Title: Genetic control of variability in subcortical and intracranial volumes

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Abstract

Sensitivity to external demands is essential for adaptation to dynamic environments, but comes at the cost of increased risk of adverse outcomes when facing poor environmental conditions. Here, we apply a novel methodology to perform genome-wide association analysis of mean and variance in ten key brain features (accumbens, amygdala, caudate, hippocampus, pallidum, putamen, thalamus, intracranial volume, cortical surface area and cortical thickness), integrating genetic and neuroanatomical data from a large lifespan sample (n=25,575 individuals; 8 to 89 years, mean age 51.9 years). We identify genetic loci associated with phenotypic variability in thalamus volume and cortical thickness. The variance-controlling loci involved genes with a documented role in brain and mental health and were not associated with the mean anatomical volumes. This proof-of-principle of the hypothesis of a genetic regulation of brain volume variability contributes to establishing the genetic basis of phenotypic variance (i.e., heritability), allows identifying different degrees of brain robustness across individuals, and opens new research avenues in the search for mechanisms controlling brain and mental health.

Introduction

Phenotypic variability is key in evolution, and partly reflects inter-individual differences in sensitivity to the environment [1]. Genetic studies of human neuroanatomy have identified shifts in mean phenotype distributions (e.g., mean brain volumes) between groups of individuals with different genotypes [2], and have documented genetic overlaps with common brain and mental disorders [3]. Despite the evolutionary relevance of phenotypic dispersion evidenced in multiple species and traits [1,4], the genetic architecture of variability in human brain morphology is elusive.

Phenotypic variance across genotypes can be interpreted in relation to robustness, i.e., the persistence of a system under perturbations [1,4] and evolvability, the capacity for adaptive evolution [5]. High phenotypic robustness is indicated by low variation in face of perturbations, i.e. phenotypes are strongly determined by a given genotype. In contrast, lack of robustness corresponds to high sensitivity, yielding phenotypes with overall larger deviations from the population mean in response to environmental, genetic or stochastic developmental factors. Neither increased or decreased robustness confers evolutionary advantages *per se* [1], and their consequences for adaptation need to be understood in view of the genotype-environment congruence. Reduced robustness (and thus increased variability of trait expression) can be a conducive to adaptive change [5], and increased variability of phenotypic expression can in itself also be favored by natural selection in fluctuating environments [6]. Thus, recognizing genetic markers of sensitivity can aid in identifying individuals who are more susceptible to show negative outcomes when exposed to adverse factors –either genetic or environmental– and otherwise optimal outcomes in the presence of favorable factors. Such variance-controlling genotypes may be conceived as genomic hotspots for gene-environment and/or gene-gene

interactions, with high relevance for future genetic epidemiology studies [7]. Data from this type of approaches may complement existing genetic association methods and contribute to establish the genetic basis of phenotypic variance (i.e., heritability), allow identifying different levels of brain robustness across subjects, and open new research avenues in the search for mechanisms controlling brain and mental health.

To provide a proof-of-principle of the hypothesis of a genetic regulation of brain volume variability, we conducted a genome-wide association study of intragenotypic variability in seven key subcortical regions and intracranial volume (ICV) using a harmonized genotype and imaging data analysis protocol in a lifespan sample (n=25,575 individuals; 8 to 89 years, mean age 51.9 years; 48% male, Methods and Supplementary Table S1 and Supplementary Table S2).

Materials and Methods

Participants

Data from 25,575 unrelated European-ancestry individuals were included (mean age 51.9 years, ranging from 8 to 89 years old; 48% male), recruited through 16 independent cohorts with available genome-wide genotyping and T1-weighted structural MRI. Extended information on each cohort reported in Supplementary Methods and Supplementary Table 1 includes recruitment center, genotyping and brain imaging data collection, sample-specific demographics, distribution of brain volumes and, when relevant, diagnoses (795 individuals had a diagnosis). Written informed consent was provided by the participants at each recruitment center, and the protocols were approved by the corresponding Institutional Review Boards.

