carriers, whereas it started in the third decade at 6 and 8 kHz, fourth decade at 4 kHz and fifth decade at the remaining frequency range in female carriers.

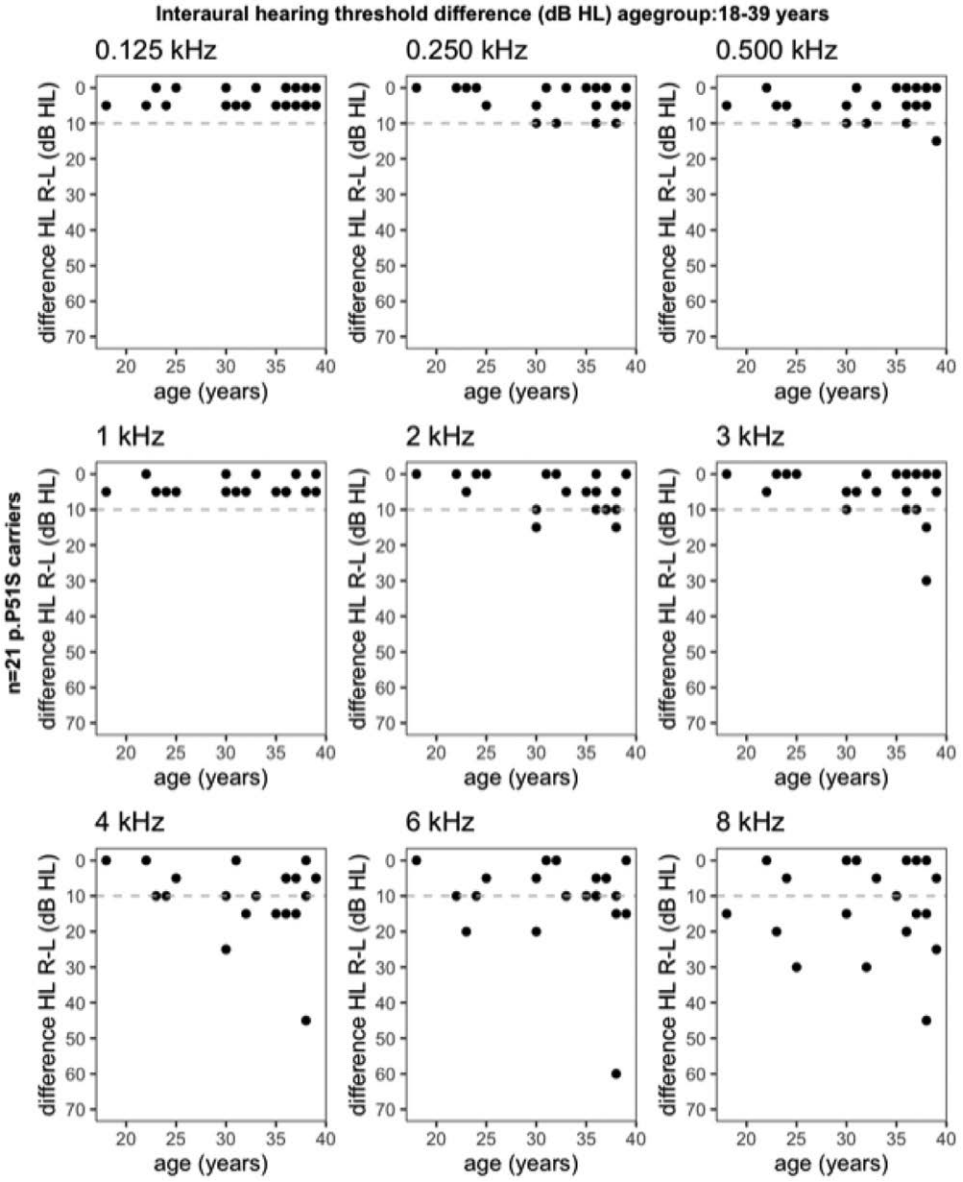

Fig. 6. Interaural hearing threshold difference (dB HL) at all frequencies (0.125 to 8 kHz) in 21 P51S carriers (42 ears) (male and female) aged from 18 to 39 years. Note the higher level of threshold difference at highest frequencies (dotted line = 10 dB interaural difference).

Age of Onset in Years and Annual Threshold Deterioration

Frequency-specific (range: 0.125 to 8 kHz) nonlinear sigmoid-shaped plots of hearing thresholds against age were obtained from auditory data of 222 ears of 111 individual hearing thresholds, also including presymptomatic carriers, as shown in Figure 4 for male and Figure 5 for female carriers. Similar graphs were also built using the three PTAs for both genders separately (Figures 7 and 8 in Supplemental Digital Contents 7 and 8, http://links.lww.com/EANDH/A857).

