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Evidence for gene-smoking interactions for hearing loss and deafness in Japanese American families

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Abstract

Background: This study investigated the relationship between smoking and hearing loss and deafness (HLD) and whether the relationship is modified by genetic variation. Data for these analyses was from the subset of Japanese American families collected as part of the American Diabetes Association Genetics of Non-insulin Dependent Diabetes Mellitus study. Logistic regression with generalized estimating equations assessed the relationship between HLD and smoking. Nonparametric linkage analysis identified genetic regions harboring HLD susceptibility genes and ordered subset analysis was used to identify regions showing evidence for gene-smoking interactions. Genetic variants within these candidate regions were then each tested for interaction with smoking using logistic regression models.

Results: After adjusting for age, sex, diabetes status and smoking duration, for each pack of cigarettes smoked per day, risk of HLD increased 4.58 times (odds ratio (*OR*)=4.58; 95% Confidence Interval (CI): (1.40,15.03)), and ever smokers were over 5 times more likely than

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nonsmokers to report HLD ($OR=5.22$; 95% CI: (1.24, 22.03)). Suggestive evidence for linkage for HLD was observed in multiple genomic regions (Chromosomes 5p15, 8p23 and 17q21), and additional suggestive regions were identified when considering interactions with smoking status (Chromosomes 7p21, 11q23, 12q32, 15q26, and 20q13) and packs-per-day (Chromosome 8q21).

Conclusions: To our knowledge this was the first report of possible gene-by-smoking interactions in HLD using family data. Additional work, including independent replication, is needed to understand the basis of these findings. HLD are important public health issues and understanding the contributions of genetic and environmental factors may inform public health messages and policies.

Keywords

gene-environment interaction; genome-wide linkage; hearing loss; smoking; family; linkage

INTRODUCTION

In the United States, hearing loss is the most common sensory impairment affecting about 1 in every 8 Americans and is the most prevalent sensory disorder in developed countries (Lin, et al., 2011; Uchida, et al., 2012). Furthermore, Americans with diabetes are at an increased risk of hearing loss (Bainbridge, et al., 2008), with evidence that Japanese with diabetes are twice as likely to have hearing loss (Horikawa, et al, 2013). Severe to profound hearing loss is expected to cost society \$297,000 over the lifetime of an individual, with most of those losses due to reduced work productivity (Mohr, et al., 2005). Typical risk factors for acquired hearing loss are trauma to the head and ear, excessive noise exposure (occupational and recreational), and aging (Mayo Clinic, 2015). Additionally, several studies have demonstrated that smoking is positively associated with risk of hearing loss disorder (HLD) (Agrawal et al., 2008; Siegelau et al., 1974; Nomura et al., 2005), especially hearing loss at high frequencies with the attenuation of the association after smoking cessation among Japanese (Hu et al., 2019): however, the results are not consistent (Karlslose et al., 2000; Lin et al., 2011). One potential explanation for these inconsistent findings is that the relationship between smoking and HLD may be modified by other factors, including underlying genetic variation.

More than 50% of prelingual deafness, defined as hearing loss which begins prior to the onset of speaking, is genetic, most often autosomal recessive, and not associated with other physical symptoms (nonsyndromic) (Ito et al., 2010). While acquired hearing loss is typically attributed to environmental factors, genetic factors also play a role. For example, family history is significantly associated with hearing loss (Smith et al., 2014; McMahon et al., 2008), and approximately 36% of the variance is attributable to genetic variation ($h^2=0.36$) (Kvestad et al., 2012). However, the genetic transmission of postlingual hearing impairment is predominantly autosomal dominant (Shearer et al, 2014). In a family-based study, Huyghe and colleagues (Huyghe et al, 2008) identified a linkage peak at 8q24.13-q24.22, representing the first locus identified for an age-related hearing impairment trait. Furthermore, O'Neil et al (O'Neill, 1998) performed linkage analysis on a large multi-generational family in which a gene for autosomal dominant late-onset progressive nonsyndromic hearing impairment was segregating, and identified *DNFB10* on

Chromosome 6q. Lavinsky and colleagues (Lavinsky et al., 2015) conducted a genome-wide association study (GWAS) and identified *NADPH-oxidase 3*, located on chromosome 6, as a critical gene for susceptibility to hearing loss. In another GWAS, Friedman and researchers (Friedman et al., 2009) identified common alleles of *GRM7*, located on Chromosome 3, that contribute to an individual's susceptibility of developing age-related hearing impairment. To date, over two dozen genes have been identified for autosomal recessive nonsyndromic hearing loss (Kvestad et al., 2012; Hilgert et al., 2009; Dickson et al., 2010). Moreover, among the Japanese, certain genes were identified as 1) being causal: GJB2 on chromosome 13q12.11, mitochondrial DNA 12SrRNA; 2) having an association with type 2 diabetes (T2D) and audiovestibular phenotypes (Rohayem et al., 2011): WFS1 on chromosome 4p16.1 (Kobayashi et al., 2018); and 3) being related to abnormal enlargement of the vestibular aqueduct in the inner ear: SLC26A4 on chromosome 7q22.3, EYQ1, and SIX1 on chromosome 14q23.1 (Ito et al., 2010).

THEORY

While both environmental and genetic factors are involved in HLD, to our knowledge, interactions have not been investigated, particularly those between smoking and genetic susceptibility to HLD. Therefore, the main purpose of this study was to investigate this question using existing data from a sample of Japanese American families recruited or studies of T2D.

METHODS AND METHODS

1.1 Study Subjects

From 1993–2003, the American Diabetes Association established the GENNID (Genetics of Non-insulin Dependent Diabetes Mellitus) study, a multicenter family-based resource to identify the genetic components of Type 2 Diabetes (T2D) (Raffel et al., 1996). The proportion of those with HLD was significantly different across the GENNID ethnic groups ($\chi^2_{df=3} = 22.65$, $p\text{-value} = 4.78 \times 10^{-5}$) with 7.4% HLD in African Americans, 17.1% HLD in European Americans, 23.3% HLD in Japanese Americans, and 13.9% HLD in Mexican Americans. Because the greatest prevalence of HLD was seen among Japanese Americans families, our study focused on this group.

1.2 Data Collection

Extensive data were collected as described previously (Ehm et al, 2000), including self-reported information on HLD and age of deafness. To determine HLD status, participants were asked “Have you been told by a health care worker that you have hearing loss/deafness?” Information on smoking, including ever smoking cigarettes, age began smoking, age stopped smoking, and packs-per-day were also self-reported. In particular, smoking characteristics of interest were ever-smoking and packs-per-day, although differences in duration of smoking and age at smoking were also evaluated between non-HLD and HLD subjects. Moreover, each pack of cigarettes can be roughly quantified as 20 cigarettes as standard in the U.S. at the time of data collection.

