Polygenic risk scores implicate genetic pathways involved in neurodevelopmental disorders in hearing thresholds and hearing asymmetry in children

Running title:

Genetics of hearing, asymmetry and neurodevelopment

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Abstract

An efficient auditory system contributes to cognitive and psychosocial development. A right ear advantage in hearing thresholds (HT) has been described in adults and atypical patterns of left/right hearing threshold asymmetry (HTA) have been described for psychiatric and neurodevelopmental conditions. Previous genome-wide association studies (GWAS) on HT have mainly been conducted in elderly participants whose hearing is more likely to be affected by environmental effects. We analysed HT and HTA in a children population cohort (ALSPAC, n = 6,743, 7.6 years). Better hearing was associated with more advanced cognitive skills and higher socioeconomic status (SES). Mean HTA was negative (-0.28 dB), suggesting a left ear advantage in children but mainly driven by females (-0.48 dB in females v -0.09 dB in males). We performed the first GWAS on HT in children and the very first GWAS on HTA (n = 5,344). Single marker trait association analysis did not yield significant hits. Polygenic risk score (PRS) analysis revealed associations of PRS for schizophrenia with HT, which remained significant after controlling for SES and cognitive skills, and of PRS for autism spectrum disorders (ASD) with HTA. Gene-based analysis for HTA reached genome-wide significance for *MCM5*, which is implicated in axon morphogenesis. This analysis also highlighted other genes associated with contralateral axon crossing. Some of these genes have previously been reported for ASD. These results further support the hypothesis that pathways distinguishing the left/right axis of the brain (i.e. commissural crossing) contribute to both different types of asymmetries (i.e. HTA) and neurodevelopmental disorders.

Keywords: hearing threshold, left/right asymmetry, audiometry, air conduction, autism spectrum disorder, schizophrenia, axonogenesis, neuronal migration, axon guidance, laterality, GWAS, ALSPAC

Introduction

The auditory system involves a series of complex distributed cerebral networks and its impairment affects psychosocial, emotional and cognitive development[1]. Hearing-impaired children are at increased risk for learning disabilities[2] and even within the normal range, better hearing has been associated with better reading skills, working memory and nonverbal IQ in a sample of 1,638 UK school children[3].

Hearing ability is usually defined as the threshold in decibel (dB) at which a tone is perceived, so that lower values indicate better hearing. An age-related hearing decline is well documented. In a Korean general population sample (n > 15,000) the hearing threshold (HT) for medium frequencies declined from 3 dB in adolescents to 38 dB in elderly participants[4]. A sex difference in favour of women was found in adults (n = 10,145; 30-69 years old), but not in children and young adults (n = 3,458).

Successful hearing requires transformation of changes in air pressure into vibrations in the basilar membrane that is transferred onto sensory hair cells of the inner ear, whose depolarization is initiated by deflection of mechano-sensitive hair bundles[5]. The auditory nerve transmits these signals to the cochlear nucleus in the brainstem. The majority of the input is transmitted to the contralateral superior olivary complex, while a minor part of the input is transmitted ipsilaterally[6,7]. Greater contralateral medial olivonuclear suppression in the right compared to the left ear[8] has been suggested as the underlying correlate of a fundamental functional asymmetry between the left and right ear. This hearing threshold asymmetry (HTA) has typically been reported as HT left – HT right so that positive values indicate an advantage of the right and negative values indicate an advantage of the left ear. In a study of more than 50,000 adults[9], a right ear advantage (HTA between 1 dB and 4 dB) has been reported with more pronounced HTA in males than in females. In a children sample of n = 1,191, a right ear advantage has been reported, albeit to a smaller extent than in adults[10]. Other authors found a general right ear advantage in males (n = ~400, HTA between 0.1 dB and 0.5 dB) and a left ear advantage in females for specific frequencies (n = ~400, HTA between -0.1 and -0.4 dB)[11]. Smaller studies reported a general left ear advantage in children[12,13].

An absence of HTA has been reported in schizophrenia[14,15] and ADHD[16]. Moreover, symmetrical contralateral suppression in the olivary complex in the left and the right ear in schizophrenia[17] is in contrast with the right ear advantage typically found in controls. In children and adolescents, a right ear advantage has been reported in a sample of n = 22 with autism spectrum disorder (ASD) while no asymmetry was found in the control group[18]. A developmental effect towards stronger HTA has been

reported in controls that was absent in ASD children (n = 24)[19]. Reduced laterality in processing auditory stimuli was reported in ASD and bipolar disorder (BIP) suggesting that HTA is linked to neurodevelopmental disorders[20,21].

Twin and family studies estimated the heritability for HT to range from .26 to .75 with larger environmental effects for the non-dominant ear[22–24]. Genome-wide association studies (GWAS) focused on age-related hearing loss in subjects ranging from 45 to 75 years of age and identified genes including *GRM7* and *ESRRG*[25–28]. GWAS for normal hearing included subjects from 18 to 92 years of age[29–31] and implicated several genes (i.e. *DCLK1*, *PTPRD*, *GRM8*, *CMIP*, *SIK3*, *PCDH20*, *SLC28A3*), which have partly been associated with neurodevelopmental traits[32–37]. A case control design based on electronical health records on age-related hearing loss identified SNPs near *ISG20* and *TRIOBP*[38], which had previously been associated with prelingual nonsyndromic hearing loss[39]. In the UK Biobank, 41 and 7 independent loci have been identified for hearing difficulty and hearing aid use, respectively, implicating genes such as *CDH23*, *EYA4*, *KLHDC7B* and again *TRIOBP*[40]. Mutations in *CDH23* have been associated with early-onset hearing loss and Usher syndrome causing early-onset deafness[41].

