Genome-wide meta-analysis of cognitive empathy: heritability, and correlates with sex, neuropsychiatric conditions and brain anatomy

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We conducted a genome-wide meta-analysis of cognitive empathy using the 'Reading the Mind in the Eyes' Test (Eyes Test) in 88,056 Caucasian research participants (44,574 females and 43,482 males) from 23andMe Inc., and an additional 1,497 Caucasian participants (891 females and 606 males) from the Brisbane Longitudinal Twin Study (BLTS). We confirmed a female advantage on the Eyes Test (Cohen's d = 0.21, P < 0.001), and identified a locus in 3p26.1 that is associated with scores on the Eyes Test in females (rs7641347, $P_{meta} = 1.57 \times 10^{-8}$). Common single nucleotide polymorphisms (SNPs) explained 20% of the twin heritability and 5.6% (± 0.76 ; P = 1.72×10^{-13}) of the total trait variance in both sexes. Finally, we identified significant genetic correlation between the Eyes Test and measures of empathy (the Empathy Quotient), openness (NEO-Five Factor Inventory), and different measures of educational attainment and cognitive aptitude, and show that the genetic determinants of striatal volumes (caudate nucleus, putamen, and nucleus accumbens) are positively correlated with the genetic determinants of performance on the Eyes Test.

Cognitive empathy, defined as the ability to recognize what another person is thinking or feeling, and to predict their behaviour based on their mental states, is vital for interpersonal relationships, which in turn is a key contributor of wellbeing. Cognitive empathy is distinct from affective empathy, defined as the drive to respond to another's mental states with an appropriate emotion1. Difficulties in cognitive empathy have been found in different psychiatric conditions, particularly autism[2]. The dissociation between cognitive and affective empathy (the latter often being intact in autism, for example) suggests these have independent biological mechanisms. Little is known about the genetic correlates of cognitive empathy. Here, we investigated the genetic architecture of this aspect of social cognition using a well-validated test, the 'Reading the Mind in the Eyes' Test (Eyes Test). The Eyes Test is a brief online test where participants are shown photographs of the eye regions and have to identify the appropriate emotion or mental state they express[1]. It has been widely used to investigate differences in cognitive empathy in several neuropsychiatric conditions including autism[3], schizophrenia[4], bipolar disorder[5], anorexia nervosa[6], and major depressive disorder[7].

In collaboration with 23andMe, Inc. we conducted three separate genome-wide association studies (GWASes) of the Eyes Test: a males-only GWAS (n = 43,482), a females-only GWAS (n = 44,574**)**, and a non-stratified GWAS (n = 88,056). Study protocol is provided in Figure 1. All participants completed the full version of the Eyes Test online, comprising 36 questions (mean score = 27.47±3.67) (Supplementary Note Section 1). GWAS of the non-stratified and the males-only GWAS did not identify any significant loci. In the

females-only analysis, we identified one locus at 3p26.2 that was significant at a threshold of $P < 5x10^{-8}$. The leading SNP, rs114076548, had $P = 6.49x10^{-9}$. We did not identify any inflation in the P-values due to population stratification using LDSC (intercept = 1.00 ± 0.008).

Insert Figure 1 here

Subsequently, we performed sex-stratified and non-stratified meta-analysis using data from 1,4971 Caucasian participants (891 females and 606 males) from the BLTS[8] who had completed a short version of the Eyes Test comprising 14 questions (Supplementary Note section 1). We did not identify any significant loci in males-only or the non-stratified analyses. However, the females-only meta-analysis identified 21 significant SNPs in 3p26.2 locus (Figure 2), with concordant effect direction for 19 SNPs in the 23andMe and BLTS datasets. rs114076548 had a discordant effect direction between the two datasets and subsequently had a less significant P-value in the meta-analysis than in 23andMe cohort. The leading SNP in the meta-analysis was rs7641347 ($P_{meta} = 1.57 \times 10^{-8}$) and explained 0.067% of the total variance. This SNP was nominally significant in the non-stratified analysis ($P_{meta} = 1.1x10^{-5}$) but non-significant in the males-only analysis ($P_{meta} = 0.4954$). In addition, SNPs in high LD ($r^2 > 0.8$) were also not nominally significant in the males-only analysis. Together, all 21 SNPs span a region of approximately 77kb in 3p26.2 (Supplementary Table 1). The locus is an intergenic region, and the closest genes are Leucine Rich Neuronal 1 (*LRRN1)*, Sulfatase Modifying Factor 1 (*SUMF1)*, and SET Domain And Mariner Transposase Fusion Gene (*SETMAR)*. *LRRN1* is highly expressed in brain tissues[9], with median expression the highest in the putamen, nucleus accumbens and the caudate nucleus, all three of which are part of the striatum. Deletion of 3p26.1 and 3p26.2 can cause developmental delay, hypotonia and epileptic seizures and has been implicated in autism[10]. The most significant SNP in the males-only GWAS meta-analysis (rs4300633 in 16p12.3, $P = 9.11x10^{-8}$) explained 0.062% of the variance, and the most significant SNP in the non-stratified GWAS meta-analysis (rs149662397 in 17q21.32 $P = 1.58x10^{-7}$) explained only 0.029% of the variance. All LD pruned SNPs with $P < 1x10^{-6}$ are provided in Supplementary Table 2. The QQ-plot and locus-zoom plot for the females-only meta-analysis, and the Manhattan and QQ-plots for the males-only and non-stratified analyses are provided in the Supplementary Note section 5. Gene-based analyses MetaXcan[11] for ten neural tissues (Online Methods) and functional enrichment analyses for the non-stratified GWAS did not identify any significant results (Supplementary Tables 3 and 4).

