The Convergence of eQTL Mapping, Heritability Estimation and Polygenic Modeling: Emerging Spectrum of Risk Variation in Bipolar Disorder

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It is widely held that a substantial genetic component underlies Bipolar Disorder (BD) and other neuropsychiatric disease traits. Recent efforts have been aimed at understanding the genetic basis of disease susceptibility, with genome-wide association studies (GWAS) unveiling some promising associations. Nevertheless, the genetic etiology of BD remains elusive with a substantial proportion of the heritability – which has been estimated to be 80% based on twin and family studies – unaccounted for by the specific genetic variants identified by large-scale GWAS. Furthermore, functional understanding of associated loci generally lags discovery. Studies we report here provide considerable support to the claim that substantially more remains to be gained from GWAS on the genetic mechanisms underlying BD susceptibility, and that a large proportion of the variation in disease risk may be uncovered through integrative functional genomic approaches. We combine recent analytic advances in heritability estimation and polygenic modeling and leverage recent technological advances in the generation of -omics data to evaluate the nature and scale of the contribution of functional classes of genetic variation to a relatively intractable disorder. We identified \( \text{cis} \) eQTLs in cerebellum and parietal cortex that capture more than half of the total heritability attributable to SNPs interrogated through GWAS and showed that eQTL-based heritability estimation is highly tissue-dependent. Our findings show that a much greater resolution may be attained than has been reported thus far on the number of common loci that capture a substantial proportion of the heritability to disease risk and that the functional nature of contributory loci may be clarified \textit{en masse}.

Recent advances have facilitated high-throughput explorations of function-related aspects of the genome\(^1-3\). These developments have yielded comprehensive maps of functional elements, providing an unparalleled resource to infer the phenotypic consequences of genetic variation. Our group has been particularly interested in clarifying the impact of regulatory variation on pathophysiology\(^4\) and in integrating heterogeneous genomic datasets to expand on GWAS findings.

For many complex traits, there is an enormous, much-lamented gap between estimates of heritability derived from population studies (e.g., family and twin studies) and the proportion of variation explained by specific genetic loci identified by genome-wide scans\(^5,6\). A second equally fundamental lacuna exists, between the set of genetic variants identified by GWAS and the functional role of the implicated variants in mediating the trait.

Recent reports from our group and others have evaluated the utility of expression quantitative trait loci (eQTL)\(^4,7,8\), and more recently methylation quantitative trait loci (mQTL)\(^9\) and microRNA quantitative
trait loci (miRNAQTL)\textsuperscript{10}, to enhance discovery of the genetic basis of complex traits. All together, these studies have demonstrated that trait associations from a comprehensive catalog of published GWAS\textsuperscript{11} as well as from the top ranks of GWAS results, at least for some complex traits, are significantly enriched for functional quantitative trait loci of molecular-level phenotypes, raising the possibility of utilizing functional genomics to increase the power of a genome-wide scan to detect true associations as well as to clarify the nature of the identified associations. However, it remains to be seen to what extent the findings emerging from functional genomic studies (e.g., eQTLs) may explain the so-called missing heritability characteristic of GWAS findings.

A flurry of recent studies have utilized random effects models (linear mixed modeling)\textsuperscript{12,13} to estimate the “chip” heritability due to causal loci tagged by SNPs interrogated in GWAS and a weighted risk score analytic approach (polygenic modeling)\textsuperscript{14,15} to quantify the contribution of SNPs that fail to reach genome-wide significance in GWAS scans. However, the findings from the application of these methods to GWAS thus far have yielded little insight into the nature of the polygenic component to trait variation. Bipolar Disorder (BD) has been seen as especially intractable and has contributed little to illuminate the nature of the genetic architecture of disease risk. We hypothesized that the convergence of functional genomic approaches and these recently developed techniques for analysis of GWAS data may shed light on the number of contributory loci for BD and provide a global molecular perspective on their mode(s) of functional mediation. Although a recent study\textsuperscript{14} concluded that BD and Schizophrenia share a polygenic component with tens of thousands of common genetic variants of small effect size likely to be contributory, studies we report here show that a much greater resolution may be attained on the number of common loci that capture a substantial proportion of the heritability to disease risk and that the functional nature of contributory loci may indeed be clarified en masse.