However, older subjects have had more exposure to environmental factors which might affect hearing, such as extensive noise[42], medication[43], chemicals[44] and medical conditions[45]. An investigation of HT in 250 monozygotic (MZ) and 307 dizygotic (DZ) twin pairs from 36 to 80 years of age suggests that environmental effects become more significant with age[46]. Despite this age effect, no study has ever investigated genetic factors involved in hearing function in children.

We analysed HT and HTA in children from the Avon Longitudinal Study of Parents and Children (ALSPAC) (n = 6,743). Consistent with previous studies we found that better hearing is associated with enhanced cognitive skills and higher SES. We report the first GWAS for HT in children and the very first GWAS for HTA (n = 5,344). In addition to single marker trait associations, we conducted gene-based and gene set analyses and tested the effects of polygenic risk scores (PRS) for a range of neurodevelopmental disorders, IQ and educational attainment (EA). Our results suggest that PRS for schizophrenia are associated with HT, while PRS for ASD are associated with HTA. Genes involved in contralateral axon crossing are associated with HTA, linking genetic factors involved in axonogenesis and left/right axis to different types of asymmetries and neurodevelopmental disorders.

Materials and Methods

Cohort

ALSPAC is a longitudinal cohort representing the general population living in the Bristol area. Pregnant women resident in the county of Avon, UK, with expected dates of delivery from 1st April 1991 to 31st December 1992 were invited to take part in the study, resulting in 14,062 live births and 13,988 children who were alive at 1 year of age[47,48]. From age seven, all children were invited annually for assessments on a wide range of physical, behavioural and neuropsychological traits. Informed written consent was obtained from the parents after receiving a complete description of the study at the time of enrolment into ALSPAC, with the option to withdraw at any time. Ethical approval for the present study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committees. The ALSPAC study website contains details of all the data that is available through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/).

Phenotypes

Audiometry was performed according to British Society of Audiologists standards. Hearing tests were carried out in a room with minimal external noise. Testing was stopped if the background noise level exceeded 35 dBA. The air conduction threshold, i.e. the lowest intensity in decibels at which a tone is perceived 50% of the time (dBHL, decibel hearing level), was tested using either a GSI 61 clinical audiometer or a Kamplex AD12 audiometer. Lower dBHL values indicate better hearing. For each ear, the air conduction threshold level was tested at 500 Hz, 1 kHz, 2 kHz and 4 kHz. For each frequency, stimuli were first presented on the right and then on the left ear. The average threshold across different frequencies was derived for each ear.

After applying exclusion criteria (supplementary methods), a sample of n = 6,743 was available for phenotypic analysis (3,344 females, 3,391 males, 8 missing values for sex, mean age = 7.59 years, SD = 0.32 years).

HT was defined as the average air conduction threshold on the better ear. HTA was defined as the absolute difference in air conduction threshold between the left and right ear. Thus, positive values indicate a right ear advantage, while negative values indicate a left ear advantage. Handedness was assessed in terms of writing hand (5,805 right-handers, 787 left-handers, 151 missing values).

Cognitive skills were assessed using tests for reading ability[49], communication skills[50], listening comprehension[51], short term memory[52], verbal and performance IQ[53] and EA measured as capped General Certificate of Secondary Education (GCSE) scores (for

detailed descriptions, see supplementary methods). Maternal highest educational qualification during pregnancy was used as a proxy for SES[54]. Educational qualification was grouped into 'CSE and no education', 'vocational', 'O level', 'A level' and 'Degree'.

Children were assigned to neurodevelopmental and control subgroups as defined for the ALSPAC sample previously[37]. We specified subgroups for specific language impairment (SLI) (n = 155), reading disability (RD) (n = 141), ASD (n = 35), ADHD (n = 21), comorbidity (n = 49) and a control sample matched for sex (n = 2,071). The strategies for subgroup assignments are reported in the supplementary methods.

Genotype quality control (QC) and imputation

Genotypes were generated on the Illumina HumanHap550-quad array at the Wellcome Trust Sanger Institute, Cambridge, UK and the Laboratory Corporation of America, Burlington, NC, US. Standard QC was performed as described elsewhere[55]. In total, 9,115 subjects and 500,527 SNPs passed QC filtering. Haplotypes were estimated using ShapeIT (v2.r644). QC-filtered autosomal SNPs were imputed using Impute v3 using the HRC 1.1 reference data panel. Poorly imputed SNPs (Info score < 0.8) and SNPs with low minor allele frequency (MAF < 0.05) were excluded from further analysis.