According to guidelines from the World Health Organization (WHO, 2006), diabetes status was defined as having a 2-hour plasma glucose ≥ 200 mg/dl (11.1 mmol/l) during an OGTT. The genetic data consisted of a genome-wide linkage panel with 374 polymorphic microsatellite markers yielding average marker spacing of approximately 9.42 cM and median marker spacing of 9.10 cM. Descriptions of the genetic data cleaning and pedigree checking have been summarized previously (Edwards et al., 2008). The microsatellite markers were used in the genome-wide linkage analysis and OSA. In addition, all samples were also genotyped using Illumina's Multi-Ethnic Global BeadChip (v1.0). After quality control, imputation was also performed after phasing using the 1000 Genomes Phase 3 panel and was corrected for relatedness (unpublished). Both imputed and genotyped single nucleotide variants (SNVs) were available for evaluating gene-by-environment (GxE) interaction under linkage regions of interest.

There were a total of 17 pedigrees, of which 15 families had at least one HLD case. However, because we were interested in evaluating GxE, we selected those families with at least two biologically related cases of HLD reported in the pedigree to increase the likelihood of underlying genetic influences. Eight families with a total of 97 family members met this criterion, of which 75 members had genetic and phenotypic data available for our analyses. These eight families were used for determining candidate genetic regions with GxE among subsets of families using the ordered subset analysis (OSA) method on genome-wide microsatellite linkage scan results. In order to follow-up within these candidate regions, all Japanese American GENNID families (N=121 samples) were used to test for GxE at imputed and genotyped SNVs.

1.3 Statistical Analysis

We first evaluated the association between smoking and HLD. HLD was coded as a binary "yes/no" variable for all analyses. Logistic regression models were used with generalized estimating equations (GEE) and independence correlation structure to account for between-family-member correlations. Two sets of adjusted models for each smoking variable (ever-never smoking and packs-per-day) were examined. Model 1 was adjusted for age, sex, and diabetes status. Model 2 included all the covariates included in Model 1, plus duration of smoking, calculated as the difference in age stopped smoking and age began smoking. Odds ratios (ORs) and their corresponding 95% Confidence Intervals (CIs) were also calculated and shown. Non-smokers had a smoking duration of 0 years. Statistical significance was based on the Wald's Z-test, and p-values < 0.05 were considered statistically significant. Analyses were performed using the gee package (Carey, 2015) in R software version 3.2.5 (Team, 2015).

Next, we performed a genome-wide linkage scan for HLD susceptibility regions using a nonparametric two-point linkage (NPL) analysis at each marker (Whittemore et al, 1994; Kong et al., 1967) with the Cox and Kong exponential model. Because we were looking genome-wide, we used a non-parametric model as this approach makes the fewest assumptions regarding mode of inheritance. Analyses were based on data from 374 microsatellite markers across the genomes of 75 subjects from the eight families with at least two HLD cases. The NPL routine in MERLIN software (Abecasis et al., 2002) was used.

LOD scores greater than 3 and 1.9 provide significant and suggestive evidence of linkage, respectively.

Next, ordered subset analysis (OSA) (Hauser et al., 2004) was implemented to identify those chromosomal regions with evidence for differences in linkage by smoking status (i.e., GxE). That is, in the presence of etiologic heterogeneity based on smoking, we located chromosomal regions that have maximal evidence of linkage with a subset of families based on smoking. Here, a genome-wide OSA was conducted at each marker as implemented in FLOSS software (Browning, 2006). Based on prior studies, smoking appears to increase risk of HLD; hence, we used a one-sided test. Specifically, families were ranked in descending order (high to low) using per-family summary values of the given smoking covariate (i.e., percentage of ever smokers in the family or average packs-per-day smoked among family members). Then, families were added one-at-a-time in descending smoking order into the two-point NPL analysis until a maximum resulting LOD (LOD-OSA) was achieved at each marker. P-values were calculated as the proportion of random family rankings that gave permuted LOD-OSA scores greater than the original LOD-OSA using a permutation test. At least 100 random rankings were needed to adequately determine the p-value by the Besag-Clifford sequential stopping rule (Besag et al., 1991). Significance of the gene-by-smoking interaction in the subset of families is determined if the OSA p-value < 0.002 which corresponds to a Bonferroni correction for separate tests on 22 chromosomes (i.e., 0.05/22). Furthermore, because our results are exploratory, suggestive evidence of GxE interaction is determined if the OSA p-value < 0.05 per chromosome.

Finally, logistic regression models using GEE were used to evaluate individual SNVs for GxE within the putative genetic regions identified by OSA. The independence correlation structure in GEE was used to adjust for within family correlations, to maximize power as well as reduce type I error and bias in OR estimation (Hancock et al., 2007). Analyses using the entire Japanese-American GENNID sample (N=121 subjects) were adjusted for age, sex, and diabetes status, and were performed using the GWAF package (Chen et al., 2015) in R (R Core Team, 2019). To correct for the multiple testing of 311,989 SNVs in 6 candidate regions (Table 4), the conservative Bonferroni method was used to adjust the significance threshold in which p-values less than 1.6×10^{-7} (i.e., 0.05/311,989) were considered as significant evidence of GxE interaction. All results were converted in the human genome from GrCh37/hg19 to build GrCh38/hg38 using the LiftOver tool (Hinrichs et al., 2006) and then annotated using SeattleSeq version 151 (Ng et al., 2009), and interaction p-value plots of select candidate regions were made using Locus Zoom (Pruim et al., 2010).

RESULTS

The characteristics of 75 Japanese American individuals in 8 families are presented in Table 1; there was a median of 10 subjects per family with a minimum of 9 subjects and a maximum of 19 subjects per family. Individuals with HLD were significantly older than those without HLD (67 ± 11 years compared to 51 ± 15 years, respectively; $p=1.23 \times 10^{-9}$). The total sample was 53% female, and there was a significantly higher proportion of women among HLD cases compared to non-HLD individuals. Specifically, 71% of HLD cases were female compared to 45% of non-HLD individuals ($P=0.030$). Additionally, there was

evidence of increased smoking exposure in the HLD group compared to the non-HLD group ($P=0.001$), longer duration of cigarette smoking ($P=0.014$), and higher average packs-per-day ($P=0.049$).

Table 2 describes demographic characteristics for each of the eight families, which are ordered based on decreasing percentages of ever-smokers per-family. Families differed significantly by age (mean ranging from 47.57 years to 67.31 years) and gender (at least 50% of five of eight families were predominantly female). In addition, smoking characteristics differed significantly among families, with the number of ever smokers ranging from 86% to 18%, the duration of smoking ranging from 29.33 years to 2.82 years, and the number of packs-per-day smoked ranging from 1.17 packs-per-day to 0.05 packs-per-day.

