Heritability of Age-Related Hearing Loss in Middle-Aged and Elderly Chinese
A Population-Based Twin Study
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Published in:
Ear and Hearing

DOI:
10.1097/AUD.0000000000000610

Publication date:
2019

Document version
Accepted manuscript

Citation for published version (APA):

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Manuscript Number: EANDH-D-16-00324R1

Full Title: Heritability of Age-Related Hearing Impairment in Middle-Aged and Elderly Chinese: a Population-Based Twin Study

Article Type: Research Article

Section/Category: Epidemiology of Auditory and Vestibular Disorders

Keywords: age-related hearing impairment; heritability; better ear hearing level; pure tone average; better hearing level; twin study

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**Heritability of Age-Related Hearing Impairment in Middle-Aged and Elderly Chinese: a Population-Based Twin Study**

Haiping Duan¹, ², ³, Dongfeng Zhang¹*, Yajun Liang⁴, Chunsheng Xu¹, ², Yili Wu¹, Xiaocao Tian², Zengchang Pang², Qihua Tan⁵, ⁶, Shuxia Li⁵ and Chengxuan Qiu³

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**Abbreviations:** ARHI, age-related hearing impairment; PTA, pure tone average; BEHL, better ear hearing level.

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ABSTRACT

Objectives: Age-related hearing impairment (ARHI), or presbycusis, is the most prevalent sensory disorder in the elderly. The genetic and environmental influences on ARHI have been studied mostly in the developed countries.

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Conclusion: This twin study suggests that genetic factors contribute to ARHI at the low and high frequencies among middle-aged and elderly Chinese.
1. Introduction

Hearing impairment affected more than 50% of middle-aged and elderly people in China (Liu et al. 2006; Liu et al. 2013). Age-related hearing impairment (ARHI), or presbycusis, is the most common type of sensorineural hearing loss (SNHL) caused by the natural aging of the auditory system. In the early stages, it typically affects audibility at high frequencies, which interferes with normal speech comprehension in both quiet and noisy conditions; then the impairment extends to the medium and low frequencies over time. ARHI is usually detected at the stage when speech comprehension is already impaired; early stage ARHI is often under-recognized (Panza et al. 2015).

The impact of genetic factors on ARHI has been frequently reported in developed countries. Familial aggregation and twin studies as well as genome-wide linkage analysis suggest that genetic factors may contribute substantially to ARHI, with the heritability varying between 25% and 75% (Bogo et al. 2015; Christensen et al. 2001; DeStefano et al. 2003; Gates et al. 1999; Kvestad et al. 2012a; Momi et al. 2015; Raynor et al. 2009; Viljanen et al. 2007; Wingfield et al. 2007). However, the heritability of ARHI among middle-aged and elderly Chinese population has not yet been quantified. In addition, previous studies have consistently shown the gender difference in hearing loss such that men are more likely than women to have hearing problems regardless of race (McMahon et al. 2008; Mitchell et al. 2011; Pratt et al. 2009; Wallhagen et al. 1997).

Currently, there is no gold standard method for defining ARHI. Different studies have used pure tone average (PTA) at different frequencies (DeStefano et al. 2003; Gates et al. 1990; Kvestad et al. 2012b; Wingfield et al. 2007), the better or worst ear hearing level thresholds (Viljanen et al. 2007), principal component scores (Huyghe et
al. 2008), standardized Z-scores, speech-in-noise (SIN) (Momi et al. 2015), speech to noise ratio hearing test (Wolber et al. 2012), and self-reported reduced hearing (Christensen et al. 2001) to evaluate ARHI. As the sub-phenotypes of ARHI, better ear hearing level (BEHL) thresholds and PTA are more proximate to direct genetic control than the composite phenotype itself, and provide a quantitative measure of hearing impairment. The population-based twin studies focusing on these measurements should exhibit more power in helping understand the etiology and development of ARHI.

In this study, using data from a population-based twin sample of middle-aged and elderly Chinese people, we sought to quantify the variations of hearing impairment due to genetic influences at different frequencies, and further to estimate their genetic correlations for the possible common genetic background.

2. Materials and Methods

2.1. Subjects

Our study sample was derived from the Qingdao Twin Registry (Duan et al. 2013), which is part of the Chinese National Twin Registry established in 2001. The twin registry was aimed at providing data resources for long-term genetic epidemiological research of complex diseases and related phenotypes. All twins in the registry were identified through local disease control and prevention network, neighborhood/village committees, and residence registry, and then appointments for face-to-face interview were made through telephone. In 2012-2013, middle-aged and elderly twins (age range, 33-80) were invited to undertake clinical examination if both co-twins were
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The Qingdao twin study was approved by the Ethics Committee of Medicine at Qingdao Center for Disease Control and Prevention (Qingdao CDC). Written informed consent was obtained from all participants. Research within the Qingdao Twin Registry was conducted according to the ethical principles for medical research involving human subjects, as expressed in the Declaration of Helsinki.

2.2. Zygosity identification

We conducted face-to-face interviews, anthropometric measurements, and laboratory test either at Qingdao CDC or at clinics in local towns and villages. We first identified potential MZ and DZ twins through sex and ABO blood group system. Twins with opposite sex and/or different blood groups were classified as DZ twins. Venous blood was drawn for zygosity identification for like-sex twin pairs using 16 multiple short tandem sequence repeat DNA markers (Becker et al., 1997; Jackson et al., 2001) at the central laboratory of the Qingdao Blood Center, with the probability
of correct zygosity assignment being 99.99%.