Insert Figure 2 here

We used LD score regression to calculate the heritability explained by all the SNPs in the HapMap3 with minor allele frequency > 5%. We identified a significant narrow sense heritability of 0.056 ± 0.0076 ($P = 1.72x10^{-13}$) in the non-stratified GWAS. We calculated the twin heritability from 749 twin individuals (including 122 complete monozygotic pairs and 176 complete dizygotic pairs) in the BLTS. Heritability, from the best-fitting additive genes/unique environment (AE) model, was 0.28 (95% CI: 0.13–0.42) (Supplementary Note Section 4). All SNPs cumulatively accounted for approximately 20% of the heritability.

We next investigated how the non-stratified Eyes Test is genetically correlated to psychiatric conditions and specific psychological and cognitive traits for which summary GWAS data were available (Supplementary Table 5). After correcting for multiple testing, we identified significant positive genetic correlations between Eyes Test scores and three cognitive traits – self-reported empathy measured using the Empathy Quotient (r_g = 0.18±0.07; P= 0.009), college years (r_g = 0.40±0.6; P= $1.76x10^{-11}$)[12], and the NEO-Five Factor Inventory measure of openness (r_g = 0.50±0.14; P= $3x10^{-4}$)[13]. We were able to confirm the association with education years in a larger cohort of educational attainment (0.34±0.04; P=$3.7x10^{-18}$)[14]. To investigate if this association was independent of word knowledge, we also conducted post-hoc genetic correlation analysis with cognitive aptitude (calculated as Spearman's *g*)[15]. We identified similar genetic correlation (r_g = 0.34±05; P=$6.6x10^{-9}$). A meta-analysis has identified a significant positive correlation between scores on the Eyes Test and IQ (n = 3583; r = 0.24; 95% CI: 0.16 – 0.32)[16]. Other tests of theory of mind are also positively correlated with cognitive aptitude and measures of intelligence[17–19]. In addition, we identified nominally significant positive genetic correlations between the Eyes Test scores and anorexia nervosa (r = 0.24; P = 0.02) (Figure 3). We did not identify a significant genetic correlation between autism and scores on the Eyes Test. Recent research suggests that this may be due to heterogeneity in performance in the Eyes Test, with only a subset of individuals with autism showing impaired performance on the Eyes Test[20]. A recent meta-analysis has reported global or selective deficits in performance on the Eyes Test in individuals with schizophrenia, anorexia, bipolar disorder, and clinical depression but preserved or even enhanced performance for individuals with non-clinical depression and borderline personality disorder[21]. However, these studies are typically conducted in small sample sizes and presence of psychiatric conditions may impair performance on the Eyes Test.

We also investigated if subcortical brain volumes are correlated with performance on the Eyes Test. We used data from the ENIGMA consortium for six subcortical regions and

intracranial volume[22]. We excluded the amygdala, even though it is relevant for social cognition, as the low heritability of the amygdala could not be accurately quantified using LDSC[23]. After correcting for multiple testing, we identified a significant positive correlation between the Eyes Test scores and the volumes of the caudate nucleus[22] (r_g = 0.25±0.09; P= 0.009). We also identified a nominally significant positive correlation between Eyes Test scores and volume of the putamen (r_g = 0.20±0.08; P = 0.019). Imaging studies have identified activation in both the putamen[24] and caudate nucleus[25] on tasks of social cognition. In humans, the ventral striatum is composed of the nucleus accumbens and olfactory tubercle, whereas the dorsal striatum is composed of the caudate nucleus and putamen. There is some evidence to support the role of the striatum in theory of mind[26], though there is no clear consensus that cognitive and affective empathy utilize different neural circuits. Our results suggest that variants that contribute to volumes of regions in the striatum, the dorsal striatum in particular, also contribute to cognitive empathy. It is difficult to delineate the mechanism behind this observed pleiotropy, but we can hypothesize that volumes of striatal structures influence scores on the Eyes Test. All genetic correlations are provided in Supplementary Table 5.