In tables 4 and 5, ages of onset of hearing deterioration, expressed in years, together with ages at which the progression of the hearing deterioration was estimated to reach 40 dB HL, 70 dB HL and maximal hearing deterioration as well as ATD were summarized, with 95% confidence intervals. The estimates were computed for each frequency as well as the three PTAs, for both male and female carriers, respectively.

Hearing deterioration started at about 46 years of age on average (range: 42 to 49 years) in male carriers and it was estimated at 38 years on average in female carriers (range: 27 to 43 years). At the highest frequency, onset was estimated at 28 years of age on average in female carriers. Hearing deterioration of 120 dB HL was achieved approximately one decade earlier in female (80 years on average; range: 67 to 108 years) versus male carriers (94 years on average; range 92 to 111 years). The decline rate was estimated at about 2.97 dB/y on average in female carriers (range: 1.99 to 4.56 dB/y) and 2.68 dB/y in male carriers (range: 2 – 3.29 dB/y).

Estimation of Treatment Eligibility

Tables 4 and 5 also give an overview of milestone ages at which p.P51S carriers in theory might become eligible for conventional

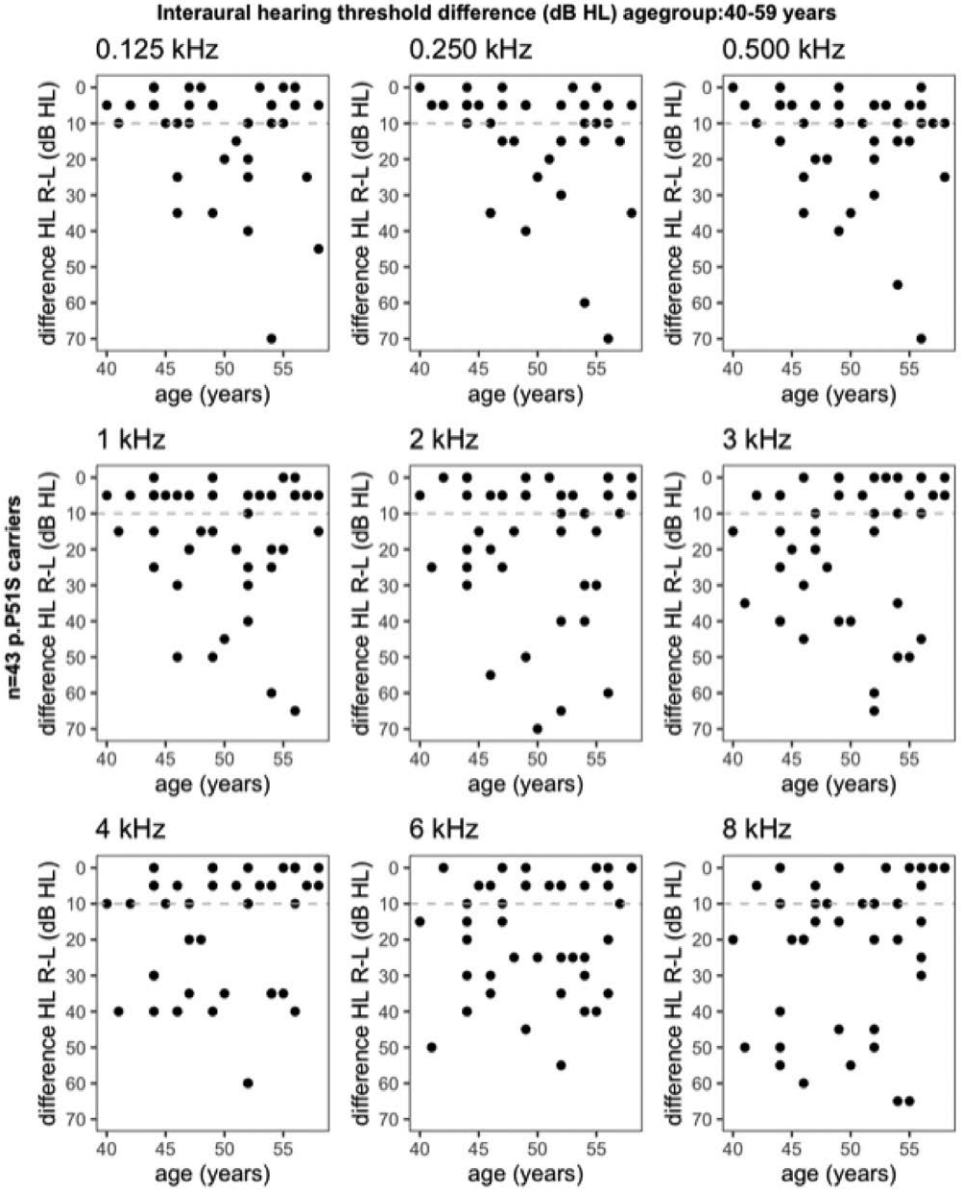

Fig. 7. Interaural hearing threshold difference (dB HL) at all frequencies (0.125 to 8 kHz) in 43 P51S carriers (86 ears) (male and female) aged from 40 to 59 years. Note the high level of threshold differences across all frequencies (dotted line = 10 dB interaural difference).

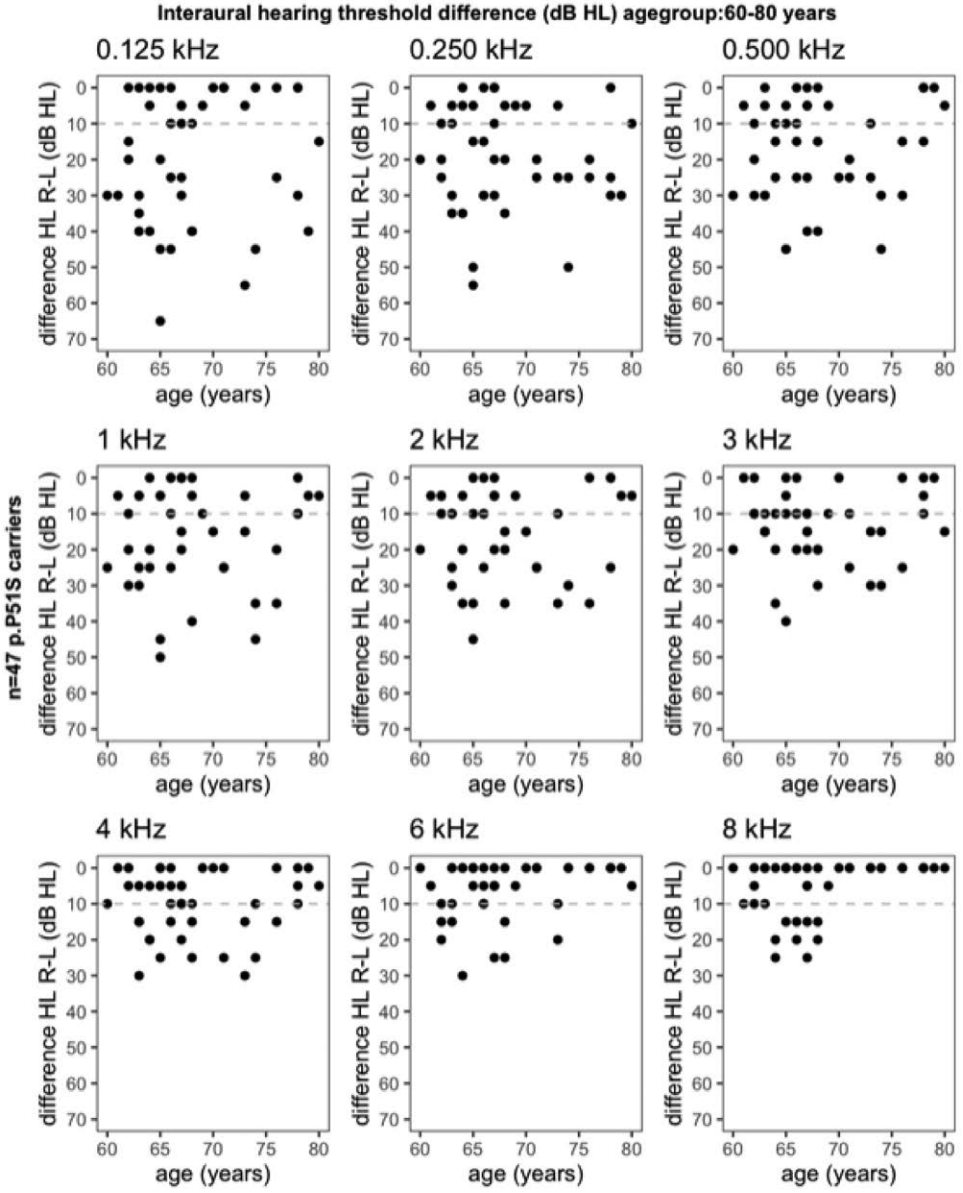

Fig. 8. Interaural hearing threshold difference (dB HL) at all frequencies (0.125 to 8 kHz) in 47 P51S carriers (94 ears) (male and female) aged from 60 to 80 years. Note the higher level of threshold differences at lower frequencies (dotted line = 10 dB interaural difference).