2.3. Audiometric examination

Audiometric examination was performed in a dedicated sound isolating room. First, otoscopy was given to ensure that the ear canals of the participants were clear of debris and were not impacted by cerumen. Pure-tone air-conducted hearing thresholds in each ear were measured separately at the frequencies of 0.5, 1, 2, 4, 8 and 12.5 kHz by a diagnostic audiometer (EN.60645-1,-2 type2A, MadsemIteral 2; GN Otometrics, Denmark) for all individuals. Pure-tone thresholds of each ear for each audiometric frequency ranging from -10 to 120 dB were selected with higher threshold reflecting lower hearing level.

BEHL was defined as hearing level of the ear with the lowest average threshold over each frequency from the better ear. Because 12.5 kHz is an ultra-high frequency, pure-tone thresholds of either ear could not be detected for nearly half of twin pairs, PTA was calculated using BEHLs of the other five frequencies.

2.4. Statistical analysis

We used SPSS 22.0 for Windows (SPSS, Chicago, IL, USA) for descriptive statistics and correlations, and Mx (Neale et al. 2006) for genetic model fitting of twins. Due to skewed distribution, we made square root transformation for BEHLs, and log transformation for PTA. The maximum likelihood estimation (MLE) approach was used to estimate phenotypic correlations and twin correlations in all model-fitting procedures.

The classic twin methodology is based on the fact that MZ twins share 100% of
their genes, whereas DZ twins share, on an average, 50% of their segregating genes and no more genetically related than ordinary siblings. Twins reared in the same family are assumed to share their environments to the same extent. Thus, the population-based twin study is considered a powerful approach to quantify the relative contribution of genetic and environmental variances to the phenotypes. Structural equation modeling (SEM) was fitted using the full information maximum likelihood (FIML) method implemented in Mx software (Neale et al. 2006). Twin design and analytical methods were described elsewhere (Neale et al., 1992). The ACE model (A represents the additive genetic influences, C indicates the common or shared environmental influences and E refers to the unique environmental influences) was compared with simpler AE and CE models. The fit of each model was assessed by the differences in log likelihood between the sub-models and the full-models. The Akaike information criterion (AIC) gives a measure of model fit, taking the balance of the $\chi^2$ statistic and number of degrees of freedom into consideration. We carried out the variance components analysis, assuming only linear relations given our small sample size. Heritability is defined as the proportion of the total variance attributable to genetic variance. Age, sex and educational level were included in all models as covariates. Age was categorized into <45, 45-49, 50-54, 55-59, and ≥60 years, and educational level into illiterate, primary school, middle school, high school, and university.

To determine whether genetic influences on BEHLs specific to hearing at each of the six frequencies still existed after taking into account those influences on hearing at
other frequencies, a Cholesky decomposition model was fitted to BEHLs at six
frequencies, including factors A, C, and E, as sources of covariation. We put BEHLs
at frequencies 0.5, 1, 2, 4, 8, and 12.5 kHz as the first, second, third, fourth, fifth, and
the sixth latent factors (unobserved factors), respectively. The multivariate twin
models were fitted using the Mx software package (Neale et al. 2006).

3. Results

3.1. Descriptive

Of the 358 twin pairs and 1 triplet (719 persons), the frequencies of the
participating twins in the age groups of <45, 45-49, 50-54, 55-59, and ≥60 years were
140, 191, 136, 144, and 108 twins, respectively. The mean age of the participating
twin-pairs was 51.55 years (standard deviation [SD], 7.65), and the male to female
ratio (M/F) was 1:1.024. Table 1 shows the basic statistics for BEHLs and PTA. After
transformations all variables had nearly normal distribution. Education and age were
both related to PTA, but in the model that included both educational level and age as
covariates, only age had significant effects on PTA ($p = 0.021$). There were J-shaped
audiometric patterns of ARHI assessed by BEHLs at different audiometric frequencies
by age groups and sex (Figure 1).

3.2. Univariate twin correlation and heritabilities

Table 2 shows intra-pair correlations of BEHLs and PTA in MZ and DZ pairs.
Except for 0.5 kHz, intra-pair correlations were higher in MZ twins than in DZ twins
at all other five audiometric frequencies ($p <0.05$).
To avoid overestimation of genetic influence, the ACE model was used for heritability estimation. In all ACE models, the estimated heritability ranged from 1.65% to 54.20%, with the low heritability (1.65% to 18.68%) for BEHLs at low frequencies (0.5 and 1 kHz) and 27.71% for PTA, and the moderate heritability (47.08% to 54.20%) for BEHLs at other frequencies measured.

3.3. Phenotypic correlations among BEHLs and PTA

There were moderate to strong phenotypic correlations between BEHLs (0.5-8 kHz) and PTA, with the correlation coefficients ranging from 0.60 to 0.75 (Table 3). For BEHLs, strong phenotypic correlations were found both among the low frequencies (0.5, 1, and 2 kHz) and among the high frequencies (4, 8, and 12.5 kHz). However, the correlations of BEHL at 12.5 kHz with BEHLs at the frequencies of 0.5, 1.0 and 2.0 kHz were weak and not statistically significant (p >0.05).