In all logistic regression models, there was a significant and positive relationship between HLD and both ever-smoking and packs per-day (Table 3). Being an ever-smoker increased the odds of HLD by 5.0-fold (unadjusted), 2.83-fold when adjusted for age, sex and history of diabetes (Model 1), and 5.76-fold when additionally adjusted for smoking duration (Model 2). Furthermore, for every additional pack-per-day smoked, the odds of HLD increased 2.04-fold (unadjusted) yet increased 4.97-fold after adjusting for age, sex, diabetes, and smoking duration (Model 2).

Results from the nonparametric linkage analysis indicated suggestive evidence for linkage to putative HLD loci ($LOD > 1.9$) for three chromosomal regions (Supplemental Figure 1, grey line). On chromosome 5p15.3 at D5S2505 (13.6 cM), the LOD was 2.18, while on 8p23.1 at D8S277 (11.3 cM), the LOD was 2.19. The highest evidence for linkage was found on chromosome 17q21.3 at ATC6A06 (68.3 cM) where the LOD was 2.31.

Table 4 and Supplemental Figure 1 (blue, red, and magenta lines) show the regions of the strongest evidence for gene-environment interaction in the families with the highest per-family percentage of smoking and average packs-per-day. Analyzing the top 4 families ranked by their average packs-per-day values, chromosome 8q21 at D8S1119 (89 cM) had an OSA max LOD score of 3.54 ($P=0.04$), providing evidence for gene-smoking interaction nearby the HLD suggestive linkage region ($LOD=2.19$ at 11.3 cM). These four families (Families 1,2,3,5 in Table 2) also had the highest duration of smoking, highest percentage of ever smoking, and higher prevalence of T2D. The other GxE regions based on OSA p-values of less than 0.05 were not in the proximity of regions showing suggestive evidence for linkage to HLD (Supplemental Figure 1). The OSA max LOD score of 2.9 ($P=0.01$) and 2.6 ($P=0.01$) for the top 5 and top 3 ranked families based on percentage of smokers supports a gene-environment interaction on chromosome 15q26 at D15S816 (96.4 cM) and on chromosome 12q32 at D12S1045 (145.8 cM), respectively. Additionally, on chromosome 20q13 at D20S171 (92.2 cM) there was also evidence of GxE using OSA with the top 3 families based on percentage of ever smoking yielding an OSA LOD score of 3.03 ($P=0.02$). Furthermore, there was evidence of gene-by-smoking interaction for chromosomes 7p21 ($P=0.04$) and 11q23 ($P=0.03$) in the top 2 families based on percentage of ever smoking.

Evaluating GxE among the single nucleotide variants (SNVs) underlying the candidate regions in Table 4, there was evidence of hearing loss risk associated with SNVs on chromosome 8 being modified by smoking. The 23 variants that significantly modified the association between hearing loss and packs per day are summarized in Supplementary Table 1. In particular, intronic SNVs within PDE7A, MTFR1, ZBTB10 and PAG1 genes as well as novel intergenic SNVs were shown to interact with smoking on the risk of HLD.

DISCUSSION

While it is well-established that smoking is harmful to one's health, our findings lend support to the observation that smoking is related to HLD based on this sample of Japanese American families. Further, we also identified three regions that appear to harbor HLD susceptibility genes, and these regions appear to be novel. We extend these linkage findings and provide the first evidence to our knowledge for gene x smoking interactions. Importantly, additional potential susceptibility regions for HLD appear to have been detected only after considering heterogeneous evidence of linkage based on smoking, in particular for the region on chromosome 8 between 66Mb and 107.3 Mb. These results demonstrate the importance of considering GxE even in the context of family and linkage based studies.

In agreement with others, our study results support an association between smoking and HLD, that remains significant after adjustment for age, gender, history of diabetes and duration of smoking. Similarly, our findings are consistent with others showing an elevated risk of HLD with ever smoking and with packs-per-day. For example, in a cross-sectional study Cruickshanks and colleagues (Cruickshanks et al., 1998) showed that current smokers have a 69% increased risk of developing hearing loss compared to non-smokers, adjusting for age, history of cardiovascular disease, alcohol consumption, occupational noise exposure, and education. Similarly, Helzner and colleagues (Helzner et al, 2005) demonstrated that current smokers have a 57% increased risk of developing hearing loss compared to non-smokers through age-adjusted analyses. A cohort study conducted by Nakanishi and colleagues (Nakanishi et al., 2000) showed that the multivariate-adjusted risk for high-frequency hearing impairment was between 1.21–1.82 for current smokers, depending on the amount of cigarettes smoked per day. While our estimates are somewhat higher, it is possible that this may be due to uncontrolled confounding or possibly because we focused on families with multiple cases of HLD.

Results from the nonparametric linkage analysis indicated suggestive evidence for linkage to putative HLD loci for three chromosomal regions: chromosomes 5p15, 8p23, and 17q21. Bykhovskaya et al. observed that a highly suggestive locus on chromosome 8 near D8S277, position 6,516,725 – 6,516,870, associated with progressive hearing loss in carriers of the A1555G mitochondrial mutation in Spanish, Italian, and Arab-Israeli families (Bykhovskaya et al., 2000). In the Arab-Israeli/Spanish/Italian families, the combined maximized allele-sharing LOD score was 3.1. Although there is evidence of linkage on chromosomes 8 and 17 in other studies, these nominated regions did not overlap with the regions of suggestive linkage in this study. Also, there are no reported HLD or related traits for the region on chromosome 5.

Moreover, results from the OSA indicated six novel genomic regions that appeared to be involved in gene-by-smoking interactions. Consequently, these regions on chromosomes 7, 8, 11, 12, 15 and 20 did not contain any known HLD genes and appear to be novel regions for HLD. This is likely if genes in these regions only operate in the presence of smoking and are otherwise undetectable. These results are consistent with a recent large multi-ancestry genome-wide smoking interaction study of over 380,000 individuals showing that when considering interaction with smoking, 13 new loci associated with serum lipids were identified (Bentley et al, 2019). Aside from this recent study, there have been relatively few gene-environment-wide interaction studies (GEWIS) examining complex diseases Hutter et al., 2012; Cornelis et al., 2012), and even fewer that have addressed GxE in HLD. Studies that have addressed this have primarily focused on noise-induced hearing loss (NIHL). For instance, Carlsson and researchers (Carlsson et al., 2005) investigated whether genetic variability in the human antioxidant system is associated with high or low susceptibility to NIHL. However, no significant differences were found between susceptible and resistant groups. Yang and colleagues (Yang et al., 2006) determined whether there was an association of polymorphisms of the Hsp70 genes with susceptibility to NIHL among Chinese automobile workers. Their results suggested that some haplotypes of the Hsp70 gene cluster may be associated with a higher susceptibility to NIHL.