Insert Figure 3 here

We also investigated sex-differences in the Eyes Test. There was a significant female advantage on the scores of the full Eyes Test (males = 27.08±3.75; females = 27.85±3.55; cohen's d = 0.21, $P < 2x10^{-16)}$, replicating previous results[27] (Figure 4). There was no significant difference in males-only or females-only SNP heritability estimates (males = 0.060±0.012, females = 0.0674±0.013; P = 0.715). There was a reasonably high but imperfect genetic correlation between males and females (r_g = 0.683±0.1242; $P = 3.79x10^{-8}$). Binomial sign test of LD-pruned nominally significant SNPs in the sex-stratified analyses identified that 61% (95% CI: 59% - 62%) of the SNPs had a concordant effect direction ($P < 2.2x10^{-16}$). We further investigated the effect direction and statistical significance of all independent SNPs with $P < 1x10^{-6}$. SNPs that were of suggestive significance in one sex were not nominally significant in the other. However, SNPs that were of suggestive significance in the non-stratified GWAS were nominally significant in both the sexes (Supplementary Table 2 and Supplementary Note section 5). Using MetaXcan[11] we identified the top cortically expressed genes (P < 0.05) for both sexes and calculated the overlap in the genes. We did not find any enrichment in gene overlap (Fold difference = 1.2, P = 0.264). We also investigated if there was an enrichment of female-overexpressed or male-overexpressed cortical genes for

the Eyes Test (Online Methods) and did not find any significant enrichment (Supplementary Note section 3, and Supplementary Tables 6 - 8).

Insert Figure 4 here

In conclusion, we identify a genetic locus that is associated with scores on the Eyes Test in females. The closest gene, *LRRN1*, is highly expressed in striatum according to the GTEx database. Phenotypic sex-differences for the Eyes Test may be partly due to different genetic architectures in males and females, interacting with postnatal social experience. We identify significant positive genetic correlations between scores on the Eyes Test and empathy, cognitive aptitude and educational attainment, and openness to experience. We also identify positive correlation between striatal volumes and scores on the Eyes Test.

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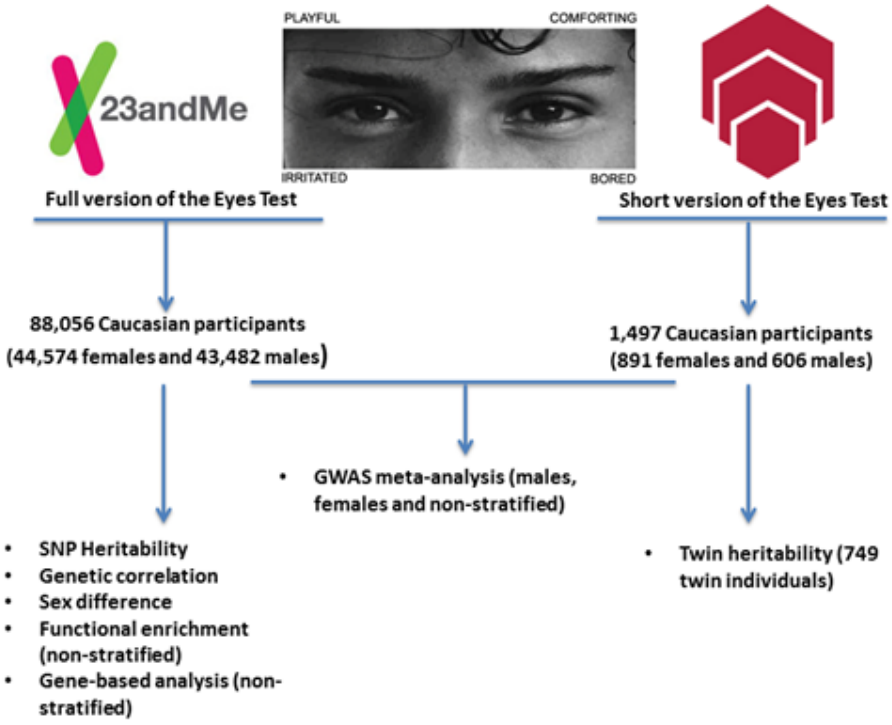
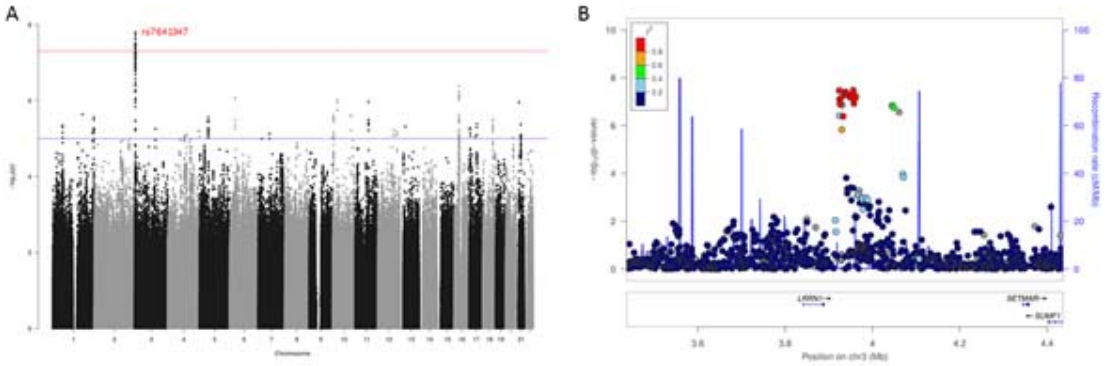
Figure 1: Schematic diagram of the study protocol



