A Genome-Wide Association Analysis of a Broad Psychosis Phenotype Identifies Three Loci for Further Investigation

Supplemental Information

Supplemental Methods

Principal Component Analysis

After the quality control, for the 4,835 individuals remaining with 695,193 single nucleotide polymorphisms (SNPs), we applied the following further SNP pruning filters: a 10% minor allele frequency, 10^{-3} Hardy-Weinberg equilibrium deviation threshold, and all SNPs within a 1500 SNP window had to have r^2 below 0.2 (window shift of 150 used). Thus a subset of 71,677 SNPs was selected for principal component analysis using EIGENSOFT version 3.0 (1). Three covariate vectors were obtained and a total of 356 individuals with non-European ancestry were removed (HapMap2 data and the average difference in the probe intensities across SNPs were used).

Bayesian Meta-Analysis

The Bayesian related effects model assumes a multivariate normal distribution with zero mean and covariance matrix $s^2 R$ as the prior distribution for the genetic effects on the log-odds scale. In this study, we have used a value $s = 0.2$ for the standard deviation of the effect size (see (2) for a justification) and in the correlation matrix R we have set the correlation between our data and the two replication data sets to 0.9375 and between the two replication data sets to 0.99. This reflects the fact that the phenotype definition is more similar between the replication data sets than between our data and the replication data sets. The Bayes factor between this related effects model and the null model (all effects are a priori equal to zero) can be approximated by the ratio of two multivariate normal densities,

$$\frac{f(b \mid mean = 0, \text{var} = s^2 R + S)}{f(b \mid mean = 0, \text{var} = S)},$$

evaluated at the effect size estimates from the three studies (collected in vector b) and where S is the diagonal matrix whose elements are the squared standard errors of those effect size estimates.

Polygenic Score Analysis

As explained in the main text, we performed a polygenic score analysis using the SNPs associated with schizophrenia in the Psychiatric GWAS Consortium (PGC) study. The 19,434 SNPs that were chosen as proxies for some PGC SNPs (see the main text) were aligned by the allele frequencies.

A logistic regression model regressing the case-control status on the three principal components covariates and the polygenic score had pseudo R^2 of 0.166 (Nagelkerke's pseudo R^2) and 0.0984 (McFadden's pseudo R^2), whereas for the model without polygenic score the corresponding values were 0.124 (Nagelkerke's) and 0.0723 (McFadden's). Here we interpret these values by saying that the variance explained by the polygenic scores is 4.2% (Nagelkerke's scale) or 2.6% (McFadden's scale). The P value for the polygenic score was 6×10^{-25}.

