Continuous inflation analysis: a threshold-free method to estimate genetic overlap and boost power in imaging genetics

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Abstract. Methods to quantify genetic overlap may elucidate relationships between disparate traits, and provide Bayesian priors to guide the search for genetic influences on brain measures. Here we describe a threshold-free method called continuous inflation analysis (CIA), which we used to compare genome-wide association statistics (GWAS) for the volumes of eight brain regions, computed from brain MRI. Our goal was to understand the extent of pleiotropy (overlap in genetic influences) and concordance for the volumes of brain regions with different biological functions. We found significant pleiotropy among seven of the subcortical brain volumes. We found positive concordance across the seven subcortical structures and negative concordance between genetic influences on each subcortical structure and intracranial volume (ICV). Using a conditional FDR approach, we showed that a given brain volume GWAS could act as a Bayesian prior and improve the power to detect novel associations in a related brain volume. When conditioning the putamen volume GWAS on the caudate volume GWAS, we identified 17 novel loci associated with putamen volume.

1 Introduction

Recent imaging genetics work in the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) Consortium has focused on discovering common genetic variants associated with the volumes of seven subcortical brain structures (nucleus accumbens, amygdala, caudate, hippocampus, globus pallidus, putamen, thalamus) and one measure of global head size (intracranial volume; ICV) [1]. Hibar et al. examined individual SNP associations with each of the eight brain volumes – each considered as a single trait – but did not examine the overlapping genetic influence of the full set of common variants across structures. By examining the pleiotropy (common genetic influences) and concordance[1] across subcortical structures we should be able to (1) define Bayesian approaches to guide the search for genetic influences on the

[1] Concordance is an extension to the concept of pleiotropy that includes the *direction* of a pleiotropic SNP effect (i.e. a positive or negative correlation).

brain, and (2) better understand the underlying genetic pathways that may partially explain volumetric variations across different brain regions.

Twin and family studies can estimate the *genetic correlation* between subcortical brain volume traits [2], i.e., the fraction of the observed correlation that is due to genetic factors. However, there may be little or no publicly available twin or family data for a given pair of traits. If genome-wide SNP data is available for a cohort, we can use GWAS summary statistics (i.e., regression coefficients relating each SNP to the traits of interest) to estimate the common genetic overlap. This is perhaps surprising, because almost all SNPs have no detectable effects, and even significantly associated SNPs generally have weak effects. So, without vast samples of data, it can be challenging to pick up genetic overlap from SNP association data. A recent method called LDSC regression [3] uses GWAS summary statistics from two traits to estimate a genetic correlation driven by common genetic determinants. One limitation of this method (and genetic correlations calculated from twin and family studies as well) is that it is not possible to identify which *specific* variants overlap and contribute to the correlation. A related method, SNP Effect Concordance Analysis (SECA)[4], looks at pleiotropy and concordance and predefined, arbitrary thresholds. Even so, it is of great interest to try to narrow the search for genetic variants associated with brain measures, to avoid heavy multiple comparisons corrections and the vast sample sizes they currently imply (often requiring tens of thousands of subjects, e.g. in the ENIGMA studies).

Here we describe a novel method to quantify the global enrichment (pleiotropy) and concordance between GWAS summary statistics from two traits. We apply this method to examine the genetic overlap between brain structures examined in the ENIGMA Consortium. Our hypothesis is that brain regions will show genetic overlap with structures similar to their functional groupings: limbic system (hippocampus, amygdala, thalamus) and basal ganglia (putamen, caudate, nucleus accumbens, and globus pallidus). Further, we examine whether a conditional FDR framework can be used to boost power to detect novel associations.

2 Methods

2.1 Estimating the genetic overlap between two traits

We developed a data-driven, threshold-free method, called continuous inflation analysis (CIA), to assess global enrichment (pleiotropy) and concordance based on GWAS summary statistics from any two pairwise traits. Here we were interested in assessing the genetic overlap across the volumes of eight different brain regions: the nucleus accumbens, amygdala, caudate, hippocampus, globus pallidus, putamen, thalamus, and intracranial volume (ICV). We performed all pairwise combinations of overlap tests between the eight traits. Before comparing two traits, we designated one dataset the *reference* dataset and the other the *test* dataset. This designation is important because the CIA procedure is not symmetric. To begin, we performed a clumping pro-