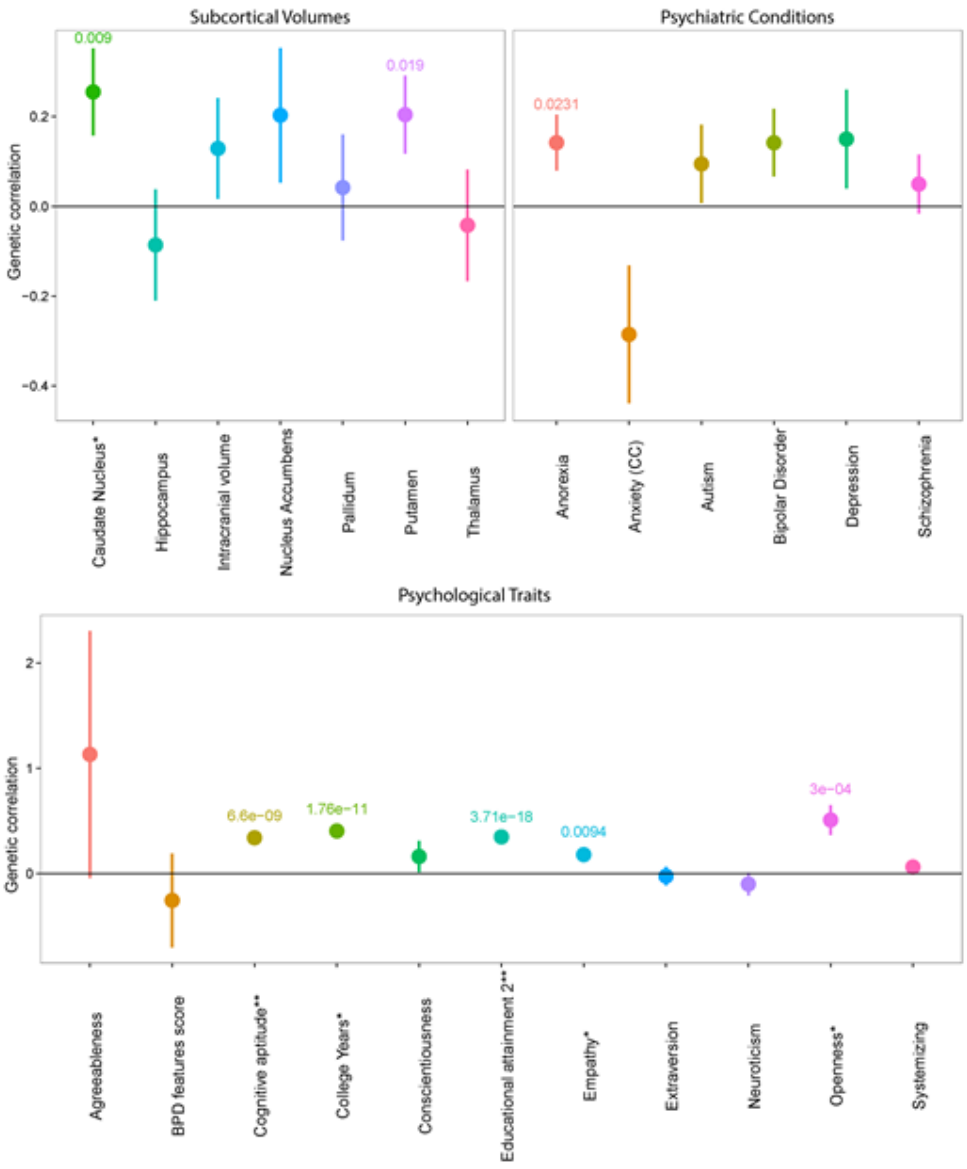
88,056 Caucasian participants from 23andMe, Inc. completed the full version of the Eyes Test and were genotyped. An additional 1,497 Caucasian participants from the Brisbane Longitudinal Twin Study completed the short version (14 questions) of the Eyes Test and genotyped. Genome-wide association meta-analysis was performed on the combined cohort of 89553 participants. Three separate meta-analyses were performed: males-only, females-only, and non-stratified. Subsequently, functional enrichment and gene-based analysis was performed for the non-stratified meta-analysis GWAS using the 23andMe dataset. SNP heritability and genetic correlation using LDSC was performed for the 23andMe GWAS dataset. Sex differences were also investigated using the same dataset. In parallel, twin heritability was calculated from 749 twin individuals from the Brisbane Longitudinal Twin Study who had completed the short version of the Eyes Test.