Statistical analysis

Genetic association tests: Genome-wide genotype data were available for 5,344 children with phenotypes (2,691 males, 2,653 females, mean age = 7.58 years, SD = 0.31 years). HT and HTA were inverse rank-transformed to achieve a normal distribution for GWAS. Association testing was performed in Plink v2 under a generalised linear modeling (GLM) framework specifying sex and the first two ancestry-informative principal components as covariates. Overall, 4,875,234 SNPs that were either directly genotyped or imputed and passed QC were tested for association. The genomic inflation factor (λ) was calculated for all SNPs and revealed no evidence of population structure (HT: λ = 1.02, HTA: λ = 1.00). For HT, we specifically tested for replication of markers associated with quantitative hearing phenotypes in previous studies ($p < 10^{-5}$ [28,31], $p < 10^{-6}$ [29,30]). We also tested for replication of markers showing genome-wide significance in case-control GWAS on age-related hearing loss ($p < 5 \times 10^{-8}$ [38,40]). This procedure resulted in 644 unique markers, 502 of which overlapped with the markers tested in this study. The Bonferroni-corrected significance level was thus set to 0.05/502 = .0001.

Annotation and gene mapping: We applied FUMA v1.3.6[56] on the GWAS summary statistics. Functional consequences of SNPs were obtained by performing ANNOVAR [57] using Ensembl genes (build 85). SNPs were mapped to genes based on positional mapping. Intergenic SNPs were annotated to the closest genes upstream and downstream. Input SNPs were mapped to 18,024 protein-coding genes.

Gene-based and gene set analyses: FUMA implements MAGMA v1.07[58] to summarise SNP associations at the gene level (gene-based analysis) and associate the set of genes to biological pathways (gene set analysis). Gene-based *p* values were computed using an F-test in a multiple linear principal components regression while accounting for LD between SNPs. Genome-wide significance was defined as $p = 0.05/18{,}024 = 2.8 \times 10^{-6}$. For gene set analysis, MAGMA converts gene-based *p* values into *z* values, which reflect the strength of association. MAGMA performs a competitive gene set analysis, comparing the mean association of genes within a gene set with the mean association of genes not in the gene set while correcting for gene size and density. Gene set *p* values were computed for 7,343 gene ontology (GO) terms for biological processes obtained from MsigDB v5.2. The Bonferroni-corrected significance level was set to $0.05/7{,}343 = 6.8 \times 10^{-6}$.

PRS: We carried out PRS analyses to investigate the genetic overlap of markers associated with HT/HTA and relevant traits and conditions using PRSice 2.2.11.b[59]. PRSice uses GWAS summary statistics as training GWAS to build PRS, which are then tested as predictors in the target GWAS. Psychiatric Genomics Consortium summary statistics were downloaded (https://www.med.unc.edu/pgc/data-index/) for schizophrenia[60], ADHD[61], ASD and BIP[62] as these are the psychiatric and neurodevelopmental conditions often found to be associated with reduced laterality[15,17,18,21]. Based on associations of HT with cognitive skills, GCSE scores and SES, we downloaded summary statistics for IQ[63] from the Complex Trait Genetics lab website (https://ctg.cncr.nl/software/summary_statistics) and EA[64] from the Social Science Genetic Association Consortium (https://www.thessgac.org/data).

SNPs were clumped based on LD ($r^2 >= 0.1$) within a 250 kb window. PRS were derived as the weighted sum of risk alleles based on odds ratios or beta values from the training GWAS summary statistics. Sex and first two principal components were included as covariates. Results are presented for the optimal training GWAS *p* value threshold (explaining the highest proportion of phenotypic variance in the target GWAS). For training GWAS *p* value thresholds and number of SNPs included in the PRS, see Supplementary Table S1. For six training GWAS and two target GWAS (HT and HTA), the Bonferroni-corrected significance level was set to .05/12 = .004. For significant

effects, analyses were repeated for males and females separately and including only the first two principal components as covariates.

Data preparation and visualization was performed using R v.4.0.0. All analysis scripts are available through Open Science Framework (https://osf.io/gewj2/).

Results

Phenotypes

We first analysed the distribution of HT and HTA in the overall sample (n = 6,743) (results for the subset used in the GWAS, n = 5,344, are shown in Supplementary Figure S1). HT ranged from -8.75 to 20.00. Mean HT was 6.14 (SD = 3.97) (Figure 1A). Females (n = 3,344) showed slightly higher HT (mean = 6.30, SD = 4.06) than males (n = 3,391, mean = 5.98, SD = 3.88), $t_{(6709.2)}$ = -3.31, p = .001 (Figure 1B).

HTA ranged from -18.75 to 20 and revealed a significant left ear advantage for the overall sample (mean = -0.28, SD = 3.75) as determined by one-sample t-test against zero ($t_{(6742)}$ = -6.17, $p = 7.37 \times 10^{-10}$) (Figure 1C). Females showed stronger HTA (mean = -0.48, SD = 3.81) than males (mean = -0.09, SD = 3.68), $t_{(6716.9)}$ = 4.33., $p = 1.54 \times 10^{-5}$ (Figure 1D). We thus performed one-sample t-tests against zero for females and males separately. While females showed a significant left ear advantage ($t_{(3343)}$ = -7.30, $p = 3.50 \times 10^{-13}$), there was no ear advantage in males ($t_{(3390)}$ = -1.37, p = .172).