In particular, the variants of the PDE7A on chromosome 8 implicated in GxE interaction with smoking are also involved with the purine metabolism pathway. There is some evidence the purine metabolism pathway is involved with X-linked hearing loss with gene PRPS1 (Liu et al., 2013; Jimenez et al, 2012; Yang et al, 2017; Jinnah et al., 2013). This pathway is also correlated with smoking (Mouhamed et al., 2011; Jones et al, 2016). In addition, studies have also evaluated that hearing loss is twice as high among those with diabetes compared those without, and there is a 30% higher rate of hearing loss among those with pre-diabetes (Cullen et al., 1993; Kim et al, 2017). Specifically, because GENNID families were selected based on multiple family members with T2D, and there is also evidence of uric acid being associated the MetS (Kushiyama et al., 2014), perhaps both MetS and hearing loss are mediated through an overlap of similar causal pathways, and the uric acid and purine metabolism is one of such pathways (Bainbridge et al., 2008; Simmonds et al., 1985; Kim et al., 2007). More studies will have to be performed to evaluate this hypothesis. In addition, although significant GxE variants were nominated with low p-values and account for multiple comparisons using a conservative Bonferroni correction, these findings should be considered suggestive until they are replicated and confirmed in independent samples.

There is increased motivation for the use of GEWIS or other methods that incorporate environmental factors when marginal effects of genetic factors are small and our results are the first to evaluate GxE in relation to HLD, but there were limitations of this study. First, HLD was self-reported. While the survey questioned whether they had been told by a healthcare worker that they had HLD, measured deafness using audiometric tests would have likely improved the accuracy of our phenotype. Furthermore, by not measuring hearing loss, it is likely that some cases with early hearing loss were missed. Our inability to subtype by severity also extends to our inability to subclassify or exclude known subtypes such as non-syndromic or syndromic status and by etiology (i.e., congenital, noise-induced). However, the resulting misclassification would likely result in a decrease in power to detect

association, linkage, and GxE. Our approach in using a per family average definition of smoking status for the family unit may have also resulted in misclassification of the exposure and reduced the power to detect effects. Additionally, we did not have information on the participants' occupational exposure, including both ototoxic chemicals or excessive noise working environments and were unable to account for those potential confounders. Finally, it is possible that our results which were based on families selected for type 2 diabetes may not generalizable to families without diabetes.

CONCLUSIONS

Although, it has been estimated that over 16.8% of US adults were current smokers, defined as smoking 100 cigarettes or more during one's lifetime and currently smoking every day or some days (Center for Disease Control and Prevention, 2015), differences in smoking patterns among the Japanese American population may differentially affect the risk of developing HLD. Thus, clarification of the association between smoking and HLD may have a large impact on the public health and help motivate significant behavioral change among at-risk adults.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- Abecasis GR, Cherny SS, Cookson WO, Cardon LR, 2002 Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet* 30, 97–101. doi:10.1038/ng786. [PubMed: 11731797]
- Agrawal Y, Platz EA, Niparko J, 2008 Prevalence of hearing loss and differences by demographic characteristics among US adults: data from the National Health and Nutrition Examination Survey, 1999–2004. *Arch Intern Med* 168, 1522–1530. [PubMed: 18663164]
- Bainbridge KE, Hoffman HJ, Cowie CC, 2008, Diabetes and hearing impairment in the United States: Audiometric evidence from the National Health and Nutrition Examination Surveys, 1999–2004. *Ann Intern Med* 149, 1–10. [PubMed: 18559825]
- Bentley AR, Sung YJ, Brown MR, Winkler TW, Kraja AT, et al., 2019 Multi-ancestry genome-wide gene-smoking interaction study of 387,272 individuals identifies new loci associated with serum lipids. *Nat Genet* 51, 636–648. doi: 10.1038/s41588-019-0378-y. [PubMed: 30926973]
- Besag J and Clifford P, 1991 No Title Sequential Monte Carlo p-values. *Biometrika* 78, 301–304.

