SUPPLEMENTARY METHODS AND RESULTS

This document contains supplementary material for:

Shi et al. Genomewide association study of recurrent early-onset major depressive disorder (GenRED GWAS); and

Shyn et al: Novel loci for major depression identified by genome-wide association study of STAR*D and meta-analysis of three studies.

Table of Contents

Supplementary files (association test results). Six supplementary datafiles are provided online:

genred_supplementary_data.txt
stard_supplementary_data.txt
meta-analysis_broad_supplementary_data.txt (as zip file)
meta-analysis_narrow_supplementary_data.txt (as zip file)
candidate_gene_results.xls
SNP_intensity_cluster_plots.pdf

The GenRED, STAR*D and meta-analysis files contain data for all SNPs that produced P<0.001 in at least one of the three analyses (males+females, males, females) for that dataset. There is a key in each file to the content of the data columns, to the right of the actual data. Frequencies of the tested allele are rounded to the first decimal place. Thus, "0" indicates 1-5%, "0.1" indicates 5-15%, etc. The frequencies have been "binned" in this way in order to prevent identification of individual subjects based on individual genotypes from some other source and pooled genotypes from this study.[1]

1. QUALITY CONTROL ANALYSES

Table S1 summarizes the final datasets that results from the QC analyses described in this section (this is an expanded version of the top part of Table 2 in the manuscript, with X chromosome counts added):

Table S1: Numbers of subjects and genotyped SNPs passing quality control (3 datasets)

		----------AUTOSOMAL----------				---------X CHROMOSOME---------			
		GAIN	**GenRED**	**STAR*D**	**Total**	**GAIN**	**GenRED**	**STAR*D**	**Total**
ALL	**Broad cases**	1716	1020	1221	**3957**	1683	1004	1211	**3898**
	Narrow cases	469	1020	702	**2191**	462	1004	696	**2162**
	Controls	1792	1636†		**3428**	1753	1607†		**3360**
MALES	**Broad cases**	524	298	506	**1328**	493	291	506	**1290**
	Narrow cases	113	298	276	**687**	106	291	271	**668**
	Controls	681	918†		**1599**	644	918†		**1562**
FEMALES	**Broad cases**	1192	722	715	**2629**	1190	713	715	**2618**
	Narrow cases	356	722	426	**1504**	356	713	425	**1494**
	Controls	1111	718†		**1829**	1109	689†		**1798**
	SNPs (N)	427,874	646,431	254,857		6,438	22,546	5,617	

† GenRED and STAR*D analyses used the same screened controls; the meta-analysis corrected for this overlap.

a. Genetics of Recurrent Early-Onset Depression (GenRED) dataset and MGS controls

This section describes QC procedures for the GenRED dataset for both the GWAS report and for the meta-analysis report. The two datasets are exactly the same except: (1) both reports include both genotyped and imputed SNPs, but the GenRED report includes genotyped SNPs that are not in HapMap II, whereas the meta-analysis report includes only HapMap II SNPs (whether genotyped or imputed) to permit imputation in other datasets where needed; and (2) thus, GenRED QQ plots are slightly different for the GWAS and meta-analysis reports, because the non-HapMap SNP data are missing from the latter.

Subjects. Subject recruitment and selection were as described in the main text of the GenRED paper, and characteristics of the sample are summarized in Table 1. The GenRED GWAS consisted of 1,110 MDD cases (of whom 1,020 were available post-QC) and 1,636 controls selected from a larger MGS European-ancestry control sample after all QC procedures were applied to that dataset (see below). All subjects are of self-reported European ancestry, and ancestry was further checked based on genotypes as described below. MGS subjects were genotyped in two phases (as described elsewhere[2, 3]). The MGS-GAIN (Genetic Association Information Network) subjects were genotyped during November-December of 2007 and the remaining "NonGAIN" MGS subjects were genotyped during May-July 2008. The European-ancestry genotyped sample of the MGS GWAS project (GAIN + NonGAIN, pre-QC) included 2,838 schizophrenia cases, 2,817 controls, 61 duplicates and 62 trios (for quality control analyses). We selected our 1,636 controls (863 from MGS-GAIN and 773 from MGS-NonGAIN) based on absence of lifetime major depressive disorder as described in the text. All MGS control data have been deposited in dbGAP. The NonGAIN control data are still under the dbGAP embargo period, and are used here as part of a collaboration with the MGS GWAS project. GenRED cases were genotyped in the winter of 2007-8. All subjects were genotyped using the same platform and procedure. Because the cases and controls

were genotyped seperately, we included QC procedures designed to filter out false positive results that could result from technical differences in genotyping quality for cases and controls.

Genotyping. All genotyping for case and control samples was carried out by the National Center for Genotyping and Analysis at the Broad Institute. All subjects were genotyped using the Affymetrix 6.0 array, which assays 906,600 SNPs and 946,000 monomorphic copy number probes (the latter are not considered here). Genotype calls were made by the BIRDSEED module of the BIRDSUITE package (version 2).[4] Genotypes were called separately for each plate (i.e., for each set of ~ 92 DNAs aliquoted onto the same plate and thus assayed at approximately the same time) as recommended by the authors of BIRDSUITE. Genotypes with confidence score > 0.1 were set as missing. [4]

MGS controls. We selected 1,636 MGS controls that passed MGS QC filters, and the same control genotypes were used for the STAR*D GWAS analysis as described below, while the meta-analysis of GenRED, STAR*D and GAIN-MDD corrected for this overlap. Note that the same analytic group was responsible for the MGS and GenRED GWAS analyses, so we were familiar with all aspects of the MGS QC procedures and results. Here we briefly describe the MGS QC criteria and results that are relevant to the GenRED GWAS and meta-analysis of the GenRED and STAR*D samples.

MGS control subjects were removed if any of the following criteria were met:

(1) genotyping call rate <97%;
(2) the gender predicted by X chromosome genotypes was inconsistent with the gender reported by the site or control data file;
(3) heterozygous genotypes > 28.5% or < 26.0% (based on all SNPs, prior to QC), a criterion that was established by inspection of the distribution of values in relation to call rates as an indicator of poor genotyping quality;
(4) the subject was an outlier in the distributions of scores for ancestry-informative Principal Components 1 and 2 from the EigenStrat analysis;
(5) the subject had estimated kinship > 10% with more than 50 subjects, based on pairwise IBD estimates computed by PLINK (we considered this an indicator of poor genotyping quality, given that we did not observe any unusual unusual clusters of ancestry which coul also explain such a finding);
(6) in the case of unexpected duplicates or close relatives, the subject with the lower call rate was removed from each such pair.

After autosomal QC, 1,636 MGS controls were qualified for GenRED GWAS. We then performed additional QC of X chromosome data and removed 27 additional subjects from X chromosome analysis because of low X chromosome call rate or unusual heterozygosity proportion (for females).

GenRED cases. Of 1,110 depression cases (one per family) submitted for genotyping, 1,092 were genotyped successfully. The following is the summary of the QC filters for subjects:

(1) Fifteen subjects were removed for call rates less than 97.4%, a threshold chosen according to the observed distribution of call rates and evaluation of the effect of alternative thresholds on Genomic Control λ values.

(2) Twenty-five additional subjects were removed who were outliers of the distribution of proportion of heterozygous genotypes. For a homogenous population, unusually high or low heterozygosity values are thought to occur primarily because of genotyping/DNA quality problems. For cases, outliers had heterozygosity < 31.06% or > 33.21% because on inspection of the distribution in relation to call rates. (Note that the thresholds are higher than for controls because for cases we excluded rare SNPs before computing heterozygosity.) An additional 13 were heterozygosity outliers but were already excluded because of low call rates.
(3) Five subjects were excluded as ancestry outliers. We performed Principal Components Analysis as described in the main text using EIGENSTRAT[5] using the genotypes (for SNPs typed successfully in these groups) of the GenRED case subjects, 1,636 selected controls and the 269 Hapmap II subjects. Ancestry outliers were removed by visual check of the first two principal component scores.
(4) Six subjects were removed because the gender predicted by X chromosome genotypes was not consistent with that reported by the recruitment site.
(5) Twelve subjects were excluded because of cryptic relatedness or unexpected duplicates. According to the estimated kinship coefficients, we found two unexpected duplicates, five sibling pairs, two parent-child pairs and one grandparent-child pairs.
(6) Nine subjects were excluded from association analysis because they had an ineligible family history of depression or had an ineligible proband diagnosis.

After subject quality control, 1,020 GenRED cases remained for association analysis for autosomal SNPs.

Sixteen GenRED cases (7 males and 9 females) were removed from association analysis for SNPs on the X chromosome because of low call rate for X chromosome genotypes (less than 99% for males and 97% for females). Twenty-nine MGS controls were also removed from X chromosome association analysis. So, 1,004 GenRED cases and 1,607 MGS controls remained for X chromosome association analysis.

SNP QC. For the controls, to correct for any technical differences in the genotyping of the GAIN and NonGAIN controls, we performed separate SNP QC for MGS-GAIN, MGS-NonGAIN, and the combined MGS GAIN+NonGAIN datasets. We applied SNP QC criteria to each of these analyses separately, and excluded any SNP that failed any criterion in any of these three analyses. Criteria for each parameter were selected based in part on extensive evaluations of the effects of alternative criteria on GC λ values. SNPs were excluded that met any of the following criteria:

(1) SNP call rate < 95%;
(2) P-value for deviation from Hardy-Weinberg Equilibrium $<10^{-6}$ (in controls);
(3) MAF<1%;
(4) failed plate effect test: for each SNP, allele frequencies of each plate were tested against all other plates combined, and a SNP failed if one plate had a $\chi^2[1]$ test with $P < 10^{-8}$ or 2 plates had $P < 10^{-4}$;
(5) >2 trios with a Mendelian error;
(6) >1 discordant genotype in duplicate pairs;
(7) >2 discordant genotypes in 93 subjects (31 trios) genotyped twice (MGS-GAIN and NonGAIN).

Based on control sample QC only, 671,424 autosomal SNPs and 25,068 X chromosome SNPs were eligible for further analysis.

Next, we removed SNPs with call rate less than 97% or MAF less than 1% in GenRED cases. Then, because the GenRED cases and MGS controls were genotyped separately, we applied an additional filter based on case-control call rate difference (CCCRD) between GenRED cases and MGS controls. The rationale here was that if the call rate was much lower in one experiment, this could have been due to a technical factor that could produce differential (rather than random) missingness and thus could produce a spurious case-control allele frequency difference (false positive test). This effect has been reported previously by Clayton et al.[6] The CCCRDs were computed for all cases and the 1,378 control samples (from the GAIN sample) available in March 2008. We computed the correlation between CCCRD and -log(p) of the association test for that SNP; there was a highly significant correlation, which was eliminated when SNPs were excluded that had CCCRD > 2%. This was therefore adopted as a filter criterion. Subsequently, when all MGS control samples (GAIN+NonGAIN) were available, we re-checked the CRD and found that an additional 535 SNPs exceeded the 2% threshold. Because imputation had already been carried out using these SNPs, we did not exclude them. We manually checked the SNPs with the lowest p-values among these 535 SNPs to make sure that the p-values were consistent with other SNPs in the region. For example, the lowest p-value was 0.000075 (rs17689334), which has neighboring SNPs with p = 0.00026 (rs12986994) and p = 0.00038 (rs11894180).

