A Novel Z-Score–Based Method to Analyze Candidate Genes for Age-Related Hearing Impairment

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Objective: **Approximately half of the variance of Age-Related Hearing Impairment (ARHI) is attributable to environmental risk factors, and the other half to genetic factors. None of these genes has ever been identified, but the genes involved in monogenic nonsyndromic hearing impairment are good candidates. Here we define and validate a quantitative trait value for ARHI, correcting for age and gender, to allow the genetic study of ARHI as a quantitative trait.**

Design: **Based on the ISO 7029 standard, we convert audiometric data into a Z-score, an age- and gender-independent value expressing to what extent a person is affected by ARHI. The validity of this approach is checked using a test population of randomly collected subjects. The power to evaluate the contribution of a candidate gene to ARHI is assessed using simulated populations. As an example, one ARHI candidate gene is analyzed.**

Results: **In our test population, Z-scores were normally distributed although the mean did not equal zero. Z-scores were independent of age, and there was no difference between men and women. Power studies using simulated populations indicated that to detect moderate genetic effects, sample sizes of at least 500 random subjects are necessary.**

Conclusion: **The Z-score conversion appears to be a valid method to describe to what extent a subject is affected by ARHI, allowing to compare persons from different age and gender. This method can be the basis of future, powerful studies to identify ARHI genes.**

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Epidemiology, Pathology, and Risk Factors

It is well documented that hearing thresholds increase with aging. At the age of 80, approximately half of the population suffers from a hearing impairment that affects their communication skills (Davis, 1995). Men are more severely affected than women. Age of onset, progression and severity of Age-Related Hearing Impairment (ARHI) show great variation. The most common type of ARHI is bilaterally symmetrical, sensorineural and most pronounced in the high frequencies. Variation is at its largest in the high frequencies and increases with age.

Several environmental factors have been reported to lead to hearing loss, but it is unknown how important they are and to what extent they influence hearing at a later age. Excessive noise may lead to either mechanical or metabolic cochlear damage (Flock, Flock, Fridberger, Scarfone, & Ulfendahl, 1999; Luz & Hodge, 1971; Mulroy, Henry, & McNeil, 1998). At a lower level of noise, cochlear damage is predominantly metabolic and probably related to the excitotoxicity of the neurotransmitter gamma amino-butyric acid, and to the presence of free radicals and other reactive endogenous substances (Pujol & Puel, 1999; Yamasoba, Nuttall, Harris, Raphael, & Miller, 1998). The effect of tobacco smoking on hearing loss is controversial. Some authors reported that smoking can cause hearing loss (Cruickshanks et al., 1998), whereas other studies could not demonstrate smoking to be a risk factor (Drettner, Hedstrand, Klockhoff, & Svedberg, 1975; Fuortes, Tang, Pomrehn, & Anderson, 1995). Many drugs and chemicals have been reported to have an ototoxic effect, mainly reflected by a high frequency sensorineural hearing loss, but only few of these effects are well documented and most are reversible (Govaerts et al., 1990; Palomar-Garcia, Abdulghani-Martinez, Bodet-Agusti, Andreu-Mencia, & Palomar-Asenjo, 2001).

Genetics of ARHI

No genetic factors contributing to ARHI have been identified so far. Using mice, two loci have been mapped: *Ahl1* has been mapped to mouse chromosome 10 and is the major contributor to age-related hearing loss in at least 10 mouse strains (Johnson, Zheng, & Erway, 2000). *Ahl2* was recently mapped to mouse chromosome 5 and modulated the hearing loss in mice that were homozygous for the *Ahl1* genotype (Johnson & Zheng, 2002). However, the responsible genes have not yet been identified and it is unknown whether these genes contribute to ARHI in humans.

