

Association of common genetic variants in GPCPD1 with scaling of visual cortical surface area in humans

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Visual cortical surface area varies two- to threefold between human individuals, is highly heritable, and has been correlated with visual acuity and visual perception. However, it is still largely unknown what specific genetic and environmental factors contribute to normal variation in the area of visual cortex. To identify SNPs associated with the proportional surface area of visual cortex, we performed a genome-wide association study followed by replication in two independent cohorts. We identified one SNP (rs6116869) that replicated in both cohorts and had genome-wide significant association ($P_{\text{combined}} = 3.2 \times 10^{-8}$). Furthermore, a metaanalysis of imputed SNPs in this genomic region identified a more significantly associated SNP (rs238295; $P = 6.5 \times 10^{-9}$) that was in strong linkage disequilibrium with rs6116869. These SNPs are located within 4 kb of the 5' UTR of *GPCPD1*, glycerophosphocholine phosphodiesterase GDE1 homolog (*Saccharomyces cerevisiae*), which in humans, is more highly expressed in occipital cortex compared with the remainder of cortex than 99.9% of genes genome-wide. Based on these findings, we conclude that this common genetic variation contributes to the proportional area of human visual cortex. We suggest that identifying genes that contribute to normal cortical architecture provides a first step to understanding genetic mechanisms that underlie visual perception.

allometry | brain morphometry | imaging genetics | V1

Primates, including humans, rely on vision to navigate their environment, find food, and avoid predators. Visual performance varies between primate species partly because of genetic variation, and better vision may have provided an evolutionary fitness advantage. For example, allelic diversity of visual pigments in the eye evolved convergently in apes, Old World monkeys, and howler monkeys (1) and enabled red–green color discrimination, enhancing these primates' ability to identify sources of food (2).

Visual performance also varies within primate species, such as between human individuals (3), and this performance may be correlated with the number of neurons available to process visual information. Indeed, two studies of healthy human subjects found that increased surface area of primary visual cortex (V1) and thus, more neurons in V1 (4) were associated with increased Vernier acuity (5) and decreased susceptibility to two optical illusions (6). It is striking that visual cortical surface area is associated with optical illusion strength, because this result implies

that the number of neurons in V1 can explain human variation in the conscious perception of seemingly physically identical stimuli.

Individuals have highly variable portions of their brains devoted to visual processing, because the surface areas of visual cortical regions (e.g., V1, V2, and V3 in the occipital lobe) are correlated and vary two- to threefold in humans (7, 8). This variation is significantly greater than variation in total cortical area, and therefore, both the absolute area and proportion of the cortical sheet allocated to processing vision varies between individuals. Moreover, a recent human twin study showed strong genetic correlations between the area of V1 and the remainder of occipital cortex but not other cortical lobes (9), suggesting that occipital visual areas share common genetic influences. However, it is still largely unknown what specific genetic and environmental factors contribute to normal variation in the absolute and proportional size of occipital cortex. To address this question, we performed a genome-wide association study (GWAS) to identify SNPs associated with the proportional surface area of occipital cortex in two independent human cohorts.

Human twin studies have shown a significant genetic component to cortical volume (10–12) and surface area (>80% heritable) (13–16), and the occipital proportion of cortex is also quite

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heritable (25–50%) (11, 13). These studies reveal that genes have both global and regional effects on cortical surface area, which also seems to be true in mice. For example, the work by Airey et al. (17) reported that two strains of inbred mice with different genetic backgrounds have different proportions of cortex allocated to primary visual and somatosensory cortex, and these strains can be reliably discriminated based on these regional as well as global measures of cortical surface area.