We had conducted genome-wide expression profiling to map eQTLs (and mQTLs) in the cerebellum cortex, as previously described\textsuperscript{9}. For the present study, we also utilized the results of eQTL mapping in parietal cortex. We classified eQTLs into \textit{cis} and \textit{trans} regulators of gene expression phenotypes on the basis of the SNP’s distance to its target gene and on the strength of the evidence for association with gene expression (see Methods). Using the Wellcome Trust Case Control Consortium (WTCCC) GWAS study in BD\textsuperscript{16}, we observed a highly significant enrichment for parietal cortex \textit{cis} eQTLs and cerebellum \textit{cis} eQTLs ($p < 0.001$ for each) among the top SNPs (defined as $p < 0.001$) (Figure 1) relative to random sets of SNPs (n = 1000) matched on minor allele frequency and location with respect to nearest gene. In contrast, no enrichment was found ($p > 0.05$) for \textit{cis} eQTLs identified in lymphoblastoid cell lines (LCLs).

We therefore quantified the contribution of eQTLs in the separate tissues to the heritability of disease risk. Following Yang \textit{et al.}\textsuperscript{12}, we utilized a linear mixed model (LMM):
\[ Y = X\beta + g + e \]

\[ \text{var}(Y) = A\sigma_g^2 + I\sigma_e^2 \]

where \( Y \) is a phenotype vector of size \( N \times 1 \), \( \beta \) is a vector of fixed effects, \( g \) is a vector of random additive genetic effects (the “polygenic component” of trait variation) from the set of eQTLs under study (more generally, any set of QTLs with a priori support from functional genomics, e.g., mQTL \(^9,17,18\) or miRNAQTL \(^10,19\)), and \( e \) is a vector of residuals. This model leads to two variance components, namely the additive genetic variance \( \sigma_g^2 \) captured by the tested SNPs and the residual variance \( \sigma_e^2 \). We estimated the (narrow-sense) heritability, \( \sigma_g^2 / (\sigma_g^2 + \sigma_e^2) \), explained by the cis eQTLs and, separately, the trans eQTLs (despite differential power) \(^20\). These estimates, derived from the genotyped SNPs, quantify the proportion of phenotypic variance attributable to causal variants in linkage disequilibrium (LD) with the tested eQTL SNP sets. A genetic relationship matrix (GRM), denoted by \( A \) (with entries \( A_{ij} \) between pairs of individuals \( i \) and \( j \) ), was estimated from each SNP set, and variances were estimated using restricted maximum likelihood (REML) \(^20\). We were interested in the heritability captured by the cis eQTLs (and the trans eQTLs) as a proportion of the heritability captured by all SNPs interrogated in the GWAS and passing QC filters (see Methods), \( h^2_{eqtl} / h^2_{all} \), hereafter referred to as the heritability concentration index. We demonstrated, on theoretical grounds (see Methods) and empirically, that the heritability concentration index is the same whether calculated on the observed scale or estimated on the liability scale (the latter premised on a continuous liability threshold model). Furthermore, it is independent of disease prevalence (\( K \)) or ascertainment bias (\( P \), the proportion of cases in the samples, which may be \( > K \)) (see Methods).

To evaluate the contribution of eQTLs to the (narrow-sense) heritability of BD, we utilized the TGen+GAIN dataset, which consists of data from the Bipolar Genome Study (TGen) and from an earlier study (GAIN) conducted under the GAIN initiative (Table 1). All together, the sample set included in our analysis consists of 2,191 cases and 1,434 controls. Selecting samples so that \(|A_{ij}| < 0.025\) for all pairs \( i \) and \( j \) (\( n = 3,189 \) individuals) and conducting extensive QC on the genotype data (see Methods), we performed the heritability estimation analysis in the TGen+GAIN dataset with and without the study ID as covariate, with no substantial difference in the estimates. Indeed, consistent with a previous report \(^21\) on the WTCCC study of BD \(^16\), the genome-wide SNPs (\( n > 600,000 \)) explained 35\% (s.e. = 6\%) of the phenotypic variance on the liability scale (assuming disease prevalence \( K = 0.01 \)). Remarkably, we found that the cerebellum cis eQTLs (\( n = 27,107 \)) and parietal cortex cis eQTLs (\( n = 26,979 \)) included in our analysis had heritability concentration index \( h^2_{eqtl} / h^2_{all} \) of 0.57 and 0.58, respectively. The heritability captured by the cis eQTLs
previously identified in LCLs was less than 1%, which demonstrates the tissue dependence of eQTL-based heritability estimation. The inclusion of 20 principal components in the LMM as fixed effects yielded similar estimates (Supplemental Table 1).