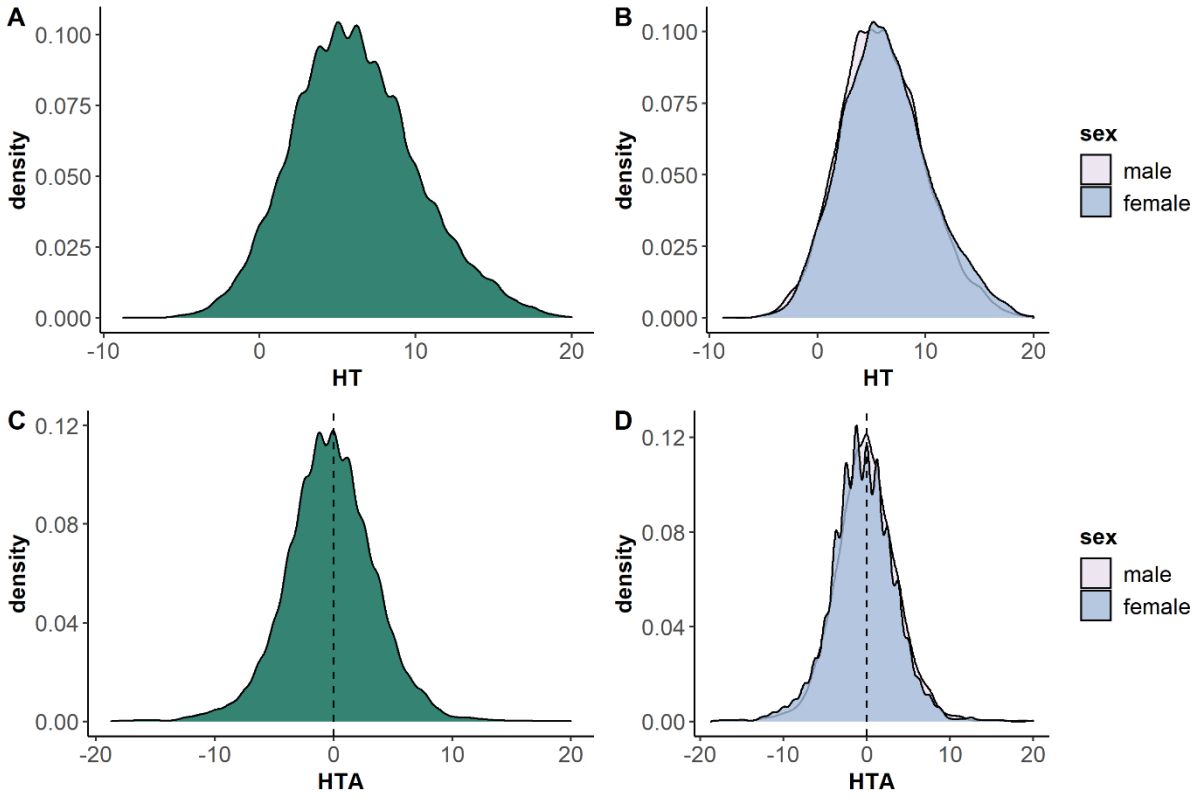

Figure 1: Distribution of HT and HTA. A) Distribution of HT (better ear) in the overall sample (n = 6743), and B) as a function of sex. C) Distribution of HTA in the overall sample, and D) as a function of sex. The dotted line represents no asymmetry between the left and right ear.

There was no evidence for an effect of handedness on HTA (right-handers: n = 5,805, M = -0.28, SD = 3.75; left-handers: n = 787, M = -0.24, SD = 3.72, $t_{(1015)}$ = -0.29, p = .776).

Bivariate Pearson correlations revealed significant positive correlations among the different cognitive measures as previously reported[37]. There were significant negative correlations after Bonferroni correction (36 comparisons, p < .0014) for all cognitive measures but listening comprehension with HT (Figure 2), indicating that lower HT (better hearing) is associated with better cognitive performance (correlation plots are shown in Supplementary Figure S2) and higher GCSE scores. There was no association between HTA and cognitive measures.