Figure 2: Manhattan plot and regional association plot for the Eyes Test (females) meta-analysis GWAS



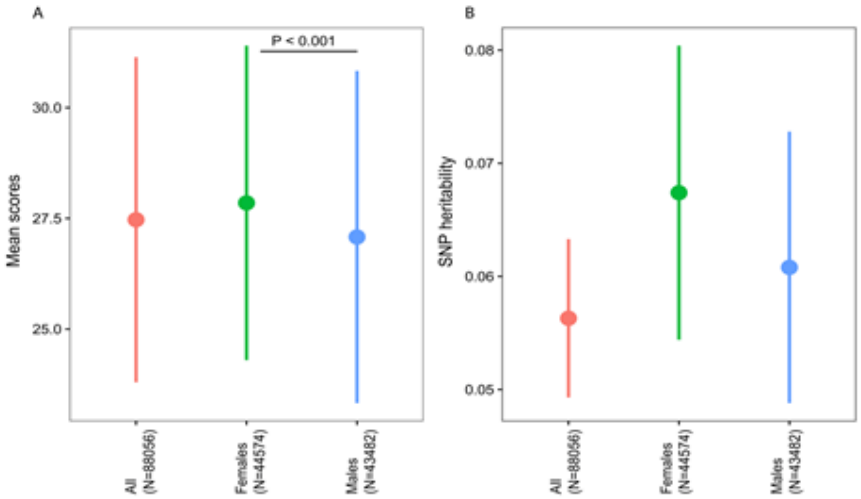
A. Manhattan plot of the Eyes Test meta-analysis (female). X axis is the chromosomal position of the SNP, and Y axis is the negative logarithm of the P-value. The red line indicates genome-wide significant threshold of $5x10^{-8}$. Lead SNP for all loci with $P < 1x10^{-6}$ is provided. n = 44,574, and λ_{gc} = 1.05. LDSC intercept = 1.05. Regional association plot of the significant locus for the Eyes Test (females) meta-analysis.

Figure 3: Genetic correlations between the Eyes Test and psychiatric conditions, psychological traits and subcortical brain volumes



*Genetic correlations and standard errors for the Eyes Test in the 23andMe cohort. All P-values with P < 0.05 provided. * represents significant P-values after FDR correction. ** represents quasi-replication analysis (Educational attainment2 and cognitive aptitude) which were not included in FDR correction. Point estimate represents the genetic correlation, and the error bars represent the standard errors.*

Figure 4: Mean scores and SNP heritability



3a. Mean phenotypic scores and standard deviations for the Eyes Test in the 23andMe cohort. Point estimate provides the mean score, and the error bars represent standard deviations. Difference in mean scores between males and females was highly significant ($P < 0.001$; Cohen's $d = 0.21$). 3b. Mean SNP heritability estimates and standard errors for the Eyes Test in the 23andMe cohort. Point estimate provides mean SNP heritability, and error bar represents standard errors. There was no significant difference in SNP heritability estimates between males and females ($P = 0.715$).