Furthermore, specific homeobox transcription factors (e.g., *EMX2* and *PAX6*) have been identified that are expressed in gradients across the surface of the mouse brain during neural development and control the anterior–posterior distribution of cortical areas (18, 19). Cortex-specific overexpression of *EMX2* in mice resulted in an expansion of occipital areas and a corresponding reduction in sensory and motor areas that led to dysfunctional tactile and motor behaviors (20). Similarly, *EMX2* and *PAX6* may be expressed in gradients during neural development in human subjects (21), and individuals with protein coding mutations in *PAX6* (22, 23) exhibit cortical malformations. In addition, *EMX2* mutations have been associated with schizencephaly, a rare cortical developmental disorder (24, 25), although these mutations likely explain a small fraction of this disease burden (26, 27). Finally, mutations in the laminin gene *LAMC3* have been associated with cortical malformations solely within the occipital lobe (28), providing additional evidence for genetic control over regional cortical development.

Genetic variants may also mediate more subtle variation in human cortical structure. For example, two candidate gene studies recently identified SNPs in microcephaly genes (29) and *MECP2* (30) that explained a small but statistically significant amount of variation in total cortical surface area between human individuals and were replicated in independent study populations.

In this study, we extend the analysis of the datasets used in those studies in two ways. First, we performed an unbiased GWAS rather than selecting candidate genes to identify genetic loci that contribute to normal variation in human cortical structure. Second, we analyzed the scaling of occipital cortical surface area with total cortical area because of the evidence from mice and human twin studies that this scaling relationship may be under independent genetic control from overall brain size.

Results

In a sample of 421 human subjects with Norwegian ancestry from the Thematic Organized Psychoses (TOP) study, we found that occipital cortical surface area is highly correlated with total cortical area ($r = 0.85$, $P = 3.1 \times 10^{-118}$). The occipital cortex occupied, on average, 12.1% of total cortical surface area regardless of brain size, and the occipital fraction of cortex ranged from 11.1% to 14.8% in all subjects. We hypothesized that this variation was partly because of genetic differences between subjects. Therefore, we tested SNPs genome-wide for their effect on the scaling relationship between occipital and total cortical surface area. Specifically, we tested each SNP in a GWAS for the strength of the interaction between the SNP minor allele and total cortical surface area in predicting occipital cortical surface area, while controlling for sex, age, and diagnosis. This interaction effect would reflect the degree to which the SNP accentuated the influence of overall cortical surface area on occipital cortical surface area (i.e., modified the scaling relationship between total and occipital cortical surface area).

One SNP (rs6116869; minor allele frequency = 0.36) showed strong interaction association ($P = 7.75 \times 10^{-8}$, $\beta = 0.0285$, $SE = 0.0052$, $n = 413$) with occipital cortical surface area, although this SNP did not quite reach genome-wide significance ($P < 5 \times 10^{-8}$). A quantile–quantile plot of $-\log_{10}(P)$ values from the GWAS revealed moderate genomic inflation ($\lambda_{GC} = 1.25$), and this inflation suggested that the original SNP P value may have been artificially low. Thus, we sought a more accurate estimate of

the P value in two ways: (i) accounting for genetic relatedness between subjects and (ii) permutation testing. In addition, we tested the SNP for association in two independent replication cohorts and interaction association with surface area across the whole cortex. Finally, we investigated the cortical expression pattern of *GPCPDI*, a nearby gene, in two human brains.

First, despite the fact that subjects were unrelated and self-reported Norwegian ancestry, we hypothesized that subtle population structure or cryptic relatedness could have compromised the statistical independence of subjects. A lack of independence would have decreased SNP variance estimates and associated P values, resulting in genomic inflation. We approximated population structure in our study by using principle components analysis (PCA) to estimate major axes of variation of genome-wide allele frequencies. We repeated the GWAS and controlled for sex, age, and diagnosis as well as population structure along the first four axes from the PCA, which has been shown to help correct for population stratification (31). We found that genomic inflation was slightly reduced ($\lambda_{GC} = 1.23$), and rs6116869 association was now genome-wide significant ($P = 4.95 \times 10^{-8}$, $\beta = 0.0289$, $SE = 0.0052$). We performed genomic control, and rs6116869 still showed markedly stronger association ($P_{GC} = 8.79 \times 10^{-7}$, $\beta = 0.0289$, $SE_{GC} = 0.0058$) than other SNPs (Fig. 1 and Figs. S1 and S2).