Genotypes

Only participants with European ancestry (as determined by multidimensional scaling) were included in the final set of analyses, in recognition that the inclusion of subjects from other ethnicities can potentially add genetic and phenotypic confounding. Except for the UK Biobank cohort, all directly genotyped data were imputed in-house using standard methods with the 1000 Genomes European reference panel. After imputation, each genotyping batch underwent a quality control stage (MAF < 0.01; Hardy-Weinberg equilibrium $p < 10^{-6}$; INFO score < 0.8). When all samples were combined, over 5 million distinct markers passed quality control genome-wide. Additional filters on genotyping frequencies were applied to the final merged dataset based on statistical considerations for genotype frequency in variance-controlling detection, as described below. Genetic data analysis was conducted using PLINK [8], with R [9] plugin functions when appropriate (https://www.cog-genomics.org/plink/1.9/rserve).

Brain features

Three-dimensional T1-weighted brain scans were processed using FreeSurfer [10] (v5.3.0; http://surfer.nmr.mgh.harvard.edu). Mean cortical thickness and eight well-studied volumetric features were selected for analysis moving forward, as literature findings on large datasets show that their mean population value is influenced by common genetic variation [2]: accumbens, amygdala, caudate, hippocampus, pallidum, putamen, thalamus and ICV. Cohort-wise distribution of values is summarized in Supplementary Figure S1 and Supplementary Figure S2. Generalized additive models (GAM) were implemented in R (https://www.r-project.org) to regress out the effects of scanning site, sex, age, diagnosis and ICV (for subcortical volumes and cortical surface only), and outliers (±6 standard deviations from the mean) were removed. Hereafter, brain volumes correspond to residuals from those GAM fits unless otherwise specified. As noticed on violin plots of brain features stratified by sample (Supplementary Figure S1), some of the traits display a wider distribution in the UK Biobank than in the other cohorts, which may be related to a broader phenotypic repertoire captured by a much larger sample size; in addition, at each cohort, individuals on the very ends of the distributions might have been considered outliers/anomalous, and were probably removed at each imaging center during early quality control stages.

Statistical analyses

Genome-wide association statistics were computed for genetic effects on the mean and variance of the volumetric feature distributions in multiple stages. The data were analyzed and visualized in R with the aid of appropriate packages. Although the current dataset includes subjects across a

broad range of ages, the cross-sectional nature of the data (one image per individual) makes the

main focus of the models below the analysis of gene-gene (epistasis) and gene-environment

interactions. For each marker, an additive genetic model was computed with

$$y = \beta_0 + \beta_1 \text{sample} + \beta_2 C_1 + \beta_3 C_2 + \beta_4 C_3 + \beta_5 C_4 + \beta_6 \text{SNP} + \varepsilon$$

where y is the normalized phenotype variable; SNP is the relevant marker coded additively and ε

stands for regression residuals. Four genomic principal components (C_1-C_4) were included, to

control for population stratification and cryptic relatedness, and to make the results

consistent/comparable with a previous large-scale analysis of genetic variation and brain

volumes [2]. Then, the distribution of residuals (ε) was normalized via rank-based inverse normal

transformation (INT) to prevent statistical artifacts. Scale transformations like INT have been

shown to aid genetic discovery by constraining mean-effects and reducing the effect of

phenotypic outliers, which reduces Type I error rates without sacrificing power [11, 12].

Specifically, INT was applied to transform each subject's residualized phenotype (ε_i) as

$$\text{INT}(\varepsilon_i) = \phi^{-1}\left[\frac{\text{rank}(\varepsilon_i) - 0.5}{n}\right]$$

where $\text{rank}(\varepsilon_i)$ is the rank within the distribution, n stands for sample size (without missing

values) and ϕ^{-1} denotes the standard normal quantile function. Intuitively, all phenotype values

are ranked and the ranks are mapped to percentiles of a normal distribution. Those INT-

transformed residuals were passed as input for the variance-model using Levene's test centered

at the median, implemented with R's car package (https://cran.r-project.org/package=car). In

short, $z_{ij} = |\varepsilon_{ij} - \tilde{\varepsilon}_j|$ is calculated from the transformed residuals, with $\tilde{\varepsilon}_j$ as the median of

group j (here, genotype) and these, in turn, to compute the F statistic:

$$F = \left(\frac{N-p}{p-1}\right) \frac{\sum_{j=1}^{p} n_j (\bar{z}_{.j} - \bar{z}_{..})^2}{\sum_{j=1}^{p} \sum_{i=1}^{n_j} (\bar{z}_{ij} - \bar{z}_{.j})^2},$$

where n_j is the number of observations in group j, p is the number of groups (2 or 3 different genotypes), and $\bar{z}_{.j}$ denotes the mean in group j. For potentially significant associations, between-sample differences in minor allele frequencies were assessed, to avoid spurious results due to issues with genotype imputation. Also, to prevent increases in false positive rates arising from small groups [13], only markers with at minimum (non-zero) genotype count of at least 100 were included. This value was chosen based on literature about power and statistical considerations of genome-wide association studies for phenotypic variability [13].