3.4. Multivariate model fit

The best fitting multivariate model was AE model, and the C matrix could be dropped off the model without worsening fit (AIC = 839.104, p = 0.719, Δdf = 21). Figure 2 shows the standardized path correlation coefficients with 21 pathways for each of A and E in the AE Cholesky decomposition (these must be squared to obtain variance components), remembering that, to a small extent, the A estimates were confounded with some shared environmental effects. We found that the genetic correlations of phenotypes were degraded from lower to higher frequencies. For example, the first genetic factor (A1), which was loaded primarily at the frequencies of 0.5, 1, 2, 4, 8, and 12.5 kHz, accounted for 48.73%, 23.98%, 12.40%, 9.01%, 8
5.93%, and 1.13% of variance, respectively (Figure 2). There was a phenomenon towards increased heritability with increased frequency among different phenotypes.

Interestingly, additive genetic factor loadings related to 1.0 kHz showed no significant correlation with those of 4.0, 8.0 and 12.5 kHz. Similarly, no significant correlation was found for additive genetic factor loading related to 12.5 kHz. These loadings could be dropped without significantly affecting the model fitting. However, the unique environmental factor loadings were all significant in the model.

3.5. Genetic and environmental correlations among BEHLs

The additive genetic and unshared environmental correlation coefficients between these BEHLs, derived from the AE Cholesky analysis, were shown in Table 4. The bivariate analysis revealed high genetic correlations between BEHLs at the frequencies of 0.5 and 1 kHz ($r_G = 0.83$), 1 and 2 kHz ($r_G = 0.76$), and 4 and 8 kHz ($r_G = 0.75$). There were modest genetic correlations of BEHLs at 2 and 4 kHz ($r_G = 0.56$), 0.5 and 2 kHz ($r_G = 0.48$), and 4 and 12.5 kHz ($r_G = 0.46$). The BEHL at 12.5 kHz only showed significant correlation with BEHLs at 4 kHz. These results suggested that the closer the frequencies, the higher the genetic correlations. For both phenotypic and latent genetic correlations, high correlations were found between low frequencies (0.5, 1 and 2 kHz) or between high frequencies (4, 8 and 12.5 kHz). The correlations among those BEHLs were also strongly genetic rather than environment. Interestingly, most of the genetic correlations appeared to be stronger than phenotypic correlations among BEHLs.
Data from our twin study of middle-aged and elderly Chinese people showed relatively low heritabilities of PTA and BEHLs at the frequencies of 0.5 and 1 kHz, and the moderate heritabilities of BEHLs at 2, 4, 8 and 12.5 kHz.

These results were comparable with the findings from some of the previous studies. For instance, the Framingham Heart Study comparing aggregation of hearing levels in genetically unrelated and related people, revealed a familial aggregation for ARHI. The heritability was estimated to be 26%, 27%, and 32% from PTA hearing thresholds at the low (0.25-1 kHz), medium (0.5-2 KHz), and high (4-8 kHz) frequencies (Gates et al. 1999). A twin study among 2076 twins aged 18–87 years in UK that explored the genetic and environmental contributions to ARHI determined by SIN found the heritability of 25% after taking into account age (Momi et al. 2015).

Another study of twins aged 75 years and older in Denmark assessed at intake interview revealed a substantial heritability of 40% for self-reported reduced hearing (Christensen et al. 2001). In the Framingham Heart Study, the heritability of age-adjusted PTA at low (0.25, 0.5 and 1 kHz) and medium (0.5, 1 and 2 kHz) frequencies was 31% and 38%, respectively (DeStefano et al. 2003). A study of 11263 sibling pairs aged 20-101 years from Norway suggested that the upper limit of the heritability of hearing loss measured with PTA was 36% (Kvestad et al. 2012a).

However, other studies found a higher heritability of ARHI than our report. The Swedish study of 583 males twins aged 34-79 years who were examined at baseline and again two decades later found a moderate heritability (53%–65%) for PTA at both
low and high frequencies, and the genetic influences were of equal magnitude at baseline and follow-up (Bogo et al. 2015). The Finnish study of 217 female twin pairs aged 63-76 years indicated a 75% heritability using the hearing threshold level of the better ear at 0.5 – 4 kHz (Viljanen et al. 2007). Also a US familial aggregation study found that heritability estimates for ARHI was 68%, after adjusting for age, sex, education, and exposure to work noise (Raynor et al. 2009).

The heritability of ARHI across these previous studies ranges from 25%-75%, the large variation in heritability of ARHI may be partly due to use of different measurements of hearing loss such as tonal frequencies utilized to obtain PTA, BEHL, worse ear hearing level (WEHL), and SIN auditory test (Wolber et al. 2012), use of various audiometric cutoffs to define hearing loss, and use of different statistical approaches in estimating heritability. The differences in characteristics of the study sample (e.g., age, race, volunteer or population-based sample, or twins) may also contribute to the variations of heritability estimation.