- Browning BL, 2006 FLOSS: flexible ordered subset analysis for linkage mapping of complex traits. *Bioinformatics* 22, 512–513. doi:10.1093/bioinformatics/btk012. [PubMed: 16368771]
- Bykhovskaya Y, Estivill X, Taylor K, et al., 2000 Candidate locus for a nuclear modifier gene for maternally inherited deafness. *Am J Hum Genet.* 66, 1905–1910. doi:10.1086/302914. [PubMed: 10788333]
- Carey VJ. Ported to R by TL (versions 3. 1. and 4. 4. and BR (version 4. 13. gee: Generalized Estimation Equation Solver. R package version 4.13–19. <https://cran.r-project.org/package=gee>. Published 2015.
- Carlsson P– I., Van Laer L, Borg E, et al., 2005 The influence of genetic variation in oxidative stress genes on human noise susceptibility. *Hear Res* 202, 87–96. doi:10.1016/j.heares.2004.09.005. [PubMed: 15811702]
- Center for Disease Control and Prevention (2015) Current Cigarette Smoking Among Adults—United States, 2005–2014. *Morb Mortal Wkly Rep* 64, 1233–1240.
- Chen M-H and Yang Q, 2015 GWAF: Genome-Wide Association/Interaction Analysis and Rare Variant Analysis with Family Data. R package version 2.2.n <https://CRAN.R-project.org/package=GWAF>.
- Cornelis MC, Tchetgen EJT, Liang L, et al., 2012 Gene-environment interactions in genome-wide association studies: a comparative study of tests applied to empirical studies of type 2 diabetes. *Am J Epidemiol* 175, 191–202. doi:10.1093/aje/kwr368. [PubMed: 22199026]
- Cruikshanks KJ, Klein R, Klein BE, Wiley TL, Nondahl DM, Tweed TS (1998) Cigarette smoking and hearing loss: the epidemiology of hearing loss study. 279:1715–1719. doi:10.1001/jama.279.21.1715.
- Cullen Jr., Cinnamon MJ, 1993 Hearing loss in diabetics. *J Laryngol Otol* 107, 179–82.. [PubMed: 8509689]
- Dickson SP, Wang K, Krantz I, Hakonarson H, Goldstein DB, 2010 Rare variants create synthetic genome-wide associations. *PLoS Biol* 8, e1000294. doi:10.1371/journal.pbio.1000294. [PubMed: 20126254]
- Edwards KL, Hutter CM, Wan JY, Kim H, Monks SA, 2008 Genome-wide linkage scan for the metabolic syndrome: the GENNID study. *Obesity (Silver Spring)* 16, 1596–1601. doi:10.1038/oby.2008.236. [PubMed: 18421265]
- Ehm MG, Karnoub MC, Sakul H, et al., 2000 Genomewide search for type 2 diabetes susceptibility genes in four American populations. *Am J Hum Genet* 66, 1871–1881. doi:10.1086/302950. [PubMed: 10793009]
- Friedman RA, Van Laer L, Huentelman MJ, et al., 2009 GRM7 variants confer susceptibility to age-related hearing impairment. *Hum Mol Genet* 18,785–796. doi:10.1093/hmg/ddn402. [PubMed: 19047183]
- Hancock DB, Martin ER, Li YJ, Scott WK, 2007). Methods for interaction analyses using family-based case-control data: conditional logistic regression versus generalized estimating equations. *Genet Epidemiol* 31, 883–893. DOI:10.1002/gepi.20249. [PubMed: 17565751]
- Hauser ER, Watanabe RM, Duren WL, Bass MP, Langefeld CD, Boehnke M, 2004 Ordered subset analysis in genetic linkage mapping of complex traits. *Genet Epidemiol* 27, 53–63. doi:10.1002/gepi.20000. [PubMed: 15185403]
- Helzner EP, Cauley JA, Pratt SR, et al., 2005 Race and sex differences in age-related hearing loss: the Health, Aging and Body Composition Study. *J Am Geriatr Soc* 53, 2119–2127. doi:10.1111/j.1532-5415.2005.00525.x. [PubMed: 16398896]
- Hilgert N, Smith RJH, Van Camp G, 2009 Forty-six genes causing nonsyndromic hearing impairment: which ones should be analyzed in DNA diagnostics? *Mutat Res* 681, 189–196. doi:10.1016/j.mrev.2008.08.002. [PubMed: 18804553]
- Hinrichs AS, Karolchik D, Baertsch R, Barber GP, Bejerano G, et al., 2006 The UCSC Genome Browser Database: update 2006. *Nucleic Acids Res.* 1, 34(Database issue):D590–8. [PubMed: 16381938]
- Horikawa C, Kodama S, Tanaka S, Fujihara K, Hirasawa R, et al., 2013, Diabetes and risk of hearing impairment in adults: a meta-analysis. *J Clin Endocrinol Metab* 98, 51–58.

- Hu H, Sasaki N, Ogasawara T, Nagahama S, Akter S, Kuwahara K, Kochi T, Eguhi M, et al., 2019 Smoking, smoking cessation, and the risk of hearing loss: Japan Epidemiology Collaboration on Occupational Health Study. *Nicotine Tob Res* 21, 481–488. [PubMed: 29547985]
- Hutter CM, Chang-Claude J, Slattery ML, et al., 2012 Characterization of gene-environment interactions for colorectal cancer susceptibility loci. *Cancer Res* 72, 2036–2044. doi:10.1158/0008-5472.CAN-11-4067. [PubMed: 22367214]
- Huyghe JR, Van Laer L, Hendrickx J-J, et al., 2008). Genome-wide SNP-based linkage scan identifies a locus on 8q24 for an age-related hearing impairment trait. *Am J Hum Genet* 83, 401–407. doi:10.1016/j.ajhg.2008.08.002. [PubMed: 18760390]
- Ito T, Noguchi Y, Yashima T, Ohno K, Kitamura K, 2010 Hereditary hearing loss and deafness genes in Japan. *J Med Dent Sci* 57, 1–10. [PubMed: 20437760]
- Jimenez RT, Puig JG, 2012 Purine metabolism in the pathogenesis of hyperuricemia and inborn errors of purine metabolism associated with disease. *Gout and Other Crystal Arthropathies*. 36–50.
- Jinnah HA, Savina RL, Van Den Berghe G (2013) Metabolic disorders of purine metabolism affecting the nervous system. *Handb Clin Neurol* 113:1827–1836. doi: 10.1016/B978-0-444-59565-2.00052-6. [PubMed: 23622405]
- Jones DP, Walker DI, Uppal K, Rohrbek P, Mallon TM, Go YM, 2016 Metabolic pathways and networks associated with tobacco use in military personnel. *J Occup Environ Med* 58, S111–S116. doi: 10.1097/JOM.0000000000000763. [PubMed: 27501098]
- Karlslose B, Lauritzen T, Engberg M, Parving A, 2000 A five-year longitudinal study of hearing in a Danish rural population aged 31–50 years. *Br J Audiol* 34, 47–55. <http://www.ncbi.nlm.nih.gov/pubmed/10759077>. Accessed April 21, 2016. [PubMed: 10759077]
- Kim HJ, Sohn KM, Shy ME, Krajewski KM, et al., 2007 Mutations in PRPS1, Which Encodes the Phosphoribosyl Pyrophosphate Synthetase Enzyme Critical for Nucleotide Biosynthesis, Cause Hereditary Peripheral Neuropathy with Hearing Loss and Optic Neuropathy (CMTX5). *Am J Hum Genet* 81, 552–558. DOI:10.1086/519529 [PubMed: 17701900]
- Kim MB, Zhang Y, Chang Y, Ryu S, Choi Y, et al., 2017 Diabetes mellitus and the incidence of hearing loss: a cohort study. *Int J Epidemiol* 46, 717–726. DOI:10.1093/ije/dyw342. [PubMed: 27818377]
- Kobayashi M, Miyagawa M, Nishio S, Moteki H, Fujikawa T, et al., 2018 WFS1 Mutation screening in a large series of Japanese hearing loss patients: Massively parallel DNA sequencing based analysis. *Plos One* 13, e0193359. [PubMed: 29529044]
- Kong A, Cox NJ, 1967 Allele-sharing models: LOD scores and accurate linkage tests. *Am J Hum Genet* 61, 1179–1188. doi:10.1086/301592.
- Kushiyama A, Tanaka K, Hara S, Kawazu S (2014) Linking uric acid metabolism to diabetic complications. *World J Diabetes* 5:787–795. doi: 10.4239/wjd.v5.i6.787. [PubMed: 25512781]
- Kvestad E, Czajkowski N, Krog NH, Engdahl B, Tambs K, 2012). Heritability of hearing loss. *Epidemiology* 23, 328–331 doi:10.1097/EDE.0b013e318245996e. [PubMed: 22249243]
- Lavinsky J, Crow AL, Pan C, et al., 2015 Genome-wide association study identifies nox3 as a critical gene for susceptibility to noise-induced hearing loss. *PLoS Genet* 1, e1005094. doi:10.1371/journal.pgen.1005094.
- Lin FR, Niparko JK, Ferrucci L, 2011 Hearing loss prevalence in the United States. *Arch Intern Med* 171, 1851–852. [PubMed: 22083573]
- Lin FR, Thorpe R, Gordon-Salant S, Ferrucci L, 2011 Hearing loss prevalence and risk factors among older adults in the United States. *J Gerontol A Biol Sci Med Sci* 66, 582–590. doi:10.1097/EDE.0b013e318245996e. [PubMed: 21357188]
- Liu XZ, Xie D, Yuan HJ, de Brouwer APM, Christodoulou J, Yan D, 2013 Hearing loss and PRPS1 mutations: Wide spectrum of phenotypes and potential therapy. *Int J Audiol* 52, 23–28. doi: 10.3109/14992027.2012.736032. [PubMed: 23190330]
- Matise TC et al., 2007 A second-generation combined linkage physical map of the human genome. *Genome Res* 17, 1783–6. [PubMed: 17989245]
- McMahon CM, Kifley A, Rohtchina E, Newall P, Mitchell P, 2008 The contribution of family history to hearing loss in an older population. *Ear Hear* 29, 578–584. doi:10.1097/EDE.0b013e318245996e. [PubMed: 18469720]