For X chromosome SNPs, we first excluded SNPs failing MGS QC analyses. Then we computed the genotype call rate for male controls, male cases, female controls and female cases. Any SNPs with call rate <99% in male cases or <98% in female cases were removed. The CCCRDs were computed separately for males and females. Any SNPs with CCCRDs > 1% between cases and controls in females or in males were removed from analysis (based on examination of the correlation between CCCRD and -log(P).

After completion of these additional QC steps involving cases, there were 646,431 autosomal SNPs and 22,546 chromosome X SNPs that passed all QC filters and were used in association testing and for imputation of additional HapMap II SNP data for GenRED cases and for the controls for GenRED analyses. (Note that in the STAR*D section, below, we describe a separate SNP QC procedure to select a set of SNPs genotyped in STAR*D cases and GenRED controls; this much smaller set of SNPs was used for imputation of case and control data for the STAR*D GWAS analysis.)

The imputation procedure described below was applied to the GenRED dataset. Based on a criterion of MAF > 1% and imputation $R^2 > 0.3$ (as recommended by the authors of MACH 1.0[7], and see discussion in the main text of each paper), an additional 1,849,062 autosomal SNPs and 43,124 chromosome X SNPs (HapMap II) were available for analysis based on imputed genotypic data. Thus, analyses considered 2,495,493 autosomal and 65,670 chromosome X SNPs, for a total of 2,561,163 genotyped or imputed SNPs. The QQ plot for the GenRED GWAS analysis is shown below (**Figure S7**).

b. Sequenced Treatment Alternatives to Relieve Depression (STAR*D) dataset

QC analyses of this dataset have been described in the main text of this article, and resulted in selection of 1,221 Caucasian STAR*D cases for inclusion in the meta-analysis. Note that these cases were genotyped with two different platforms as described in the text: 606 with Affymetrix 5.0 and 639 with Affymetrix 500K. The 1,636 MGS/GenRED controls were genotyped with the Affymetrix 6.0 array. The same STAR*D data have been used for the STAR*D GWAS analysis and for the meta-analysis reported in the main text.

SNP QC. We carried out a new SNP QC procedure, starting with 378,549 SNPs that had been genotyped in both the STAR*D and GenRED datasets and that had passed QC for the MGS controls (see above for MGS filter criteria). Then, we used three procedures to filter out SNPs with possible case-control differences in genotyping quality, to avoid spurious findings:

Rate of discordant genotypes in cross-platform duplicates. There were 806 control subjects who had Affy 6.0 genotypes from MGS, and who also had been genotyped with the Affymetrix 500K array in a previous study, with results made available through the NIMH repository.[8] We verified that the two sets of genotypes for these 806 subjects represented true duplicates by estimating pairwise IBS/IBD with PLINK. Then, for each SNP, discordancy rates within the duplicate pairs were examined, and SNPs were excluded if they had >8 discordant genotypes out of 806 (~1% discordancy rate), because this indicated that genotyping was of low quality for at least one platform. For example, discordancy rate was strongly related with association test p-values with all SNPs included, but there was no significant correlation after excluding the SNPs with >8 discordant genotypes.

Minor allele frequency. Rare SNPs are more vulnerable to genotyping errors due to the nature of clustering algorithm for genotyping calling and because for a given number of errors, the trend test will be more greatly affected for rare SNPs; thus the risk of spurious findings is greater for rare SNPs when using genotypes from two different platforms, where there is potential for genotyping error in either platform. Therefore, we excluded SNPs with MAF < 5% in either cases or controls in STAR*D. However, we imputed genotypic data for SNPs with MAF between 1-5% in this dataset, and used the results in the meta-analysis if the imputation R^2 was > 0.3.

Call rate difference between cases and controls. We excluded SNPs with call rate < 98% in either MGS controls or STAR*D cases, and we also excluded SNPs with case-control call rate difference > 2%.

X chromosome QC. For X chromosome analysis, 10 additional STAR*D cases (plus 29 MGS controls as noted above) were excluded for average call rates < 97%. We then filtered out SNPs failing MGS SNP QC or with > 7 discordant genotypes based on the MGS controls genotyped twice. For the remaining SNPs, we computed QC metrics separately for males and females and excluded SNPs with call rate < 98%, or MAF < 5%, or case-control call rate difference > 2%.

Marker coverage estimation. Because a smaller number of genotyped SNPs was available for the STAR*D analysis, we estimated genome-wide coverage of common SNPs empirically. The results are reported in the main text. We downloaded the ten 500 kb genomic regions studied in HapMap ENCODE

(http://www.hapmap.org/downloads/encode1.html.en) and used the Tagger algorithm in Haploview to determine what proportion of HapMap ENCODE CEU SNPs were captured by the markers remaining in our panel, post-QC, within each respective ENCODE interval plus an additional 500 kb upstream and downstream. These numbers were the basis for the figures we reported in Results at r^2 levels of 0.50 and 0.80. We used this approach to estimate coverage in order to reduce the bias of using only HapMap SNPs, which leads to upward biases in the extent of coverage[9].

c. GAIN-MDD dataset

Genotyping. Genotyping procedures using the Perlegen platform have been described in the published GWAS report for this dataset.[10] This dataset was made available to qualified scientists through the dbGAP website. We obtained approved access to carry out genetic association analyses of major depressive disorder and related disorders, and carried out our own QC procedures to ensure consistency with the other two datasets in our meta-analysis and because no post-QC data had been made available through dbGAP when we initially accessed the data. There are some small differences between our results for this dataset and the previously published results[10], but these are minor and are mainly due to our exclusion of a slightly larger number of ancestry outliers (individuals with higher proportions of non-European ancestry according to our principal components analysis).

The original dataset downloaded from dbGap had genotypes and quality scores for 598,123 SNPs in 3,761 subjects (1,821 cases, 1,822 controls, 73 parents forming 36 complete trios and 20 duplicates of whom 9 were controls and 11 were cases. For the Perlegen quality score for each genotype, larger scores imply better quality, with a recommendation by the company to remove SNPs with quality score < 7. For consistency with the published report, however, we applied a filter of < 10 which was recommended by consultants to the GAIN program. Such a high threshold lowered the call rate (sometimes to as low as 89%,)compared to a mean call rate of 98% <7 as the criterion.

Sample QC. Pairwise kinship was estimated for all pairs of subjects using PLINK based on 59K SNPs (every 10^{th} SNP along each chromosome). We excluded thirty-eight subjects (excluding the pair member with the lower call rate) who were related to other subjects: we found fourteen parent-child pairs, one affected sibling trio, thirteen sibling pairs and eleven second degree relative pairs. In addition, we found a set of nine subjects, each pair of which had an estimated kinship larger than 0.1. Ancestry analysis based on principal components suggested that these nine subjects had similar ancestry and were likely to have a high Asian admixture, but they were unlikely to be relatives. These subjects were excluded as ancestry outliers as discussed below.

Additional exclusions, based where appropriate on inspection of distributions and evaluation of effects of alternative criteria on GC λ scores, included:

(1) One subject was removed because of low heterozygosity proportion of 24.6% which was a clear outlier to the distribution of values, indicating likely genotyping problems.
(2) Eight subjects were excluded due to call rate < 97%.
(3) Seven subjects were removed because the gender predicted by X chromosome genotypes was in consistent with the gender in the downloaded datafile.

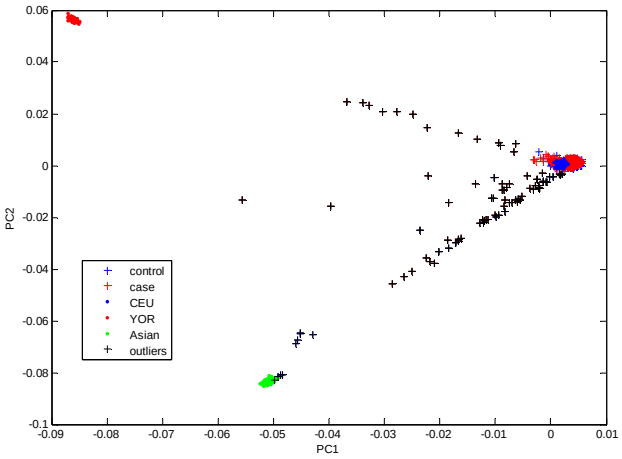


Figure S1. The first two principal components for GAIN-MDD and Hapmap II subjects. Outliers were defined as having PC1 < -0.005, or (PC1 < 0 and PC2 < -0.0015), or (PC1<0.002 and PC2<-0.002).

(4) We merged the genotypes of the GAIN MDD subjects with Perlegen genotypes for 269 Hapmap II subjects and then computed principal components (EIGENSTRAT[5]). Based on visual inspection of scores for the first two principal component scores (see **Figure S1**), we excluded 81 subjects as ancestry outliers.

After sample QC, 3,508 subjects (1,716 cases and 1,792 controls) remained for association analysis.

SNP QC. We filtered out SNPs that met at least one of the following criteria:

(1) MAF <1%;
(2) The number of discordant genotypes > 1 out of 20 duplicate pairs;
(3) Mendel error > 1 in 36 complete trios;
(4) Call rate < 95%;
(5) P-value for deviation from (in controls) < 10^{-6}.
(6) CCCRD > 2%.

Removing or SNPs for HWE deviation remains controversial. For GAIN MDD, ~ 2,500 SNPs passed other QC filters but failed the HWE test. Sullivan et al.[10] chose not to remove these SNPs from analysis on the grounds that deviation from HWE did not necessarily reflect poor genotyping quality. Here, however, the meta-analysis required imputation of data for most SNPs, and the imputation algorithms assume HWE. Therefore we did exclude SNPs based on deviation from HWE in controls. After QC, 427,874 (out of 585,874) autosomal SNPs remained for association analysis.

For the ***X chromosome***, many SNPs had low call rates in the GAIN-MDD dataset. Therefore we filtered out these SNPs before computing call rates for samples. We computed the SNP QC metrics separately for males and female, but the final exclusion thresholds were the same as 1-6 above. After SNP QC, 6,438 SNPs (out of 11,540) remained for association analysis. (Note that a less stringent quality score criterion would result in a larger number of X SNPs for analysis.) Based on these SNPs, we computed the

call rate and heterozygosity proportion for the 3,508 subjects who passed QC for autosomal SNP analysis; 33 cases and 39 controls were excluded from X chromosome analysis because of call rate.

d. Population stratification

Analysis of population stratification was carried out using the 81,913 SNPs autosomal SNPs (post-QC) that were genotyped in all three studies, after removing 448 SNPs from the MHC region (chr6:25-35 Mb) and the common 8p inversion region (chr8:8-12 Mb) because these regions produce highly local components that do not appear to reflect geographical ancestry (data not shown). Principal component (PC) scores were computed (using EIGENSTRAT) for the combined three-study dataset. We used the top 10 PC scores as covariates in logistic regression association analysis to control for the effects of population stratification.