TABLE 6. The IQR of hearing thresholds from 111 P51S carriers separated into three different age groups

Frequency (kHz)	Age Group 1 (18–39 years) n = 21	Age Group 2 (40–59 years) n = 43	Age Group 3 (60–80 years) n = 43
0.125	10	35	58.75
0.250	5	35	33.75
0.500	8.75	40	38.75
1	5	40	35
2	5	40	35
3	15	40	33.75
4	15	40	25
6	13.75	33.75	25
8	15	30	20
Mean	10.28	37.08	33.89

The mean IQR scores are highest in the age group 40–59 years and are the lowest in the age group 18–39.
IQR, interquartile range.

hearing aids and cochlear implants (CI), based only on PTA 0.5 to 4 kHz, for male and female carriers, respectively. Hearing deterioration of at least 40 dB HL (corresponding to 33.33% of progression of the drc hearing deterioration curve) was estimated at about 50 years on average in male carriers (range: 46 to 54 years) and 48 years on average in female carriers (range: 41 to 54 years), whereas 70 dB HL (corresponding to 58.33% of total progression of the drc hearing deterioration curve) was estimated at about 59 years on average in male carriers (range: 55 to 64 years) and 56 years on average in female carriers (range: 51 to 57 years).

Individual Asymmetry (Interaural Difference) and Interindividual Variability of Hearing Thresholds

Figures 6 to 8 give an overview of the interaural differences between right and left hearing thresholds for each individual male and female carrier, who were pooled in subsets of three different age groups: 18 to 39, 40 to 59, and 60 to 80 years.

TABLE 7. Correlation coefficients between individual right and left hearing thresholds in 111 P51S carriers, separated into three age groups, showing higher coefficient values being consistent with less interaural asymmetry

Age Group	Frequency	Spearman
Age 18–39	**125 Hz**	0.723
	250 Hz	0.637
	500 Hz	0.451
	1 kHz	0.521
	2 kHz	0.082
	3 kHz	0.522
	4 kHz	0.316
	6 kHz	0.415
	8 kHz	0.271
Age 40–59	**125 Hz**	0.715
	250 Hz	0.703
	500 Hz	0.752
	1 kHz	0.665
	2 kHz	0.598
	3 kHz	0.543
	4 kHz	0.544
	6 kHz	0.589
	8 kHz	0.544
Age 60–80	**125 Hz**	0.653
	250 Hz	0.591
	500 Hz	0.564
	1 kHz	0.522
	2 kHz	0.549
	3 kHz	0.475
	4 kHz	0.511
	6 kHz	0.741
	8 kHz	0.812

Note the same pattern compared with Figures 6–8, with lower degrees of interaural differences at lower frequencies in age group 18–39 and at higher frequencies in age group 60–80.

The dotted line represents an interaural difference of 10 dB HL, which is considered to be acceptable. Table 6 represents the interquartile range (IQR) scores per decade at all frequencies in three age groups: age group 18 to 39 years, age group 40 to 59 years, and age group 60 to 80 years.

Correlation coefficients were calculated between right and left hearing thresholds in the same individual in 111 carriers for the three age groups separately, as summarized in Table 7.

Age-related Hearing Deterioration

ARTA allow a clear overview of the hearing progression over decades. Figure 9 represents measured or observed hearing threshold progression in male (Fig. 9A) and female (Fig. 9B) carriers (observed ARTA), whereas predictions based on frequency-specific hearing thresholds using nonlinear 'drc"-regression model (predicted ARTA) were illustrated in Figure 10 (A: male and B: female carriers). This was also applied for male and female carriers together (Figures 9 in Supplemental Digital Content 9, http://links.lww.com/EANDH/A857).

The pattern with which differences between medians of each frequency evolves with aging is illustrated in Figure 10 in Supplemental Digital Content 10, http://links.lww.com/EANDH/A857).