An additional validation stage was implemented in recognition that, despite the conservativeness of the previous approach, variables like sex and batch might still confound the variance test results [14, 15]. Generalized additive models for location scale and shape (GAMLSS) were applied to the top significant results from Levene's tests. Variants were selected for additional inspection based on their *p*-values in the variance analysis, using linkage disequilibrium-based clumping with default parameters on PLINK (Levene's test p-values below 1×10^{-4} over 250kb windows and an LD threshold of $r^2 = 0.5$, http://www.cog-genomics.org/plink/1.9/postproc#clump). As indicated next, this resulted in 1,799 genotype-phenotype associations that were further examined via GAMLSS. For consistency with Levene's test (which does not have a pre-specified inheritance model), GAMLSS tests were evaluated under 4 relevant paradigms: additive scale (A1 allele coded as continuous: 0, 1 or 2), dominant-like (0 copies of A1 allele vs either 1 or 2 copies), recessive-like (2 copies of A1 allele vs either 1 or 0 copies) and heterozygote (dis)advantage-like (1 copy of A1 vs either 0 or 2 copies). This resulted in 1,799 times 4 = 7,196 tests.

Briefly, GAMLSS are semi-parametric regression type models that allow the parameters of the distribution of the response variable (mean, variance, skewness and kurtosis) to be

modelled as linear/non-linear or smooth functions of the explanatory variables [16]. Here, mean

and dispersion in the distribution of raw brain phenotypes (as extracted from FreeSurfer) were

modeled as a function of genotype, sex, scanner, diagnose, ICV (when relevant) and an

additional P-spline term for age as function of sex, through penalized varying coefficients with

10 intervals [16, 17]. GAMLSS were fitted through a normal distribution with the default *identity*

link for location (μ) and *log* link for scale (σ). Unless otherwise specified, all other parameters

were set to default from R's GAMLSS implementation. It is worth noting that Levene's test was

here adopted as main discovery strategy as it provides some advantages over the GAMLSS' R

implementation, e.g.: GAMLSS are computationally more expensive (from seconds to minutes

per marker-phenotype test), and do not allow different inheritance models to be directly tested in

the same run.

Results

Genome-wide association statistics were computed for genetic effects on the variance and mean of the volumetric feature distributions. Consistent with previous large-scale analyses on genetics of neuroimaging volumetric measures [2, 18], features included bilateral (sum of left and right) amygdala, caudate nucleus, hippocampus, nucleus accumbens, pallidum, putamen and thalamus, as well as ICV and mean cortical thickness. 96.9% of the included participants were healthy controls (n=24,780); the remaining 3.1% were diagnosed with a brain disorder (n=795; including psychosis, depression, and attention deficit hyperactivity disorder, Supplementary Table S2). The analyses were conducted in a two-stage protocol. For each genotype, we conducted a standard association test for the GAM-transformed brain volumes [11], adjusting for scanning site, sex, age, diagnosis, and ICV (for the subcortical volumes only). Residuals from that model were then INT-transformed and submitted to genome-wide Levene's tests to investigate if specific alleles associate with elevated or reduced levels of phenotypic variability. For relevant markers, variances explained by mean and variance models were estimated from the INT-transformed volumes before fitting regression models using a previously reported approach [7].

A mega-analysis of 25,575 unrelated subjects of European ancestry identified candidate loci associated with differential levels of phenotypic variability overall on two out of the ten volumetric features (mean thickness and thalamus) (Figure 1). A loci displayed an association with Pallidum volume (Supplementary Figure S3) but was discarded as between-cohort allelic frequencies showed significant differences, indicating potential uncertainty in genotype imputation. Genomic inflation factors (lambda) ranged between 1.01 and 1.07 for the ten different variance-GWAS (Supplementary Figure S3). A conventional mean phenotype GWAS with additive model on the same set of variants, with INT-transformed phenotypes, showed 70

significant loci influencing four volumetric traits after adjustment for genomic inflation (accumbens [2], amygdala [5], caudate [10], hippocampus [12], pallidum [7], putamen [6], thalamus [4], ICV [9], cortical surface area [7] and mean cortical thickness [8]) (Supplementary Figure S3). Manhattan plots for both mean- and variance-GWAS are displayed as Supplementary Figure S3.