Principal components were computed separately for the X chromosome, using 1,232 post-QC SNPs that were genotyped in all three studies. The top 10 PC scores from this analysis were used as covariates in logistic regression analysis for X chromosome SNPs. **Figure S2** illustrates PC scores 1-5 for a subset of GenRED subjects and MGS controls who self-reported a single ancestry in all grandparents.

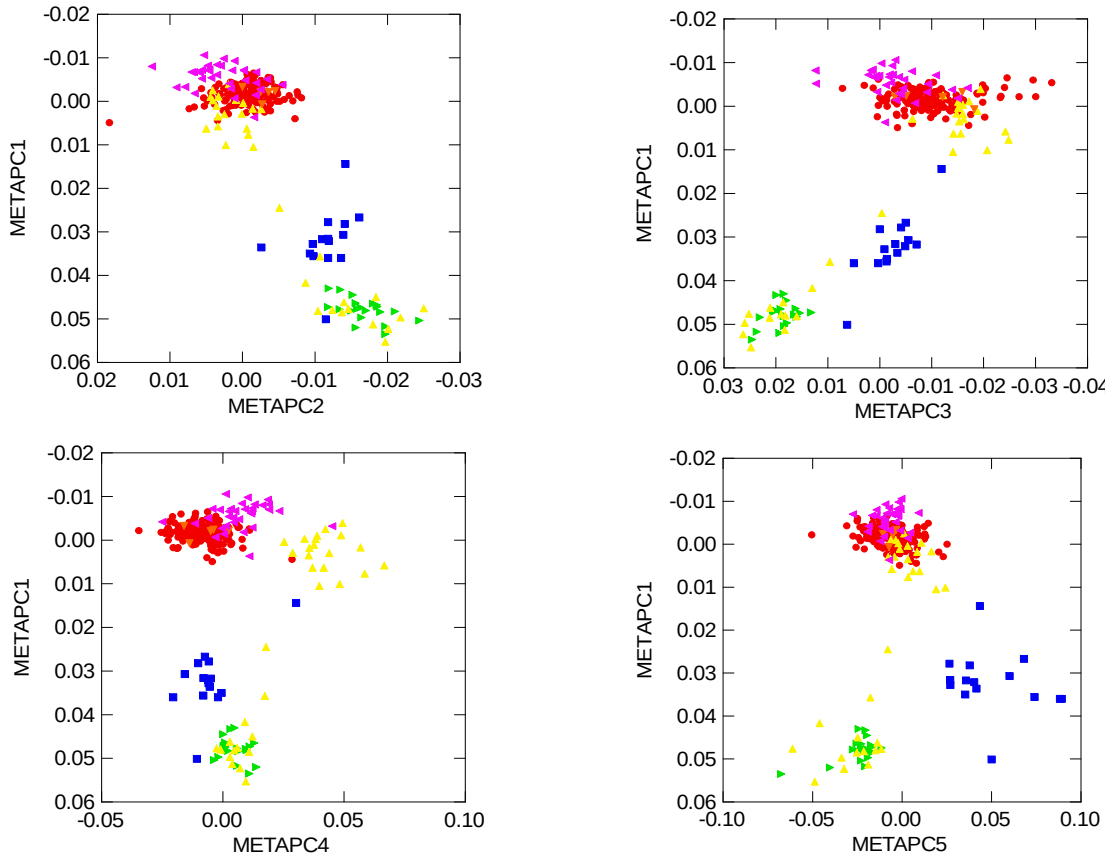


Figure S2. PC Scores for subjects self-reporting one ancesty. ***Red=Anglo-Saxon, Blue=Mediterranean, Green=Ashkenazi, Yellow=Slavic, Orange=No.European, Purple=W.European***

The plots illustrate the top 5 PC scores for a subset of GenRED and MGS subjects who self-reported a single European ancestry, to inform the interpretation of the geographical origin of the components. The PC scores were computed as described above for all meta-analysis subjects together. Note that these are within-Europe variations; if inspected in relation to HapMap CEU, YRI and CHB/JPT data, all of these subjects are in the "European" cluster. The geographical distribution of the top 5 PCs is as follows:

PC	Geographical distribution
1	North-South - Anglo-Saxon and N/W Eur at, top, Ashkenazi at bottom. Note: the Ashkenazi cluster includes a subset of Ashkenazi Jewish subjects who self-report as "Russian/Slavic" ancestry.
2	Also primarily North-South in this subset (differentiates Asian in other datasets).
3	Separates Ashkenazi from other ancestries.
4	East-West - non-Ashkenazi Slavic subjects are separated to the right.
5	Separates Mediterranean (mostly Italian and Greek) from other ancestries.

2. IMPUTATION OF GENOTYPIC DATA FOR HAPMAP II SNPS

There were ~ 90,000 post-QC SNPs common to all datasets -- too few for a meta-analysis. Therefore, we used MACH 1.0[7] (http://www.sph.umich.edu/csg/abecasis/mach/index.html) to impute autosomal SNPs and IMPUTE[11] (http://www.stats.ox.ac.uk/~marchini/software/gwas/impute.html) for X chromosome SNPs for the three datasets. The training data consisted of phased autosomal haplotypes of 60 Hapmap II CEU subjects and 90 sets of phased X (CEU) haplotypes. MACH and IMPUTE estimated genotypes conditioning on the flanking markers and linkage disequilibrium, as a "dosage" of one of the alleles (i.e., the estimate is non-integer, reflecting the weighted probabilities of each possible genotype); and, for each SNP, the imputation information content r^2. This is an estimate of the squared correlation that would be observed between the imputed and real genotypes, if the real genotypes were known. MACH computes r^2 from the variance of the imputed genotypes divided by the expected variance (which is 2p(1-p), where p = allele frequency). For the X chromosome, we computed the expected variance as 4p(1-p) for males. The effective sample size of a test for the SNP is proportional to r^2. Hence, SNPs with r^2<0.3 or MAF < 0.01 in any of the three data sets were excluded from meta-analysis because of insufficient power (see discussion in text of each paper). The meta-analysis thus included 2,339,408 autosomal and 51,795 X chr SNPs with rsq>0.3 and MAF>1% in all three studies, totaling 2,391,203 SNPs.

For STAR*D, we used the same 254,857 SNPs to impute case and control genotypes; but 646,431 SNPs were used to impute genotypes for these same controls for GenRED. Thus an imputed SNP could have a different estimated genotypic dosage for the same subject in the two analyses (as shown in Table S12). This was necessary because we observed an inflated variance in the empirical distribution of STAR*D test statistics when imputation was carried out using different sets of markers for cases and controls, suggesting that this sometimes resulted in systematically different genotype calls in cases vs. controls.

The distributions of imputation r^2 values in the three studies are illustrated in **Figures S3 and S4** for autosomal and X chromosome SNPs. For autosomal SNPs, average r^2 is higher for GAIN and GenRED because STAR*D had fewer genotyped SNPs as a basis for imputation. For X chromosome SNPs, GenRED r^2 is higher because of the larger number of genotyped X chromosome SNPs. Males have slightly higher average r^2 for X chromosome SNPs in all studies because more SNPs passed QC filters for males.

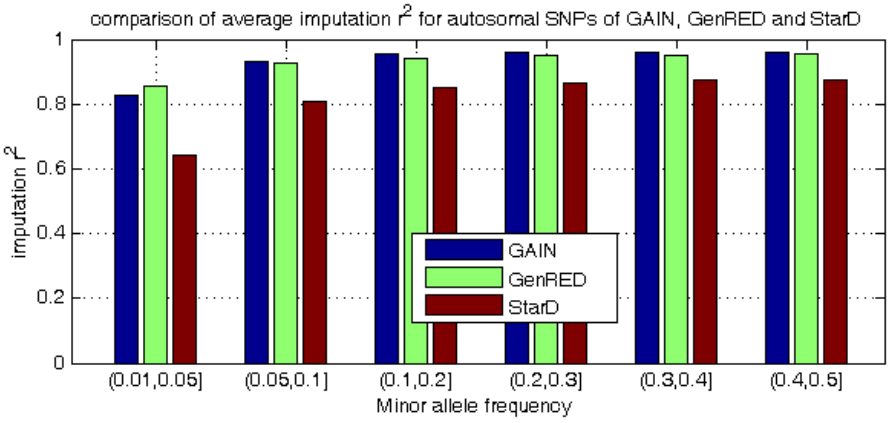


Figure S3. Average imputation r^2 of the three datasets (autosomal SNPs).

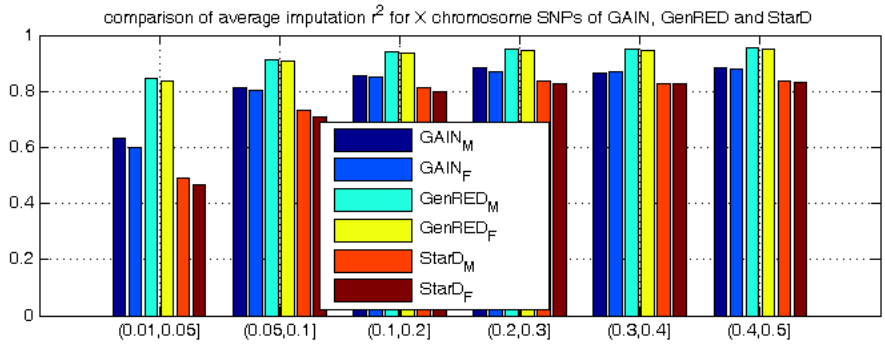


Figure S4. Average imputation r^2 of the three studies (X chromosome SNPs).

In **Figures S5 and S6**, we also compared the datasets for coverage (the proportion of all common Hapmap SNPs with imputation r^2 > a threshold). STAR*D has a lower coverage for autosomal SNPs, while males have slightly higher coverage than females for X chromosome SNPs in all three studies.

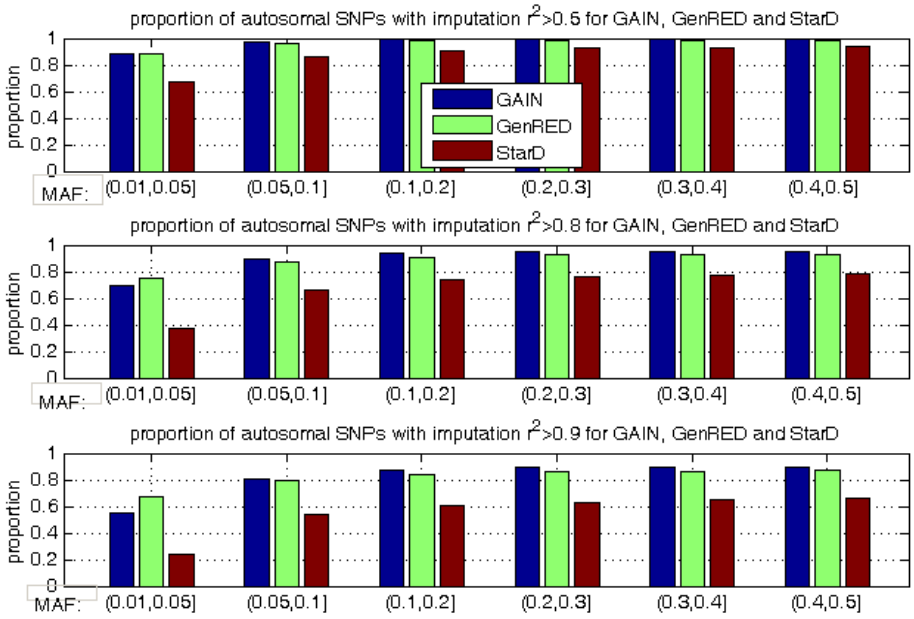


Figure S5. HapMap coverage for the three studies (autosomal SNPs).