In our study, multivariate Cholescky decomposition model was fitted to explore the underlying genetic correlation among BEHLs at various frequencies. We found strong overlaps in genetic influences of BEHLs at all six frequencies. However, there were much weak genetic correlations of BEHLs at 12.5 kHz (A6) and other frequencies (A1-A5). In addition, we found that regardless of phenotypic or latent genetic correlations, there were high correlations of BEHLs among low frequencies (0.5, 1 and 2 kHz) and among high frequencies (4, 8 and 12.5 kHz). This may be explained as neighboring trend patterns that magnitude the effects followed by closeness. This
implies that there might be pleiotropic genes that affect hearing specifically at low frequencies, and other unknown genes that influence hearing specifically at high frequencies. Population-based twin studies that used multivariate model to analyze genetic correlation of phenotypes on ARHI were rare. Using bivariate genetic analysis, the Finish female twin study revealed that a genetic component in common accounted for 75% of the variance in hearing threshold level of the better ear and 54% in speech recognition threshold level of the better ear. In addition, 10% of the variance in the better ear’s speech recognition threshold level was explained by specific genetic component (Viljanen et al. 2007).

Our study indicates that genetic factors play an important role in determining hearing ability, especially at high frequencies, which provides a basis for genetic studies aiming at identifying potential candidate genes that may contribute to age-related hearing loss. This suggests that clinical and epidemiological studies of age-related hearing loss should include not only information on environmental exposures but also on family history of hearing loss and, if possible, biological samples for identifying potential candidate genes related to hearing loss.

In this cross-sectional study of middle-aged and elderly Chinese twins, the effects of age on BEHLs and PTA were expected, especially on high pitch tone. Our results were consistent with previous reports from other studies (Cooper et al. 1991; Gates et al. 1990; Lee et al. 2005; Wiley et al. 2008). In addition, our data showed that hearing threshold was increased at the frequency of 12.5 kHz, and hearing thresholds at both 4 and 8 kHz were higher for twins older than 50 years compared to those aged 50 years.
or younger. This supports the view that ARHI mainly occurs at high frequencies.

To the best of our knowledge, this is the first twin study in China to investigate the heritability of ARHI measured by BEHLs at different frequencies, and to explore the relative influence of genetic and environmental factors on ARHI. However, our study has limitations. First, our study sample was relatively small and thus limited by statistical power. Large-scale studies are needed to replicate and validate our estimates. Second, the study participants are relatively young. As a result, the findings may not reflect the heritability of BEHLs in the older population. Third, similar to all classical twin studies, our study also suffered from the equal-environment assumption, upon which the classical twin methodology is based, which could affect our heritability estimates. In any case, our significant results encourage genetic association studies using high-throughput techniques for finding genetic variants responsible to the phenotypic variation and covariation in age-related hearing impairment.

5. Conclusions

In conclusion, we found that genetic factors partially contribute to the hearing ability at different frequencies in the middle-aged and elderly Chinese people. These results encouraged us to extend our study to older twins with the hope of capturing the genetic and environmental regulation patterns in ARHI in the aging process. Given that little is known about the potential role of susceptible genes in ARHI, additional studies with large sample size are required to identify the candidate genes that might be involved in ARHI.
Acknowledgements

This study was supported by the National Natural Science Foundation of China (grant #30872170), China Postdoctoral Science Foundation (2016M590622), Qingdao Postdoctoral Application Research Project (2015150), Qingdao Key Health Discipline Development Fund, Qingdao Outstanding Health Professional Development Fund, and Shandong Medical and Health Technology Development Program (2015wso329).

Conflict of interest

None declared.
References


FIGURE 1 Mean (standard error, SE) for BEHLs at different frequencies by age groups and sex.

FIGURE 2 Cholesky decomposition for AE model. Latent factor loadings are standardized to unit variance and must be squared to obtain standardized variance components. A1-A6 referred to additive genetic factors, and E1-E6 specific environmental factors. Dash lines indicated that loading could be dropped without significantly worsening the model fitting. A, additive genetic influences; E, unique environmental influences.
ABSTRACT

Objectives: Age-related hearing impairment (ARHI), or presbycusis, is the most prevalent sensory disorder in the elderly. The genetic and environmental influences on ARHI have been studied mostly in the developed countries.

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1. Introduction

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of correct zygosity assignment being 99.99%.

2.3. Audiometric examination

Audiometric examination was performed in a dedicated sound isolating room. First, otoscopy was given to ensure that the ear canals of the participants were clear of debris and were not impacted by cerumen. Pure-tone air-conducted hearing thresholds in each ear were measured separately at the frequencies of 0.5, 1, 2, 4, 8 and 12.5 kHz by a diagnostic audiometer (EN.60645-1,-2 type2A, MadsemIteral 2; GN Otometrics, Denmark) for all individuals. Pure-tone thresholds of each ear for each audiometric frequency ranging from -10 to 120 dB were selected with higher threshold reflecting lower hearing level.

BEHL was defined as hearing level of the ear with the lowest average threshold over each frequency from the better ear. Because 12.5 kHz is an ultra-high frequency, pure-tone thresholds of either ear could not be detected for nearly half of twin pairs, PTA was calculated using BEHLs of the other five frequencies.

2.4. Statistical analysis

We used SPSS 22.0 for Windows (SPSS, Chicago, IL, USA) for descriptive statistics and correlations, and Mx (Neale et al. 2006) for genetic model fitting of twins. Due to skewed distribution, we made square root transformation for BEHLs, and log transformation for PTA. The maximum likelihood estimation (MLE) approach was used to estimate phenotypic correlations and twin correlations in all model-fitting procedures.

The classic twin methodology is based on the fact that MZ twins share 100% of
their genes, whereas DZ twins share, on an average, 50% of their segregating genes and no more genetically related than ordinary siblings. Twins reared in the same family are assumed to share their environments to the same extent. Thus, the population-based twin study is considered a powerful approach to quantify the relative contribution of genetic and environmental variances to the phenotypes.