Similar analyses were run adding further covariates accounting for the seven centers involved in the study and the P value for polygenic score remained small (5×10^{-14}). With the centers as covariates, the pseudo variances explained by the polygenic score were 1.7% (Nagelkerke's pseudo R^2) and 1.4% (McFadden's pseudo R^2).
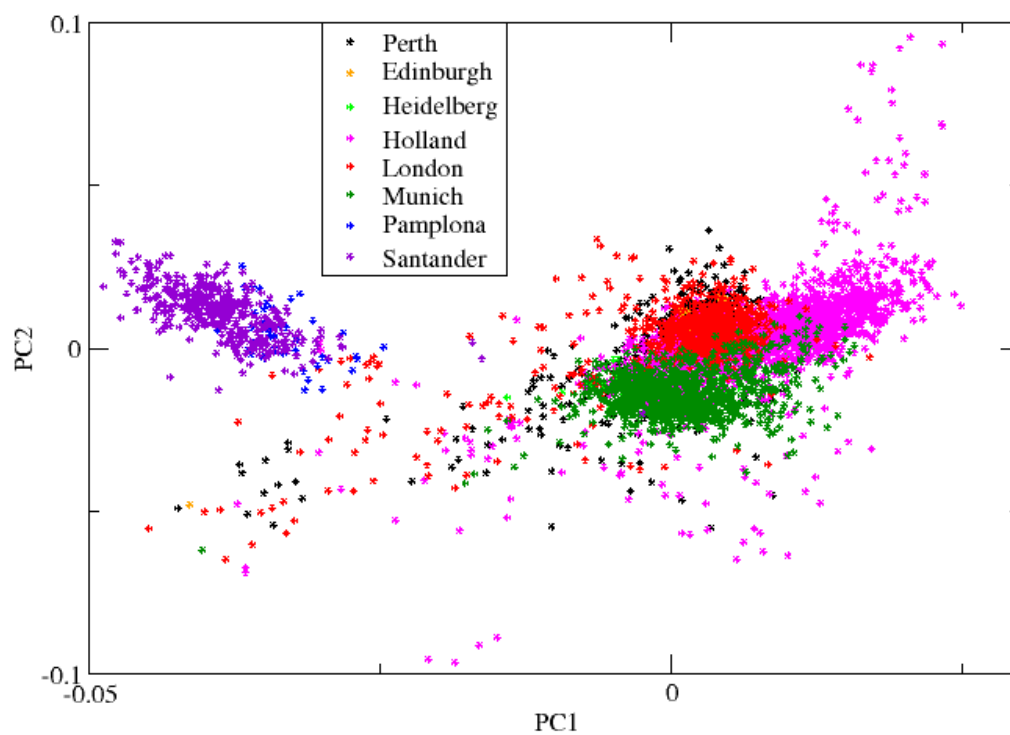
Figure S1. Principal components analysis of discovery data. Plotted is the projection of the study individuals on to the first two principal components (PC) of genetic structure. Individuals are colored according to recruitment locations as given in legend.
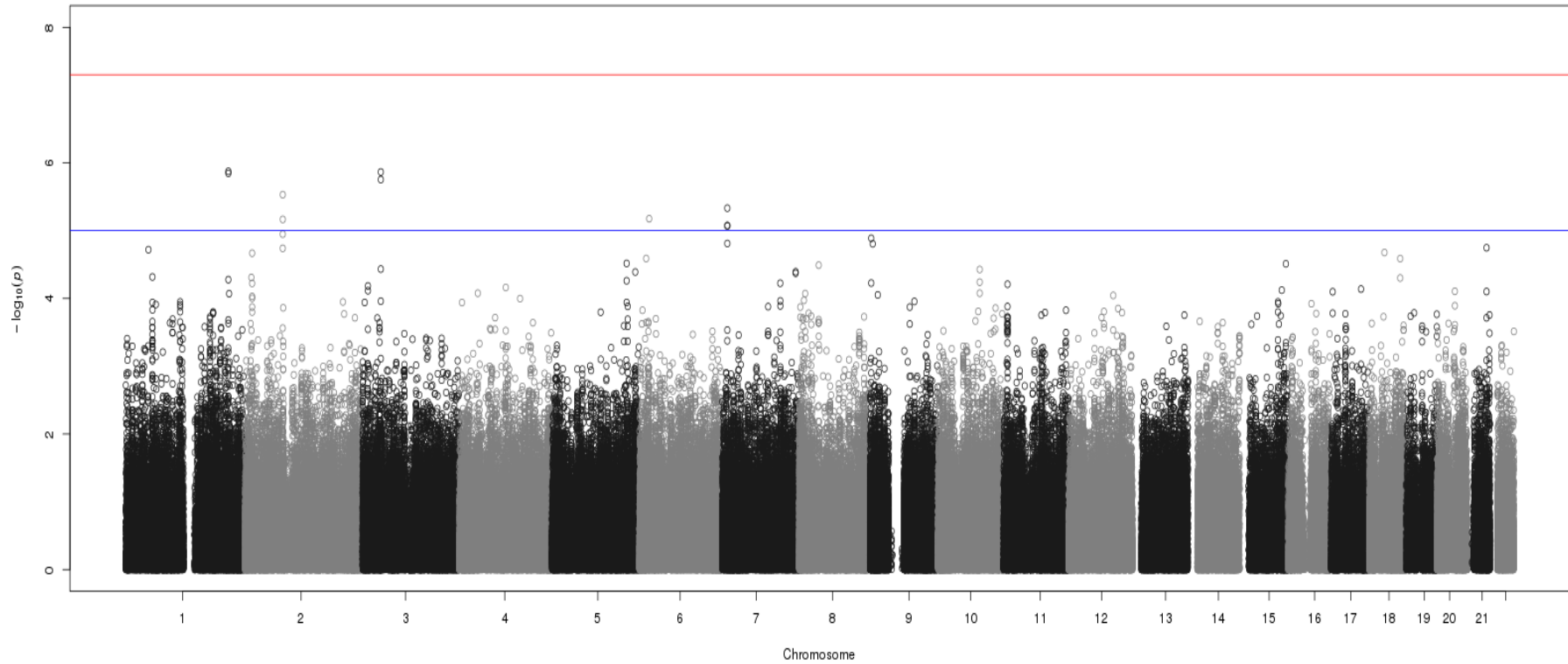
Figure S2. This Manhattan plot shows the evidence for association at all autosomal single nucleotide polymorphisms that passed quality control. Red line indicates a P value of 5×10^{-8} and blue line 1×10^{-5}. This analysis includes all samples passing quality control in our discovery study: 1,239 cases, 857 unaffected relatives and 2,739 healthy controls.