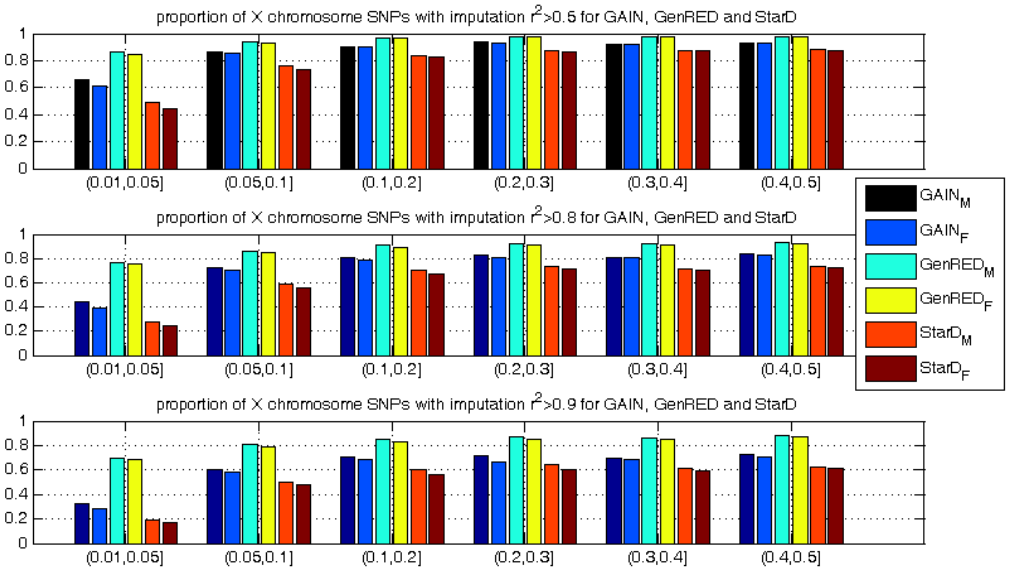


Figure S6. HapMap coverage for the three studies (X chromosome).

3. STATISTICAL ANALYSIS OF GENETIC ASSOCIATION

a. Statistical analysis of individual datasets. For each study, we performed logistic regression analyses to derive a Wald Z test statistic for each SNP, correcting for potential population stratification using the top 10 ancestry-informative principal components. If a SNP was genotyped and passed SNP QC filters, we used the Z statistic based on real genotypes without imputing the missing genotypes (using PLINK), because for these SNPs, imputing the missing genotypes tends to shrink larger *Z* scores slightly (data not shown). If a SNP was not genotyped or did not pass QC filters, the logistic regression analysis was carried out based on the imputed genotypic dosages (using custom software implementing the same procedure as PLINK, but permitting input files with non-integer dosage values). For X chromosome SNPs, we also corrected for sex in the logistic regression to avoid potential bias that could result because there were unequal numbers of cases and controls of each gender, and there are likely to be some technical differences in genotype calling and imputation for males (homozygous) and females (hemizygous). For SNPs on male X chromosomes, the two homozygous genotypes were coded as 0 and 2. For each SNP in each study, we determined the Z statistic value, the tested allele, the allele frequency of the control subjects, the number of cases/controls and the imputation information content. These summary statistics were then used to construct a test for the meta-analysis as described below.

Genomic control (GC) λ values will be > 1 when systematic genotyping differences (e.g., call rate, genotyping error rate) exist between cases and controls or in the presence of population substructure.

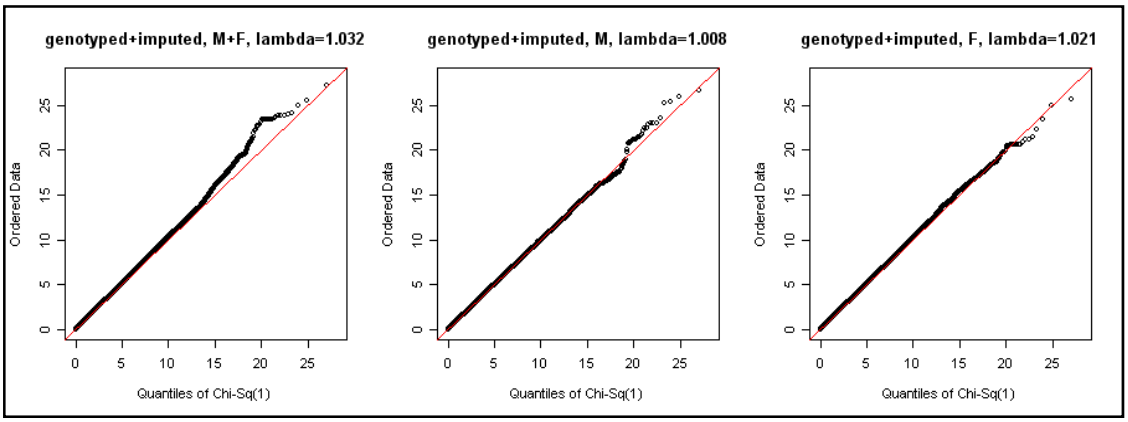


Figure S7. QQ plots for GenRED (genotyped and imputed SNP data used for GenRED GWAS analysis).

Non-HapMap genotyped SNPs are included above but not in the meta-analysis (below) (because they could not be imputed in other datasets). Thus, Figures S7 and S8 are slightly different.

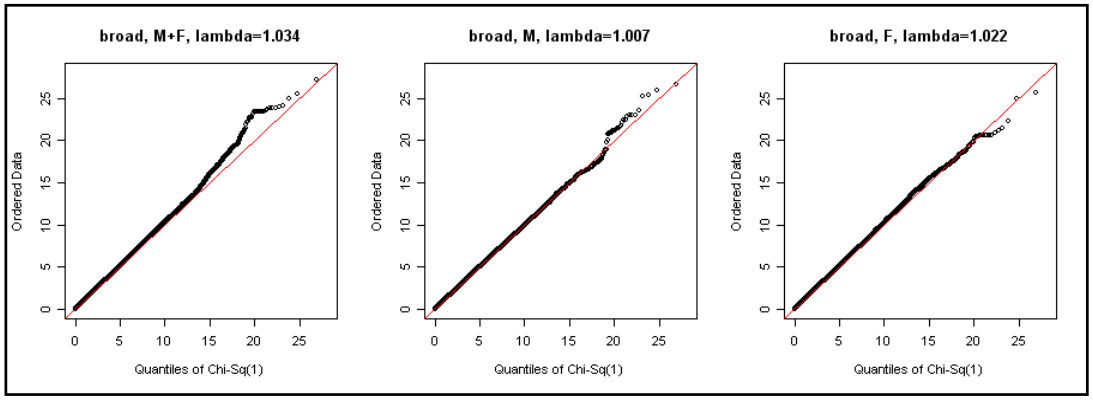


Figure S8: QQ plots of GenRED analysis for meta-analysis (non-HapMap SNPs excluded).

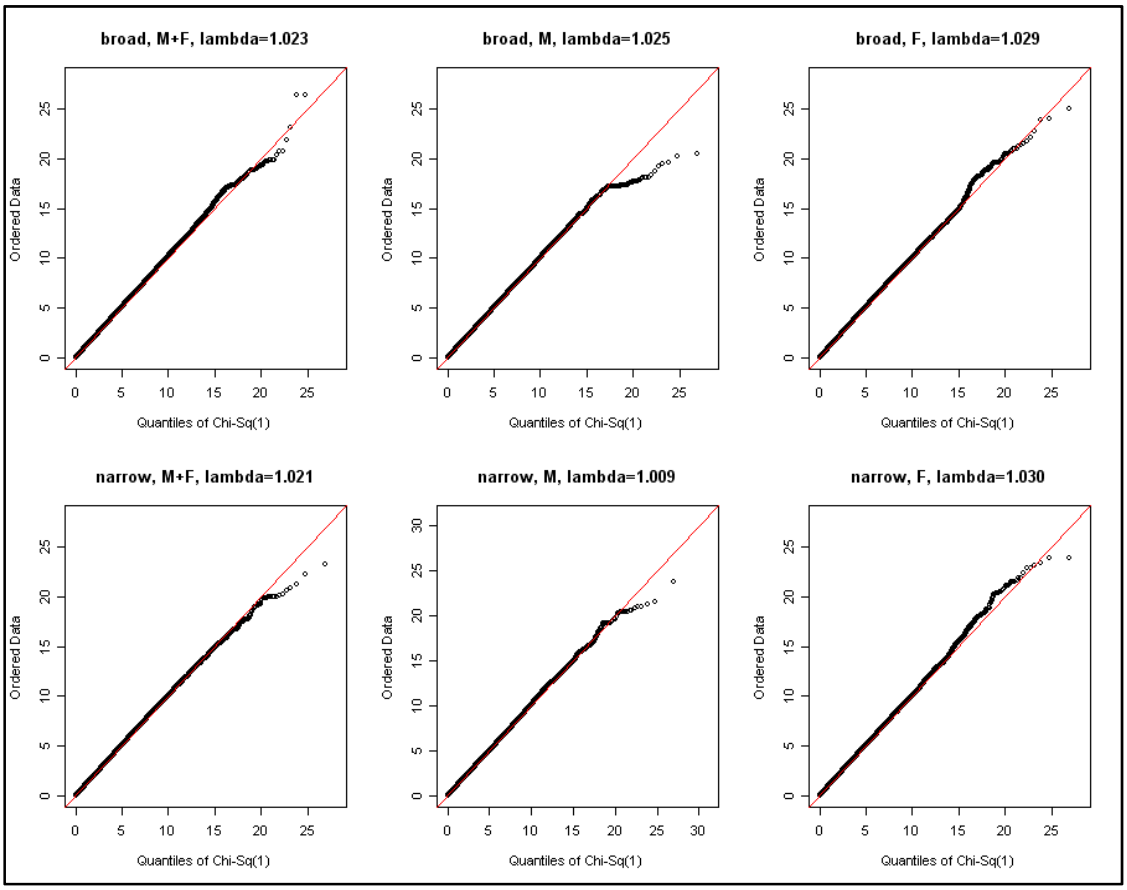


Figure S9. QQ plots for STAR*D analysis for meta-analysis.