Structural equation modeling (SEM) was fitted using the full information maximum likelihood (FIML) method implemented in Mx software (Neale et al. 2006). Twin design and analytical methods were described elsewhere (Neale et al, 1992). The ACE model (A represents the additive genetic influences, C indicates the common or shared environmental influences and E refers to the unique environmental influences) was compared with simpler AE and CE models. The fit of each model was assessed by the differences in log likelihood between the sub-models and the full-models. The Akaike information criterion (AIC) gives a measure of model fit, taking the balance of the $\chi^2$ statistic and number of degrees of freedom into consideration. We carried out the variance components analysis, assuming only linear relations given our small sample size. Heritability is defined as the proportion of the total variance attributable to genetic variance. Age, sex and educational level were included in all models as covariates. Age was categorized into <45, 45-49, 50-54, 55-59, and $\geq$60 years, and educational level into illiterate, primary school, middle school, high school, and university.

To determine whether genetic influences on BEHLs specific to hearing at each of the six frequencies still existed after taking into account those influences on hearing at
other frequencies, a Cholesky decomposition model was fitted to BEHLs at six 
frequencies, including factors A, C, and E, as sources of covariation. We put BEHLs 
at frequencies 0.5, 1, 2, 4, 8, and 12.5 kHz as the first, second, third, fourth, fifth, and 
the sixth latent factors (unobserved factors), respectively. The multivariate twin 
models were fitted using the Mx software package (Neale et al. 2006).

3. Results

3.1. Descriptive

Of the 358 twin pairs and 1 triplet (719 persons), the frequencies of the 
participating twins in the age groups of <45, 45-49, 50-54, 55-59, and ≥60 years were 
140, 191, 136, 144, and 108 twins, respectively. The mean age of the participating 
twin-pairs was 51.55 years (standard deviation [SD], 7.65), and the male to female 
ratio (M/F) was 1:1.024. Table 1 shows the basic statistics for BEHLs and PTA. After 
transformations all variables had nearly normal distribution. Education and age were 
both related to PTA, but in the model that included both educational level and age as 
covariates, only age had significant effects on PTA (p = 0.021). There were J-shaped 
audiometric patterns of ARHI assessed by BEHLs at different audiometric frequencies 
by age groups and sex (Figure 1).

3.2. Univariate twin correlation and heritabilities

Table 2 shows intra-pair correlations of BEHLs and PTA in MZ and DZ pairs. 
Except for 0.5 kHz, intra-pair correlations were higher in MZ twins than in DZ twins 
at all other five audiometric frequencies (p <0.05).
To avoid over estimation of genetic influence, the ACE model was used for heritability estimation. In all ACE models, the estimated heritability ranged from 1.65% to 54.20%, with the low heritability (1.65% to 18.68%) for BEHLs at low frequencies (0.5 and 1 kHz) and 27.71% for PTA, and the moderate heritability (47.08% to 54.20%) for BEHLs at other frequencies measured.

3.3. Phenotypic correlations among BEHLs and PTA

There were moderate to strong phenotypic correlations between BEHLs (0.5-8 kHz) and PTA, with the correlation coefficients ranging from 0.60 to 0.75 (Table 3). For BEHLs, strong phenotypic correlations were found both among the low frequencies (0.5, 1, and 2 kHz) and among the high frequencies (4, 8, and 12.5 kHz). However, the correlations of BEHL at 12.5 kHz with BEHLs at the frequencies of 0.5, 1.0 and 2.0 kHz were weak and not statistically significant ($p > 0.05$).

3.4. Multivariate model fit

The best fitting multivariate model was AE model, and the C matrix could be dropped off the model without worsening fit ($AIC = 839.104$, $p = 0.719$, $\Delta df = 21$). Figure 2 shows the standardized path correlation coefficients with 21 pathways for each of A and E in the AE Cholesky decomposition (these must be squared to obtain variance components), remembering that, to a small extent, the A estimates were confounded with some shared environmental effects. We found that the genetic correlations of phenotypes were degraded from lower to higher frequencies. For example, the first genetic factor ($A_1$), which was loaded primarily at the frequencies of 0.5, 1, 2, 4, 8, and 12.5 kHz, accounted for 48.73%, 23.98%, 12.40%, 9.01%, 8.76%, and 2.10%, respectively.
5.93%, and 1.13% of variance, respectively (Figure 2). There was a phenomenon
towards increased heritability with increased frequency among different phenotypes.
Interestingly, additive genetic factor loadings related to 1.0 kHz showed no significant
correlation with those of 4.0, 8.0 and 12.5 kHz. Similarly, no significant correlation
was found for additive genetic factor loading related to 12.5 kHz. These loadings
could be dropped without significantly affecting the model fitting. However, the
unique environmental factor loadings were all significant in the model.