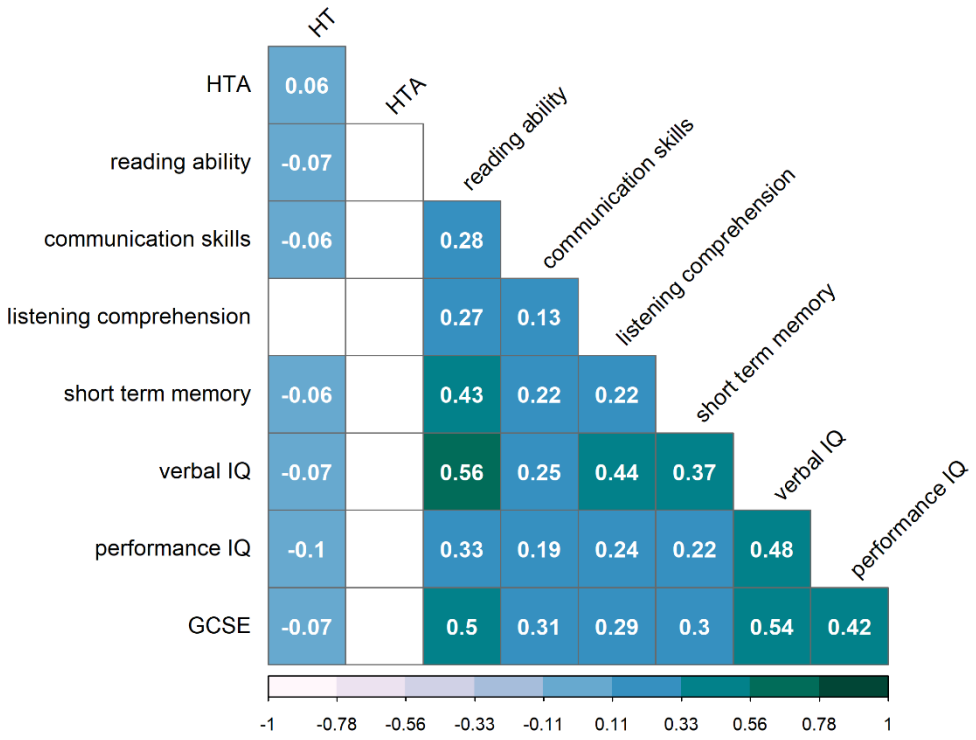

Figure 2: Correlation matrix for HT, HTA and cognitive measures. Correlation coefficients are shown if passing the Bonferroni-corrected significance level (p < .0014). Sample sizes range from n = 4730 to n = 6743 depending on data availability.

One-way between-subjects ANOVAs were conducted to compare the effect of SES on HT and HTA. There was a significant effect of SES on HT ($F_{(4,6137)}$ = 7.25, p = 8.1 × 10^{-6}). Post hoc comparisons using the Tukey test indicated significantly lower HT for 'A level' (mean = 6.12, SD = 3.95), 'O level' (mean = 5.91 , SD = 3.93) and 'Degree' (mean = 5.92, SD = 4.05) compared to 'CSE' (mean = 6.69, SD = 4.12) and significantly lower HT for 'O level' and 'Degree' compared to 'Vocational' (mean = 6.52, SD = 3.84), indicating higher SES is associated with better hearing (Supplementary Figure S3). There was no significant effect of SES on HTA ($F_{(4,6137)}$ = 1.78, p = .130).

Two-sample *t*-tests revealed no difference between children affected by neurodevelopmental disorders and sex-matched controls in HT (Supplementary Table S2) or HTA (Supplementary Table S3). However, there was a consistent pattern across neurodevelopmental subgroups with more negative HTA compared to the control group, indicating more leftward asymmetry.

Genetic association tests

GWAS was performed in n = 5,344 children. No individual SNP reached genome-wide significance for HT (Supplementary Table S4). The strongest association was for marker rs11644235 on chromosome 16 ($p = 1.51 \times 10^{-6}$) with each copy of the minor allele (MAF = 0.42) shifting an individual 0.09 standard deviations towards lower HT (i.e. better hearing). In the replication analysis, one marker reached Bonferroni-corrected significance (rs12955474, ß = -0.17, $p = 5.59 \times 10^{-5}$). This marker had been previously reported in a GWAS for the third PC on HT over different frequencies (0.25, 0.5, 1, 2, 3, 4, 6, 8 kHz) (ß = -0.10, $p = 3.57 \times 10^{-7}$)[28]. Another eight SNPs reached nominal significance with effects in the same direction (Supplementary Table S5), among those five reported on HT at 2 kHz and 4kHz[31], one reported on PC3[29] and two markers reported as genome-wide significant for hearing loss[40].

No individual SNP reached genome-wide significance for HTA (Supplementary Table S6). The marker rs10434985 on chromosome 7 was the most strongly associated SNP ($p = 7.27 \times 10^{-7}$) with each copy of the minor allele (MAF = 0.21) shifting an individual 0.12 standard deviations towards better hearing on the left ear. Manhattan and QQ plots are shown in Supplementary Figure S4-S6.

Gene-based analysis highlighted three suggestive associations for HT (*AE000662.92*: association $p = 2.15 \times 10^{-5}$; *C6orf201*: association $p = 4.10 \times 10^{-5}$; *FAM217A*: association $p = 6.95 \times 10^{-5}$) (Figure 3).

The *MCM5* gene reached the genome-wide significance level for HTA ($p = 4.30 \times 10^{-7}$) (Figure 3). Other four genes reached the suggestive significance level (*LHX6*, $p = 1.77 \times 10^{-5}$; *NRP1*, $p = 4.50 \times 10^{-5}$; *RUVBL1*, $p = 5.89 \times 10^{-5}$; *EPHA8*,$p = 7.67 \times 10^{-5}$). QQ plots are shown in Supplementary Figures S7-S8.