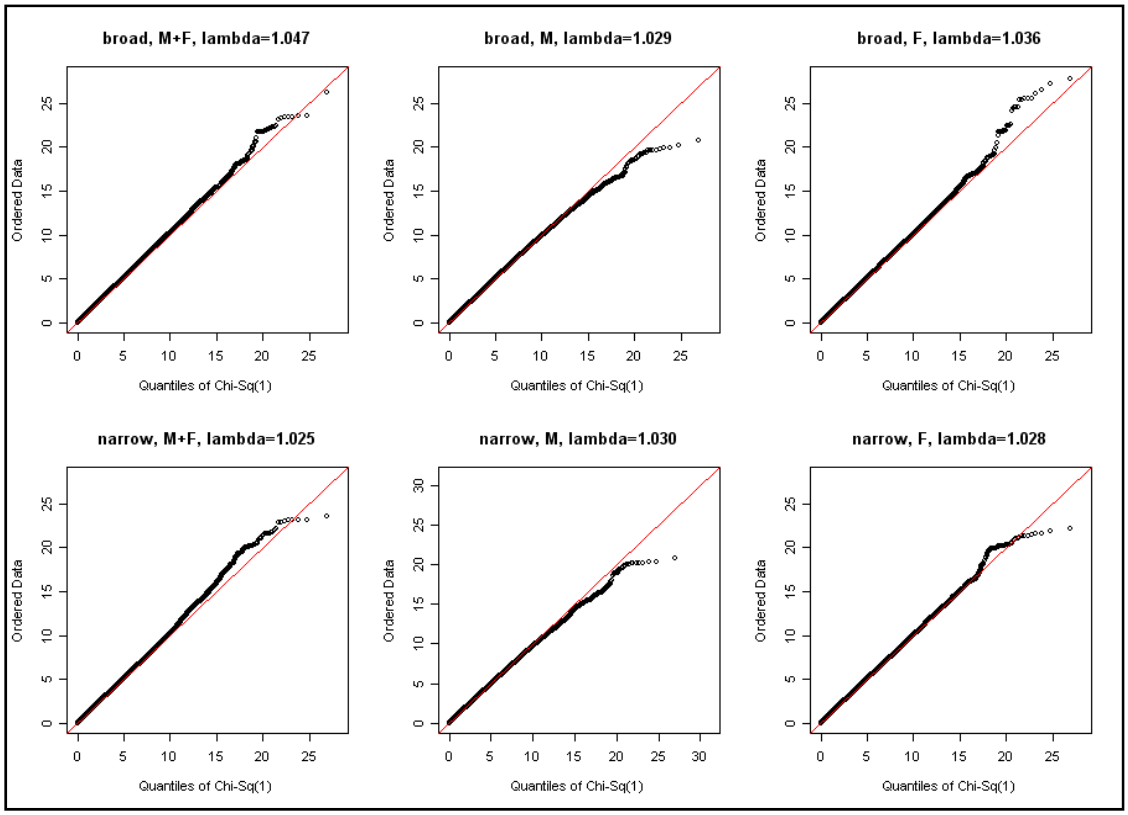


Figure S10. QQ plots for GAIN-MDD analysis for meta-analysis.

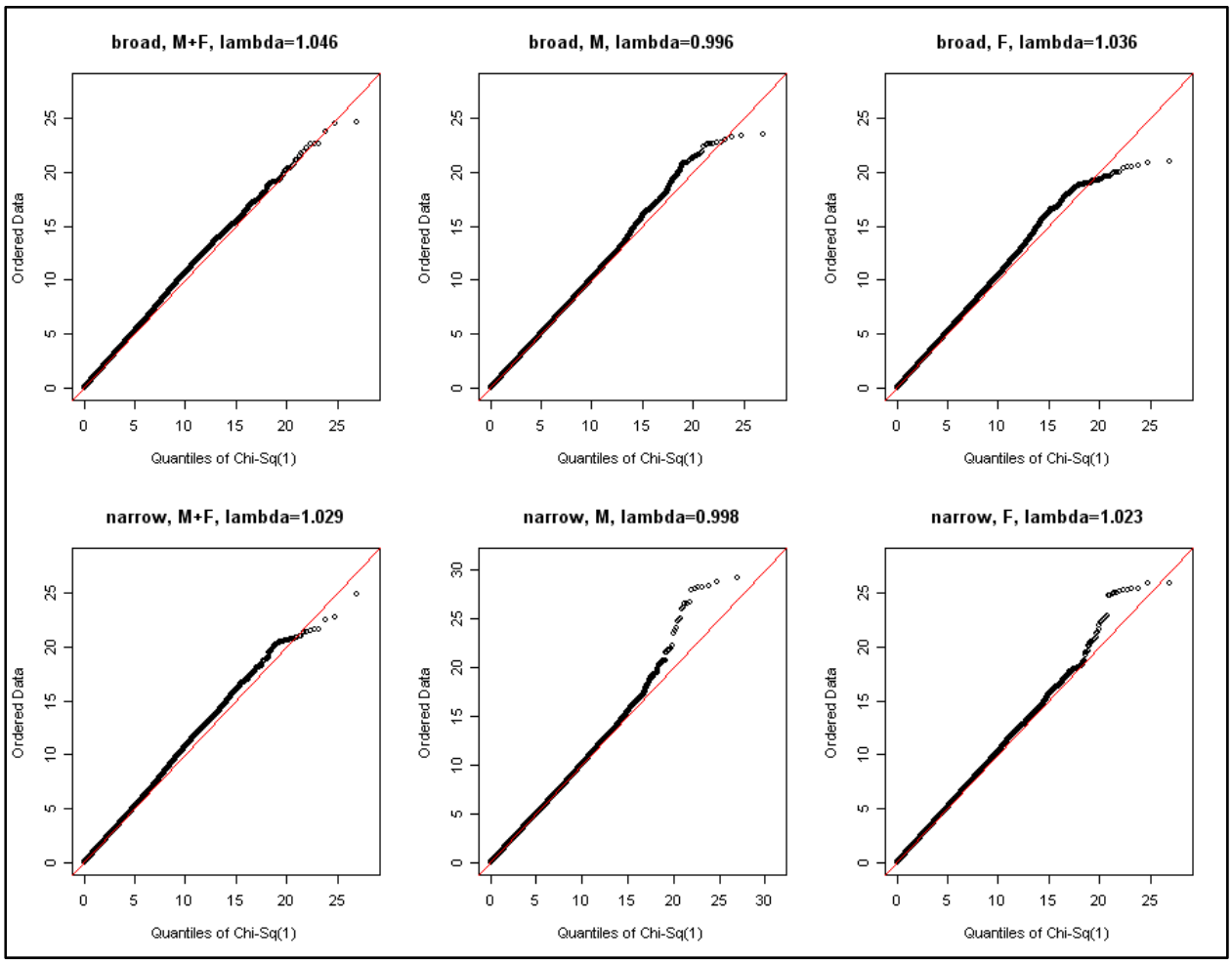


Figure S11. QQ plots for the meta-analysis of 3 datasets.

b. Meta-analysis based on Z-scores. We considered whether to perform a "mega-analysis" with the combined genotypic data for three datasets. This would require imputing data for non-genotyped HapMap SNPs for each dataset, and then performing an association test for each SNP based on the genotypic dosages, with covariates for population stratification and perhaps also using an indicator for each study to reduce artifacts. This approach is computationally intensive but manageable. However, because 1,636 common controls were used in the GenRED and STAR*D analyses, these analyses are correlated. This can be handled more easily in the context of a meta-analysis (see below). We have (1) computed the Wald Z-statistic for each study with appropriate correction for population stratification, and (2) combined the Z-statistic values from three studies with appropriate correction for the sample size of each study, the correlation between test statistics due to shared controls, and the imputation r^2 for each study. The proposed meta-analysis approach is the most powerful under local alternatives and asymptotically equivalent to the score statistic based on a stratified logistic regression framework.

Meta-analysis for autosomal SNPs. For each SNP, let $Z_{GAIN}, Z_{GenRED}, Z_{StarD}$ denote the Wald Z test statistics from the logistic regression analyses for GAIN-MDD, GenRED and STAR*D respectively,

correcting for population stratification appropriately. Under the null hypothesis, these *Z* statistics follow the standard normal distribution *N(0, 1)*. Because GenRED and STAR*D share control samples, Z_{GenRED} and Z_{StarD} are correlated with asymptotic correlation

$$\rho = \frac{1}{\left[\frac{N_{share}}{n_{StarD}} + 1\right]^{1/2}\left[\frac{N_{share}}{n_{GenRED}} + 1\right]^{1/2}} \quad (1)$$

Here, $N_{share} = 1636$ is the number of overlapping controls, n_{StarD}, n_{GenRED} are the numbers of cases in STAR*D and GenRED, respectively. **Table S2** gives the asymptotically correlation between Z_{GenRED} and Z_{StarD} based on (1). The correlations are slightly different for X chromosome SNPs and for autosomal SNPs because additional subjects were excluded from X chromosome analysis.

Table S2: Asymptotic correlation between Z-score test statistics of GenRED and STAR*D due to shared controls

		males+females	males only	females only
Autosomal SNPs	**Broad case analysis**	0.405	0.295	0.500
	Narrow case analysis	0.340	0.238	0.434
X chromosome SNPs	**Broad case analysis**	0.406	0.291	0.508
	Narrow case analysis	0.341	0.234	0.440

We then define a correlation matrix

$$\Sigma = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & \rho \\ 0 & \rho & 1 \end{pmatrix}.$$

One can show that the three test statistics $(Z_{GAIN}, Z_{GenRED}, Z_{StarD})$ follow a multivariate normal distribution with correlation matrix Σ asymptotically.

The meta-analysis statistic is then computed as:

$$Z = \frac{(\eta_{GAIN,}\eta_{GenRED}, \eta_{StarD})\Sigma^{-1}(Z_{GAIN,}Z_{GenRED}, Z_{StarD})^t}{\left[(\eta_{GAIN,}\eta_{GenRED}, \eta_{StarD})\Sigma^{-1}(\eta_{GAIN,}\eta_{GenRED}, \eta_{StarD})^t\right]^{1/2}}. \quad (2)$$

Here,

$$\eta_k = [n_k\phi_k(1-\phi_k)]^{1/2}[2p_k(1-p_k)]^{1/2}r_k$$

is proportional to the asymptotic non-centrality parameter (NCP) of the Wald Z test statistic for study k; n_k is the total sample size of study k, ϕ_k is the proportion of cases, p_k is the allele frequency of the

controls and $r_k \in [0,1]$ is the correlation between imputed genotype dosage and real genotypic value for the SNP. Under the null hypothesis that the SNP is unassociated with the disease, the statistic *Z* follows the standard normal distribution. Under local alternative hypothesis that the relative risk is close to one, the proposed meta-analysis statistic is the most powerful asymptotically.

The genomic control λ values for each individual study and for the meta-analysis are shown in **Figures S8-S11** above. The lack of any substantial inflation of test statistics demonstrates the validity of our approach.

The β value (note that OR=exp(β)) for the meta-analysis is again the weighted average of βs of three studies, with weights depending on sample sizes, allele frequencies, imputation information content and the correlation structure. Formally, we have approximately:

$$\beta = \frac{(\eta_{GAIN,}\eta_{GenRED},\eta_{StarD})\Sigma^{-1}(\eta_{GAIN}\beta_{GAIN,}\eta_{GenRED}\beta_{GenRED},\eta_{StarD}\beta_{StarD})^t}{\left[(\eta_{GAIN,}\eta_{GenRED},\eta_{StarD})\Sigma^{-1}(\eta_{GAIN,}\eta_{GenRED},\eta_{StarD})^t\right]}.$$

The OR for meta-analysis is then OR=exp(β).