3.5. Genetic and environmental correlations among BEHLs

The additive genetic and unshared environmental correlation coefficients between
these BEHLs, derived from the AE Cholesky analysis, were shown in Table 4. The
bivariate analysis revealed high genetic correlations between BEHLs at the
frequencies of 0.5 and 1 kHz ($r_G = 0.83$), 1 and 2 kHz ($r_G = 0.76$), and 4 and 8 kHz ($r_G$
$= 0.75$). There were modest genetic correlations of BEHLs at 2 and 4 kHz ($r_G = 0.56$),
0.5 and 2 kHz ($r_G = 0.48$), and 4 and 12.5 kHz ($r_G = 0.46$). The BEHL at 12.5 kHz
only showed significant correlation with BEHLs at 4 kHz. These results suggested
that the closer the frequencies, the higher the genetic correlations. For both
phenotypic and latent genetic correlations, high correlations were found between low
frequencies (0.5, 1 and 2 kHz) or between high frequencies (4, 8 and 12.5 kHz). The
correlations among those BEHLs were also strongly genetic rather than environment.
Interestingly, most of the genetic correlations appeared to be stronger than phenotypic
correlations among BEHLs.
4. Discussion

Data from our twin study of middle-aged and elderly Chinese people showed relatively low heritabilities of PTA and BEHLs at the frequencies of 0.5 and 1 kHz, and the moderate heritabilities of BEHLs at 2, 4, 8 and 12.5 kHz.

These results were comparable with the findings from some of the previous studies. For instance, the Framingham Heart Study comparing aggregation of hearing levels in genetically unrelated and related people, revealed a familial aggregation for ARHI. The heritability was estimated to be 26%, 27%, and 32% from PTA hearing thresholds at the low (0.25-1 kHz), medium (0.5-2 KHz), and high (4-8 kHz) frequencies (Gates et al. 1999). A twin study among 2076 twins aged 18–87 years in UK that explored the genetic and environmental contributions to ARHI determined by SIN found the heritability of 25% after taking into account age (Momi et al. 2015). Another study of twins aged 75 years and older in Denmark assessed at intake interview revealed a substantial heritability of 40% for self-reported reduced hearing (Christensen et al. 2001). In the Framingham Heart Study, the heritability of age-adjusted PTA at low (0.25, 0.5 and 1 kHz) and medium (0.5, 1 and 2 kHz) frequencies was 31% and 38%, respectively (DeStefano et al. 2003). A study of 11263 sibling pairs aged 20-101 years from Norway suggested that the upper limit of the heritability of hearing loss measured with PTA was 36% (Kvestad et al. 2012a).

However, other studies found a higher heritability of ARHI than our report. The Swedish study of 583 males twins aged 34-79 years who were examined at baseline and again two decades later found a moderate heritability (53%–65%) for PTA at both
low and high frequencies, and the genetic influences were of equal magnitude at baseline and follow-up (Bogo et al. 2015). The Finnish study of 217 female twin pairs aged 63-76 years indicated a 75% heritability using the hearing threshold level of the better ear at 0.5 – 4 kHz (Viljanen et al. 2007). Also a US familial aggregation study found that heritability estimates for ARHI was 68%, after adjusting for age, sex, education, and exposure to work noise (Raynor et al. 2009).

The heritability of ARHI across these previous studies ranges from 25%-75%, the large variation in heritability of ARHI may be partly due to use of different measurements of hearing loss such as tonal frequencies utilized to obtain PTA, BEHL, worse ear hearing level (WEHL), and SIN auditory test (Wolber et al. 2012), use of various audiometric cutoffs to define hearing loss, and use of different statistical approaches in estimating heritability. The differences in characteristics of the study sample (e.g., age, race, volunteer or population-based sample, or twins) may also contribute to the variations of heritability estimation.

In our study, multivariate Cholescky decomposition model was fitted to explore the underlying genetic correlation among BEHLs at various frequencies. We found strong overlaps in genetic influences of BEHLs at all six frequencies. However, there were much weak genetic correlations of BEHLs at 12.5 kHz (A6) and other frequencies (A1-A5). In addition, we found that regardless of phenotypic or latent genetic correlations, there were high correlations of BEHLs among low frequencies (0.5, 1 and 2 kHz) and among high frequencies (4, 8 and 12.5 kHz). This may be explained as neighboring trend patterns that magnitude the effects followed by closeness. This
implies that there might be pleiotropic genes that affect hearing specifically at low frequencies, and other unknown genes that influence hearing specifically at high frequencies. Population-based twin studies that used multivariate model to analyze genetic correlation of phenotypes on ARHI were rare. Using bivariate genetic analysis, the Finish female twin study revealed that a genetic component in common accounted for 75% of the variance in hearing threshold level of the better ear and 54% in speech recognition threshold level of the better ear. In addition, 10% of the variance in the better ear’s speech recognition threshold level was explained by specific genetic component (Viljanen et al. 2007).

Our study indicates that genetic factors play an important role in determining hearing ability, especially at high frequencies, which provides a basis for genetic studies aiming at identifying potential candidate genes that may contribute to age-related hearing loss. This suggests that clinical and epidemiological studies of age-related hearing loss should include not only information on environmental exposures but also on family history of hearing loss and, if possible, biological samples for identifying potential candidate genes related to hearing loss.

In this cross-sectional study of middle-aged and elderly Chinese twins, the effects of age on BEHLs and PTA were expected, especially on high pitch tone. Our results were consistent with previous reports from other studies (Cooper et al. 1991; Gates et al. 1990; Lee et al. 2005; Wiley et al. 2008). In addition, our data showed that hearing threshold was increased at the frequency of 12.5 kHz, and hearing thresholds at both 4 and 8 kHz were higher for twins older than 50 years compared to those aged 50 years.
or younger. This supports the view that ARHI mainly occurs at high frequencies.