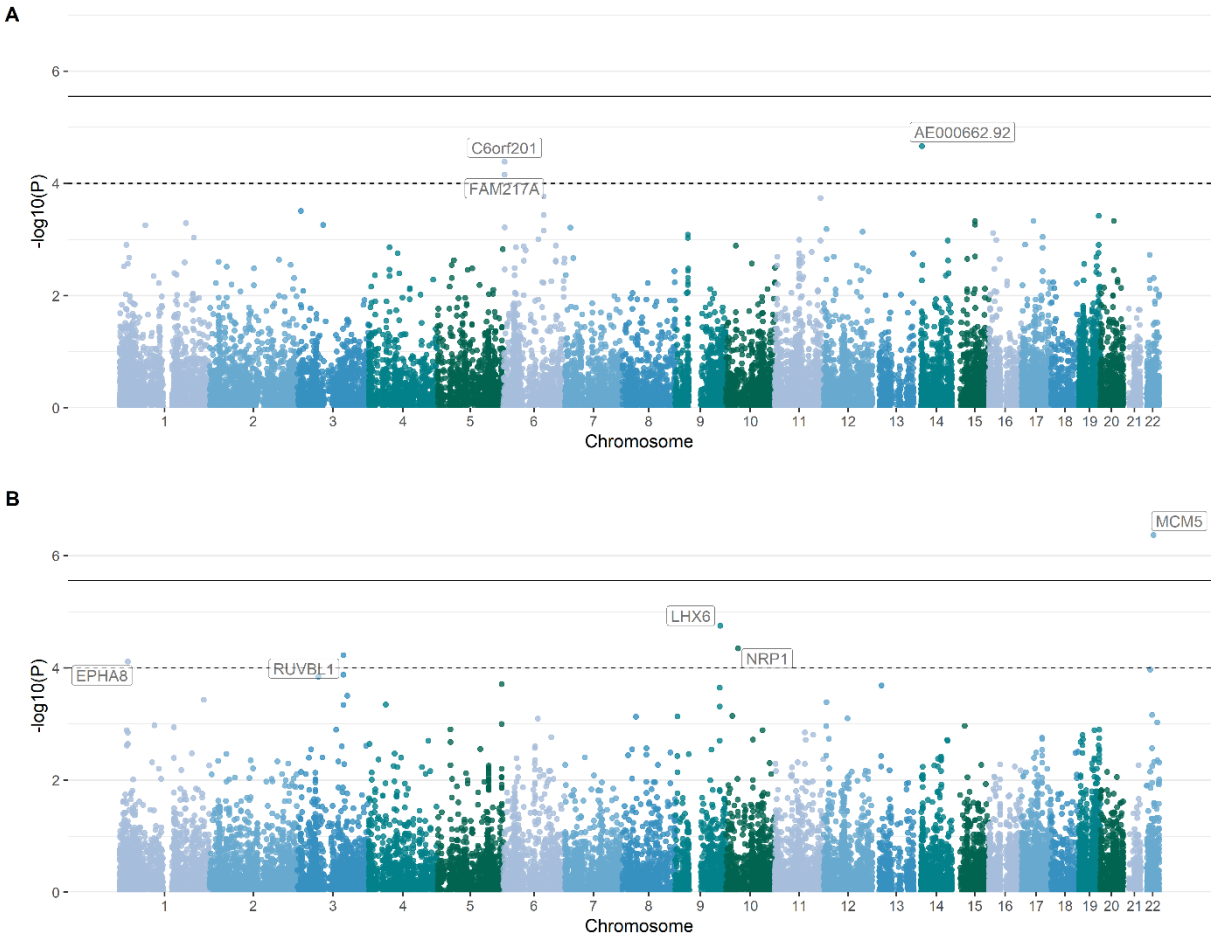

Figure 3: Gene-based analysis results. Manhattan plots are shown for A) HT and B) HTA. Gene-based association p values are plotted against chromosome and position. The solid line represents the genome-wide significance level ($p = 2.8 \times 10^{-6}$), the dotted line represents the suggestive significance level ($p = .0001$).

In gene set enrichment analysis no GO term reached the Bonferroni-corrected level of significance ($p = 6.8 \times 10^{-6}$) for either HT or HTA. "Translational termination (GO: 0006415)" ($p = 1.17 \times 10^{-5}$) and "cerebral cortex tangential migration (GO:0021800)" ($p = 4.17 \times 10^{-5}$) were the strongest associations detected for HT and HTA, respectively.

PRS

PRS were tested for IQ and EA based on the correlations between HT and cognitive measures and GCSE scores (Figure 2). PRS for four neurodevelopmental conditions were tested on the basis of previously reported associations with HTA in the literature [15,17,18,21]. PRS for ADHD showed an association with HT ($R^2 = 0.003$, $\beta = 314.98$, SE = 102.78, $p = .002$, Figure 4), indicating that higher genetic risk for ADHD is associated with higher HT, i.e. worse hearing. This effect was stronger in females than in males (females: $R^2 = 0.004$, $\beta = 571.67$, SE = 268.11, $p = .033$; males: $R^2 = 0.003$, $\beta = 275.60$, SE =

97.83, $p = .005$). In contrast, schizophrenia PRS showed a negative association with HT, suggesting that higher genetic risk for schizophrenia is associated with lower HT, i.e. better hearing ($R^2 = 0.003$, $\beta = -280.16$, SE = 85.70, $p = .001$, Figure 4). This effect was stronger in males than in females (females: $R^2 = 0.003$, $\beta = -100.73$, SE = 54.99, $p = .067$; males: $R^2 = 0.004$, $\beta = -408.09$, SE = 128.37, $p = .001$). PRS for EA reached borderline significance for HT ($R^2 = 0.003$, $\beta = -1593.45$, SE = 588.06, $p = .007$, Figure 4). The negative association suggests that a genetic predisposition towards higher EA is associated with better hearing.