Epidemiological studies have shown that genetic factors account for approximately 50% of the vari-

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ance encountered in ARHI. Using a combination of questionnaire and audiometric data, a Swedish male twin study showed a heritability of 47% for the population above 65 (Karlsson, Harris, & Svartengren, 1997). Across all ages, both environmental and hereditary factors were important sources of variation, with the environmental factors becoming more influential with increasing age. A second study (Gates, Couropmitree, & Myers, 1999) compared the auditory status in genetically unrelated people (spouse pairs) and genetically related people (sibling pairs, parent-child pairs), revealing a clear familial aggregation for age-related hearing levels. In the latter study, the heritability of the condition was estimated at 35 to 55%. These studies suggest that ARHI is a complex trait, caused by interplay between genetic and environmental factors.

Research into the genetic factors leading to hearing impairment has up to now concentrated on monogenic forms of syndromic and nonsyndromic hearing impairment. At the moment, the genes for the most common syndromic forms of deafness have been identified. Nonsyndromic hearing impairment (NSHI) turned out to be genetically extremely heterogeneous. Almost 100 loci for monogenic NSHI have been reported (51 dominant, 39 recessive, 8 X-linked) but only 32 of the genes have been identified. The ever-growing list of identified genes includes developmental proteins, ion channels, extracellular matrix components, cytoskeletal proteins, and genes with an unknown function (Van Camp and Smith, Hereditary Hearing Loss Home Page, http://dnalab-www.uia.ac.be/dnalab/hhh/). Generally, mutations leading to monogenic hearing impairment are very rare variants, but they have a large effect on protein functioning. These mutations lead to a severe phenotype in all the people carrying them. In contrast to these rare mutations leading to monogenic disorders, it has been proposed that genetic factors contributing to complex traits are common genetic variants having a more subtle influence on protein functioning or expression.

Studying ARHI as a Complex Trait

The nucleotide sequence of the human genome is not the same in all individuals, and the sites in the genome that vary between individuals are called polymorphisms. There are different types of polymorphisms in the genome, but the type that is held responsible for most of the phenotypic variation between individuals, are variations altering one single base pair or single nucleotide polymorphisms (SNPs). It is estimated that about one out of every thousand base pairs in the genome is polymorphic. SNP databases have been generated as part of the Human Genome Project. Moreover, SNP analysis is relatively cheap and the technology to routinely screen SNPs at high-throughput is developing quickly.

Under the hypothesis that complex diseases are caused by common genetic variants, susceptibility genes for complex disorders can be searched by screening candidate genes for SNP variants that are more frequent among affected persons compared with controls (Risch & Merikangas, 1996). Alternatively, the test population can be stratified according to the genotype at a particular candidate locus, after which the different strata are compared with each other (Boerwinkle et al., 1987). While the former case-control approach is more applicable to binary traits, quantitative traits (QTs) like ARHI are better studied using the latter approach, since dichotomizing a QT leads to loss of statistical power (Page & Amos, 1999).

Treating ARHI as a QT requires the definition of a value that describes to what extent an individual is affected. Hearing thresholds, recorded using pure-tone audiometry, are not suitable since the median thresholds for each frequency are age- and gender- dependent. To assess the relative normality of an audiogram, an adjustment for age and gender is required.

Here we describe a method that converts the frequency-specific thresholds to a gender- and age-independent value referred to as the Z-score. The Z-score expresses the difference with the median value for a particular age and gender in standard deviation units. A similar approach has previously been used to adjust for age and gender in audiological analysis of monogenic hearing impairment (Govaerts et al., 1998; Wuyts, Van de Heyning, & Declau, 1998). We validate the method using a real-world test population of randomly collected subjects, and assess the power of the method using simulated populations. As an illustration, we analyze the contribution of a common SNP in the *COCH* gene, one of the genes responsible for nonsyndromic late-onset hearing impairment.

MATERIALS AND METHODS

Calculation of the Z-Score

For the otologically normal population between age 18 and 70, the International Standard (ISO) 7029 describes the median (P50) threshold of hearing by air conduction as a function of age for men and women using the formula:

$$H_{md,Y} = \alpha(Y\text{-}18)^2 \quad (1)$$

where the subscript md,Y stands for the median at a given age, Y stands for the age, and α being a gender- and frequency- specific constant. The

value of α can be found in ISO7029 tables and is larger in men than in women, and larger in the higher frequencies than in the lower frequencies. To check whether a person belongs to the better or worse hearing part of the population, we compared his/her recorded hearing thresholds at each frequency to the age- and gender-specific median given by the above formula.