DISCUSSION

The scope of this prospective cross-sectional study was to evaluate hearing function in the largest prospective series of the p.P51S variant in the *COCH* gene. DFNA9 caused by the p.P51S variant is fully penetrant and all carriers will eventually develop clinical signs. Audiological phenotypic expression is similar in all carriers, however, with variable timing of milestone ages and with a high degree of interaural asymmetry (Fransen and Van Camp 1999; Bom et al. 2003). The phenotypic characteristics were provided by genotype-phenotype correlation studies that were conducted 15 years ago. These studies on family pedigrees were focused on finding a locus with linkage analysis and later the mutation with Sanger sequencing (de Kok et al. 1999; Bom et al. 2001; Fransen et al. 2001; Verstreken et al. 2001; Bom et al. 2003; Lemaire et al. 2003).

In this prospective cross-sectional study, presymptomatic mutation carriers were also enrolled to define the natural course of the disease, including 22 under the age of 40 years in a total of 111 p.P51S carriers.

The World Health Organization defines disabling hearing loss with hearing thresholds greater than 40 dB in the better hearing ear in adults and greater than 30 dB in the better hearing ear in children (https://www.who.int/news-room/fact-sheets/detail/deafness-and-hearing-loss). In this study, however, one of the objectives was to delineate the starting point at which hearing would deviate from age-referenced limits, which expectedly would occur in the presymptomatic period of the DFNA9 disease. By applying the p97.5th besides the p95th percentile, the cutoff value represents the upper 1.98% of the Gaussian curve of normative auditory values, which are even more stringent criteria than those used previously in literature.

Because age-referenced limits are more pronounced in men, determination of age of onset (whether in decades or years) and ARTAs were established for both male and female p.P51S carriers separately. As a result, the onset of hearing deterioration was systematically estimated at older ages for male compared with female carriers (Tables 2 to 5), with a difference in onset age of about 8 years (46 years for male and 38 years for female carriers). This was even more striking at 8 kHz, with an onset age observed in the third decade (27 years) in female carriers compared with fifth decade for their male peers (49 years).

The sequence of the hearing deterioration across all hearing frequencies tended to evolve from high to low frequencies in female carriers, both visually and quantitatively, as shown in Figures 9 and 10 as well as Tables 4 and 5. However, for male carriers, onset was observed nearly simultaneously in all frequencies. Different conclusions can be drawn if we observe the intersection point between age-referenced limits and the drc-regression line in Figures 4 and 5. The intersection was consistently observed in the fifth decade for all frequencies below 3 kHz, whereas these limits were already exceeded in the third decade at the higher frequencies (4 to 8 kHz) in both male and female subjects, without any intersection at all, suggesting the hearing thresholds were, in reality, already deviating from age-referenced limits at the earliest ages in p.P51S carriers, regardless of gender. This apparent discrepancy between estimations and visual representation of the hearing deterioration progression can be explained by the more stringent age-referenced limits in male population, which were the key to estimations of the y-variable in Tables 3 and 4 (see Materials and Methods section). Respective ATDs of both male and female carriers were relatively comparable (male/female ratio: 0.9 dB/y), even though progression of hearing