As the results for parietal cortex and cerebellum were found to be similar (and to significantly differ from the results for LCLs), we illustrate our approach in the remainder of the paper with the cerebellum eQTLs unless explicitly stated. When we evaluated each GWAS (TGen and GAIN) separately and partitioned the genetic variance captured by the cis eQTLs onto the individual autosomes (by fitting the GRMs of the chromosomes simultaneously in a joint analysis), we found that the estimates of variance explained by each chromosome (Figure 2) were highly correlated between the two studies (Pearson correlation = 0.60). Furthermore, the signals in the chromosomal estimates in each GWAS (Figure 2) are not driven by the large chromosomes. Notably, each GWAS study showed a peak in heritability estimate on chromosome 11. Supplemental Table 2 provides a list of the chromosome 11 transcripts implicated by this analysis, including some with a number of independent \( r^2 < 0.10 \) cis eQTLs. In particular, using gene set enrichment analysis, we found that the chromosome 11 transcripts with 5 or more cis eQTLs contributing to our estimate of heritability are significantly enriched for Immunoglobulin C-2 Type (IGc2) proteins (Benjamini Hochberg adjusted p = 6.2 x 10^{-3}; Supplemental Table 3), which raises the question of an infectious/inflammatory etiology to BD or a novel CNS mechanism for this immunoglobulin.

Although our study was not sufficiently powered to reliably identify trans eQTLs, we found that the set of SNPs (n = 6,892) included in our analysis with association p < 1.9 x 10^{-6} (= 0.05/# of genes tested) with at least one gene expression trait in cerebellum had heritability concentration index of 0.11.

Since experimental or genotyping artifacts as case-control differences may appear as “heritability” (more so than in the case of quantitative traits, which are less likely to be correlated with these artifacts), we investigated the effect of QC thresholds on the estimates of heritability captured by the genome-wide SNPs as well as the cis eQTLs and the trans eQTLs among the interrogated SNPs (see Methods) and found these estimates to be stable.

To test for the presence of any inflation in these estimates, we conducted heritability estimation using the genome-wide SNPs and, separately, the subset of cis eQTLs on permuted traits (n=1000) while conditioning on the same corresponding genetic relationship matrix \( A \) (one for each SNP set). This analysis showed that estimates of heritability of simulated phenotypes derived from the genome-wide SNPs and from the cis eQTLs, given their standard error, were consistent with zero heritability, as we would expect from a simulated trait with no real association with genotype. Supplemental Figure 1 illustrates the (null) distribution of the heritability estimate for the cis eQTLs.

SNP-based heritability estimation is susceptible to confounding by population stratification. The use of
principal components (as fixed effects) in the LMM model may not adequately correct for such confounding, and indeed principal component-adjusted estimates may yield substantially similar values as the non-adjusted ones in the presence of fine-scale population structure. To quantify the effect of population structure, we estimated heritability from the sum of heritabilities obtained from two disjoint sets of chromosomes (chr 1-10 and chr 11-22), which we then compared with the estimate derived from genome-wide SNPs, following Yang et al. We found that the heritability concentration index was largely unaffected (0.57 [joint] vs. 0.55 [disjoint]).