The ISO 7029 standards describe the distribution of hearing thresholds around the median by two halves of a normal (Gauss) distribution, with the half above the median having a larger standard deviation than the half below the median. Standard deviations of the upper and lower part of the distribution are given by these formulae (ISO 7029):

$$s_u = b_u + 0.445\ H_{md,Y} \text{ for thresholds} > H_{md,Y} \quad (2)$$

$$s_l = b_l + 0.356\ H_{md,Y} \text{ for thresholds} < H_{md,Y} \quad (3)$$

The constants b_u and b_l (u = upper, l = lower) are gender- and frequency- specific. Standard deviations are larger in men compared with women, and in the high frequencies compared with the low frequencies.

To calculate a frequency-specific Z-score, we calculated how many standard deviations the recorded hearing threshold diverged from the median threshold value for this age, gender and frequency:

$$Z_f = (\text{threshold} - H_{md,Y})/s_u \text{ for thresholds} > H_{md,Y} \quad (4)$$

$$Z_f = (\text{threshold} - H_{md,Y})/s_l \text{ for thresholds} < H_{md,Y} \quad (5)$$

As ARHI typically affects the high frequencies, we used the average of the Z-scores from 2, 4, and 8 kHz (referred to as Z_{248}) in all our further calculations. An illustration of this method is given in Figure 1.

Collection of the Test Population of Random Subjects

In previous projects, we have collected several large families with autosomal dominant NSHI. In addition to affected persons, we also systematically collected blood and audiological data from their spouses. In families with late-onset hearing loss, where assortive mating is absent, the spouses of the affected and unaffected family members represent a random set of unrelated subjects. A total of 126 unrelated spouses from various families was collected.

Only subjects between ages 40 and 70 were included, as the ISO 7029 standards are only applicable up to the age of 70. Below age 40, threshold increases due to ARHI are too small compared with the error of an audiometer. Pure-tone thresholds with air and bone conduction were registered at

TABLE 1. Power of the MGG or MGA ANOVA test for an additively-acting trait (increaser) allele T, as a function of trait allele frequency, effect size and sample size*

Simulation parameters	Population						
	1	2	3	4	5	6	7
Trait allele freq†	0.5	0.5	0.5	0.2	0.2	0.5	0.2
Mean Z-score‡							
NN	−0.25	−0.25	−0.25	−0.25	−0.25	−0.25	−0.25
NT	0	0	0	0	0	0	0
TT	+0.25	+0.25	+0.25	+0.25	+0.25	+0.25	+0.25
Residual SD	1	1.2	1.5	1	1.2	0.5	0.5
Genetic variance§	0.028	0.02	0.01	0.02	0.013	0.115	0.07
Estimated Power‖							
MGA							
100	22	23	12	23	15	94	65
500	95	76	52	82	66	100	100
1000	100	99	90	99	91	100	100
MGG							
100	33	30	23	32	21	97	75
500	99	84	55	89	77	100	100
1000	100	100	100	100	99	100	100

* $\alpha = 0.05$.

† *A hypothetical biallelic SNP with a normal allele (N) and a trait (increaser) allele (T).*

‡ *Effect size of the SNP was specified by defining a mean Z-score and residual SD for a homozygous normal (NN) subpopulation, a heterozygous (NT) subpopulation, and a subpopulation homozygous for the increaser allele (TT). The size of the three subpopulations was determined by the trait allele frequency and the Hardy-Weinberg law. The three subpopulations were pooled to obtain one simulated population.*

§ *Genetic variance was calculated by regressing the Z-scores from the entire simulated population on the genotype (as a continuous trait).*

‖ *Two hundred simulations were performed whereby 100, 500, or 1000 subjects were selected at random from the simulated population. We counted the number of times a significant effect ($p < 0.05$) of the genotype on the Z-score could be detected.*

ANOVA: analysis of variance.