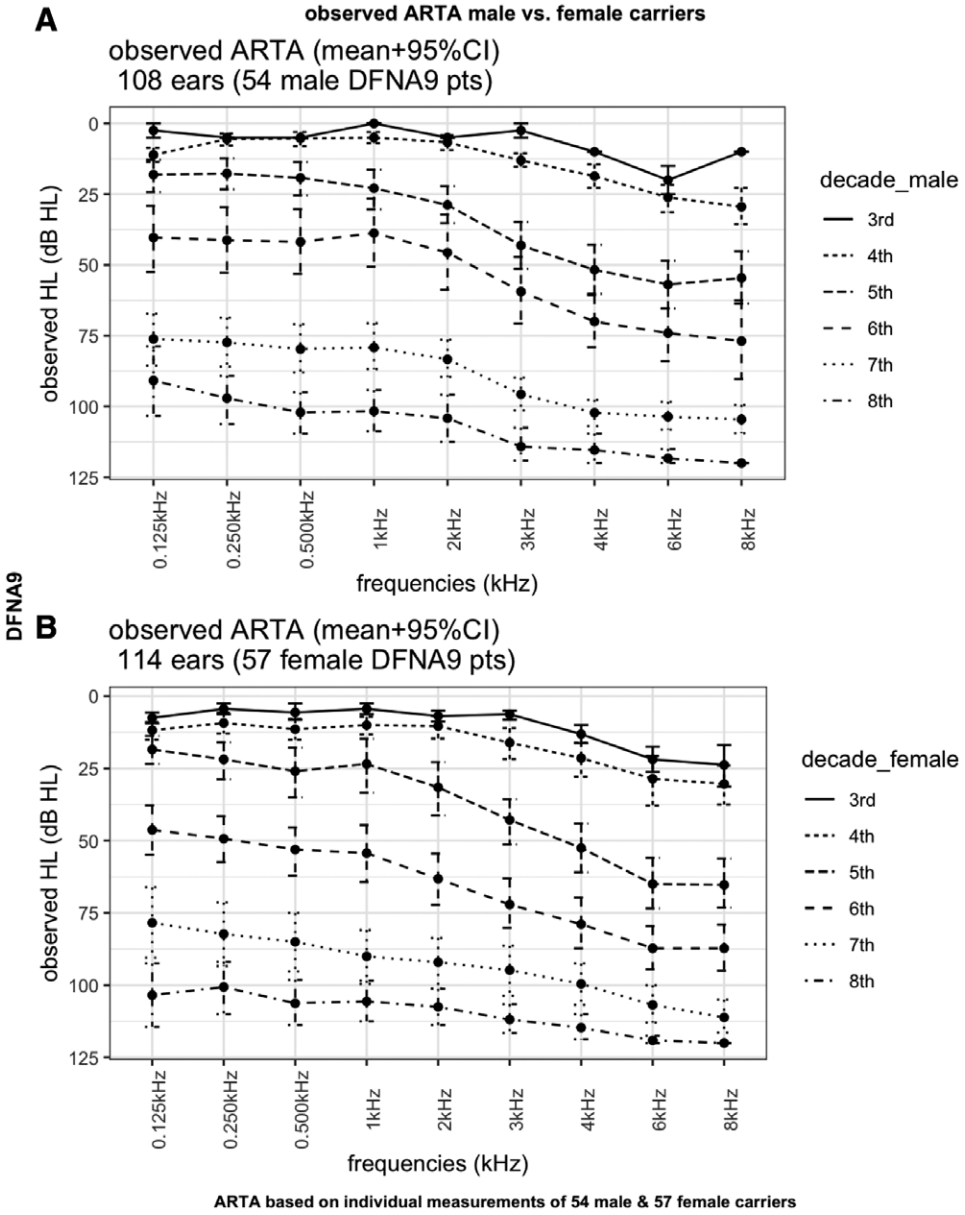

Fig. 9. ARTA based on observed data from 54 male (A) and 57 female (B) p.P51S carriers (observed ARTA).

deterioration was approximately 10% slower in male carriers than in female carriers.

More interesting, when comparing male with female predicted ARTA's (i.e., derived from prediction models), as depicted in Figure 10, it appears that the progression of hearing deterioration was indifferent to gender, even though the deterioration seemed more important at higher frequencies between third and fifth decade in female carriers, whereas the same was observed at the middle and lower frequencies during the time lapse between fifth to sixth decade with observed data (Fig. 9). Also, unpaired two-sample t tests to compare the means of hearing thresholds between male with female carriers were insignificant, independently of frequency and age (Table 1 in Supplemental Digital Content 1, http://links.lww.com/EANDH/A857). Therefore, the p.P51S *COCH* variant shows similar phenotypic expression in both genders, in contrast with the estimations earlier. Once again, this discrepancy is probably the reflection of more stringent ISO 7029 age-referenced limits in male subjects compared with female. In daily practice, if only hearing deterioration is to be considered (disregarding vestibular symptoms), this would probably result in higher chance of false negative genetic testing in case of high-frequency deterioration for male carriers aged under 50 years.

To summarize, although the P51S *COCH* variant expresses similarly in male and female carriers, it is clinically more difficult to attribute the early adult-onset hearing deterioration to DFNA9 in male carriers. As a consequence, DFNA9 disease may very well be associated with early hearing deterioration, probably even before 30 years of age. The