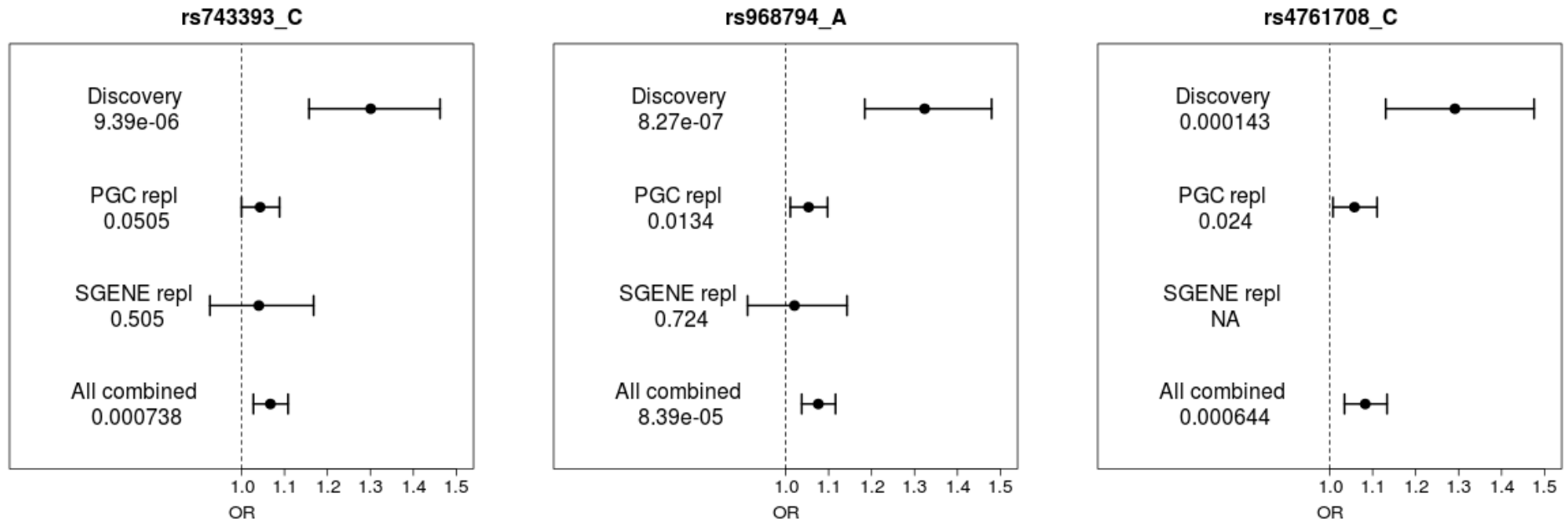
Figure S3. Forest plot showing the evidence of association in the discovery and replication cohorts. The plots show the estimated odds ratio (OR) and the 95% confidence interval at the three single nucleotide polymorphisms presented in Table 2 and Figure 3. Combined estimates use fixed effects meta-analysis. PGC, Psychiatric GWAS Consortium.
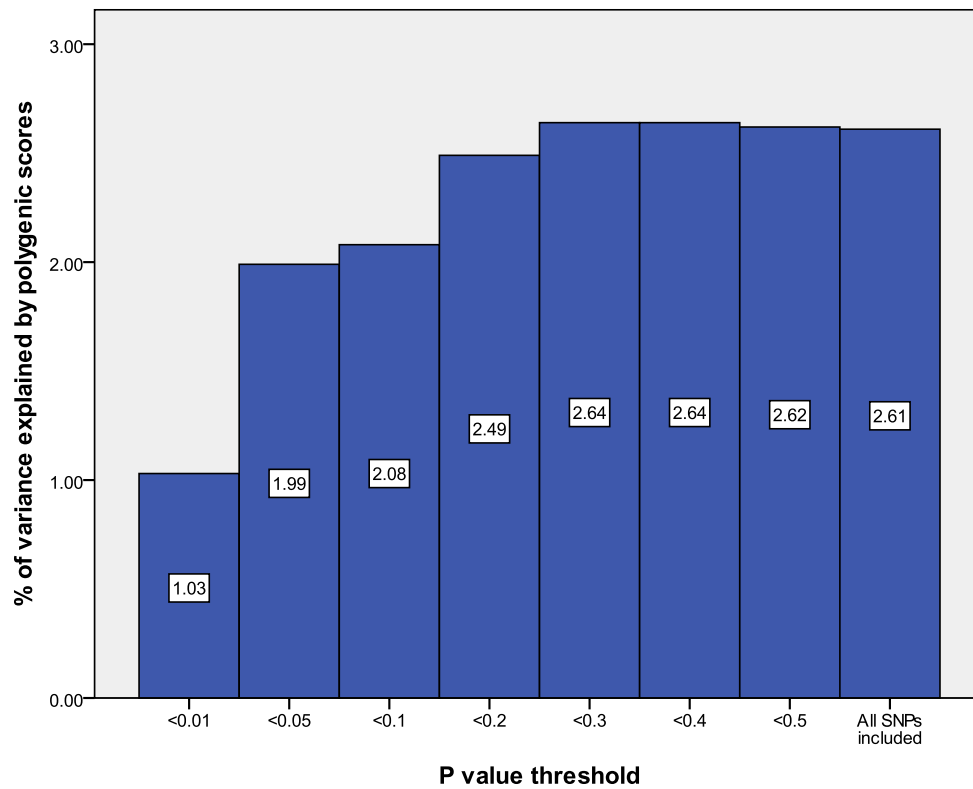
Figure S4. Variance explained (McFadden's pseudo R^2) by the polygenic scores by *P*-value threshold. The variance explained is plotted by *P* value threshold applied to the discovery Psychiatric GWAS Consortium schizophrenia sample (3) from a logistic regression model whose only covariates are the 3 principal components. The corresponding significance of the polygenic score at each threshold ranged between 4.0×10^{-11} and 5.7×10^{-25}. SNPs, single nucleotide polymorphisms.