TABLE 2. Power of the MGG or MGA ANOVA test for a recessive trait (increaser) allele T, as a function of trait allele frequency, effect size and sample size*

Simulation parameters	Population										
	8	9	10	11	12	13	14	15	16	17	18
Trait allele freq†	0.5	0.5	0.5	0.5	0.5	0.2	0.2	0.8	0.8	0.2	0.8
Mean Z-score‡											
NN	−0.25	−0.25	−0.25	−0.25	−0.25	−0.25	−0.25	−0.25	−0.25	−0.25	−0.25
NT	−0.25	−0.25	−0.25	−0.25	−0.25	−0.25	−0.25	−0.25	−0.25	−0.25	−0.25
TT	+0.25	+0.25	+0.25	+0.25	+0.25	+0.25	+0.25	+0.25	+0.25	+0.5	+0.5
Residual SD	1	1.2	1.5	0.5	0.7	1	0.5	0.5	1	0.5	1
Genetic variance§											
Total	0.045	0.033	0.019	0.154	0.089	0.012	0.036	0.19	0.055	0.081	0.117
Additive	0.03	0.021	0.013	0.104	0.059	0.002	0.009	0.168	0.049	0.027	0.104
Estimated power‖											
MGA											
100	49	34	22	97	76	15	33	100	53	70	87
500	99	91	83	100	100	57	97	100	100	100	100
1000	100	100	100	100	100	86	100	100	100	100	100
MGG											
100	45	26	20	91	71	10	24	100	59	41	90
500	99	98	75	100	100	21	54	100	100	94	100
1000	100	100	95	100	100	29	84	100	100	100	100

* $\alpha = 0.05$.

† *A hypothetical biallelic SNP with a normal allele (N) and a trait (increaser) allele (T).*

‡ *Effect size of the SNP was specified by defining a mean Z-score and residual SD for a homozygous normal (NN) subpopulation, a heterozygous (NT) subpopulation, and a subpopulation homozygous for the increaser allele (TT). The size of the three subpopulations was determined by the trait allele frequency and the Hardy-Weinberg law. The three subpopulations were pooled to obtain one simulated population.*

§ *Additive and total genetic variance were calculated by regressing the Z-scores from the entire simulated population on the genotype (as a continuous trait), and adding heterozygosity as a covariate.*

‖ *Two hundred simulations were performed whereby 100, 500, or 1000 subjects were selected at random from the simulated population. We counted the number of times a significant effect ($p < 0.05$) of the genotype on the Z-score could be detected.*

0.125, 0.25, 0.5, 1, 2, 4, and 8 kHz. Persons with a conductive component, defined as a mean air-bone gap at 0.5, 1, and 2 kHz exceeding 10 dB in the better-performing ear, were excluded. People with a dip at 4 kHz (most likely due to noise exposure) in the better-performing ear were excluded if the 4 kHz threshold exceeded the 8 kHz threshold by 20 dB or more. In addition, we excluded a small number of people having or having had a disease that could have an influence on hearing (including chronic otitis media, auto-immune disease, chemotherapy, rheumatoid arthritis). A total number of 104 persons met the inclusion criteria. All subjects originate from Belgium (Flanders) or the Netherlands.

Simulated Populations and Power Analyses

Eighteen different simulated populations were constructed with the Z-score determined by the combination of random effects and a biallelic causative variant. Effect size of the trait allele, standard deviation of the residuals, allele frequencies and the additive/dominant nature of the trait allele for each population are given in Table 1 (populations 1–7) and Table 2 (populations 8–18). Populations 1–7 have a trait (increaser) allele acting additively, whereas for populations 8–18, the trait allele was recessive. For each population, we calculated the locus-specific total genetic variance and the additive genetic variance, by regressing the Z-score on genotype (as a continuous variable) and adding heterozygosity as a dichotomous covariate.