[Insert Figure 1]

In the variance analysis, the top association was a locus in chromosome 20 between *SNAP25*, *PAK7* and *ANKEF1*, linked to mean cortical thickness variability (rs6039642; chr20:9940475:G:A; MAF=0.17; p=2.3×10^{-8}; variance explained variance model: 0.129%; variance explained mean model: 0.003%). In addition, an intergenic locus near *LINC00347* showed a borderline significant association with variance in thalamus volumes (rs9543733; chr13:75211673:C:T; MAF=0.34; p=6.8×10^{-8}; variance explained mean model: 0.002%; variance explained variance model: 0.054%) (Figure 1 and Supplementary Figure S4). Between cohorts, no clear differences in allelic frequencies were observed at these loci (MAF between 0.15 and 0.199 at rs6039642, and MAF between 0.35 and 0.37 at rs9543733). Results were consistent when re-analyzing the data from healthy controls only (excluding participants with neuropsychiatric diagnoses), and when validating via GAMLSS: p=6.8×10^{-5} (rs6039642-cortical thickness), p=4.8×10^{-4} (rs9543733-thalamus). Males displayed higher variability than females in thalamus volumes according to those GAMLSS models (B=0.047, SE=0.011, p=1.9×10^{-5}), and a trend-significant association for higher variability in cortical thickness (B=0.036, SE=0.021, p=0.082). Figure 2 shows the relevant phenotype distributions for the top hits for the two models

grouped by genotypes generated via the shift function [19]. In short, the adopted shift function procedure was implemented in three stages: deciles of two phenotype distributions were calculated using the Harrell-Davis quantile estimator, followed by the computation of 95% confidence intervals of decile differences with bootstrap estimation of deciles' standard error, and multiple comparison control so that the type I error rate remained close to 5% across the nine confidence intervals. Decile-by-decile shift function analysis confirmed increased thalamus variance among TC heterozygotes than rs9543733 homozygotes (TT, CC). Similarly, major allele homozygous subjects for rs6039642 (GG genotype) showed lower cortical thickness variance than carriers of the minor allele A.

[Insert Figure 2]

As mentioned on Methods, associations from the variance models were further examined to discard potential artifacts, as variables like sex and batch might still confound the test results. Using standard clumping methods on the ten variance-GWAS, the top 1,799 genotype-phenotype associations were selected for additional analysis using GAMLSS. As observed above for the the pallidum variance-GWAS hit, some of the top markers obtained with Levene's test can be susceptible to genotype imputation quality problems that influence variability in between-cohort genotype frequencies and might artificially introduce bias. Hence, markers that were both unavailable for one or more cohorts and had strong between-cohort differences in genotype frequencies (indexed by a chi-square test $p<10^{-25}$) were excluded from the plots, giving a total of 1,526 loci clumped from Levene's test p-values. Results from Supplementary Figure S5 suggest that Levene's test p-values are not particularly skewed towards false positives and, in some cases

(e.g., thalamus, Figure S5i), GAMLSS p-values might be smaller than the output from their

Levene's test-based counterpart. In view of those observations, we believe that a conservative

two-stage approach combining Levene's test with GAMLSS validation is adequate.

Discussion

To our knowledge, this is the first evidence of genetic loci influencing variability of brain

volumes beyond their mean value. A conceptually and methodologically similar approach

revealed genetic control of the variance in body height and body mass index [12]. Adding to the

notion that phenotypic spread in a population is related to genetic variability, the current results

show that the population variance of cortical thickness and thalamus volume is partly under

genetic control. Importantly, our findings on brain structure and the previous work on body mass

index [12] provide converging evidence supporting the notion that common genetic variants

affecting the mean and the variance of a trait need not be correlated and may influence

phenotypes through complementary mechanisms.

Variants associated with volumetric dispersion were at loci that have previously been

linked to neuropsychiatric traits. The significant variance locus for cortical thickness on

chromosome 20 was located next to *PAK7* - a gene conferring risk for psychosis and involved in

oxytocin gene networks of the brain [20, 21] - and the synaptosome associated protein 25 gene

(*SNAP25*) -which participates in synaptic function and increases susceptibility for severe

psychiatric conditions [22, 23]. Moreover, the 13q22 locus related to thalamus variance was near

LINC00381, a non-protein coding RNA-gene that has recently been associated with hand

preference in the UK Biobank [24].

