Estimating the Genetic Variance of Major Depressive Disorder Due to All Single Nucleotide Polymorphisms

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Genome-wide association studies of psychiatric disorders have been criticized for their lack of explaining a considerable proportion of the heritability established in twin and family studies. Genome-wide association studies of major depressive disorder in particular have so far been unsuccessful in detecting genome-wide significant single nucleotide polymorphisms (SNPs). Using two recently proposed methods designed to estimate the heritability of a phenotype that is attributable to genome-wide SNPs, we show that SNPs on current platforms contain substantial information concerning the additive genetic variance of major depressive disorder. To assess the consistency of these two methods, we analyzed four other complex phenotypes from different domains. The pattern of results is consistent with estimates of heritability obtained in twin studies carried out in the same population.

Key Words: Fasting glucose, genome-wide association studies (GWAS), genome-wide complex trait analysis (GCTA), height, major depressive disorder (MDD), missing heritability, smoking

A limited number of meta-analyses combining multiple genome-wide association (GWA) studies of psychiatric disorders have yielded replicated associations between such disorders and single nucleotide polymorphisms (SNPs) (e.g., schizophrenia) (1). In contrast, analyses of major depressive disorder (MDD) lack genome-wide significant results (2,3). Previously, we conducted a GWA study in 1738 MDD cases and 1802 controls selected to be at low liability for MDD (2). None of the SNPs reached genome-wide significance. Subsequent GWA and meta-analysis studies of MDD were equally unsuccessful in detecting significant SNPs (3). The lack of breakthrough results has spurred a discussion concerning the utility of GWA studies of psychiatric phenotypes. Part of this discussion focuses on whether SNPs analyzed in GWA studies are the relevant polymorphisms or whether psychiatric disorders traits are more likely influenced by rare variants.

Yang *et al.* (4) and So *et al.* (5) added to this discussion by proposing two methods that focus on the estimation of phenotypic variance explained by currently genotyped SNPs rather than on the detection of specific SNPs that contribute to a trait or disorder. Using human height as an example, Yang *et al.* (4) demonstrated that SNPs that are currently included on genotyping platforms explain a substantial proportion of the heritability of the phenotype. So *et al.* (5,6) reached similar conclusions. The two approaches differ substantially, with Yang *et al.*'s approach falling under the broad umbrella of variance decomposition methods and So *et al.*'s being a density estimation method. Both methods focus on estimating variance due to additive genetic effects.

The rationale of Yang *et al.*'s method is to decompose the variance of the phenotype into a component that is due to the additive effects of all measured SNPs and a residual component that is due to random noise, unmeasured environmental influences, or effects of unmeasured genetic variants that are not captured by the measured variants. Their two-step method involves first obtaining the genetic similarity between all pairs of subjects, which is calculated as a correlation. For each pair of subjects, their minor allele counts on a given SNP are subtracted from the SNP mean, and the cross-product of the resulting deviance scores is divided by the SNP variance. Summing these terms over all SNPs gives a measure of genetic similarity between two subjects that is due to additive genetic effects. The second step of the method uses this measure as a random effect to predict the phenotype in a linear mixed model. The original approach for continuous traits has been extended to case–control studies (7,8).

The method proposed by So *et al.* is entirely different and is applied subsequent to a GWAS. The basic idea is to compare the distribution of *z* statistics of the regression coefficients of genome-wide SNPs in a GWAS to the theoretical null distribution of *z* statistics representing no effects. Explained variance will differ from zero if more *z* statistics from the GWAS have larger values than expected under the null. Specifically, the observed *z* statistics, which contain error due to sampling fluctuation, are first corrected to obtain *z* statistics representing "true" effect sizes. The estimate of variance explained for continuous phenotypes can then be computed by summing contributions of all nonnull "true" *z* statistics using sums of squares as in analysis of variance. For case–control phenotypes, So *et al.* (6) have described a method using the odds ratio, allele frequency, and disease prevalence.

Our aim was to estimate the joint effect of all SNPs in explaining the variance in MDD. In addition, we investigated four other phenotypes that are currently targets of large GWA projects—namely, height, fasting glucose, smoking initiation, and current smoking. The estimates of explained variance of all five phenotypes using the two methods were compared with heritability estimates obtained in twin and family studies that were carried out in the same population.