To the best of our knowledge, this is the first twin study in China to investigate the heritability of ARHI measured by BEHLs at different frequencies, and to explore the relative influence of genetic and environmental factors on ARHI. However, our study has limitations. First, our study sample was relatively small and thus limited by statistical power. Large-scale studies are needed to replicate and validate our estimates. Second, the study participants are relatively young. As a result, the findings may not reflect the heritability of BEHLs in the older population. Third, similar to all classical twin studies, our study also suffered from the equal-environment assumption, upon which the classical twin methodology is based, which could affect our heritability estimates. In any case, our significant results encourage genetic association studies using high-throughput techniques for finding genetic variants responsible to the phenotypic variation and covariation in age-related hearing impairment.

5. Conclusions

In conclusion, we found that genetic factors partially contribute to the hearing ability at different frequencies in the middle-aged and elderly Chinese people. These results encouraged us to extend our study to older twins with the hope of capturing the genetic and environmental regulation patterns in ARHI in the aging process. Given that little is known about the potential role of susceptible genes in ARHI, additional studies with large sample size are required to identify the candidate genes that might be involved in ARHI.
Acknowledgements

This study was supported by the National Natural Science Foundation of China (grant #30872170), China Postdoctoral Science Foundation (2016M590622), Qingdao Postdoctoral Application Research Project (2015150), Qingdao Key Health Discipline Development Fund, Qingdao Outstanding Health Professional Development Fund, and Shandong Medical and Health Technology Development Program (2015wso329).

Conflict of interest

None declared.
References


Figure legends

FIGURE 1 Mean (standard error, SE) for BEHLs at different frequencies by age groups and sex.

FIGURE 2 Cholesky decomposition for AE model. Latent factor loadings are standardized to unit variance and must be squared to obtain standardized variance components. A1-A6 referred to additive genetic factors, and E1-E6 specific environmental factors. Dash lines indicated that loading could be dropped without significantly worsening the model fitting. A, additive genetic influences; E, unique environmental influences.
<table>
<thead>
<tr>
<th>Trait</th>
<th>Frequency (kHz)</th>
<th>Mean (dB)</th>
<th>SD (dB)</th>
</tr>
</thead>
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<td>BEHL</td>
<td>0.5</td>
<td>21.3</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>15.8</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>14.0</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>21.8</td>
<td>17.0</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>25.6</td>
<td>20.4</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>51.2</td>
<td>14.8</td>
</tr>
<tr>
<td>PTA</td>
<td>Averages</td>
<td>23.9</td>
<td>11.5</td>
</tr>
</tbody>
</table>

Abbreviations: BEHL, better ear hearing level and PTA, pure tone average.
<table>
<thead>
<tr>
<th>Traits</th>
<th>Frequency kHz</th>
<th>Monozyotic twins Coefficient (95% CI)</th>
<th>Dizygotic twins Coefficient (95% CI)</th>
<th>ACE parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A (95% CI)</td>
<td>C (95% CI)</td>
<td>E (95% CI)</td>
</tr>
<tr>
<td>BEHL</td>
<td>0.5</td>
<td>0.439(0.328-0.538)</td>
<td>0.469(0.325-0.592)</td>
<td>1.65(0.00-33.80)</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.468(0.360-0.564)</td>
<td>0.344(0.184-0.487)</td>
<td>18.68(0.00-52.42)</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0.573(0.479-0.654)</td>
<td>0.263(0.097-0.415)</td>
<td>54.20(20.53-63.26)</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>0.484(0.378-0.577)</td>
<td>0.257(0.091-0.409)</td>
<td>50.07(19.33-58.77)</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>0.569(0.474-0.651)</td>
<td>0.334(0.173-0.477)</td>
<td>47.10(15.97-64.40)</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>0.528(0.366-0.657)</td>
<td>-0.112(-0.363-0.157)</td>
<td>47.08(25.09-61.52)</td>
</tr>
<tr>
<td>PTA</td>
<td>Averages</td>
<td>0.516(0.414-0.605)</td>
<td>0.361(0.203-0.500)</td>
<td>27.71(0.00-57.76)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23.20(0.00-49.87)</td>
</tr>
</tbody>
</table>

Abbreviations: BEHL, better ear hearing level; PTA, pure tone average; A, additive genetic influences; C, common or shared environmental influences; and E, unique environmental influences.
TABLE 3 Phenotypic correlation coefficients of hearing at various frequencies adjusted for sex, age and educational level

<table>
<thead>
<tr>
<th>Traits</th>
<th>Frequency (kHz)</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
<th>4.0</th>
<th>8.0</th>
<th>12.5</th>
<th>PTA</th>
</tr>
</thead>
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<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEHL</td>
<td>0.5</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.68</td>
<td>1</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0.44</td>
<td>0.64</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>4.0</td>
<td>0.29</td>
<td>0.31</td>
<td>0.45</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>0.26</td>
<td>0.27</td>
<td>0.40</td>
<td>0.63</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>0.02*</td>
<td>0.01*</td>
<td>0.06*</td>
<td>0.10</td>
<td>0.16</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PTA</td>
<td>Averages</td>
<td>0.60</td>
<td>0.63</td>
<td>0.66</td>
<td>0.75</td>
<td>0.75</td>
<td>0.15</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviations: BEHL, better ear hearing level and PTA, pure tone average. *p > 0.05