Contributions to the heritability concentration index from causal variants tagged by the cis eQTLs may be distorted by patterns of LD. Indeed, regions of strong LD may amplify a SNP-based estimate of heritability while contributions from poorly tagged variants may be underestimated. We therefore utilized an LD-adjusted kinship matrix recently developed by Speed et al. to tease apart the impact of local LD from that of the architecture of causal variants on our estimate of the heritability concentration index. We calculated $h^2_{eQTL} / h^2_{all}$ for cis eQTLs using a modified genetic relatedness matrix derived from scaled SNP genotypes. The heritability concentration index did not change substantially with the use of LD-adjusted kinship matrix (LD-adjusted $h^2_{eQTL} / h^2_{all} = 0.572$ vs. non-adjusted $h^2_{eQTL} / h^2_{all} = 0.570$).

The concentration of heritability observed for the cis eQTLs was noteworthy. We took a second analytic approach to evaluate to what extent cis eQTLs may have a collective effect on disease susceptibility. The polygenic modeling (PM) approach has been utilized in several recent studies to demonstrate a polygenic component to an array of complex human traits, including schizophrenia, rheumatoid arthritis, myocardial infarction and coronary artery disease. We selected an LD-pruned set of cis eQTLs that meet a P-value threshold in a “discovery” GWAS (TGen) and calculated a polygenic risk score (see Methods) from this set for each individual in a “replication” GWAS (GAIN) using the risk alleles and the effects sizes from the “discovery” TGen study. We followed the LD parameters used by Purcell et al., to enable direct comparison (see Methods). We utilized several P-value thresholds for association with BD in TGen (namely, P-value $< 0.0001, 0.01, 0.05$, and $0.10$) to define a set of cis eQTLs and evaluated the polygenic risk score for association with case-control status in the second independent GWAS (GAIN) using logistic regression.

Table 2 illustrates the dependence of the association of the polygenic risk score with disease status on the P-value threshold. In particular, the set of cis eQTLs defined by P-value $< 0.05$ (n = 2,375 SNPs) showed the most significant association with case-control status. Figure 3 shows the chromosomal location of this particular set of cis eQTLs, which appear to be scattered uniformly throughout the genome.

Several observations are worth noting here. First, this set of eQTLs constitutes a much smaller number of SNPs (than has previously been reported) that underlie a polygenic variation in the trait, suggesting that
the use of eQTLs can facilitate unprecedented resolution of the polygenic component to disease. Second, the association of the polygenic risk score with case-control status is more significant for the set of cis eQTLs with P-value $\text{TGEN} < 0.05$ than it is for the larger set of interrogated SNPs that satisfy P-value $\text{TGEN} < 0.05$. Third, our approach not only tests whether a polygenic component predicts disease risk (as other PM studies do) it also highlights a potential functional mechanism for the polygenic component. Finally, both statistical approaches, LMM and PM, yield consistent findings on the effect of cis eQTLs, in aggregate, on BD susceptibility.

In summary, we have undertaken to quantify the heritability captured by a functional class of quantitative trait loci for an important complex disorder. Our study not only provides support for the role of common genetic variation in disease susceptibility, but importantly also yields a functional basis for the polygenic variation in the trait. This understanding of the genetic architecture of disease risk could have direct clinical utility and inform the design of future studies.
METHODS

eQTL Mapping

The results of our eQTL studies in cerebellum were previously reported \(^9\). Here we also present results in parietal cortex. Briefly, DNA and RNA of cerebellum samples from 153 individuals of European descent were obtained from the Stanley Medical Research Institute. Genotyping was done on the Affymetrix GeneChip Mapping 5.0 Array (Affymetrix, Santa Clara, CA, USA). Genotype data can be found in the Stanley Genomics Database. Genome-wide expression profiling was performed using the Affymetrix Human Gene 1.0 ST Array (GEO GSE35974 and GSE35977, for cerebellum and parietal cortex, respectively). SNPs with call rates less than 99% were filtered, as were SNPs that departed from Hardy-Weinberg equilibrium (P<0.001) and SNPs with MAF<10%. Principal components analysis, as implemented in Eigenstrat \(^{27}\), was used to test for the existence of population structure. We performed imputation using MACH v1.0 \(^{28}\). We excluded from analysis all probes that could be mapped to multiple genome regions as well as all probes that contain common SNPs (MAF>0.01) on the basis of 1000 Genomes and HapMap data. ComBat \(^{29}\) was used to adjust for batch effect. Surrogate variable analysis was conducted and identified surrogate variables were regressed out. Quantile normalization was used on the residuals. Linear regression with dosage of the minor allele for each SNP was then performed to identify eQTLs. In this study, a cis region is defined as within 4 MB of the probe site, while a trans region refers to the rest of the genome. A threshold of <0.01 was used for the cis analysis, while the trans analysis threshold was 0.05 divided by the number of probes. As our primary interest was in quantifying the heritability captured by these sets of SNPs (and certain subsets thereof), we started from these thresholds.