The power to detect the effect of the genotype on the phenotype was assessed by performing 3×200 simulations on each population, for three different sample sizes. In each simulation, 100, 500 or 1000 subjects were randomly chosen from the population under study, after which effect of the genotype on the phenotype is tested using two variants of the measured-genotype test: In the measured genotype test based on genotypes (MGG), phenotypic values of the selected subjects are binned into three groups according to genotype, and the between-group effect tested using a regular one-way ANOVA. In the measured genotype test based on alleles (MGA), phenotypic values are binned into two groups, i.e., one bin for each allele, whereby each phenotypic value is used twice. The phenotypic value of a heterozygote individual is put once in every bin, whereas the phenotypic value of a homozygous individual is put twice into the same bin. This latter test assumes an additive effect of the alleles, which implies that the heterozygotes are phenotypically

intermediate between the two homozygote categories (Boerwinkle et al., 1987; Page & Amos, 1999). For each population and each sample size, we counted the percentage of simulations yielding a significant p value (< 0.05).

SNP Detection and Typing

SNP T352S in the *COCH* gene (rs# 1045644) was retrieved from the SNP database (http://www.ncbi.nlm.nih.gov/SNP). Typing of the SNP in 104 random control samples was performed using the SNaPshot reaction (Applied Biosystems) according to the manufacturer's instructions. In brief, a PCR product containing the SNP is purified using Calf Intestine Alkaline Phosphatase (Amersham Pharmacia) and Exonuclease I (NE Biolabs). In the subsequent SNaPshot reaction, a primer adjacent to the SNP was extended by one, fluorescently labeled dideoxyNTP, whereby all four dideoxyNTP carry a different dye. After purification of the reaction products with Calf Intestine Alkaline Phosphatase, extension products were analyzed on an ABI Prism 3100 Genetic Analyzer to detect which one of the dideoxyNTPs had been built in.

RESULTS

Z-Score Calculation and Validation

Hearing thresholds were converted to an age- and gender-independent value termed the Z-score as described in the Methods section. The method is illustrated in Figure 1. In each patient, the average Z-score of the high frequencies (Z_{248}) was calculated separately for both ears. All subsequent calculations, as well as the exclusion criteria, were performed on the data from the better-hearing ear.

To test whether the conversion of audiometric thresholds into Z-scores appropriately corrects for age and gender, the distribution of the Z-scores in a random sample of 104 Dutch and Belgian (Flemish) subjects was studied as shown in Figure 2 and summarized in Table 3. On visual inspection, three outliers with a Z-score around +3 were excluded. Summary statistics of the remaining 101 samples are shown in Table 1. Z-scores were normally distributed ($p = 0.286$, Kolmogorov-Smirnov test), but the mean did not equal 0. Z-scores were independent of age, and men were not significantly different from women ($p = 0.556$, two-sided t-test). However, the variance in men was larger than in women ($p = 0.009$, Levene test).

Power Calculations

The influence of linked genetic variance, trait allele frequency and dominance at the trait locus on the power of a genetic association study, was estimated using simulated populations. Properties of the populations and results of the power study are given in Table 1.

Unless the gene under study accounts for at least 10% of the total genetic variance, a sample of 100 randomly selected subjects has limited power. This implies that our test population is probably too small to detect subtle genetic effects on the Z-score. Augmenting the sample size to 500 would offer a