- Mayo Clinic, 2015 Hearing Loss Risk Factors. <http://www.mayoclinic.org/diseases-conditions/hearing-loss/basics/risk-factors/con-20027684>. Accessed February 1, 2016.
- Mohr PE, Feldman JJ, Dunbar JL, et al., 2000 The Societal Costs of Severe to Profound Hearing Loss in the United States. *Int J Technol Assess Health Care* 16, 1120–1135. [PubMed: 11155832]
- Mouhamed DH, Ezaher A, Meffati F, Douki W, Gaha I., Najjar MF, 2011 Effect of cigarette smoking on plasma uric acid concentrations. *Environ Health Prev Med* 16:307–2. doi: 10.1007/s12199-010-0198-2. [PubMed: 21431788]
- Nakanishi N, Okamoto M, Nakamura K, Suzuki K, Tatara K, 2000 Cigarette Smoking and Risk for Hearing Impairment: A Longitudinal Study in Japanese Male Office Workers. *J Occup Environ Med* 42, 1045–1049. doi:10.1097/00043764-200011000-00001. [PubMed: 11094781]
- Nato AQ, Buyske S, and Matise TC The Rutgers map: A third-generation combined linkage-physical map of the human genome Human Genetics Institute of New Jersey Second Research Day. Life Sciences Building, Rutgers University, Piscataway, NJ, USA website: http://compgen.rutgers.edu/rutgers_maps.shtml.
- Ng SB, Turner EH, Robertson PD, Fluygare SD, Bigham AW, et al., 2009 Targeted capture and massively parallel sequencing of 12 human exomes. *Nature* 461(7261), 272–6. [PubMed: 19684571]
- Nomura K, Nakao M, Morimoto T, 2005 Effect of smoking on hearing loss: quality assessment and meta-analysis. *Prev Med (Baltim)* 40, 138–144.
- O’Neill M, 1996 A gene for autosomal dominant late-onset progressive non-syndromic hearing loss, DFNA10, maps to chromosome 6. *Hum Mol Genet* 5, 853–856. doi:10.1093/hmg/5.6.853. [PubMed: 8776603]
- Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, Boehnke M, Abecasis GR, Willer CJ. (2010) LocusZoom: Regional visualization of genome-wide association scan results. *Bioinformatics* 2010 September 15; 26(18): 2336.2337.
- Raffel LJ, Robbins DC, Norris JM, et al., 1996 The GENNID Study. A resource for mapping the genes that cause NIDDM. *Diabetes Care*. 19, 864–872. <http://www.ncbi.nlm.nih.gov/pubmed/8842605>. Accessed February 1, 2016. [PubMed: 8842605]
- R Core Team, 2019 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria URL <https://www.R-project.org/>.
- Rohayem J, Ehlers C, Wiedeman B, Holl R, Oexle K, et al., 2011 Diabetes and neurodegeneration in Wolfram Syndrome. *Diabetes Care* 34,1503–1510. doi: 10.2337/dc10-1937. [PubMed: 21602428]
- Shearer AE, Hildebrand MS, Smith RJH, 2014 Deafness and Hereditary Hearing Loss Overview. <http://www.ncbi.nlm.nih.gov/books/NBK1434/>. Accessed February 4, 2016.
- Siegelaub AB, Friedman GD, Adour K, Seltzer CC, 1974 Hearing Loss in Adults. *Arch Environ Heal An Int J* 29, 107–109.
- Simmonds HA, Webster DR, Lingam S, Wilson J, 1985 An inborn error of purine metabolism, deafness and neurodevelopmental abnormality. *Neuropediatrics* 16,106–108. DOI: 10.1055/s-2008-1052552. [PubMed: 2989725]
- Team R.C. R: A language and environment for statistical computing. <https://www.r-project.org/>. Published 2015.
- Uchida Y, Sugiura S, Nakashima T, Ando F, Shimokata H, 2012 Estimates of the size of the hearing-impaired elderly population in Japan and 10-year incidence of hearing loss by age, based on data from the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA). *Nihon Ronen Igakkai Zasshi* 49, 222–227. [PubMed: 23268872]
- Whittemore AS, Halpern J, 1994 A class of tests for linkage using affected pedigree members. *Biometrics*. 50,118–127. <http://www.ncbi.nlm.nih.gov/pubmed/8086596>. Accessed February 19, 2016. [PubMed: 8086596]
- WHO | Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. http://www.who.int/diabetes/publications/diagnosis_diabetes2006/en/. Accessed April 18, 2016.
- Yang H-F, Kao T-W, Peng T-C, Sun Y-S, Liaw F-Y, Want C-C, et al., 2017 Serum uric acid relation for hearing threshold shift. *Clin Exp Otorhinolaryngol* 10, 143–147. doi: 10.21053/ceo.2016.00346. [PubMed: 27604624]

Yang M, Tan H, Yang Q, et al., 2006 Association of hsp70 polymorphisms with risk of noise-induced hearing loss in Chinese automobile workers. *Cell Stress Chaperones* 11, 233–239. <http://www.ncbi.nlm.nih.gov/pubmed/17009596>. [PubMed: 17009596]

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HIGHLIGHTS

- Smoking exposure and duration greater in the hearing loss and deafness group
- Adjusted odds of hearing loss and deafness among ever-smokers was 5.76
- suggestive evidence for linkage to putative HLD loci for three chromosomal regions
- intronic SNVs within PDE7A, MTFR1, ZBTB10 and PAG1 genes as well as novel intergen