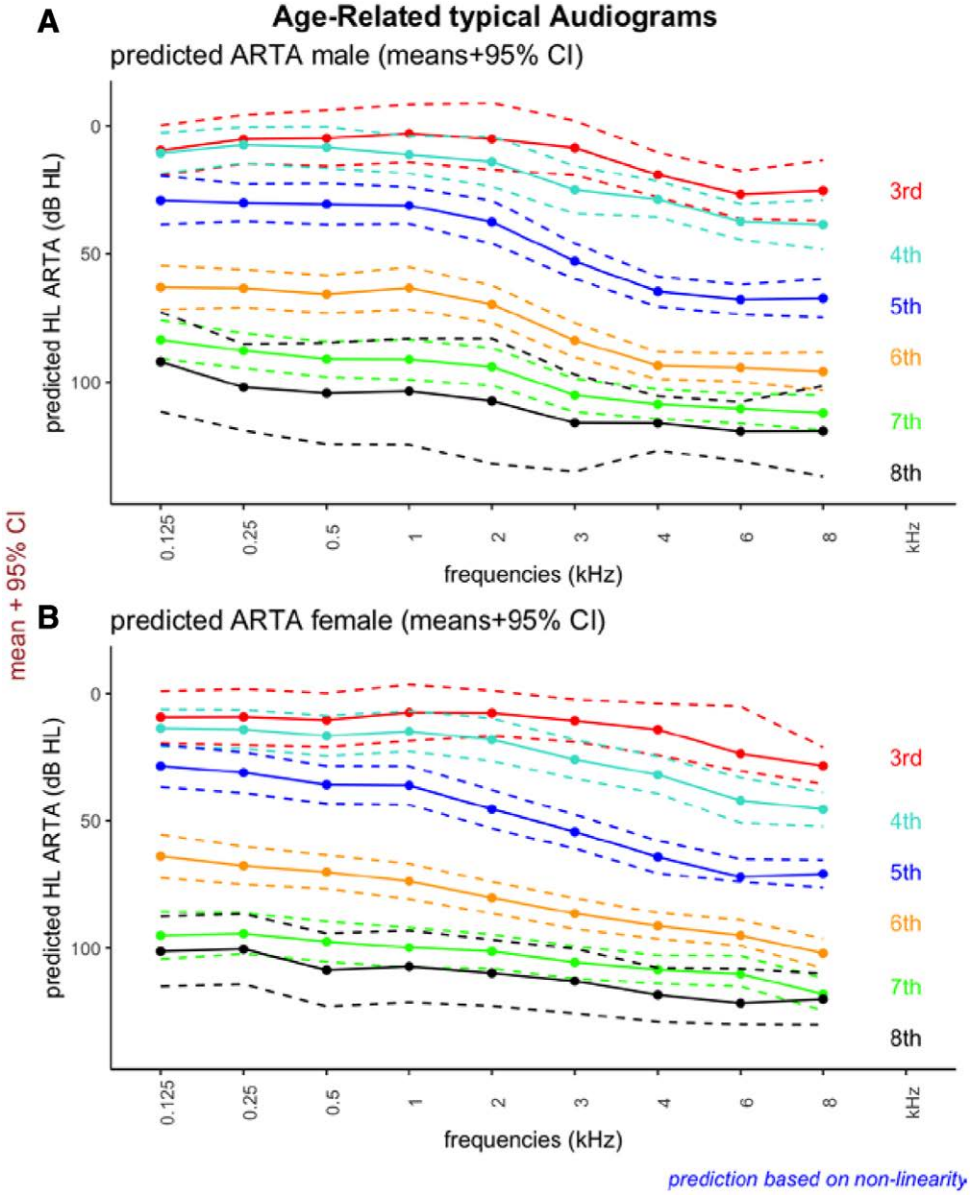

Fig. 10. Predicted ARTA derived from nonlinear dose-response regression model for male (A) and female (B) p.P51S carriers with corresponding 95% confidence intervals.

initial ISO 7029 were pooled data of 6 or 7 databases, and an algorithm was calculated. However, these databases date from the postindustrial period where most males have had noise exposure and they might be not accurate for contemporary normative data.

The early starting point of hearing deterioration, as observed in the present study, also corresponds to previous observations, where young variant carriers (<33 years of age) showed significantly higher thresholds than those in age-matched controls at 2 to 8 kHz (Bom et al. 2003). Here, this was only observed at 6 and 8 kHz and onset age was estimated at about 28 years on average compared with 32 years at 8 kHz, which is at least 5 years earlier on average. This can have several explanations: differences in defining the beginning of hearing decline, a different number of participants (111 versus 52) and whether or not averaging binaural hearing thresholds was applied. Bom et al. 2003, estimated the onset of the decline by fitting a linear regression model between 10% and 90% progression of the deterioration on the sigmoid-shaped nonlinear fit, with the assumption the slope was constant and that the segment was linear in between the boundaries. These assumptions, however, have a better chance to fit with true progression if cutoff boundaries were to be set between 20% and 80% of progression, as applied in the present study.