Variance-controlling alleles can be interpreted as underlying distinct degrees of

organismic robustness [1]. Relevance to medical genetics also comes from the observation that

several disease phenotypes emerge beyond a phenotypic threshold, which could be reached by

the influence of high variability phenotypes [25]. It is thus important to understand how the

identified markers relate to brain variability under changing environments (robustness), how they

interact with other genetic loci (epistasis) and how they relate to the clinical manifestation of disease. Similarly, variance-controlling loci can underlie variability from other genetic factors, potentially affecting evolutionary dynamics [4]. Identifying the mechanisms by which variance-controlling genotypes influence gene expression variance in relevant brain structures may provide a proof of principle for the functional relevance of the identified genotypes. This type of effect on expression has been shown in model organisms [26], and the genomic loci identified here represent suitable candidates for targeted gene expression analysis in the human brain. The identification of specific genes involved in neural evolution and mental disorders suggests that brain variability in human populations is mediated by genetic factors. In so doing it also underscores the validity of gene-gene and gene-environment interactions in explaining heritability of complex human traits.

While the present analysis does not follow an additive coding of alleles and thus may not be directly studied in relation to other GWAS datasets (e.g., through LD score regression), further research may elicit links between the variance-controlling genotypes and both mean and variance of other phenotypes. It is also important acknowledging that, even though the conventional mean-GWAS identified several hits (70 loci, as mentioned on *Results*), the variance-GWAS did not detect as many associations (2 loci). This is ostensibly due to statistical power constrains of variance tests such as Levene's and GAMLSS, and it may also be related to the adopted conservative strategies including inverse-normal transformation and removal of low-genotype count markers. Those steps, implemented to remove false positives, are probably overly conservative under some conditions; larger sample sizes may also aid the discovery of new loci.

In summary, the results indicate that beyond associations with mean trait values, genotypic architecture modulates the variance of subcortical and intracranial dimensions across individuals. The lack of overlap between genetic associations detected by the standard additive genetic model and variance-controlling loci indicate independent mechanisms. These findings contribute to establish the genetic basis of phenotypic variance (i.e., heritability), allow identifying different degrees of brain robustness across individuals, and open new research avenues in the search for mechanisms controlling brain and mental health.

Supplementary information

Supplementary information can be found with this article online.

Author contributions

Conceptualization, ACP and LTW; Methodology, ACP and LTW; Investigation, ACP, vdM, TK; Writing – Original Draft, ACP and LTW; Writing – Review & Editing, all co-authors.

Declaration of interest

The authors declare no competing interests.

Data and code availability

All scripts are available upon reasonable request to the corresponding authors. Data availability notes for each cohort can be found on Supplementary Table S1.

Acknowledgements

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Figure legends

Figure 1. Common genetic variants regulate the distribution variance of human subcortical and intracranial volumes

Locus-zoom plots show all markers on the loci (just filtered by MAF during initial QC). Related plots, after the additional filter for minor (non-zero) genotype count are included as Supplementary Figure S4.


Figure 2. Shift function plots for the top genome-wide significant associations in mean and variance model GWAS results