Genome-wide Association Studies in BD

Two genome-wide studies of Bipolar Disorder were utilized for our study of heritability estimation. The TGen GWAS \(^{30}\) consists of 1,190 cases from the Bipolar Genome Study and 401 controls. In the original GWAS study, QC procedure excluded SNPs with low MAF (<1%), significant departure from Hardy-Weinberg equilibrium in controls (p < 10\(^{-6}\)), low call rate (<95%), and other criteria. A second GWAS, from the GAIN initiative \(^{31}\), consists of 1,001 cases and 1,033 controls of European descent. As in the TGEN study, SNPs were not included in the analysis if the MAF was less than 1%, the SNP violated Hardy-Weinberg equilibrium (p < 10\(^{-6}\)) in control samples, if the call rate was low (<95%), if there were 3 or more Mendelian errors, or if there was more than one discrepancy among duplicate samples.

Following Lee et al. \(^{21}\), we performed additional QC steps to ensure the robustness of our estimates of heritability such as excluding SNPs with p < 0.05 for Hardy-Weinberg equilibrium and for missingness-difference between cases and controls. Only autosomal SNPs were included in our heritability estimation analysis.
**Heritability Estimation on the Observed Scale and on the Liability Scale**

The liability threshold model presupposes an underlying continuous random variable that defines case-control status. Cases are those subjects for which the liability exceeds a given threshold \( t \). For our purposes, suppose that the population prevalence is \( K \). Suppose \( P \) is the proportion of cases in the sample set; in general, this proportion, an ascertainment parameter, may not be a random sample from the population. Then the relationship between the heritability on the observed scale \( h^2_o \) and the heritability on the liability scale \( h^2_l \) is given by the following expression \(^{21}\):

\[
h^2_l = h^2_o \frac{K^2(1-K)^2}{P(1-P)} \frac{1}{\Phi(t)^2}
\]

where \( \Phi(t) \) is the \( y \)-value of the standard normal curve at the point \( t \). Note the same scaling factor between the observed scale and the liability scale applies to the estimate of heritability for the \textit{cis} eQTLs or \textit{trans} eQTLs as for the genome-wide SNPs:

\[
\frac{K^2(1-K)^2}{P(1-P)} \frac{1}{\Phi(t)^2}
\]

Thus, the ratio of the estimate of heritability attributable to \textit{cis} eQTLs (or to \textit{trans} eQTLs) relative to the estimate of heritability attributable to all interrogated SNPs, which we call eQTL “heritability concentration index”, is the same whether on the observed scale or on the liability scale, and is independent of disease prevalence and of ascertainment:

\[
\frac{h^2_{cis}}{h^2_{all}} \text{liability} = \frac{h^2_{cis}}{h^2_{all}} \text{observed}
\]

In our heritability estimation analysis, we selected samples so that \( |A_{ij}| < 0.025 \) for all pairs \( i \) and \( j \) (leaving \( n = 3,189 \) individuals).

**Simulation Analysis Under the Null**

We conducted simulations (\( n = 1000 \)) to test for the presence of inflation in our estimates of the heritability captured by the genome-wide SNPs and, separately, by the \textit{cis} eQTLs. In this analysis, we preserved the genotype correlation structure, utilized the genetic relationship matrix defined by each set of SNPs, and calculated the heritability for a permuted trait (\( n = 1000 \)). An empirical p-value was generated for the estimate of heritability, defined as the number of times the estimate for a simulated trait matches or exceeds the observed estimate. Additionally, we determined the number of times an estimate for a simulated trait is consistent with zero heritability, e.g., in the case of \textit{cis} eQTLs, the set of points (\( h^2_{cis}, SE(h^2_{cis}) \)) in \([0.1] \times [0.1] \) that satisfy
\[ h_{\text{cis}}^2 - 2SE(h_{\text{cis}}^2) \leq 0 \]

where \( SE(h_{\text{cis}}^2) \) is the standard error for the estimate.