Figure 4: PRS analysis results. -log10(p) values are reported for PRS analysis on six training GWAS and two target GWAS (HT and HTA). PRS for ADHD and schizophrenia contribute to HT, while PRS for ASD contributes to HTA. BIP = bipolar disorder, SCZ = schizophrenia, EA = educational attainment.

Based on this association and behavioural correlations, we next tested whether the associations between ADHD and schizophrenia PRS with HT were mediated by cognitive skills. We thus reran the PRS analysis on HT with ADHD and schizophrenia as training GWAS and using sex, the first two ancestry-informative principal components and cognitive skills as covariates. This was done separately for reading ability, communication skills, short term memory, verbal IQ, performance IQ and GCSE score. In addition, we tested whether the associations between ADHD and schizophrenia PRS with HT were mediated by SES and EA PRS by using these variables as covariates. This procedure resulted in 16 PRS analyses (two training GWAS, eight covariates). The effect of ADHD PRS on HT was reduced after adjusting for most cognitive skills, SES and EA PRS (β ranging from 2.90 to 309.32, Supplementary Table S7), suggesting that the association is mediated by these variables. In contrast, the effect of schizophrenia PRS on HT remained similar after adjusting for SES, GCSE and EA PRS and was enlarged after adjusting for cognitive skills (β ranging from -302.94 to -422.54).

PRS for ASD showed a negative association with HTA, indicating that higher genetic risk for ASD is associated with more leftward HTA ($R^2 = 0.004$, $\beta = -461.79$, SE = 158.45, $p = .0036$, Figure 4). Thus, higher PRS for ASD shift the mean towards the left of the distribution, which suggests stronger asymmetry. This effect was stronger in males than in females (females: $R^2 = 0.003$, $\beta = -404.05$, SE = 223.93, $p = .071$; males: $R^2 = 0.003$, $\beta = -982.26$, SE = 373.22, $p = .009$). There was no effect of PRS for IQ or BIP on either phenotype.

Discussion

We report a comprehensive study of HT and HTA in children to dissect their relationship with cognitive abilities and neurodevelopmental disorders both at phenotypic and genetic level. We confirm that better hearing is associated with better performance on a range of cognitive abilities (Figure 2). We also report the results of the first GWAS on HT in children. Single marker, gene-based and gene set enrichment analyses did not lead to any statistically significant results. However, PRS for both ADHD and schizophrenia were significantly associated with HT (Figure 4), with genetic risk for ADHD and schizophrenia increasing and decreasing HT, respectively. We also report the very first GWAS for HTA. Although we did not detect any single marker associations, we found that PRS for ASD were statistically associated with HTA (Figure 4), suggesting that higher genetic risk for ASD is associated with a shift towards the left ear, indicating more asymmetry with better left ear performance. Gene-based analysis for HTA highlighted several genes involved in axon guidance and commissural axon crossing in sensory cerebral networks. Some of these genes have previously been implicated in ASD.

At the phenotypic level, previous studies reported lower HT in females[65]. However, this sex difference emerged around the age of 30, while there was no sex difference in children and young adults[4]. Our data (n = 6,743) showed slightly higher HT in females (6.30 dB) compared to males (5.98 dB) (Figure 1). Therefore, our data are in agreement with a sex-specific developmental trajectory resulting in better hearing in female adults that is not detectable in children. Consistent with previous studies[3], we found that in the normal range of variation, HT is negatively associated with several cognitive skills (Figure 2). We did not detect any association between PRS for IQ and HT suggesting that this association is not mediated by shared biological pathways. A cause-effect relationship would be possible, but cannot be easily explained by our data. The analysis of PRS for ADHD and schizophrenia instead support a role of genes implicated in neurodevelopmental disorders contributing to HT. There was no association between HT and ADHD at the behavioural level, however this analysis was based on a very small sample of children meeting the criteria for ADHD (n = 21) in our dataset and therefore the results might not be conclusive (Supplementary Table S2). Hearing deficits in ADHD have been reported in terms of speech perception[66], but not in air conduction thresholds. Moreover, the effect of ADHD PRS on HT was reduced after adjusting for cognitive skills, PRS and EA PRS (Supplementary Table S7), suggesting that the effect was mediated by cognitive factors. In contrast, we found that higher genetic risk for schizophrenia is associated with better hearing. This effect was still found after adjusting for cognitive skills (Supplementary Table S7). PRS for

schizophrenia have recently been associated with better language skills, but not overall school performance[67]. Thus, the association between better hearing and language development (Figure 2) could be based on shared biological pathways which also increase the risk for schizophrenia. On the phenotypic level, there are no HT differences between individuals affected by schizophrenia and controls[68]. Although no single marker trait associations reached significance in the GWAS for HT (Supplementary Figure S4), the top marker on chromosome 15 (rs1039444) is located in an intron of *RAB8B*, which encodes for a GTPase that is expressed in inner and outer hair cells and is involved in autosomal recessive deafness[69]. Targeted analysis for markers reported in previous GWAS for HT in adults replicated association with only one marker, rs12955474, which is located in an intron of the *CCBE1* gene[28]. Other markers in this gene have been associated with depression[70] and left entorhinal cortex volume[71].