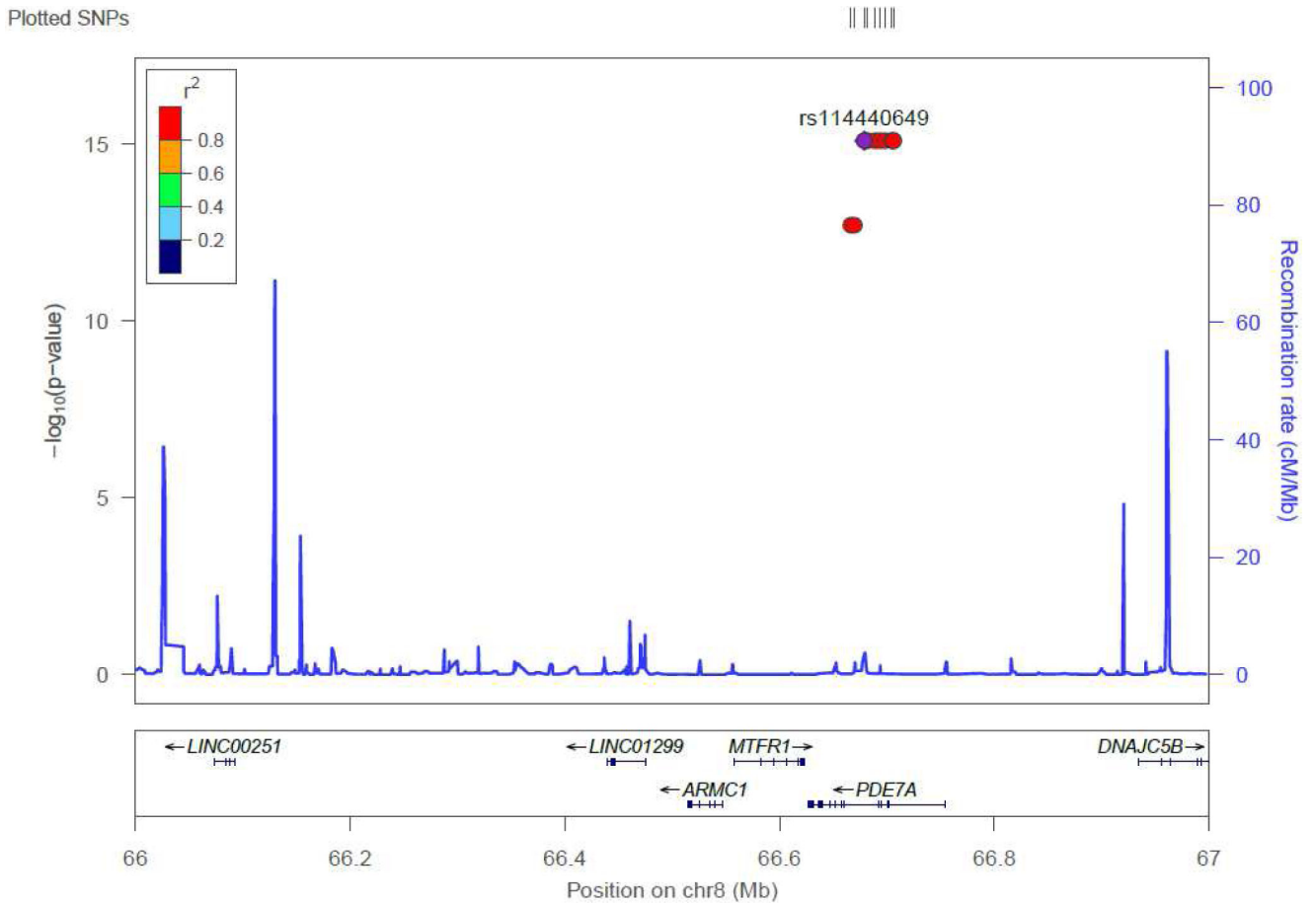


Figure 1:

Evidence of of GxE (PDE7A, MTFR1 genes by smoking packs per day) interaction (circle points of $-\log_{10}(\text{p-values})$ on lefthand vertical axis) with underlying recombination rate (blue lines with right hand vertical axis) on chromosome 8. The linkage disequilibrium (LD) or correlation between each SNV and the SNV with the highest evidence of GxE (i.e., highest $-\log_{10}(\text{p-value})$ and denoted by a purple dot) is described by r^2 with legend in the box located in the right upper corner. The red demotes high LD/correlation and dark blue denotes low LD/correlation. All variants involved in the interaction with smoking packs-per-day to modify HLD risk are all in high LD. Reference panel is hg19/1000G Nov 2014 ASN. Plot was made using Locus Zoom (Prium et al., 2010).

Table 1.

Demographic Characteristics of Japanese American Individuals

| Measure ^a | All Subjects (N=75) | HLD Cases (N=24) | Non-HLD (N=51) | P ^b |
|---------------------------|---------------------|------------------|----------------|-----------------------|
| Age (yrs) | 55.68 ± 15.9 | 66.51 ± 10.65 | 50.58 ± 15.46 | 1.23×10 ⁻⁹ |
| Sex (No. female, %) | 40 (53%) | 17 (71%) | 23 (45%) | 0.030 |
| Diabetes WHO | 30 (41%) | 13 (54%) | 17 (35%) | 0.129 |
| Age at deafness (yrs) | — | 55.24 ± 15.79 | — | |
| Ever smoked | 41 (55%) | 19 (79%) | 22 (43%) | 0.001 |
| Age began smoking (yrs) | 19.61 ± 4.25 | 19.05 ± 4.55 | 20.09 ± 4.01 | 0.334 |
| Age stopped smoking (yrs) | 42.17 ± 11.31 | 42.33 ± 11.78 | 42.05 ± 11.25 | 0.907 |
| Duration of smoking (yrs) | 11.47 ± 14.08 | 17.15 ± 14.22 | 9.2 ± 13.51 | 0.014 |
| Packs-per-day | 0.53 ± 0.71 | 0.78 ± 0.78 | 0.42 ± 0.65 | 0.049 |

^aMeans ± standard deviations of continuous measures (age, age of deafness, age began smoking, age stopped smoking, duration of cigarette smoking, packs-per-day) and the number and percentages of dichotomous variables (sex, diabetes status, cigarette ever-smoker status).

^bP is p-value from testing differences in each demographic characteristic or measure between HLD and Non-HLD subjects, we used logistic regression with GEE with independence correlation structure to account for familial correlation.