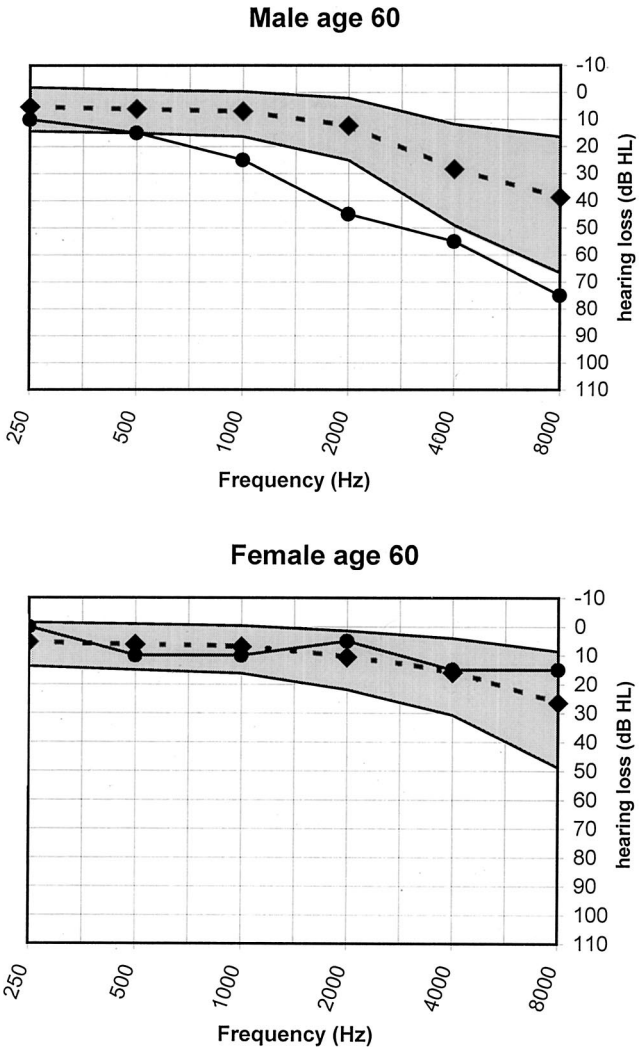

Figure 1. Illustration of the Z-scores on an audiogram. Audiograms for a 60-yr-old man and woman are shown. The dashed line indicates the p50 (median) threshold value for men and women, respectively, at age 60, calculated using Equation 1. The shaded area marks the area within 1 SD above and below the age-specific median, calculated using Equations 2 and 3. The full black lines indicate the recorded thresholds for both 60-yr-old subjects. For the male subject, the Z-scores for 2, 4, and 8 kHz are 2.57, 1.28, and 1.30, respectively (calculated using Equations 4 and 5), making a Z_{248} of 1.72. For the female subject, the Z-scores for 2, 4, and 8 kHz are -0.61, -0.07, and -0.64, respectively, making a Z_{248} of -0.44.

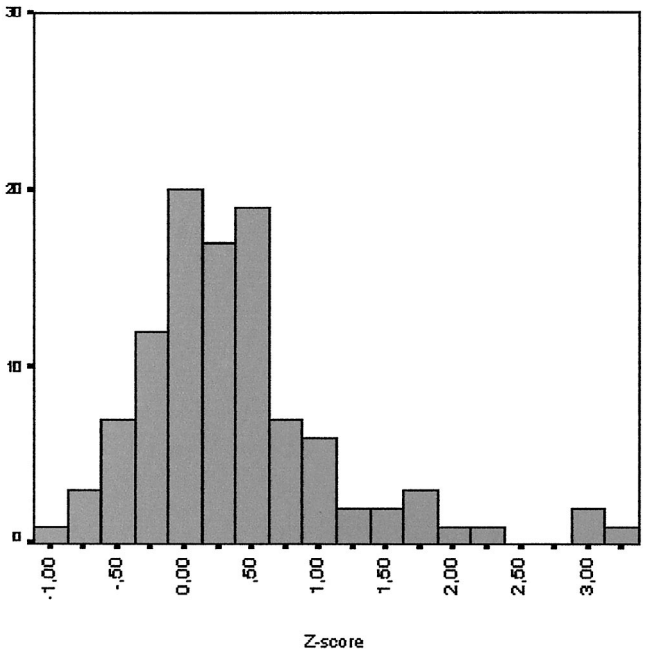

Figure 2. Distribution of Z-scores in the test population. The histogram shown an approximately normal distribution of the Z-score in the test population ($N = 104$). Three outliers with a Z-score around +3 were excluded from all further calculations. For the remaining 101 subjects, mean and SD were +0.3 and +0.61, respectively.

considerable improvement and allow the detection of more moderate effects, with a locus-specific variance around a few percent. If the alleles act additively, the highest power is reached if the trait (increaser) allele and the normal allele have the same allele frequency. The highest power for a recessive trait allele is reached if this allele has a frequency around 0.70 (i.e., 50% of the population is homozygous for the trait allele), but it quickly drops in case the trait allele gets rare. For more modest effects—a variance around 1%—or to reach a more stringent significance level, increasing the sample size to 1000 or more is necessary. For instance, the MGG test on additive population 4 for a sample size of 500 offers a power of 82% for $p < 0.05$, but only 61% power to reach $p < 0.01$. Doubling the sample size would result in a power above 95%.