The data analyzed in this study came from individuals who took part in The Netherlands Twin Register Biobank study (9) and The Netherlands Study of Depression and Anxiety (10). Genotyping on these samples was performed on the Affymetrix 6.0 (Affymetrix, Santa Clara California; $n = 298$), Affymetrix 5.0/Perlegen ($n = 3697$), Illumina 370 (Illumina, San Diego, California; $n = 290$), Illumina 660 ($n = 1439$), and Illumina Omni Express 1M ($n = 445$) platforms. Quality control was carried out per platform. Thresholds for SNPs were minor allele frequency greater than 1%, Hardy-Weinberg

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Table 1. Estimates for MDD, Smoking Initiation, Current Smoking, Fasting Glucose, and Height

	MDD	Smoking Initiation	Current Smoking	Fasting Glucose	Height
n Used for Estimation Methods	$n = 3245$ $n_{case} = 1620$ $n_{control} = 1625$	$n = 4181$ $n_{case} = 2602$ $n_{control} = 1579$	$n = 4181$ $n_{case} = 1189$ $n_{control} = 2992$	$n = 3723$	$n = 4199$
Method of Yang *et al.* (4)	.32 (.086) $p = 1.071 \times 10^{-4}$	.19 (.087) $p = .024$	.24 (.096) $p = .011$	.22 (.059) $p = 5.41 \times 10^{-5}$	.42 (.052) $p = 0$
Method of So *et al.* (5)	.28 (.058)	.28 (.084)	.44 (.063)	.19 (.036)	.29 (.035)
Heritability in Twin and Family Studies	.36 [13]	.44 [17]	.79[a]	.53[a]	.90 [18]

Variance estimates × 100 can be interpreted as percentage of explained variance. Standard errors for variance estimates are in parentheses, reference numbers are in brackets. The *p* value corresponds to the likelihood ratio test comparing the models with and without the single nucleotide polymorphism effects, and significance level is $p < .05$.

MDD, major depressive disorder.

[a]Estimated for this report using data from the Netherlands Twin Register ($n_{smoke} = 10{,}004$, $n_{glucose} = 3220$). Prevalences of MDD, current smoking, and smoking initiation were based on age-appropriate estimates for the Dutch population: .17, .34, and .50.

equilibrium greater than .00001, missing greater than 95%, and .30 < heterozygosity < .35. Samples were excluded from the data if their expected sex and identity by descent status did not match or if the genotype missing rate as greater than 10%. Subsequent to alignment using the Hapmap 2 Build 36 release 24 CEU (Utah residents with ancestry from northern and western Europe) reference set (http://hapmap.ncbi.nlm.nih.gov/), and exclusion of SNPs if allele frequencies differed more than 15% with the reference set and/or the other platforms, the data were merged into a single dataset ($n = 5856$). Imputation was done using IMPUTE v2 (http://mathgen.stats.ox.ac.uk/impute/impute.html), and SNPs were removed based on Hardy-Weinberg equilibrium less than .00001, proper info less than .40, minor allele frequency less than 1%, allele frequency difference greater than .15 against reference, and a missing rate greater than 5% assuming a 90% calling threshold. Individuals were removed with identity by state greater than .025, resulting in a sample of 4605 individuals. A second round of quality contral was carried out on the imputed data closely following steps and criteria described in Anderson *et al.* (11) and Purcell *et al.* (12). In addition, we removed SNPs that were significant in a Cochran-Mantel-Haenszel test of SNP–phenotype associations across platforms. For case–control phenotypes, we further removed SNPs that did not pass an adaptation of a two-locus test, which controls for differential case–control calling errors (13). Quality control resulted in approximately 1,140,000 SNPs for the analyses. The exact number varied slightly for the different phenotypes because the two-locus and platform association tests are phenotype-specific. The number of subjects varied between $n = 3245$ and $n = 4240$ depending on the phenotype. For MDD, we used a stringent definition of case (i.e., diagnosis of MDD) and control (i.e., no reported psychiatric illness), resulting in 1620 cases and 1625 controls. The data include a large proportion (approximately two-thirds) of the original Genetic Association Information Network MDD data set, which was selected for MDD. Note that these subjects were genotyped on the same platform, with MDD cases and controls randomly assigned to plates (2). Approximately one third of the $n = 4240$ sample that passed quality control were not selected for MDD.

For both methods, we used software provided on the respective developers' websites (http://gump.qimr.edu.au/gcta and http://sites.google.com/site/honcheongso/software/total-vg). Before applying the density estimation method, we carried out linkage disequilibrium (LD) pruning as suggested by So *et al.* (5) and used the kernel estimate because it is more stable in pruned data (5). We repeated all analyses with different pruned sets and obtained similar results, which demonstrates the robustness regarding the SNPs selected for the analysis.

Table 1 summarizes the results for all 5 phenotypes. For Yang *et al.*'s method, likelihood ratio tests comparing models with and without the SNP effects were carried out, which were significant for all phenotypes. Additional permutation tests substantiated these results. Including 4 principal components to control for population stratification did not impact results. Note that Yang *et al.* (4) and also So *et al.* (5) pointed out that their estimates are conservative because of imperfect LD of typed with causal SNPs. Results in Table 1 are uncorrected for this underestimation, the extent of which might differ for the two methods. Although point estimates vary between the two methods, all 95% confidence intervals (CIs) overlap.