With regard to the eligibility for treatment with conventional hearing aids, there is a 2-year difference between male and female carriers and a 3-year difference for CI. Even though in case of CI, other criteria, including speech perception, also need consideration to meet reimbursement criteria, the models applied in this study provide realistic estimations which correspond to daily practice. It furthermore emphasizes the rate of hearing decline during a limited time span of just a few decades (fourth to sixth decade).

Interaural asymmetry of the hearing deterioration in subjects suffering from DFNA9 is a common phenotypic feature of the p.P51S *COCH* variant. Figures 6 to 8 show relatively minor interaural differences in hearing threshold between right and left ears within the same individual in the p.P51S carriers aged from 18 to 39 years. In this age group, the higher the frequency, the higher degree of interaural difference, which is observed regardless of age (ranging from 18 to 39 years) at 8 kHz and to some extent also at 6 kHz. At the lower frequencies, the difference remains within an acceptable 10 dB HL. The pattern is completely different in the following age group, ranging from 40 to 59 years of age. At least half of the subjects present with interaural difference exceeding 10 dB HL at almost all frequencies, of which 50% of these with more than 30 to 65 dB HL. This was uniformly observed at all ages between 40 and 59 years. As aging progresses, the asymmetry tends to fade with higher frequency and with time (age group 60 to 80 years), probably due to a ceiling effect. Still, the extent of the asymmetry remains important, especially at the low frequencies and regardless of age. To some extent, the interquartile range (IQR), which is a measure of variability, as shown in Table 6, is mirroring the pattern of asymmetry across age groups and frequencies. These results suggest, even though the penetration of the p.P51S mutation in *COCH* is relatively uniform compared with other hereditary hearing losses, that once the hearing loss has started at a given frequency, it evolves with quite some variability among carriers and with moderate to severe interaural asymmetry across ages and frequencies, as long as the deterioration progresses rapidly (linear segment of the sigmoid curve). Before and after the rapid decline, variability and asymmetry are both at their lowest level. The endpoint eventually remains the same for all carriers: bilateral profound sensorineural hearing loss.

Limitations

An important limitation in this study is that it is impossible to determine whether the asymmetry would persist individually across all ages. This could be more clearly demonstrated with longitudinal data in contrast with the present study, where the hearing data were collected in a cross-sectional fashion.

Although efforts were made to enroll as many young p.P51S variant carriers as possible, more presymptomatic carriers would be beneficial to genotype-phenotype studies. Positive carrier status potentially carries a heavy mental burden since many of the young siblings already witnessed the progressive disabilities of their seniors. Furthermore, contemporary demographics—lower birth rates and aging population—constitute important limitations. Therefore, our patients were given the chance and choice to inform us about their preference to be informed or not.

Future Perspectives

These findings may have consequences in counseling (very) young patients with unknown carrier status when they present with high-frequency SNHL and a positive familial history for hereditary hearing disorders. Other examples can be found in supporting early diagnosis with the help of machine learning tools or in determining potential therapeutic windows for future treatment strategies, which seem to be relatively shorter than expected in case of DFNA9 (onset third decade instead of fourth decade as previously described) (Hildebrand et al. 2009).

CONCLUSION

This study, which was carried out on the largest number of p.P51S variant carriers in *COCH*, demonstrates that the first signs of hearing deterioration occur as early as in the third decade in female carriers, in contrast with fifth decade for their male peers. Hearing deterioration starts at the highest frequencies, followed by the lower frequencies in p.P51S carriers, whereas this deterioration exceeds the age-expected p97.5th references simultaneously across nearly all frequencies in male subjects (fifth decade), which is different from the sequence as observed for female carriers. The late-onset ages in male carriers, however, are the reflection of more stringent criteria and age-referenced limits applied to them. Moreover, prediction-based ARTAs showed similar phenotypic expression in both genders. The left-right asymmetry of the hearing levels is a typical feature of the p.P51S variant, which appears to persist across ages, with a climax from 40 to 59 years of age. At an average age of 40 to 45 years, DFNA9 patients may already need conventional hearing aids, whereas they may become eligible for CI at an average age of 57 to 62 years, depending on the gender. Even if all carriers eventually develop severe bilateral sensorineural hearing loss, there is, however, some degree of variance, which matches the interaural asymmetry across ages. This suggests DFNA9 is characterized by a full penetrance and limited variability when the endpoint status of the hearing decline is considered, however, with high inter- and intrasubject variability with regard to the timing of the phenotypic expression.

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There are no conflicts of interest to declare.

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