cedure to select independent index SNPs for each LD block in the genome. The index SNPs were chosen based on significance levels in the *reference* dataset (PLINK options: --clump-p1 1 --clump-p2 1 --clump-kb 500 --clump-r2 0.2) [5]. Next, we merged the *reference* and test dataset such that only GWAS summary statistics for the index SNPs remained in the dataset. We estimated the global enrichment (pleiotropy) by first sorting the merged dataset by the *P*-value of each SNP in the *reference* dataset (in descending order). We iterated through the sorted merged dataset at a given step size (n = 100), with each step moving down the list by n SNPs. At each step, we calculated amount of enrichment by comparing the empirical cumulative distribution function (ecdf) of *P*-values from the test dataset for SNPs from n to the end of the list with the ecdf of the full set of *P*-values from the test dataset. For example, say you are looking at the subset of SNPs in your test dataset where the subset is chosen such that it only includes SNPs with *P*-value < 0.05 in your reference dataset. Taking the ecdf of the subsetted set of SNPs and *P*-values from the test dataset you can determine if the set deviates from a null distribution. In this case the *null* distribution is the full set of SNPs and *P*-values from the test dataset without subsetting. Leftward deflections in the subsetted ecdf were considered evidence of enrichment at a given cutoff and were estimated using a one-sided, two-sample Kolmogorov-Smirnov test. The comparison of two traits was considered to have significant evidence of pleiotropy if the *P*-value vector over all cutoffs exceeded the Benjamini-Hochberg False Discovery Rate [6] (BH-FDR) threshold (set to q = (0.05/8 traits) = 0.00625).

As an extension to the pleiotropy tests, we performed a test of *concordance*, which considers the effect direction (the sign of the beta-coefficient from the regression of a given SNP against a given trait) when assessing the extent of overlap between two traits. The concordance can be negative (the effect direction in test dataset is negative when the effect direction is positive in the reference dataset, and vice versa), positive (the effect direction is positive or negative in both datasets) or null when there is no evidence for concordance. The concordance test can be simply applied by filtering out SNPs from the merged dataset (keeping either negative or positive concordant SNPs) and then continuing on with the CIA procedure described above. The significance is calculated in the same way (with the KS test) and overall global evidence of concordance was determined over all cutoffs (at BH-FDR q = 0.00625).

2.2 Examining bias in enrichment tests using a negative control

We obtained GWAS summary statistics from a skin-based trait (presence of a whorl on the left thumb [7]) to provide a negative control for our enrichment tests. The presence of whorls in fingerprints is unlikely to be related to brain volume phenotypes (and no previous link has been made in the literature) so estimating genetic overlap between brain volume GWAS and fingerprint whorl GWAS can provide evidence of Type II error bias in the CIA enrichment test model. Fingerprint data were collected from rolled ink prints and manually examined at the Queensland Institute of Medical Research and is described elsewhere [7]. The fingerprint whorl GWAS was based on

data from 3,314 participants (twins and their family members) using genotypes imputed to the 1000 Genomes phase 1, version 3 reference panel [8].

2.3 Boosting power to detect novel gene variants using conditional FDR

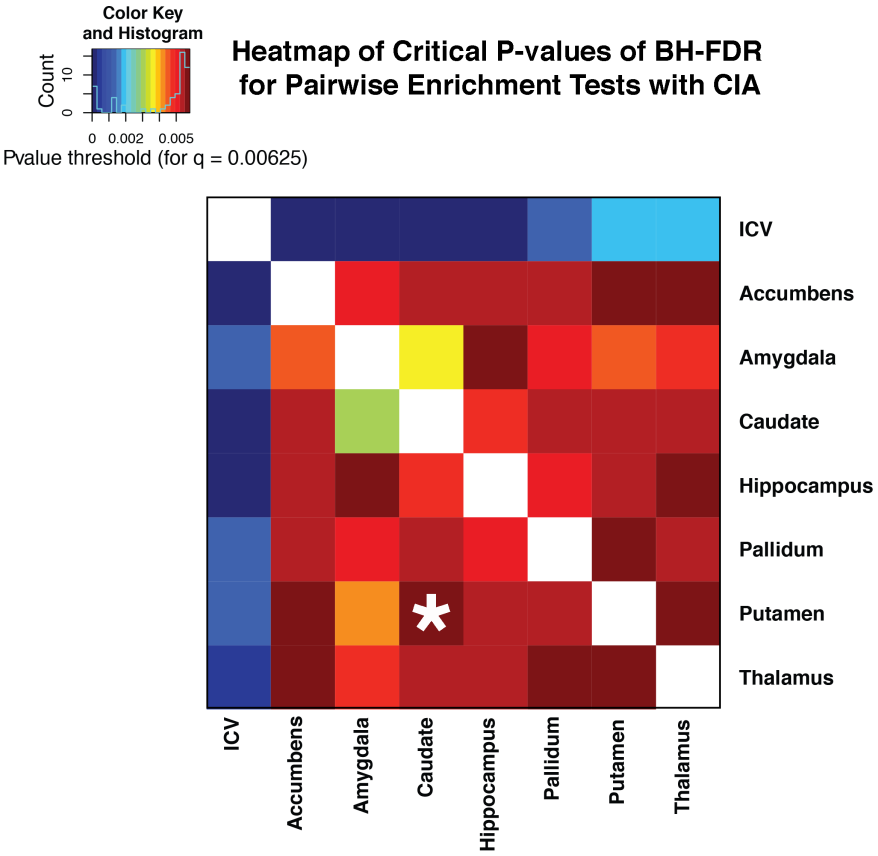
For pairwise comparisons that show significant overlap, we can boost the power to detect individual SNPs associated with a given test trait by conditioning on the reference GWAS dataset. From the CIA model for a given pairwise comparison, we can choose the step-based cutoff that results in the most significant enrichment over all possible cutoffs. Next, we can apply the BH-FDR to the SNP *P*-values from the subsetted test dataset with $q = 0.05$. For comparison, we applied the BH-FDR to the full set of SNP *P*-values from the test dataset with $q = 0.05$. SNPs that pass BH-FDR in the subsetted dataset but not in the full dataset are considered to be detected with increased power when conditioning on the reference dataset.