Comparison of the power of two meta-analysis strategies. A simpler alternative to correcting meta-analytically for the shared controls in GenRED and STAR*D would be to randomly assign subsets of controls to each of these two studies (perhaps proportionally to the case sample size). Here we compare the power of such a ***split-controls*** approach to our ***corrected meta-analysis*** approach. By theoretical power calculation, we found that the two strategies were equally powerful if a SNP's imputation information content was the same for each dataset. However, **if the SNP was imputed with high confidence in GenRED and low confidence in STAR*D, then the corrected meta-analysis method was more powerful**. Here we describe the power analysis that led to this conclusion. For convenience, we only consider meta-analysis of GenRED study and STAR*D. We compute the non-centrality parameter of the statistics in two strategies.

Strategy 1 (split controls): We randomly select 899 MGS control subjects for STAR*D and 737 MGS control subjects for GenRED such that the case proportion is 58% in both datasets. The test statistics for two studies are independent. For each study, the NCP of a trend statistic (Z) can be approximated by

$$\eta_k = [n_k\phi_k(1-\phi_k)]^{1/2}[2p_k(1-p_k)]^{1/2}r_k.$$

If the statistics are Z_1 and Z_2 in two studies, the meta-analysis test statistic is given by $Z = (\eta_1 Z_1 + \eta_2 Z_2)/\sqrt{\eta_1^2+\eta_2^2}$ and the NCP of *Z* is proportional to $\Omega_1 = \sqrt{\eta_1^2+\eta_2^2}$.

Strategy 2 (shared controls with meta-analytic correction): Both GenRED study and STAR*D study use 1,636 MGS controls. The test statistic is correlated with correlation 0.407 (see Table S2). If we define a correlation matrix

$$\Sigma = \begin{pmatrix} 1 & 0.407 \\ 0.407 & 1 \end{pmatrix}$$

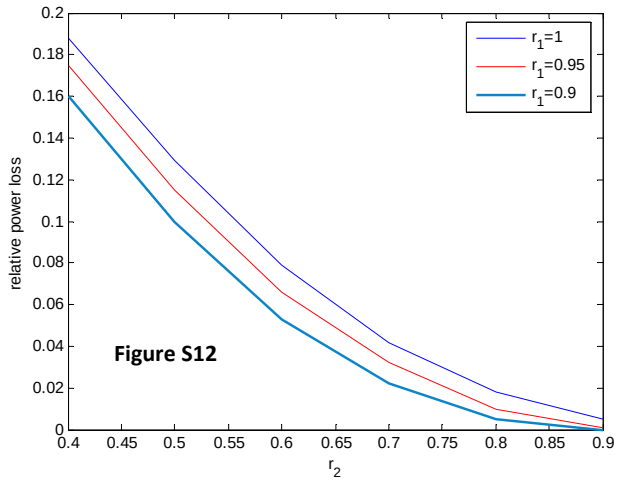
then, the same argument in the derivation of meta-analysis statistic leads to the meta-analysis test statistic:

$$Z = (\eta_1, \eta_2)\Sigma^{-1}(Z_1, Z_2)' / [(\eta_1, \eta_2)\Sigma^{-1}(\eta_1, \eta_2)']^{1/2}.$$

Its NCP is proportional to $\Omega_2 = [(\eta_1, \eta_2)\Sigma^{-1}(\eta_1, \eta_2)']^{1/2}$.

We define a metric $\Delta = 1 - [\Omega_1 / \Omega_2]^2$ which measures the relative power loss for using strategy 1 compared to strategy 2. One can show that $\Delta = 1$ if the imputation information content $r_1 = r_2$ and $\Delta < 1$ otherwise.

Figure S12 gives the relative power loss of Strategy 1 compared with Strategy 2, for each combination of (r_1, r_2), assuming that the two studies are from the same population and thus have identical allele frequencies (same ancestry). Here, r_1^2, r_2^2 represent the imputation information content of a SNP for GenRED and STAR*D, respectively. The figure gives the relative power loss for $r_1 = 1, 0.95, 0.9$ and r_2 ranging from 0.4 to 0.9. The relative power loss is small if the imputation information contents are similar. But when r_1 is large and r_2 is small which is true for many tested SNPs, the relative power loss is not negligible. Note, however, that information content is expressed here as r. For the more familiar r^2 statistic, power loss remains < 5% until r^2 for the less informative study drops well below 0.5 (r<0.7). Thus there may be situations where the strategy of splitting the control sample among studies might be acceptable.



c. Power analyses.

GenRED and Meta-analysis. For each combination of allele frequency (*p)* and allele relative risk (*R*), we computed the power of the two-side test statistic for detecting additive genetic effect assuming that relative risks and allele frequencies are consistent across three studies. In the computation, we also assume that the SNP is genotyped or imputed perfectly, so the power represents the upper limit of the real power after imputation. Because r^2 is close to 1 for most of the imputed SNPs, the real power is close to the computed power. For the meta-analysis, the power was computed based on the number of cases/controls for GAIN, GenRED and STAR*D, accounting for the fact that GenRED and STAR*D shared 1636 MGS controls.

The power is approximated under the assumption that the test statistic Z follows a normal distribution with NCP ξ and unit variance. The power is computed at significance level $\alpha = 5\times10^{-8}$: $P_{\xi}(|Z| > q_{\alpha/2} = 5.45)$. Given p, R and number of cases/controls, the asymptotic NCP of the trend test for each study is given by

$$\xi_k = \left[2n_k\phi_k(1-\phi_k)\right]^{1/2}\left[p(1-p)\right]^{1/2}\frac{(R-1)}{1+p(R-1)}.$$

One can show that the NCP of the meta-analysis statistic is reduced to

$$\xi = \left[(\eta_{GAIN,}\eta_{GenRED},\eta_{StarD})\Sigma^{-1}(\eta_{GAIN,}\eta_{GenRED},\eta_{StarD})^{t}\right]^{1/2}\frac{(R-1)}{1+p(R-1)}.$$

Table S3 shows the results of power calculations for the GenRED GWAS, while Table S4 shows the results of power calculations for the Broad and Narrow meta-analyses.

Table S3: Power of GenRED GWAS dataset

Risk allele frq→ GRR↓	0.05	0.10	0.15	0.20	0.25	0.30	0.40	0.50	0.60	0.70	0.80	0.90
ALL SUBJECTS												
1.20	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00
1.25	0.00	0.00	0.01	0.02	0.03	0.05	0.07	0.07	0.05	0.02	0.01	0.00
1.30	0.00	0.01	0.03	0.08	0.12	0.16	0.21	0.20	0.15	0.08	0.02	0.00
1.35	0.00	0.03	0.11	0.21	0.30	0.38	0.45	0.43	0.33	0.19	0.06	0.00
1.40	0.01	0.09	0.25	0.42	0.55	0.64	0.70	0.68	0.56	0.35	0.12	0.01
1.45	0.02	0.19	0.45	0.66	0.78	0.84	0.88	0.86	0.76	0.54	0.22	0.02
1.50	0.05	0.35	0.67	0.84	0.91	0.95	0.96	0.95	0.89	0.71	0.35	0.04
1.55	0.09	0.54	0.84	0.94	0.98	0.99	0.99	0.99	0.96	0.84	0.49	0.06
1.60	0.17	0.72	0.94	0.98	0.99	1.00	1.00	1.00	0.99	0.92	0.62	0.10
MALES												
1.40	0.00	0.00	0.00	0.01	0.02	0.02	0.03	0.03	0.02	0.01	0.00	0.00
1.50	0.00	0.01	0.03	0.06	0.09	0.11	0.13	0.11	0.07	0.03	0.01	0.00
1.60	0.00	0.03	0.10	0.18	0.25	0.31	0.34	0.29	0.19	0.09	0.02	0.00
1.70	0.01	0.10	0.26	0.40	0.51	0.57	0.59	0.52	0.37	0.19	0.05	0.00
1.80	0.03	0.23	0.48	0.66	0.75	0.79	0.80	0.73	0.57	0.32	0.09	0.01
1.90	0.07	0.42	0.71	0.85	0.90	0.92	0.92	0.87	0.73	0.47	0.16	0.01
2.00	0.15	0.63	0.87	0.95	0.97	0.98	0.98	0.95	0.85	0.61	0.23	0.02
FEMALES												
1.30	0.00	0.00	0.00	0.01	0.01	0.02	0.03	0.03	0.02	0.01	0.00	0.00
1.40	0.00	0.01	0.03	0.07	0.11	0.14	0.18	0.16	0.11	0.05	0.01	0.00
1.50	0.00	0.05	0.16	0.28	0.39	0.46	0.51	0.47	0.35	0.18	0.05	0.00
1.60	0.02	0.19	0.43	0.62	0.73	0.79	0.82	0.77	0.64	0.40	0.14	0.01
1.70	0.07	0.43	0.73	0.87	0.93	0.95	0.96	0.93	0.85	0.63	0.26	0.02
1.80	0.17	0.70	0.92	0.97	0.99	0.99	0.99	0.99	0.95	0.80	0.41	0.04
1.90	0.34	0.88	0.98	1.00	1.00	1.00	1.00	1.00	0.99	0.91	0.57	0.07
2.00	0.55	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.96	0.70	0.11

Table S4 shows power calculations for the meta-analyses of Broad and Narrow phenotypes.

Table S4: Power of the Broad and Narrow meta-analyses (all subjects).

Risk allele frq→ GRR↓	0.05	0.10	0.15	0.20	0.25	0.30	0.40	0.50	0.60	0.70	0.80	0.90
BROAD												
1.10	0.00	0.00	0.00	0.01	0.01	0.02	0.02	0.03	0.02	0.01	0.00	0.00
1.12	0.00	0.00	0.01	0.02	0.04	0.06	0.10	0.10	0.08	0.05	0.01	0.00
1.14	0.00	0.01	0.03	0.08	0.13	0.19	0.26	0.27	0.22	0.13	0.04	0.00
1.16	0.00	0.03	0.09	0.20	0.30	0.39	0.50	0.51	0.44	0.29	0.11	0.01
1.18	0.01	0.06	0.21	0.38	0.53	0.64	0.74	0.75	0.67	0.49	0.21	0.02
1.20	0.01	0.14	0.38	0.60	0.74	0.83	0.90	0.90	0.85	0.69	0.37	0.05
NARROW												
1.10	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00
1.14	0.00	0.00	0.01	0.02	0.03	0.05	0.07	0.07	0.06	0.03	0.01	0.00
1.18	0.00	0.01	0.05	0.11	0.19	0.25	0.34	0.34	0.28	0.17	0.05	0.00
1.22	0.01	0.07	0.21	0.38	0.53	0.63	0.73	0.73	0.64	0.46	0.19	0.02
1.26	0.02	0.21	0.50	0.72	0.84	0.90	0.95	0.94	0.90	0.76	0.43	0.06
1.30	0.07	0.46	0.79	0.93	0.97	0.99	0.99	0.99	0.98	0.93	0.68	0.13

STAR*D GWAS power analysis. A separate power analysis was carried out for STAR*D GWAS analysis (see text of STAR*D/meta-analysis paper). In Figure S13, each power curve depicted below is specific to a given MAF (minor allele frequency), as shown in the legend to the right.