Next, we used permutation tests to estimate the significance of rs6116869 association, and the permuted P value ($P_{perm} = 6 \times 10^{-7}$) supported the genomic control P value, suggesting that genomic control had effectively corrected for inflation. Thus, for the remainder of the analysis, we report the conservative genomic control SEs and P values.

Given the significant interaction effect between rs6116869 and total cortical area, we expected that the slope of the linear regression that related occipital to total cortical surface area would vary based on rs6116869 genotype. Indeed, we found that each copy of the SNP minor allele increased the slope by 28% (Fig. 2A). On average, the occipital cortex of subjects heterozygous for rs6116869 (GT genotype; $n = 184$) occupied 12.7% of total cortical surface area, and this occipital proportion was independent of total cortical area (Fig. 2B). In contrast, subjects homozygous for the major allele (GG; $n = 171$) had an occipital proportion that decreased from 13.2% to 12.6%, on average, over the range of total cortical areas observed in our study. Inversely, subjects homozygous for the minor allele (TT; $n = 58$)

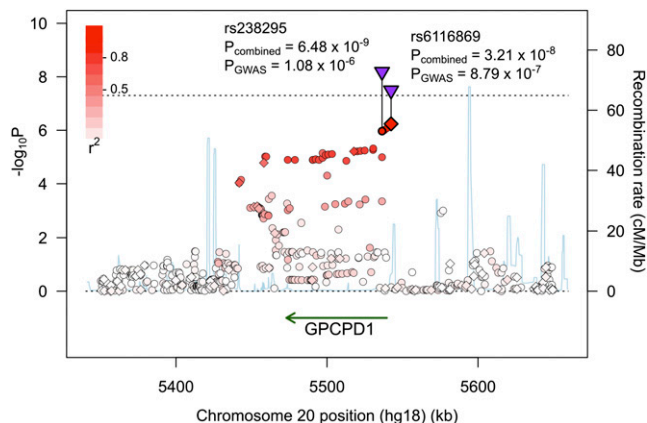


Fig. 1. Genomic region showing the strongest SNP association with occipital cortical area scaling. Genotyped (\diamond) and imputed (\circ) SNPs are colored based on linkage disequilibrium (r^2) with rs6116869. Combined P values for rs6116869 and rs238295 (\blacktriangleright) are genome-wide significant ($P < 5 \times 10^{-8}$, dotted line) based on a metaanalysis of the GWAS and two replication studies. rs238288 is not labeled and is located in between these two SNPs. P values are corrected for genomic inflation.