Analysis of a *COCH* SNP in the Test Population

COCH is an inner-ear specific protein, and mutations in the gene encoding *COCH* are responsible for a form of late-onset hearing loss (DFNA9) (Robertson et al., 1998). The *COCH* open reading frame contains one coding SNP, encoding a threonine/serine polymorphism on position 352 of the COCH

TABLE 3. Summary statistics on Z-score and age in the test population

	N	Mean	SD	Minimum	Maximum
Total					
Z_{248}	101	0.30	0.61	−0.92	2.33
Age	101	53.90	8.78	40	70
Men					
Z_{248}	50	0.34	0.74	−0.92	2.33
Age	50	55.97	9.13	40	70
Women					
Z_{248}	51	0.27	0.46	−0.87	1.48
Age	51	51.88	7.99	40	70

Z_{248}: the average Z-score at 2000, 4000, and 8000 Hz.

TABLE 4. Coding SNP in the *COCH* gene

Rs #	Amino acid change	Nucleotide change	SNaPshot primer	PCR primers
1045644	T352S	1055 C → G	CTCTGGTACAGAAGCTGTGCA	F: GTCTCTTATCTAGATTAACTTG R: CTGATGACAGCTAGGACATTC

TABLE 5. Statistical analysis of *COCH* SNP

	MGA		MGG		
Genotype	G	C	GG	GC	CC
N	123	79	35	53	13
Mean Z_{248}	0.26	0.36	0.21	0.34	0.41
SD	0.62	0.60	0.63	0.60	0.60
p value	0.273			0.504	

Z_{248}: the average Z-score at 2000, 4000, and 8000 Hz.

protein (Table 4). We have searched for a possible effect of this SNP on the Z-score using our random data set. All 101 subjects were individually genotyped for the T352S polymorphism. The results of this analysis is given in Table 5. No significant effect of the SNP on the Z-score was found, neither in the MGG test, nor in the MGA test.

DISCUSSION

It is tempting to speculate that common variants in the genes involved in monogenic NSHI explain part of the variation found in ARHI. Instead of performing a case-control study that involves dichotomization of the phenotype, we preferred to treat ARHI as a QT since it has been shown that this latter approach yields more statistical power (Page & Amos, 1999). Therefore, we needed to define a QT value that expresses to what extend a person is affected. Hearing thresholds are not suitable here since average hearing threshold values vary with age and gender. For example, a threshold of 30 dB for the high frequencies in a 40-yr-old woman is much more severe than a 30-dB threshold in a 70-yr-old man. To obtain the relative "normality" of an audiogram, a correction is required to take age and gender into account. In various fields of medicine, such adjustments are common and they are referred to as Z-scores. For instance, Z-scores in osteogenic conditions (Meema & Meema, 1982) express the difference from a normal value in standard deviation units, which is particularly useful for comparisons where the normal or median values are age- and gender-dependent.

Age corrections based on the ISO 7029 standards have already been used in the study of monogenic forms of hearing impairment. Wuyts et al. (1998) defined age-corrected audiometric inclusion and exclusion criteria for linkage analysis. Similarly, Govaerts et al. (1998) used an age-correction in the audiometric analysis of a pedigree with autosomal dominant hearing loss caused by a mutation in the TECTA gene. To quantify the effect of this mutation on hearing thresholds, an age and gender correction was used to assess the normality of the audiometric results. The authors introduced this method as Hearing Standard Deviations.