All correlation coefficients were statistically significant (p < 0.05), except 12.5 kHz with 0.5 kHz, 1 kHz and 2.0 kHz.
TABLE 4 Genetic and unique environmental correlation coefficients at different frequencies

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Unique environmental factors</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
<th>4.0</th>
<th>8.0</th>
<th>12.5</th>
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<td></td>
<td></td>
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<tr>
<td>0.5</td>
<td>-</td>
<td>0.53</td>
<td>0.38</td>
<td>0.22</td>
<td>0.23</td>
<td>0.07</td>
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</tr>
<tr>
<td>1.0</td>
<td>0.83</td>
<td>-</td>
<td>0.51</td>
<td>0.23</td>
<td>0.30</td>
<td>0.03</td>
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</tr>
<tr>
<td>Genetic factors 2.0</td>
<td>0.48</td>
<td>0.76</td>
<td>-</td>
<td>0.32</td>
<td>0.41</td>
<td>0.14</td>
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<tr>
<td>4.0</td>
<td>0.35</td>
<td>0.37*</td>
<td>0.56</td>
<td>-</td>
<td>0.50</td>
<td>0.09</td>
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<td>8.0</td>
<td>0.26</td>
<td>0.23*</td>
<td>0.37</td>
<td>0.75</td>
<td>-</td>
<td>0.20</td>
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<tr>
<td>12.5</td>
<td>0.04*</td>
<td>0.10*</td>
<td>0.15*</td>
<td>0.46</td>
<td>0.51*</td>
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* p >0.05
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<tr>
<th>Trait Frequency (kHz)</th>
<th>Model</th>
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<th>C % (95% CI)</th>
<th>E % (95% CI)</th>
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<th>df</th>
<th>AIC</th>
<th>$\chi^2$</th>
<th>p</th>
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<tbody>
<tr>
<td>0.5</td>
<td>ACE</td>
<td>1.65(0.00-33.8)</td>
<td>43.69(15.4-52.89)</td>
<td>54.67(45.11-63.65)</td>
<td>1885.287</td>
<td>711</td>
<td>1885.3</td>
<td>8.541</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>AE</td>
<td>49.00(39.45-57.35)</td>
<td>-</td>
<td>51.00(42.65-60.55)</td>
<td>1893.828</td>
<td>712</td>
<td>1893.8</td>
<td>1.65(20.53-63.26)</td>
<td>711</td>
</tr>
<tr>
<td></td>
<td>CE</td>
<td>-</td>
<td>45.00(36.33-52.9)</td>
<td>55.00(47.10-63.67)</td>
<td>1885.298</td>
<td>712</td>
<td>1885.3</td>
<td>0.011</td>
<td>0.916</td>
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<tr>
<td>1</td>
<td>ACE</td>
<td>18.68(0.00-52.42)</td>
<td>26.97(0.00-49.37)</td>
<td>54.35(45.29-64.78)</td>
<td>1863.198</td>
<td>711</td>
<td>1863.2</td>
<td>2.526</td>
<td>0.112</td>
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<tr>
<td></td>
<td>AE</td>
<td>47.31(37.73-55.78)</td>
<td>-</td>
<td>52.69(44.22-62.27)</td>
<td>1865.724</td>
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<td>1865.7</td>
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<tr>
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<td>42.55(33.68-50.68)</td>
<td>57.45(49.32-66.32)</td>
<td>1864.386</td>
<td>712</td>
<td>1864.4</td>
<td>1.88(20.53-63.26)</td>
<td>711</td>
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<tr>
<td></td>
<td>ACE</td>
<td>54.20(20.53-63.26)</td>
<td>1.43(0.00-31.93)</td>
<td>44.37(36.74-53.40)</td>
<td>1813.714</td>
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<td>1813.7</td>
<td>0.75</td>
<td>0.933</td>
</tr>
<tr>
<td>2</td>
<td>AE</td>
<td>55.69(46.91-63.27)</td>
<td>-</td>
<td>44.31(36.73-53.09)</td>
<td>1813.721</td>
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<td>0.007</td>
<td>0.933</td>
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<tr>
<td></td>
<td>CE</td>
<td>-</td>
<td>46.85(38.38-54.54)</td>
<td>53.15(45.46-61.62)</td>
<td>1824.409</td>
<td>712</td>
<td>1824.4</td>
<td>10.70</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>BEHL</td>
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<tr>
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<td>ACE</td>
<td>50.07(19.33-58.77)</td>
<td>0.00(0.00-26.2)</td>
<td>49.93(41.23-60.24)</td>
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<td>48.24(39.89-55.5)</td>
<td>51.76(44.20-60.11)</td>
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<td>0(0.00-14.67)</td>
<td>52.92(38.48-70.88)</td>
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<td>329</td>
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A, Additive genetic; C, Common environment; E, Unique environment; -2LL, twice the negative log-likelihood; df, degree of freedom; AIC, Akaike information criterion; $\chi^2$, $\chi^2$ value and p, $\chi^2$ test in model fitting.
Supplementary table 2: Multivariate model fit for all six frequencies

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<th>AIC</th>
<th>$\chi^2$</th>
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</table>

A, Additive genetic; C, Common environment; E, Unique environment; -2LL, twice the negative log-likelihood; df, degree of freedom; AIC, Akaike information criterion; $\chi^2$, $\chi^2$ value and $P$, $\chi^2$ test in model fitting.