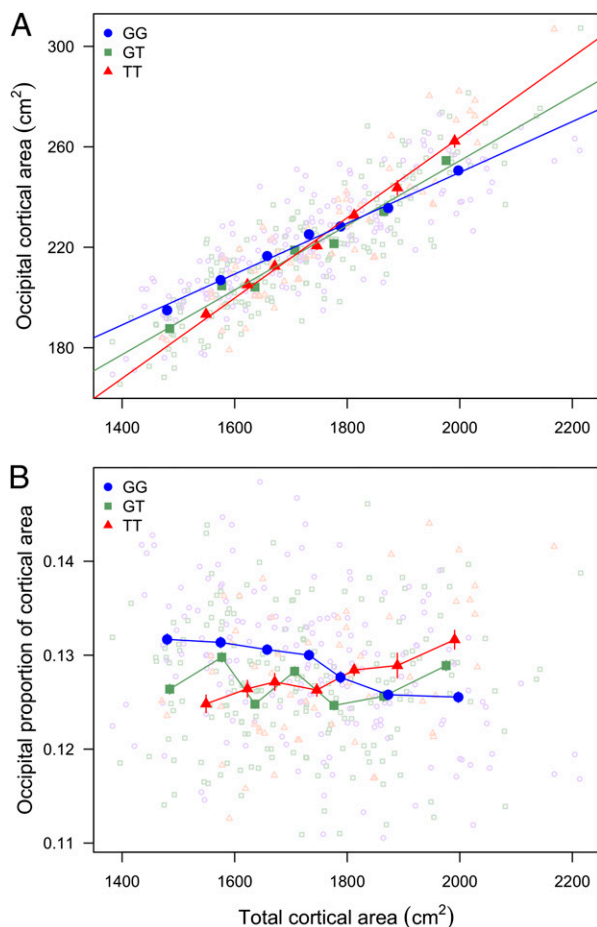


Fig. 2. Occipital cortical area scaling varies by rs6116869 genotype. (A) The slope of occipital cortical area scaling with total cortical area increases with the number of minor alleles. For each genotype, subjects are grouped into seven bins with an equal number of subjects in each bin (24, 26, and 8 subjects per bin for genotypes GG, GT, and TT, respectively). Binned averages \pm SEM (dark) and regression lines fit to individual (light) measures are plotted. (B) Occipital proportion of cortex varies based on total cortical area and genotype. Subjects are binned as in A, and occipital proportions (occipital area divided by total cortical area) are plotted for individuals (light). Binned averages \pm SEM (dark) are indicated.

had an occipital proportion that increased from 12.5% to 13.2% over this same range. These 0.6% differences in the occipital proportion of cortex based on rs6116869 genotype represented a difference in absolute occipital cortical area of ~ 11 cm², equal to almost one-half the area of V1 (6). Therefore, for example, in the subset of subjects with relatively large total cortical surface area ($\sim 2,000$ cm²), subjects with the GG genotype had an occipital cortical area of 251 cm², whereas subjects with the TT genotype had an occipital cortical area of 262 cm².

We sought to replicate the rs6116869 association with occipital cortical area in two independent cohorts. First, we used 482 subjects—healthy controls or diagnosed with mild cognitive impairment (MCI)—from the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset who clustered with European reference populations based on genome-wide genotype data. rs238288 was the closest proxy for rs6116869 that was genotyped in the ADNI sample, and this SNP was highly correlated ($r^2 = 0.96$ in HapMap CEU population with northwestern European ancestry) with and 2.5 kb upstream from rs6116869. We tested rs238288 for a significant interaction with total cortical surface area in predicting occipital cortical surface area with the identical test used in the

GWAS (i.e., controlling for sex, age, diagnosis, and population structure). We found modest genomic inflation ($\lambda_{GC} = 1.19$) (Fig. S3) in the ADNI study that was comparable with the inflation observed in the TOP study. rs238288 was significantly associated before (one-tailed $P = 0.0083$, $\beta = 0.0123$, SE = 0.0051, $n = 477$) and after ($P_{GC} = 0.015$) genomic control.

For our second replication cohort, we selected 278 subjects (aged 6–21 y old) from the Pediatric Imaging, Neurocognition, and Genetics (PING) study who clustered with European reference populations based on genome-wide genotype data. We excluded PING subjects younger than 6 y, because brain volume increases more than fourfold after birth and then, is mostly stable from age 6 y to adulthood (32). Likewise, cortical surface area decreases less than 10% during adolescence between the ages of 6 and 22 y (33), and therefore, we expected that the effect of genetic variation on the scaling of occipital cortical area would have occurred earlier in development and would be apparent in this younger replication cohort. Indeed, we found that rs238288 (the closest proxy for rs6116869) provided a second replication of the GWAS result (one-tailed $P = 0.018$, $\beta = 0.0208$, SE = 0.0098, $n = 278$). No genomic inflation was observed in this dataset ($\lambda_{GC} = 1.00$). The combined P value for rs6116869 from the GWAS and two replication studies was genome-wide significant ($P_{\text{combined}} = 3.21 \times 10^{-8}$) based on an inverse variance-weighted z score (34).

To refine the association of this genetic locus with occipital cortical area scaling, we imputed SNPs for the TOP, ADNI, and PING samples in a 300-kb window around rs6116869 and performed a metaanalysis of these SNPs. We combined the genomic control P values for the imputed SNPs from the three studies (Fig. 1), and we identified the SNP (rs238295) that was most significantly associated ($P_{\text{combined}} = 6.48 \times 10^{-9}$). rs238295 was highly correlated with and proximal to both rs6116869 ($r^2 = 0.88$, 6 kb upstream) and rs238288 ($r^2 = 0.84$, 3.5 kb upstream).