3 Results

3.1 Pleiotropic gene variants influence multiple brain regions

We found significant evidence for pleiotropy between all pairwise comparisons of seven subcortical brain volumes (see **Fig. 1**). None of the pairwise comparisons with ICV showed significant overlap. The most significant comparison showing the highest evidence of pleiotropy occurred between the putamen and caudate ($q = 0.0058$). This relationship makes intuitive sense given the strong functional and histological evidence linking the two basal ganglia brain structures together.

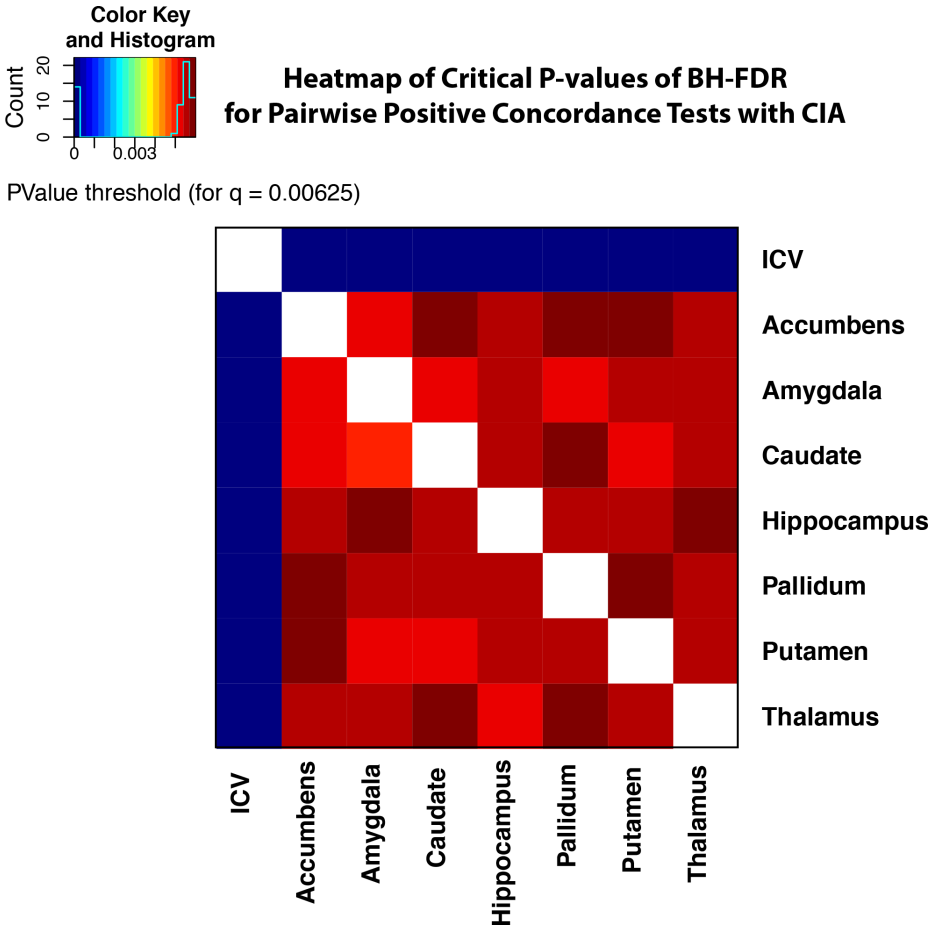
Fig. 1. Global evidence of pleiotropy for pairwise comparisons of eight brain traits. Comparisons were made using CIA and were considered significant at a BH-FDR threshold q = 0.00625). The seven subcortical brain structures were tightly linked in terms of pleiotropy, but no structures showed evidence of pleiotropy with ICV. The most significant comparison (Putamen | Caudate) is marked with a white star.