Table S1. Samples prior to quality control and centers where they were collected.

Center Location	Number of Samples				Details of Centers
	Patients	Relatives	Controls	Total (%)	
London	406	352	522	1280 (18.5)	Institute of Psychiatry – King's College London
Holland	649	649	1300	2598 (37.5)	GROUP Consortium (University of Amsterdam, University of Groningen, Maastricht University, University of Utrecht)
Perth	376	205	194	775 (11.2)	The University of Western Australia
Santander - Pamplona	309	1	323	633 (9.1)	Universidad de Cantabria
Edinburgh	48	0	48	96 (1.4)	University of Edinburgh
Heidelberg	32	17	47	96 (1.4)	Heidelberg University
Munich	0	0	1457	1457 (21.0)	Ludwig-Maximilians-Universität München
Total	1820	1224	3891	**6935**	

Table S2. Quality control and sample exclusions in the discovery cohort.

	Clinical Group				
	Patients	Relatives	Controls	Total	%
Passed quality control & genotyped	1239	857	2739	4835	69.7
Genotyping failure	204	83	735	1022	14.7
Excluded – >2% SNPs missing	67	59	88	214	3.1
Excluded – duplicate or monozygotic twin	29	19	22	70	1.0
Excluded – heterozygosity	33	13	24	70	1.0
Excluded – ancestry	139	107	110	356	5.1
Excluded – sex mismatch	22	9	26	57	0.8
Excluded – clinical reasons	21	0	0	21	0.3
Failed DNA quality control	66	77	147	290	4.2
Total	1820	1224	3891	**6935**	

Table S3. Quality control and exclusions of SNPs.

Quality Control of SNPs	SNP Count	%
Chromosomes X, Y and mitochondrial DNA exclusions	38,895	4.2
SNPs with Mendelian inheritance errors	26,858	2.9
SNP excluded if > 5% of individuals failed genotyping	11,610	1.2
SNP excluded if MAF <2%	145,097	15.6
SNP excluded if HWE p value < 1 x 10^{-6}	2,404	0.3
SNPs excluded due to poor calling (manually inspected in Evoker)	9,499	1.0
SNPs that passed all quality control filters	695,193	74.8
Total	**929,556**	

HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

Table S4. Replication of previously published loci for schizophrenia and/or bipolar disorder (sign tests). The table shows the number of previously reported loci showing an effect size in the same direction at the best tag single nucleotide polymorphism (SNP) in Table 1. The sign test is a one-sided binomial test for an increase in the fraction of SNPs where the risk allele is the same. Results are shown for all SNPs (top) and thinned to remove SNPs within 100kb and to exclude the MHC regions (bottom). Figure 1 provides a plot of these data. * indicates SNPs associated with both schizophrenia and bipolar disorder. P values in bold are significant at the 5% level.

Phenotype	All SNPs		
	Loci with published evidence of association	Loci with same risk allele in our sample (%)	Binomial test *P* value for enrichment
Schizophrenia only	24	19 (79%)	**0.0033**
Bipolar disorder only	10	5 (50%)	0.623
Schizophrenia & bipolar disorder *	10	6 (60%)	0.377
Schizophrenia (including *)	34	25 (73%)	**0.0045**
Bipolar disorder (including *)	20	11 (55%)	0.41
	Thinned SNPs excluding MHC		
Schizophrenia only	17	13 (76%)	**0.025**
Schizophrenia (including *)	27	19 (70%)	**0.026**
Bipolar disorder (including *)	18	11 (61%)	0.24

Supplemental References

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2. WTCCC (2007): Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature.* 447:661-U667.

3. Ruderfer DM, Kirov G, Chambert K, Moran JL, Owen MJ, O'Donovan MC, *et al.* (2011): A family-based study of common polygenic variation and risk of schizophrenia. *Mol Psychiatry.* 16:887-888.

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