Here we extend the use of Z-scores as a more general QT value that describes the hearing status of an individual, and we apply the method to study the variation in ARHI. The most relevant Z-scores in ARHI are the Z_2, Z_4 and Z_8, as age-related threshold shifts are most prominent in these frequencies. The average of these high-frequency Z-scores (Z_{248}) was used as a QT value in our genetic analyses. In a randomly sampled test population, Z-scores were independent of age and there was no difference in mean between men and women. This indicates that the Z-score method appropriately corrects for age and gender, and therefore allows to compare people from different age and gender. In addition, the distribution of the Z-scores was normal, which is a prerequisite for some statistical analysis methods. This indicates that we have found a valid QT value to describe the severity of ARHI independent of age and gender.

As the data compared using the Z-scores are cross-sectional, the method described here does not include the longitudinal component of ARHI, and it cannot discriminate between patients on the basis of the pattern or the rate at which their hearing declines. However, longitudinal ARHI studies suggest that differences in progression rate are probably not a major concern (Pearson et al., 1995).

In its most typical form, ARHI is nonsyndromic, bilaterally symmetrical, sensorineural, and most pronounced in the high frequencies. Many forms of monogenic NSHI show a similar audiometric pattern, albeit starting at a younger age and deteriorating faster. Therefore, the genes involved in late-onset NSHI starting in the high frequencies—like *COCH* and many more autosomal dominant genes—are excellent candidate genes for ARHI. In addition, it cannot be excluded that variations in genes involved in other types of NSHI might also play a role in ARHI, especially because mutations in the same NSHI gene can lead to different types of hearing loss (Petersen, 2002). Many genes with a known function in the inner ear can be considered functional candidate susceptibility genes for ARHI. Using the Z-score as a QT value for ARHI enables powerful statistical testing of candidate genes.

The open reading frame of the COCH gene contains one coding variant, substituting a threonine by a serine residue on 352, in the region between the two von Willebrandt type A-like domains. The conservative nature of the Thr-Ser substitution, its position in the protein and the lack of an overt T352S-associated phenotype, make it unlikely that this polymorphism has a crucial influence on the structure and integrity of the *COCH* protein. However, subtle effects on protein functioning cannot be excluded. We investigated whether this coding variant can explain part of the variance encountered in ARHI, as an illustration of our Z-score method. No significant association between the Z-score and the T352S genotype was found. However, power estimates showed that the power of this analysis, which used only 101 subjects, was very limited. The analysis of the *COCH* SNP should therefore be regarded as an illustration, rather than a firm exclusion of *COCH* as a gene for ARHI. It only excludes *COCH* as

a major ARHI gene, i.e., a gene explaining more than 10% of the variance. Such large effects due to a single gene are unlikely. Given the extreme genetic heterogeneity of monogenic hearing loss (Petersen, 2002), and the situation observed in other complex diseases (Terwilliger & Weiss, 1998), it seems more likely that many genes with small effects are involved. Our estimates indicate that, depending on allele frequencies and inheritance mode, 500 or even 1000 subjects are necessary to detect genetic effects explaining around 1 or a few percent of the variance. Alternatively, power can be raised by studying several SNPs within one candidate gene and looking for particular allelic combinations (haplotypes) in adjacent SNPs (Akey, Jin, & Xiong, 2001).

The mean Z-score in our test population did not equal 0. This finding is in line with previous reports about the discrepancy between an unselected or "typical" population and the ISO7029 standards, and has been attributed to the very stringent inclusion criteria of the ISO7029 data set (Lutman & Spencer, 1991). For the type of association studies presented here, this is not a major concern, as one is merely looking for Z-score differences attributable to a certain genotype regardless of what the mean of the population is. But it is a relevant issue if sampling is nonrandom. For instance, collecting only subjects with either a high or a low QT value (extreme sampling, as opposed to random sampling) would be another way to raise the power of a genetic association study. Extreme sampling is more economical in that it gives a higher power for the same number of subjects to be genotyped, but it requires firm and reliable cutoff values to determine who becomes included. In our test population, mean equals 0.30 and the variance is 0.61. It would be worth investigating if this situation is similar in other populations. Extreme sampling based on Z-scores is feasible, but it requires prior knowledge about the distribution of the Z-score in the population under study.

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