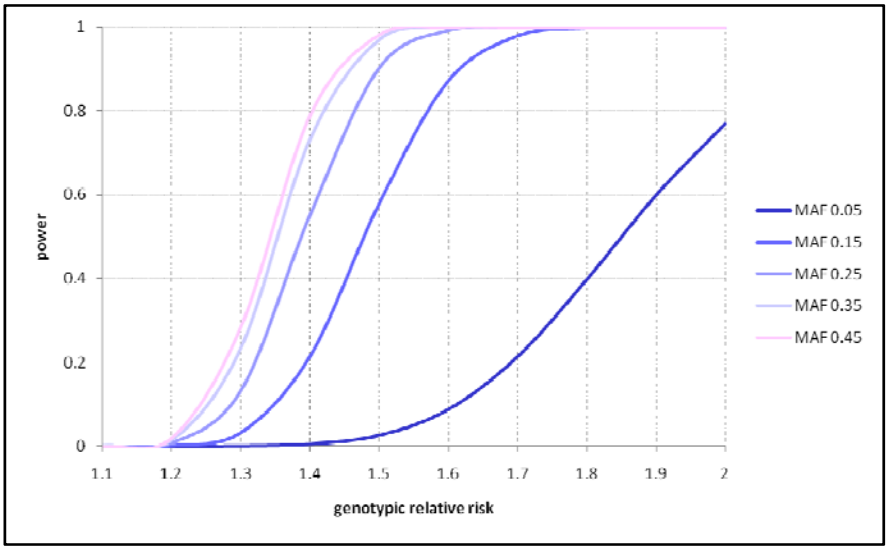


Figure S13. Power analysis of STAR*D GWAS.

4. SUPPLEMENTARY RESULTS

a. Analysis of 41 mood disorder candidate genes: methods and results. For the GenRED, Broad meta-analysis and Narrow meta-analysis, we examined results for all genotyped and imputed SNPs within (or alternatively, within 50kb of) the transcribed regions of 41 genes that have previously been mentioned in the literature as possible MDD candidate genes (listed in **Table S5**, below). These included the top GWAS findings of Muglia et al.[12] (ADCY9, CHST11, DNAJB2, EHD3, FREM3, HS6ST3, ITPR1, KLHL29,

LHFPL2, PHACTR3, PTPRR, ROS1, SLC25A21, UGT2A1, VGLL4); 6 genes identified by a recent meta-analysis as having significant support across published candidate gene studies, although without genome-wide correction (APOE, DRD4, GNB3, MTHFR, SLC6A3, SLC6A4), and additional genes identified in previous reports or literature search. [13, 14]

Table S5: 41 previously-studied candidate genes

ADCY9	adenylate cyclase 9	KLHL29	kelch-like 29 (Drosophilia)
ANK3	ankyrin 3	MMP2	matrix metalloproteinase 2 preproprotein
APOE†	apolipoprotein E	MTHFR	5,10-methylenetetrahydrofolate reductase
BDNF	brain-derived neurotrophic factor	MYO5B	myosin VB
CACNA1C	calcium channel, voltage-dependent, L type	NFE2L3	nuclear factor (erythroid-derived 2)-like 3
CCND2	cyclin D2	NFKB1	nuclear factor kappa-B, subunit 1
CD47	CD47 antigen isoform 1 precursor	NTRK3	neurotrophic tyrosine kinase, receptor, type 3
CHST11	carbohydrate (chondroitin 4) sulfotransferase	PHACTR3	phosphatase and actin regulator 3
COMT	catechol-O-methyltransferase	PTPRR	protein tyrosine phosphatase, receptor type R
DGKH	diacylglycerol kinase, eta	ROS1	proto-oncogene c-ros-1 protein precursor
DNAJB2	DnaJ (Hsp40) homolog, subfamily B, member 2	SLC25A21	solute carrier family 25 (mitochondrial
DRD3	dopamine receptor D3	SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3
DRD4†	dopamine receptor D4	SLC6A4	solute carrier family 6 (neurotransmitter transporter, serotonin), member 4
EHD3	EH-domain containing 3	SMG7	SMG-7 homolog isoform 1
FBXO8	F-box only protein 8,F-box only protein 8	SYN3	synapsin III
GABBR1	gamma-aminobutyric acid (GABA) B receptor 1	TDRD3	tudor domain containing 3
GNB3	guanine nucleotide-binding protein, beta-3	TPH2	neuronal tryptophan hydroxylase
HS6ST3	heparan sulfate 6-O-sulfotransferase 3	TSPAN8	transmembrane 4 superfamily member 3
HTR2A	5-hydroxytryptamine (serotonin) receptor 2A	UGT2A1	UDP glycosyltransferase 2 family, polypeptide
ITPR1	inositol 1,4,5-triphosphate receptor, type 1	VGLL4	vestigial like 4
KCNC2	Shaw-related voltage-gated potassium channel		

†APOE and DRD4 were not included in the aggregate analysis because there were no SNPs within the genes.

In addition, we carried an analysis to determine the gene-wide significance of the best SNP result in each gene, and also to determine whether the entire distribution of gene-wide *P*-values for all of the genes included more significant values than would be expected by chance. Because STAR*D was not independent due to the overlapping controls, we show only results for genotyped SNPs for that dataset, and did not carry out a gene-wide or aggregate analysis.

We derived the null distribution based on the 2656 GenRED samples, by permuting the case-control status of the samples 5000 times and performing a trend test for SNPs in the candidate gene regions for each permutation. (We used this null distribution as a reasonable approximation for the meta-analysis datasets as well, because the distribution depends primarily on the LD pattern among SNPs.) Then, for each dataset, we then considered the entire observed aggregate distribution of results in these regions. (The aggregate analyses of SNPs included 39 genes, because there were no SNPs within APOE and DRD4.) This requires a gene-based permutation procedure to take into account the varying gene lengths and densities of SNPs per gene, as well as possible correlation among genes. For example, the longer genes have a larger probability to have low p-values by chance. We computed an ***empirical gene-wide P-value*** for each gene and then tested whether the 39 empirical p-values ***in aggregate*** follow the uniform distribution (0, 1):

1. Let P_{ij} be the observed nominal probability of SNP *j* in candidate gene *i* in the sample. Let $q_i=min_j P_{ij}$ be the minimum nominal p-value for gene *i*.
2. Let $p_{ij}(k)$ be the nominal probability of SNP *j* in candidate gene *i* in permutation *k*. Let $q_i(k)=min_j P_{ij}(k)$ be the minimum nominal p-value for gene *i* in permutation *k*.
3. The empirical gene-wide p-value *GP(i)* for gene *i* is defined as the proportion of permutations that generates $q_i(k)$ less than the observed q_i.
4. We transformed *GP(i)* to normal quantile *Z(i)* and then computed a test statistic to test if the mean of the Z scores equals to zero. In this case, T=(Z(1)+…+Z(39))/sqrt(39var(Z)).
5. For each permutation, we can calculate a T statistic using the same procedure in step 2-4.

Results are summarized below. More detailed results are available in candidate_gene_results.xls.

GenRED candidate gene analyses. Table S6 shows GenRED results for the best SNP in each gene (witnin 50 kb) and its empirical gene-wide *P*-value (marked in red are best SNP results with $P < 0.001$, or genewide $P < 0.05$). The lowest nominal *P*-value (6.69E-5) was observed for SNP rs11614275 in CACNA1C. Its empirical gene-wide *P*-value was estimated to be 0.0198; if one corrects this value for testing 39 genes, no SNP reaches the threshold of significance in GenRED samples. Similarly, for the aggregate test of the distribution of 39 gene-wide *P*-values, the GenRED results did not deviate significantly from the null distribution, whether we considered only SNPs within transcribed regions, or SNPs within 50kb of these regions. Thus, there was no evidence that these genes contained more significant results than expected by chance.

Table S6: Candidate gene analysis of GenRED sample

Gene	#SNPs	Best SNP	*P*	Gene-wide *P*	Gene	#SNPs	Best SNP	*P*	Gene-wide *P*
CACNA1C	682	rs11614275	6.69E-05	1.98E-02	SMG7	97	rs12032165	1.40E-02	3.82E-01
ADCY9	186	rs7195913	2.14E-04	2.48E-02	PTPRR	434	rs11613970	1.43E-02	7.69E-01
SYN3	807	rs3788477	5.50E-04	1.18E-01	PHACTR3	375	rs6070855	1.50E-02	6.89E-01
TPH2	213	rs7955650	7.91E-04	5.28E-02	ITPR1	602	rs2686621	1.51E-02	7.16E-01
EHD3	224	rs13425772	9.84E-04	6.94E-02	GNB3	34	rs1075836	1.87E-02	3.30E-01
ANK3	546	rs10821712	1.52E-03	1.65E-01	HTR2A	221	rs9534518	1.91E-02	5.78E-01
NTRK3	398	rs10220910	1.75E-03	5.72E-02	SLC6A3	116	rs40180	1.97E-02	5.12E-01
ROS1	267	rs2229078	1.85E-03	8.90E-02	TSPAN8	120	rs1397558	2.16E-02	3.58E-01
CHST11	520	rs4964792	1.93E-03	2.53E-01	TDRD3	227	rs7317551	3.25E-02	8.24E-01
HS6ST3	703	rs867017	2.16E-03	4.19E-01	DGKH	404	rs1170097	3.79E-02	9.97E-01
NFKB1	193	rs4648090	2.19E-03	9.48E-02	UGT2A1	202	rs11249456	3.89E-02	7.31E-01
DRD3	142	rs9869286	3.39E-03	2.51E-01	NFE2L3	75	rs916964	4.58E-02	5.68E-01
VGLL4	290	rs6780610	3.81E-03	2.54E-01	APOE	49	rs5157	5.21E-02	6.45E-01
SLC25A21	743	rs1884777	4.04E-03	4.56E-01	GABBR1	198	rs2745412	5.49E-02	8.86E-01
CCND2	98	rs7953609	5.20E-03	1.94E-01	COMT	131	rs5993891	5.97E-02	7.91E-01
KLHL29	490	rs2288698	5.24E-03	4.74E-01	KCNC2	252	rs735637	7.07E-02	8.21E-01
MMP2	212	rs837543	8.14E-03	2.19E-01	CD47	119	rs17827026	7.30E-02	8.42E-01
MYO5B	650	rs10853589	1.06E-02	6.97E-01	SLC6A4	75	rs4493117	7.52E-02	7.34E-01
BDNF	99	rs6265	1.11E-02	2.53E-01	FBXO8	78	rs7699938	8.80E-02	8.75E-01
DNAJB2	48	rs2090163	1.14E-02	3.74E-01	DRD4	41	rs6598004	1.40E-01	9.31E-01
MTHFR	136	rs4845882	1.35E-02	2.21E-01					

STAR*D candidate gene analysis. Shown below (**Table S7**) are results in these candidate genes in the STAR*D GWAS (considering only genotyped SNPs, within the open reading frame or within 50 kb).