3.2 Evidence of a positive concordance between subcortical brain structures

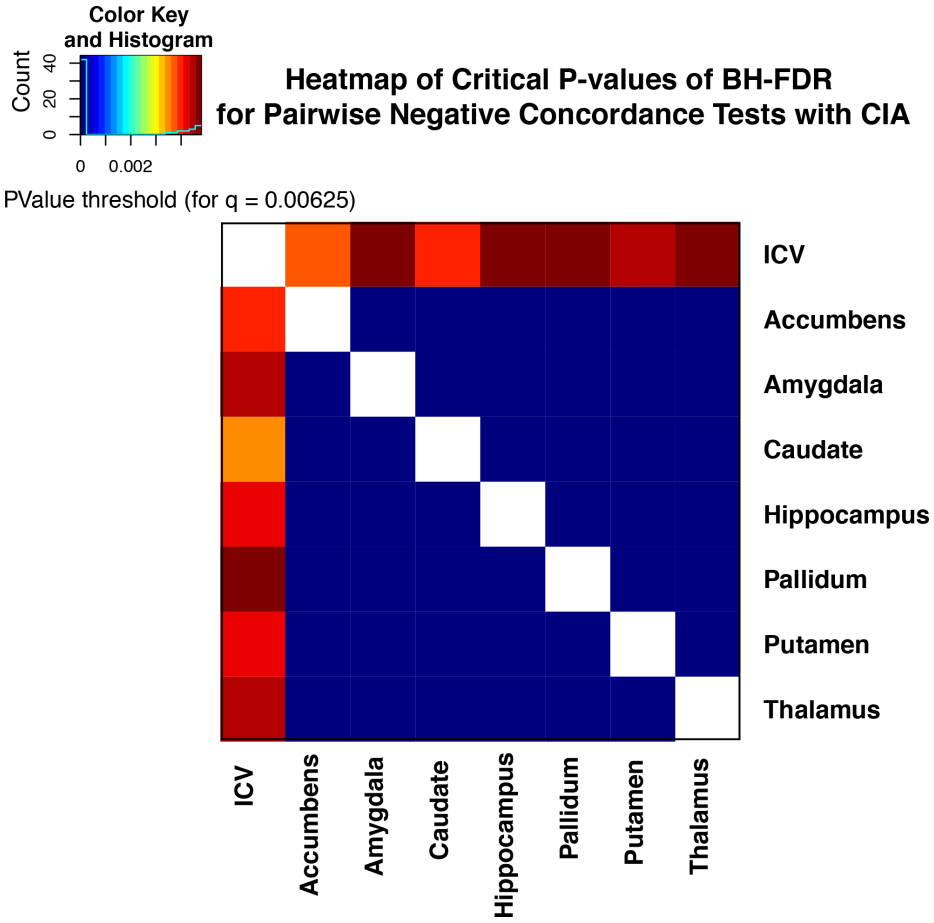
We found significant positive concordance in each of the pairwise comparisons of subcortical brain traits (see **Fig. 2**). In other words, genetic variants associated with an increase in a given brain volume also tend to be associated with an increase in the volume of another subcortical trait (and *vice versa*). Here there is no detectable evidence of positive concordance between the subcortical brain structures and ICV.

Fig. 2. Global evidence of positive concordance for pairwise comparisons of eight brain traits. Comparisons were made using CIA and were considered significant at a BH-FDR threshold q = 0.00625). The seven subcortical brain structures were tightly linked in terms of positive concordance, whereas none of the structures showed evidence of positive concordance with ICV.



We found significant negative concordance in each of the pairwise comparisons between ICV and subcortical traits (see **Fig. 3**). In other words, gene variants that are associated with an increase in ICV also tend to be associated with a *decrease* in subcortical brain volume. However, it is worth noting that the subcortical volume GWASs were corrected for ICV as a linear predictor so the relationship here likely represents any residual nonlinear relationship. In general, there is a positive phenotypic correlation between subcortical volumes and ICV.

Fig. 3. Global evidence of negative concordance for pairwise comparisons of eight brain traits. Comparisons were made using CIA and were considered significant at a BH-FDR threshold q = 0.00625). The pairwise comparisons with ICV and the other seven structures showed significant negative concordance, whereas the pairwise comparisons among the subcortical brain volume traits were not significant.