The results corresponding to the top four mean models' associations (conventional GWAS) are shown on the top rows ("A", "B"), those corresponding to the top four variance model associations are displayed on the lower sections ("C", "D"). A: Jittered marginal distribution scatterplots for the top two mean model associations, with overlaid shift function plots using deciles. Genotypes with the minor (effect) allele are shown as a single group. 95% confidence intervals were computed using a percentile bootstrap estimation of the standard error of the difference between quantiles on 1000 bootstrap samples. B: Linked deciles from shift functions on row "A". C: Jittered marginal distribution scatterplots for the top two variance model associations, grouped by reference allele(s) versus effect allele(s) carriers. 95% confidence intervals were computed as in "A". D: Linked deciles from shift functions on row "C". For the mean model associations ("A" and "B"), variances explained by mean and variance parts of the model were 0.522% and 0.0004% (hippocampus, rs77956314), and 0.437% and 0.0015% (putamen, rs715732).
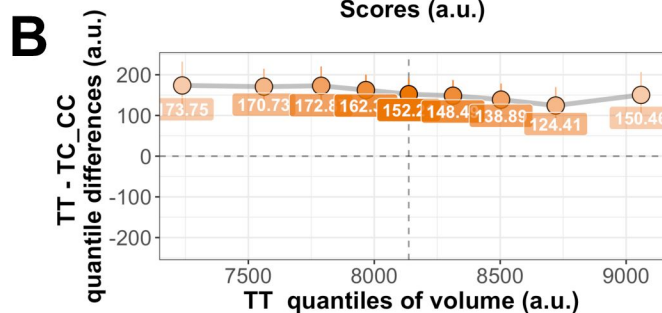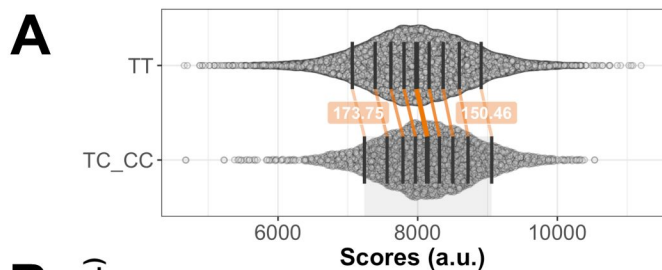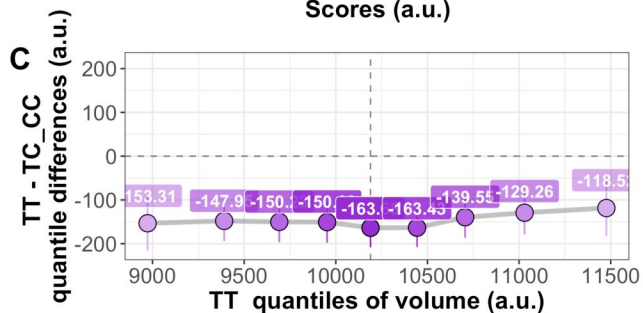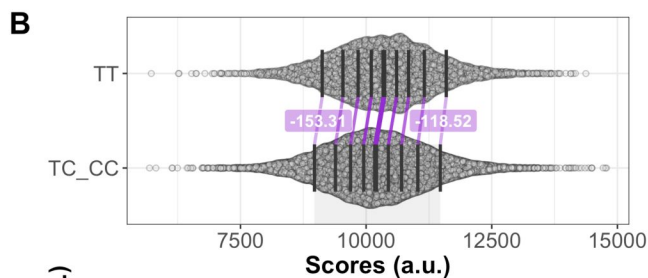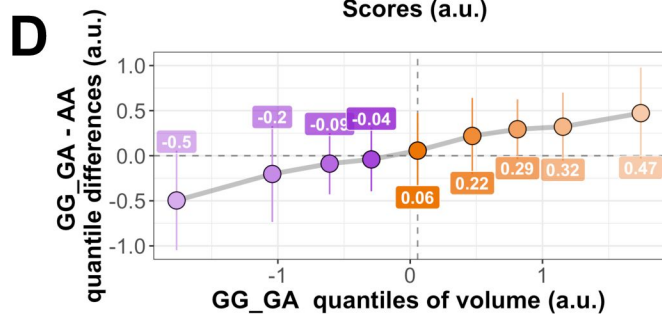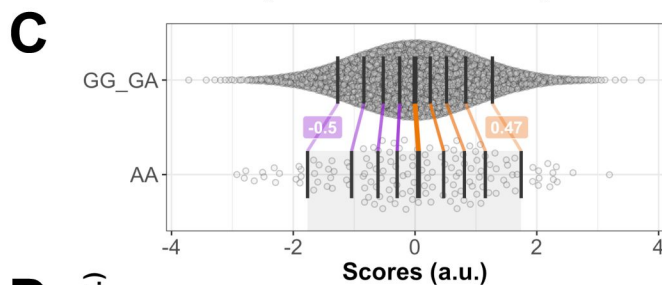
Mean thickness, rs6039642
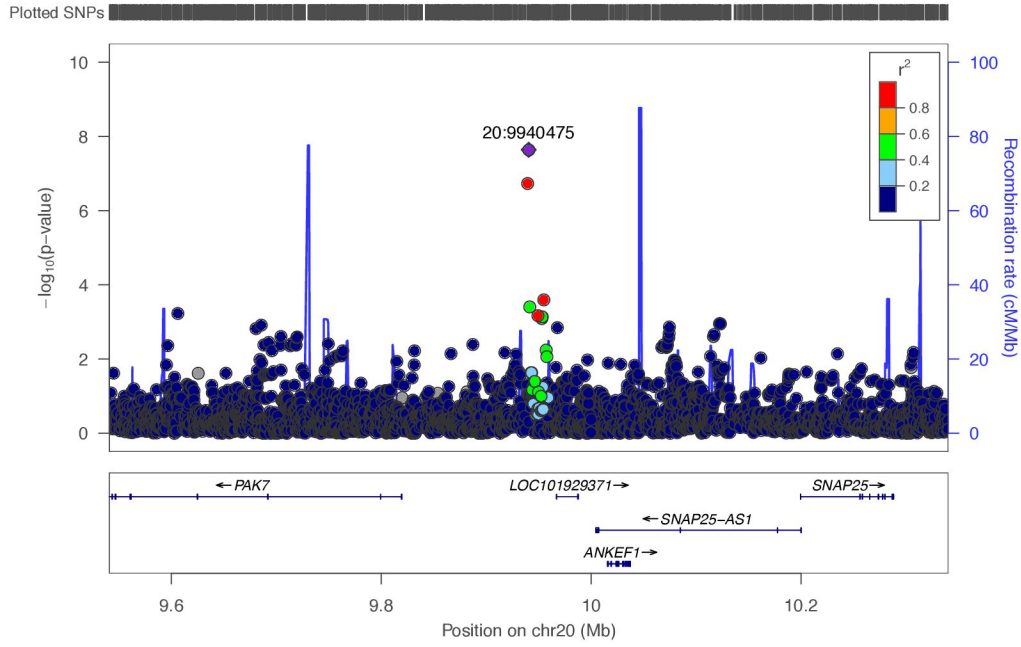
Thalamus, rs9543733
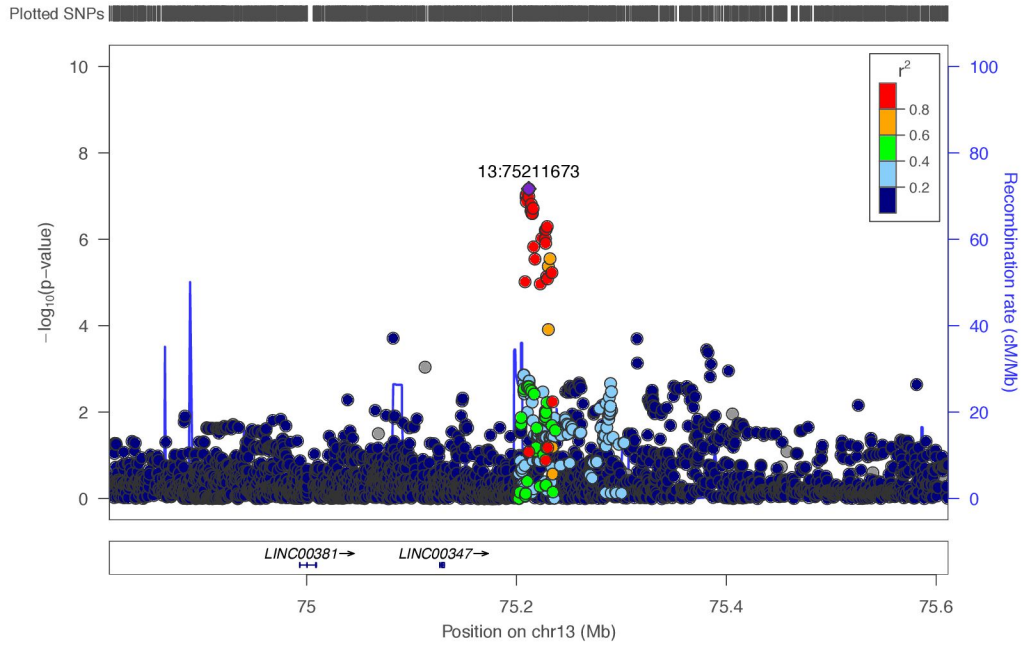
Hippocampus
(rs77956314)

Putamen
(rs7157327)

Cortical thickness
(rs6039642)

Thalamus
(rs9543733)