Demographic Characteristics of Japanese American Families

Table 2.

| Characteristic | Average Per-Family ^a | Family 1 | Family 2 | Family 3 | Family 4 | Family 5 | Family 6 | Family 7 | Family 8 |
|---------------------------|---------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|
| N | 13 ± 4 | 10 | 9 | 16 | 10 | 10 | 10 | 19 | 13 |
| HLD (N, %) | 3 ± 1 | 4 (40%) | 2 (22%) | 4 (25%) | 2 (20%) | 2 (20%) | 4 (40%) | 2 (11%) | 4 (31%) |
| Age (years) | 57.74 ± 7.79 | 66.75 | 64.95 | 53.46 | 58.21 | 53.90 | 67.31 | 49.77 | 47.57 |
| Sex (% male) | 43% ± 10% | 40% | 33% | 38% | 40% | 50% | 30% | 58% | 54% |
| Diabetes (%) | 44% ± 14% | 67% | 50% | 43% | 50% | 57% | 33% | 27% | 27% |
| Age at deafness (yrs) | 53.96 ± 6.46 | 57.75 | 55.00 | 54.67 | 41.50 | 55.00 | 59.00 | 47.50 | 61.25 |
| Ever smoked ^b | 57% ± 26% | 86% | 83% | 79% | 63% | 63% | 33% | 33% | 18% |
| Age began smoking (yrs) | 20 ± 1.57 | 19.00 | 19.60 | 18.27 | 20.60 | 19.80 | 18.50 | 21.20 | 23.00 |
| Age stopped smoking (yrs) | 42.22 ± 5.79 | 46.25 | 54.80 | 38.50 | 37.20 | 39.80 | 41.50 | 41.20 | 38.50 |
| Duration of smoking (yrs) | 13.27 ± 8.83 | 23.00 | 29.33 | 13.82 | 10.38 | 12.50 | 7.67 | 6.67 | 2.82 |
| Packs-per-day | 0.57 ± 0.4 | 1.17 | 0.75 | 0.58 | 0.31 | 1.07 | 0.25 | 0.40 | 0.05 |

Continuous covariates (age, age of deafness, age began smoking, age stopped smoking, duration of cigarette smoking, packs-per-day) were averaged within each family, and the means ± standard deviations of these per-family values are shown. Dichotomous covariates (sex, diabetes status, cigarette ever-smoker status) were summarized as the percentage within each family, and the means ± standard deviations of these per-family percentages are shown in this column of the table.

^bFamilies are ordered in descending order based on % cigarette ever smoker in family.

Table 3.

Association between HLD risk and cigarette smoking

| Cigarette Smoking exposure variable | Ever-smoker OR ^a (95% CI) ^b , p ^c | Packs-per-day OR ^d (95% CI) ^b , p ^c |
|---|--|--|
| No adjustment | 5.01 (1.88,13.32) p=0.001 | 2.04 (1.00,4.15) p=0.049 |
| Model 1: Adjusted for age + sex + diabetes | 2.83 (0.97,8.22) p=0.056 | 2.14 (0.90,5.07) p=0.084 |
| Model 2: Adjusted for age + sex + diabetes + smoking duration | 5.76 (1.46,22.66) p=0.012 | 4.97 (1.53,16.16) p=0.008 |

^a OR is odds ratio of having hearing loss disorder (HLD) in smokers compared to non-smokers. OR can also be interpreted as the multiplicative fold increase in risk of HLD comparing smokers to non-smokers.

^b 95% CI is the 95% confidence interval of the OR. This range of odds ratio values can be interpreted as 95% of all samples from the population would have an estimated OR fall in this range.

^c P-value corresponds to Wald's test; p-values < 0.05 is evidence of significant smoking effects on risk of having hearing loss disorder (HLD)

^d OR is odds ratio of having HLD for every additional packs-per-day being smoked. OR can also be interpreted as the multiplicative fold increase in risk of HLD per each additional increase of packs per day smoked.

Table 4.

Evidence of gene-by-environment interaction in subsets of families: Ordered subset analysis (OSA) results

| Smoking covariate | CHR | Location of OSA max LOD ^d (cM) | Marker at OSA max LOD | Location ^b (bp build 37) | Markers flanking 1-LOD support interval | 1-LOD support interval ^c (bp, build 37) | OSA Max LOD | Original Max LOD | p | No. Families/ Total ^d |
|-------------------|---------|---|-----------------------|-------------------------------------|---|--|-------------|------------------|------|----------------------------------|
| Packs-per-day | 8q21.3 | 89 | D8S1119 | 87172020 | D8S1136 D8S1132 | 66,053,167 107,328,872 | 3.54 | 1.83 | 0.04 | 4/8 |
| | | | | | | | | | | |
| | 7p21.1 | 21.5 | D7S3051 | 18,284,528 | D7S1819 D7S1802 | 4,493,039 20,705,304 | 3.3 | 1.77 | 0.04 | 2/8 |
| | | | | | | | | | | |
| | 11q23.1 | 111.7 | D11S1986 | 111,223,605 | D11S2002 D11S4464 | 79,965,403 123,626,627 | 2.1 | 1.12 | 0.03 | 2/8 |
| | | | | | | | | | | |
| Ever smoking | 12q32.1 | 145.8 | D12S1045 | 130,397,738 | D12S2078 D12S392 | 127,961,140 130,607,434 | 2.6 | 1.15 | 0.01 | 3/8 |
| | | | | | | | | | | |
| | 15q26.2 | 96.4 | D15S816 | 95,019,784 | D15S1015 D15S657 | 67,839,381 96,704,848 | 2.9 | 1.42 | 0.01 | 5/8 |
| | | | | | | | | | | |
| | 20q13.3 | 92.2 | D20S171 | 57,808,030 | D20S197 rs6089801 ^e | 46,160,764 62,911,391 | 3 | 1.77 | 0.02 | 3/8 |
| | | | | | | | | | | |

^aGenetic location in centiMorgans of the max LOD for the given chromosome.^bPhysical basepair positions (build 37) were determined by Rutgers Combined Linkage-Physical Map version 3 (Nato et al., 2018). If the marker was not found, the map position was extrapolated using a slope-intercept line equation and the positions of two nearby markers.^c1-LOD support intervals were determined by using locations of the nearest markers closest to flanking positions with 1-LOD difference from the max LOD.^dThe fraction indicates the numerator as the top number of families from high to low ranks based on percentage of smokers per-family ranking (see Table 2) over the denominator which is the total number of 8 families. This subset of families was found to yield significant evidence gene-by-smoking interaction ($P < 0.05$).^eThere is no right-flanking marker beyond D20S171 on chromosome 20; therefore, the marker, rs6089801, with the highest base pair position on chromosome 20 in the Rutgers' map (Matise et al., 2007) was used as the right-flanking marker.