The additive genetic variance of MDD due to all SNPs was estimated at 32% (95% CI [15.0%–48.8%] and 28% [95% CI 16.5%–39.3%]), respectively. Even when considering the lower bounds of the confidence intervals, this is a substantial proportion of the heritability estimate of 36% established in a twin study this population (14) and agrees with numerous other twin and family studies. The estimate concerning height using Yang *et al.*'s method is consistent with results obtained by Yang *et al.* (4). A limitation regarding the two smoking phenotypes is that approximately two-thirds of the sample was selected by MDD status, and both smoking phenotypes in our sample have rank correlations of approximately .24 with MDD. Height and fasting glucose have essentially zero correlation with MDD.

The results concerning MDD are in stark contrast to GWA studies that aim to detect specific SNPs (2,3). In GWAS of MDD, SNPs explain less than 1% of the variance (15). Our analyses show that SNPs typed on currently available platforms contain substantial information concerning the additive genetic variance of MDD and other complex phenotypes. The failure of MDD GWA studies is, among other reasons, likely due to small effect sizes of the involved SNPs, resulting in insufficient power to discriminate between signals and false positives (16). In addition to improving power in genome-wide analyses by increasing the number of samples in meta-analyses, novel analytical approaches including data-mining procedures can be customized to efficiently extract the information that is present in genome-wide SNP data. Given appropriate power, it should be possible to detect at least some of the locations in the genome associated with MDD and to provide a basis for the understanding of the genetic underpinnings of this devastating disorder.

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1. Kim Y, Zervas, S, Trace SE, Sullivan PF (2011): Schizophrenia genetics: Where next? *Schizophr Bull* 37:456–463.
2. Sullivan PF, de Geus EJ, Willemsen G, James MR, Smit JH, Zandbelt T, *et al.* (2009): Genome-wide association for major depressive disorder: A possible role for the presynaptic protein piccolo. *Mol Psychiatry* 14:359–375.
3. Wray NR, Pergadia ML, Blackwood DH, Penninx BW, Gordon SD, Nyholt DR, *et al.* (2012): Genome-wide association study of major depressive disorder: New results, meta-analysis, and lessons learned. *Mol Psychiatry* 17:36–48.
4. Yang J, Benyamin B, McEvoy BP, Gordon S, Henders AK, Nyholt DR, *et al.* (2010): Common SNPs explain a large proportion of the heritability for human height. *Nat Genet* 42:565–569.
5. So H-C, Li M, Sham PC (2011): Uncovering the total heritability explained by all true susceptibility variants in a genome-wide association study. *Genet Epidemiol* 35:447–456.
6. So HC, Gui AH, Cherny SS, Sham PC (2011): Evaluating the heritability explained by known susceptibility variants: A survey of ten complex diseases. *Genet Epidemiol* 35:310–317.
7. Yang J, Lee SH, Goddard ME, Visscher PM (2011): GCTA: A tool for genome-wide complex trait analysis. *Am J Hum Genet* 88:76–82.
8. Lee SH, Wray, NR, Goddard ME, Visscher PM (2011): *Am J Hum Genet* 88:294–305.
9. Willemsen G, Geus de EJC, Bartels M, Beijsterveldt van CEM, Estourgie-van Burk AI, Brooks GF (2010): The Netherlands Twin Register Biobank: A resource for genetic epidemiological studies. *Twin Res Hum Genet* 13: 231–245.
10. Penninx BW, Beekman AT, Smit JH, Zitman FG, Nolen WA, Spinhoven P (2008): The Netherlands Study of Depression and Anxiety (NESDA): Rationale, objectives and methods. *Int J Meth Psychiatr Res* 17:121–140.
11. Anderson CA, Pettersson FH, Clarke GM, Cardon LR, Morris AP, Zondervan KT (2010): Data quality control in genetic case-control association studies. *Nat Protoc* 5:1564–1573.
12. The International Schizophrenia Consortium (2009): Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* 460:748–752.
13. Lee SH, Nyholt DR, Macgregor S, Henders AK, Zondervan KT, Montgomery GW, Visscher PM (2010): A simple and fast two-locus quality control test to detect false positives due to batch effects in genome-wide association studies. *Genet Epidemiol* 34:854–862.
14. Middeldorp CM, Birley AJ, Cath DC, Gillespie NA, Willemsen G, Statham DJ, *et al.* (2005): Familial clustering of major depression and anxiety disorders in Australian and Dutch twins and siblings. *Twin Res Hum Genet* 8:609–615.
15. Demirkan A, Penninx BWJH, Hek K, Wray NR, Amin N, Aulchenko YS, *et al.* (2011): Genetic risk profiles for depression and anxiety in adult and elderly cohorts. *Mol Psychiatry* 16:773–783.
16. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, *et al.* (2009): Finding the missing heritability of complex diseases. *Nature* 461:747–753.
17. Vink JM, Willemsen G, Boomsma DI (2005): Heritability of smoking initiation and nicotine dependence. *Behav Genet* 35:397–406.
18. Silventoinen K, Sammalisto S, Perola M, Boomsma DI, Cornes BK, Davis C, *et al.* (2003): Heritability of adult body height: a comparative study of twin cohorts in eight countries. *Twin Res* 6:399–408.