Phenotypic analysis for HTA revealed an overall left ear advantage with a lower air conduction threshold of 0.28 dB on average (Figure 1), replicating results from smaller studies in children[12,13]. This result, which indicates a left ear advantage, was driven by females. Thus, a tendency towards the sex effect on HTA reported in adults seems to be established already in children. It is possible that a developmental shift towards the right ear in both sexes, resulting in reduced asymmetry in female and a rightward asymmetry in males, is driven by environmental factors[72]. This developmental shift towards stronger HTA has not been observed in children with ASD[19]. We found that PRS for ASD are associated with HTA. Higher genetic risk for ASD was linked to better hearing on the left compared to the right ear. Although not significant, this is consistent with the behavioural data, where we find a more leftward asymmetry in cases than controls across different subgroups for disorders.

We found more negative HTA in neurodevelopmental conditions including ASD compared to controls on the behavioural level that is congruent with the PRS results. Different types of asymmetry such as structural brain asymmetry[73], frontal alpha asymmetry[74], language processing[75] and handedness[76] have been implicated in ASD.

Gene-based analysis on HTA revealed genome-wide significance for the *MCM5* gene (Figure 3). *MCM5* has been linked to ASD by an algorithm modeling genomic data from cortical tissue[77]. In *C. elegans*, homozygous *mcm-5* mutants showed reduced neuron numbers and absent commissures from ventral to dorsal cord neurons[78]. Among the genes reaching the suggestive significance level, *LHX6* is required for neuronal migration[79] and survival of cortical interneurons[80]. The receptor encoded by *EPHA8* plays a critical role in axonal guidance during neurodevelopment[81]. In mice,

Epha8 has been shown contribute to correct formation of crossed commissural axons contributing to the auditory system[82]. *NRP1* encodes for a receptor associated with contralateral axon crossing at the optic chiasm in zebrafish[83] and mice[84]. NRP1 interacts with TAOK2, for which downregulation has also been reported to impair axon crossing at the midline[85] and to decrease the volumes of the *corpus callosum* and anterior commissure[86]. *TAOK2* is located on chromosome 16p11.2, which has been linked to ASD susceptibility[87]. Overall, the top hits of gene-based analysis for HTA have roles in axon morphogenesis and controlling midline crossing. Previous large-scale GWAS for handedness and structural brain asymmetries suggested a role of genes involved in axonogenesis, microtubules and cytoskeleton formation[88–91]. A smaller GWAS meta-analysis (including the ALSPAC sample) on a quantitative measure of relative hand skill implicated genes with a known role in establishing body asymmetries[55] that also play a role in neurodevelopmental disorders[92]. Of note, *RUVBL1*, one of the genes reaching suggestive significance in the GWAS on HTA, plays a fundamental role in symmetry breaking and cardiac development[93]. Moreover, *LHX6* inhibits the transcription of *PITX2*[94], which encodes for a transcription factor that is asymmetrically expressed during development of the heart and other organs and plays a direct role in their asymmetric morphology[95]. Thus, the current results are in line with the idea that shared genetic components contribute to different types of asymmetry and suggest that genes affecting structural asymmetry and contralateral axon crossing in early embryonic development might contribute to laterality and neurodevelopmental traits[96].

A possible limitation of the current study is that stimulus presentation was not randomised, but the right ear was always tested first. Testing the right ear first has been more common in previous studies than vice versa, which could either result in a learning effect (favouring the left ear) or in a fatigue effect (favouring the right ear)[97]. However, in adults, a right ear advantage is more common even in studies in which the order of stimulus presentation has been randomised[97], so the effect of stimulus presentation should be minimal. The main limitation of this study is the rather small sample size. It is of note that air conduction thresholds are not routinely collected in large-scale population studies. For example, the UK Biobank includes phenotypic information on hearing ability as self-reported hearing difficulty or use of hearing aids (n > 300,000)[40,98]. Similarly, a GWAS on age-related hearing loss in the Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort (n > 50,000) used a case control design, identifying cases based on health records (ICD-9 diagnosis)[38]. GWAS on quantitative measures of hearing ability were limited to smaller sample sizes below 6,000 subjects for individual samples[27,29]. Systematic collection of air

conduction thresholds in both ears in children would enable larger genetic studies to dissect the links between hearing, asymmetry and neurodevelopment.

In summary, our results highlight the importance of HT for cognitive development. We find that PRS for schizophrenia are implicated in HT, while PRS for ASD are implicated in HTA. This is in line with the increasing evidence supporting a role of asymmeties in ASD. Gene-based analysis highlighted genes involved in axon guidance and ASD for HTA suggesting a role of genes involved in contralateral axon crossing at the midline. The results support the hypothesis of shared pathways contributing to different types of asymmetries, neurodevelopment and disorders.

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Conflict of Interest

The authors declare no competing financial interests in relation to the work described.

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