Given the finite extent of the cortex, we expected that a relative increase in occipital cortical surface area would be associated with a compensatory decrease in surface area in other cortical regions. Therefore, we performed a region of interest analysis in the TOP, ADNI, and PING samples and tested rs238295 for association with 66 regions defined by cortical folding patterns. For each cortical region, we calculated the genomic inflation adjusted P value for the interaction between rs238295 and total cortical surface area, and we combined these P values from the three studies (Table S1). As expected, we found that rs238295 was strongly associated with the surface area of occipital cortical regions, including the bilateral pericalcarine area that is highly correlated with the V1 area (7) as well as lingual and lateral occipital areas. Intriguingly, rs238295 was also significantly associated with left superior and lateral temporal cortical areas in all three studies but in the opposite direction relative to the association with occipital cortical area. Thus, individuals homozygous at rs238295 with a relatively large occipital cortex had a relatively small left temporal cortex and vice versa.

To better visualize the SNP association with different cortical regions in the TOP cohort, we tested the interaction association of rs6116869 with total cortical area at each location across the cortical surface. A cortical map of $-\log_{10}(P \text{ values})$ broadly supported the region of interest analysis and also highlighted significant SNP associations with bilateral superior frontal cortical regions in the TOP study (Fig. 3).

The genotyped SNP that was most significantly associated with the occipital cortical area in the TOP sample (rs6116869) is located ~ 3 kb upstream of the protein coding gene *GPCPDI*, glycerophosphocholine phosphodiesterase GDE1 homolog (*Saccharomyces cerevisiae*), and the most significant imputed SNP in the combined analysis of both TOP and ADNI samples (rs238295) is located 6 kb downstream in the first intron of *GPCPDI*. These SNPs are at the end of a 100-kb linkage disequilibrium block

increased the metabolic efficiency of neurons or glia, then neurons could support larger axonal and dendritic arbors and thus, potentially, a larger visual cortex. Future work will need to establish the role of *GPCPD1* in human brain development, aging, and pathology.

In summary, we predict that rs238295 (or a closely linked functional variant) regulates expression of *GPCPD1* because of its close proximity to the 5' UTR of this gene. The timing and location in the brain of this differential expression as well as the mechanism by which this gene influences visual cortical surface area remain to be elucidated. Understanding the role of genes that contribute to normal cortical architecture in humans is an important step to understanding the genetic mechanisms of visual perception and ultimately, cortical pathology in a host of heritable neuropsychiatric disorders.

Materials and Methods

TOP Subjects. Four hundred and twenty-one subjects from the TOP study were analyzed. These subjects included 181 controls, 94 subjects with schizophrenia spectrum disorder, 97 subjects with bipolar spectrum disorder, and 49 subjects diagnosed with major depressive disorder or psychotic disorder not otherwise specified; 48.7% of the subjects were women, and the subjects were aged 35 ± 10 y (range = 18–65 y).

Genotyping. DNA was genotyped on the Affymetrix 6.0 array as previously reported (52, 53), and 597,198 SNPs passed quality control filters (SNP call rate > 95%, minor allele frequency > 5%, Hardy–Weinberg disequilibrium $P < 1 \times 10^{-6}$) and were merged with HapMap 3 reference populations. All subjects self-reported Norwegian ancestry, and PCA of an allele-sharing distance matrix across all subjects did not suggest any non-European ancestry genetic outliers.

Brain imaging. MRI scans were performed with a 1.5 T Siemens Magnetom Sonata scanner equipped with a standard head coil. Acquisition parameters were optimized for increased gray/white matter image contrast. More details are in *SI Materials and Methods* and the work by Rimol et al. (51).

ADNI Subjects. Data used in the preparation of this article were obtained from the ADNI database (<http://www.loni.ucla.edu/ADNI/>); 482 subjects who self-reported as white and non-Hispanic included 180 controls and 302 individuals with MCI (39.2% women; aged 75.3 ± 6.6 y). We included ADNI subjects with MCI but not Alzheimer's disease from the replication sample in an attempt to balance the increased power that resulted from having a larger sample size with the increased noise caused by the pathological changes in cortical surface area that have been associated with these neurological disorders.