3.3 Finger whorl pattern as a negative control for enrichment tests in brain

We found no evidence of pleiotropy between putamen volume and the dermatoglyphic negative control (presence of whorl on the left thumb) at an FDR q-value = 0.05.

3.4 Conditioning enrichment tests on another brain prior can boost power to detect effects in the original trait

Several of the pairwise comparisons of pleiotropy were significant, so, for purposes of illustration of the method, here we give the conditional FDR results for the "most significant" comparison (putamen volume GWAS conditioned on caudate volume GWAS). We identified 17 additional significant variants influencing putamen volume that were previously undetected without conditioning on the caudate volume GWAS (see **Table 1**).

Table 1. Conditional False Discovery Rate (FDR) analysis of putamen GWAS conditioned on caudate GWAS. Shown here are variants that pass FDR at $q = 0.05$ in the putamen volume GWAS when prioritizing SNPs based on their significance in the caudate GWAS, but do not pass FDR when considering the full set of putamen GWAS variants.

SNP	*Raw P-value in Putamen GWAS*	*Subset FDR*	*FDR of Full Sample*
rs4888010	4.92E-07	0.025	0.071
rs11150623	1.25E-06	0.027	0.076
rs17388257	1.41E-06	0.027	0.076
rs62394265	1.42E-06	0.027	0.076
rs76647989	7.70E-07	0.027	0.076
rs7873504	1.37E-06	0.027	0.076
rs10963102	2.77E-06	0.029	0.080
rs12487861	3.16E-06	0.029	0.080
rs184917581	3.10E-06	0.029	0.080
rs6135525	3.04E-06	0.029	0.080
rs62022639	2.12E-06	0.029	0.080
rs6869844	2.26E-06	0.029	0.080
rs7325851	2.47E-06	0.029	0.080
rs80258284	2.30E-06	0.029	0.080
rs842389	1.92E-06	0.029	0.080
rs10033333	4.72E-06	0.041	0.113
rs115186168	5.00E-06	0.041	0.113

4 Conclusions

We discovered evidence of significant pleiotropy between gene variants influencing different subcortical brain volumes, using continuous inflation analysis (CIA). This agrees with findings from twin and family heritability studies, which show that there is significant genetic correlation for volumetric measures of the subcortical structures [2]. The CIA analysis builds on the twin and family heritability estimates, because the overlap between traits is estimated from genome-wide association statistics only, and does not require a family or twin design – it can be applied to imaging genetic studies of unrelated individuals, which are more common. The most significant evidence of pleiotropy came from the putamen volume GWAS conditioned on the caudate volume GWAS. The close relationship between gene variants effecting both structures is intu-

itively reasonable, given the histological similarity of the caudate and putamen tissue [9].

Besides the known relationships, it appears that gene variants that explain variances in the volumes of subcortical structures that were previously thought to be independent do indeed have an effect (in fact, all subcortical structures showed a significant relationship with all other subcortical structures). The lack of enrichment between ICV and subcortical structures is not surprising, given that the subcortical volume GWAS is controlled for ICV [1]. Curiously though, we find evidence of *negative* concordance between ICV and subcortical structures. This is likely due to non-linear differences in ICV that are not fully accounted when adjusting subcortical brain volume GWAS with ICV as a linear predictor [10]. Among subcortical structures, we found that there is a *positive* concordance (but no effect when compared with ICV).

One distinct advantage of pairwise comparisons of traits with CIA is the ability to identify specific SNPs with pleiotropic effects. An extension of this idea is then to use the GWAS of a trait as part of a Bayesian prior for a related trait, to boost the power to detect effects. When looking at the comparison with the most significant evidence of pleiotropy (Putamen | Caudate) with a conditional FDR approach, we were able to identify 17 additional significant loci. The top loci (rs4888010) is an intergenic SNP on chromosome 16q22.3 [11], but further analysis of this and the other 16 loci is necessary, to better understand potential mechanisms that may influence putamen volume. All of these models are performed in the context of common genetic variants commonly known as SNPs. It is likely the case that further contributions to genetic overlap common from other forms of genetic variation like copy-number variants (CNVs) or insertions/deletions.

Applying CIA to other traits including those involving neuropsychiatric disease risk will help to quantify the genetic overlap between brain-related phenotypes and brain disorders and may provide a cost-effective method to screen potential endophenotypes with existing data. Further, CIA combined with conditional FDR may identify new susceptibility loci for neuropsychiatric disease risk that would have previously been undetected.

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