**Polygenic Modeling with Functional Variation**

We utilized polygenic modeling \(^4\) to evaluate the effect of large numbers of weakly associated SNPs characterized by very small allele frequency differences between cases and controls. To facilitate direct comparison with the Purcell et al. study, we pruned a given SNP set (e.g., the cis eQTLs) to filter SNPs in strong LD with other SNPs (using a pairwise \( r^2 \) of 0.25, within a 200-SNP sliding window). Using the set of risk alleles from the resulting LD-pruned set and the corresponding effect size from a “discovery” GWAS, we calculated, in a “validation” GWAS, a polygenic score from the log odds ratio-weighted sum of risk allele count \( x_{i,j} \) for each individual \( j \):

\[ S_j = \sum_i \log(OR_{i,j})x_{i,j} \]

For polygenic modeling, we evaluated the sets of cis eQTLs defined by P-value \( T_{\text{GEN}} \) < 0.0001, 0.01, 0.05, and 0.10. We tested the calculated polygenic score for association with disease status.
Figure 1. Enrichment of eQTLs among the top SNPs from the WTCCC study. The highest ranked SNPs (p<0.001) in the WTCCC study of BD were found to be enriched for cerebellum *cis* eQTLs and parietal cortex *cis* eQTLs relative to random SNPs matched on minor allele frequency and location with respect to nearest gene. The black dot represents the observed count and the histogram depicts the empirical (null) distribution of the eQTL count generated from the randomly drawn SNPs. (In contrast, no evidence for eQTL enrichment was observed in LCLs.)
Figure 2. Partitioning of variance captured by *cis* eQTLs by chromosome. The estimates of variance, from the two GWAS GAIN and TGen, captured by the cerebellum *cis* eQTLs on each chromosome were highly correlated (Pearson correlation = 0.60). Red corresponds to the GAIN study while orange the TGen study. The estimates shown here are on the observed scale.
Figure 3. eQTL-based polygenic modeling. We selected an LD-pruned set of cis eQTLs that meet a P-value threshold in a “discovery” GWAS (TGen) – P-value $T_{\text{GEN}} < 0.0001, 0.01, 0.05$, and $0.10$ were tested – and calculated a polygenic risk score from this set for each individual in a “replication” GWAS (GAIN) using the risk alleles and the effects sizes from the “discovery” TGen study. The set of cis eQTLs defined by P-value $T_{\text{GEN}} < 0.05$ ($n = 2,375$ SNPs) showed the most significant association with case-control status in GAIN using logistic regression. Each red mark indicates a cis eQTL SNP included in the polygenic model.
Table 1. Genome-wide association studies of Bipolar Disorder evaluated in our study.

| Study | Cases | Controls |
|-------|-------|----------|
| TGEN  | 1190  | 401      |
| GAIN  | 1001  | 1033     |
Table 2. Polygenic modeling with eQTLs. Using the cis eQTLs, we determined, for each individual in the “replication” GAIN study, a polygenic risk score, which is defined as the sum of the risk allele counts weighted by the log odds ratio using the risk alleles and the effect sizes from the “discovery” TGen study. The polygenic risk score was then tested for association with disease status in the GAIN study. An LD-pruned SNP set consisting of cis eQTLs with p < 0.05 in the TGEN study showed the most significant association with case control status in the GAIN study.

| TGen p-value threshold | p-value of association of polygenic score with disease status |
|------------------------|-------------------------------------------------------------|
| p < 0.0001             | 0.894                                                       |
| p < 0.01               | 0.0245                                                      |
| p < 0.05               | 0.01                                                        |
| p < 0.1                | 0.0115                                                      |
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AUTHOR CONTRIBUTIONS

ERG and NJC conceived and designed the study. ERG wrote the manuscript. NJC and DLN supervised the study. ERG, HKI, CL, and DLN contributed reagents/materials/analysis tools. The BiGS consortium participated in patient diagnosis and sample collection. All authors edited and approved the manuscript.
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