Genotyping. DNA was genotyped with the Illumina Human610-Quad Bead-Chip, and 514,073 SNPs passed quality control filters (SNP call rate > 95%, minor allele frequency > 5%, Hardy–Weinberg disequilibrium $P < 1 \times 10^{-6}$) and were merged with 34 European reference populations. PCA of an allele-sharing distance matrix was used to remove three individuals as non-European ancestry genetic outliers.

Imaging. MRI data were collected on 1.5-T scanners at many study centers across the United States. The Laboratory of Neuro Imaging (LONI) website (<http://www.loni.ucla.edu/ADNI/Research/Cores/index.shtml>) describes specific protocols. Raw digital imaging and communications in medicine MR images were downloaded from the ADNI data page of the public ADNI site at the LONI website (<http://www.loni.ucla.edu/ADNI/Data/index.shtml>) published in 2007.

PING Subjects. Data used in the preparation of this article were obtained from the PING database (<http://ping.chd.ucsd.edu/>); 278 subjects were included aged 14.2 ± 4.1 y (range = 6–21 y), and 47.8% of subjects were female.

Genotyping. DNA was genotyped with the Illumina Human660W-Quad BeadChip, and 494,082 SNPs passed quality control filters (sample call rate > 98%, SNP call rate > 95%, minor allele frequency > 5%, Hardy–Weinberg disequilibrium $P < 1 \times 10^{-6}$) and were merged with Hapmap European reference populations; 599 individuals were removed as genetic outliers based on PCA of an allele-sharing distance matrix. Additionally, 157 of the remaining subjects were removed, because they shared greater than 10% of alleles identical by descent with another subject.

Imaging. T1-weighted MRI data were collected on 3-T scanners at nine study centers across the United States. Specific MRI scanner protocols are available at the PING study website (<http://ping.chd.ucsd.edu/>).

Genotype Imputation. TOP, ADNI, and PING genotypes were independently merged with the HapMap CEU reference population, which also included genetic variant information from the sequencing by the 1,000 Genomes Project. MACH 1.0 was used to impute genotypes with the default settings, and only SNPs that passed imputation quality control ($R > 0.5$) were included for additional analysis.

Cortical Area Measurements. MRI scans were analyzed with software developed at the University of California at San Diego Multi-Modal Imaging Laboratory based on the freely available FreeSurfer software package (<http://freesurfer-software.org/>). Using cortical surface reconstruction and spherical atlas mapping procedures developed in the works by Dale et al. (52) and Fischl et al. (53), we mapped each individual's surface reconstruction into atlas space based on cortical folding patterns. Cortical folds are good predictors of the locations of functionally distinct regions (53). For example, there is close agreement between anatomical extent of primary visual cortex based on cortical folding patterns, functional MRI, and ex vivo cytoarchitecture (54).

Statistics. We tested each SNP for association using PLINK (55) to fit an additive linear model with minor allele count, sex, age, diagnosis, total cortical surface area, and a minor allele count by total cortical area interaction term as predictors of occipital cortical surface area. Genomic inflation (λ_{GC}) was estimated in the standard way by dividing the median observed χ^2 statistic from the GWAS by 0.456, the approximate median of a χ^2 distribution with one degree of freedom (56).

The permuted P value of the top SNP was calculated by shuffling subject labels ($n = 10^8$ permutations), recalculating the SNP interaction P values, and calculating the fraction of permutations that showed a more significant association than the P value derived from the original dataset. P values reported for the replication datasets are one-tailed, because we tested for an SNP effect in the same direction as in the original GWAS. In a meta-analysis of TOP, ADNI, and PING datasets, P values were combined based on inverse variance weighted z scores (34) calculated from the β -coefficients and genomic inflation-adjusted SEs (SE_{GC}). An association plot of combined P values was created using the SNAP plot online tool from the Broad Institute (57).

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