Table S7: Candidate gene results for STAR*D GWAS

Gene	chr	# SNPs	best SNP	p-value	gene	chr	#SNPs	best SNP	p-value
SYN3	22	99	rs3788477	1.64E-04	DGKH	13	36	rs10492433	8.66E-02
ROS1	6	27	rs12332810	9.37E-03	ANK3	10	59	rs3750800	8.74E-02
FBXO8	4	6	rs17060468	1.29E-02	SLC6A4	17	5	rs2020939	9.91E-02
KLHL29	2	18	rs3795933	1.56E-02	MTHFR	1	15	rs4845882	1.07E-01
PTPRR	12	47	rs17226724	2.25E-02	HS6ST3	13	60	rs7318798	1.14E-01
CACNA1C	12	78	rs2370419	2.29E-02	TSPAN8	12	11	rs10506625	1.39E-01
CD47	3	17	rs7635063	2.41E-02	TPH2	12	27	rs7955650	1.40E-01
COMT	22	14	rs5993875	2.82E-02	EHD3	2	22	rs6543616	1.48E-01
SLC25A21	14	80	rs17105237	2.92E-02	CCND2	12	14	rs3217805	1.59E-01
VGLL4	3	45	rs13075818	3.30E-02	HTR2A	13	29	rs666693	1.63E-01
MYO5B	18	76	rs4939921	3.31E-02	MMP2	16	23	rs4783894	1.74E-01
BDNF	11	11	rs6265	4.56E-02	APOE	19	5	rs519113	1.96E-01
SLC6A3	5	17	rs10053602	4.74E-02	DRD3	3	10	rs9824256	1.96E-01
ADCY9	16	31	rs2230742	4.95E-02	SMG7	1	12	rs2702182	2.22E-01
NTRK3	15	57	rs7175941	5.43E-02	KCNC2	12	20	rs1526806	2.72E-01
UGT2A1	4	26	rs7674562	6.11E-02	TDRD3	13	8	rs9538757	2.89E-01
PHACTR3	20	35	rs6070961	6.24E-02	NFKB1	4	20	rs4699030	3.60E-01
DNAJB2	2	4	rs12694457	6.68E-02	GNB3	12	5	rs1075835	4.66E-01
GABBR1	6	23	rs1233405	6.71E-02	NFE2L3	7	4	rs2391265	6.63E-01
ITPR1	3	87	rs876349	7.11E-02	DRD4	11	3	rs7123677	7.58E-01
CHST11	12	70	rs17035879	8.62E-02					

Meta-analysis. We repeated the analyses described above (for GenRED) in the Broad and Narrow meta-analysis datasets (genotyped and imputed SNPs). **Table S8** summarizes the best results (best SNP $P < 0.001$, or genewide $P < 0.05$), with further details in candidate_gene_results.xls. As was the case for GenRED, the distribution of gene-wide *P*-values did not deviate significantly from the null distribution for SNPs within genes or within 50 kb.

Table S8: Best candidate gene results in Broad and Narrow meta-analyses

SNPs within transcribed region					SNPs within 50kb of the gene				
Gene	#SNPs	Best SNP	*P*	Gene-wide *P*	Gene	#SNPs	Best SNP	*P*	Gene-wide *P*
Narrow Meta-analysis									
NTRK3	329	rs10220910	1.61E-03	4.92E-02	NTRK3	398	rs10220910	1.61E-03	5.22E-02
SLC25A21	660	rs1925685	6.70E-04	1.01E-01	SLC25A21	743	rs1925685	6.70E-04	1.15E-01
SYN3	658	rs16990831	5.18E-05	1.14E-02	SYN3	807	rs16990831	5.18E-05	1.28E-02
CHST11	400	rs7976087	3.73E-03	3.69E-01	CHST11	520	rs2248683	2.75E-04	4.64E-02
COMT	21	rs4633	5.45E-03	1.42E-02	COMT	131	rs4633	5.45E-03	1.56E-01
Broad Meta-analysis									
DGKH	303	rs369829	8.20E-03	6.59E-01	DGKH	404	rs9590678	1.42E-04	3.54E-02
EHD3	29	rs597800	7.06E-03	7.12E-02	EHD3	224	rs600980	2.23E-04	2.04E-02
SMG7	30	rs12032165	1.04E-04	1.20E-03	SMG7	97	rs12032165	1.04E-04	5.00E-03
SYN3	658	rs3788477	1.04E-04	2.18E-02	SYN3	807	rs3788477	1.04E-04	2.42E-02
TPH2	142	rs12231341	9.64E-02	9.23E-01	TPH2	213	rs7955650	1.20E-04	1.00E-02
CD47	30	rs326346	1.93E-02	1.61E-01	CD47	119	rs17232226	5.93E-04	2.44E-02

b. Supplementary association analysis results.

Manhattan plots are provided below for male and female analyses of the GenRED GWAS (**Figure S14; see text for all subjects**), the Broad meta-analysis (**Figure S15a**) and the Narrow meta-analysis (**Figure S15b**).

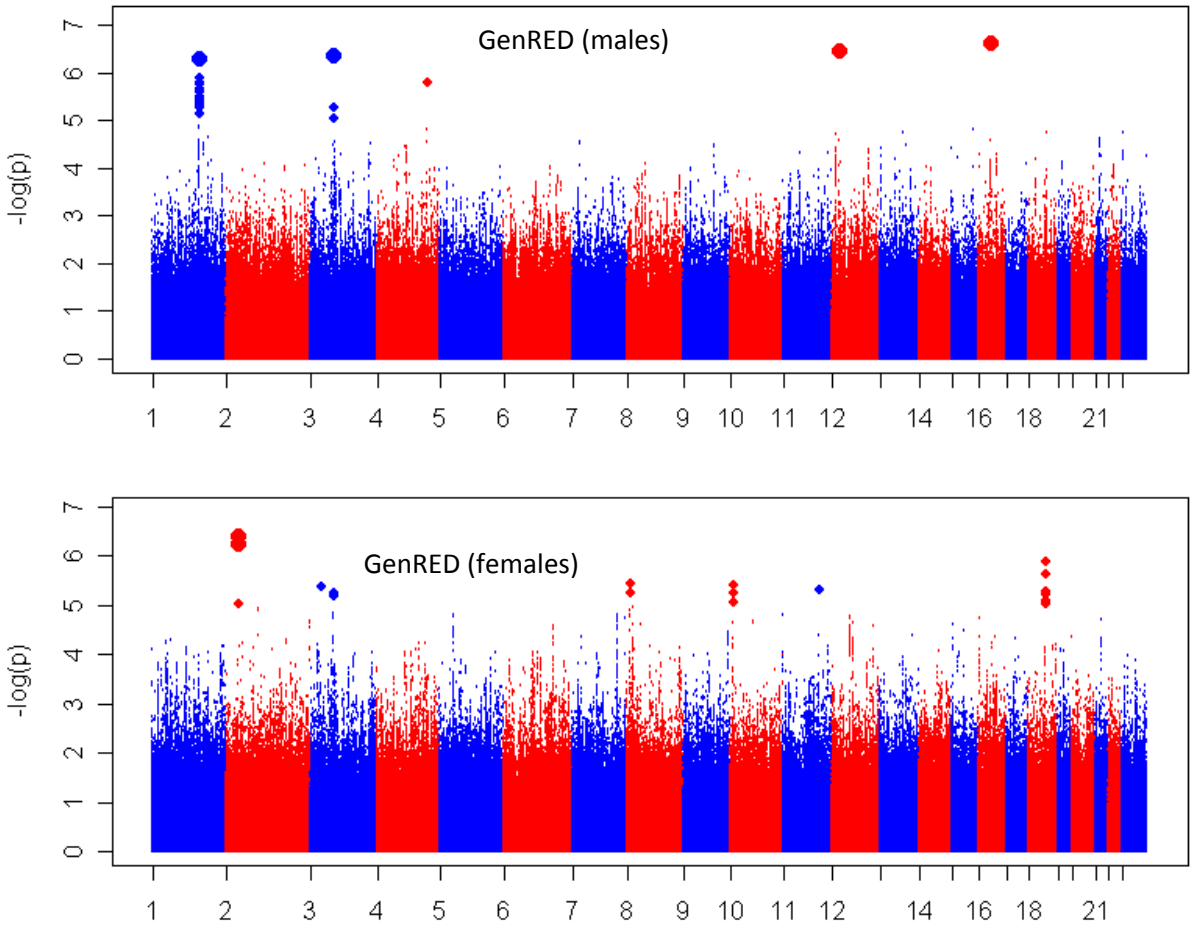


Figure S14. GenRED GWAS analysis of males and of females: Association test results ($-\log_{10}$[P-value]).

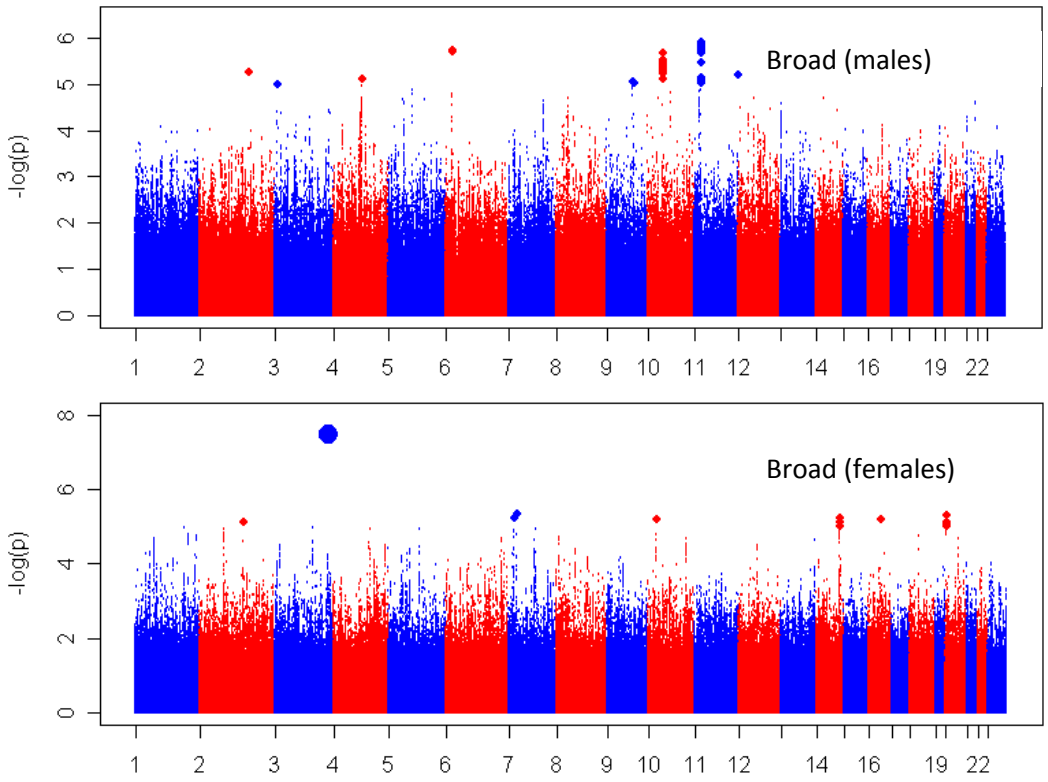


Figure S15a. Meta-analysis, Broad males and Broad females. GWAS test results (-$\log_{10}$[P-value])

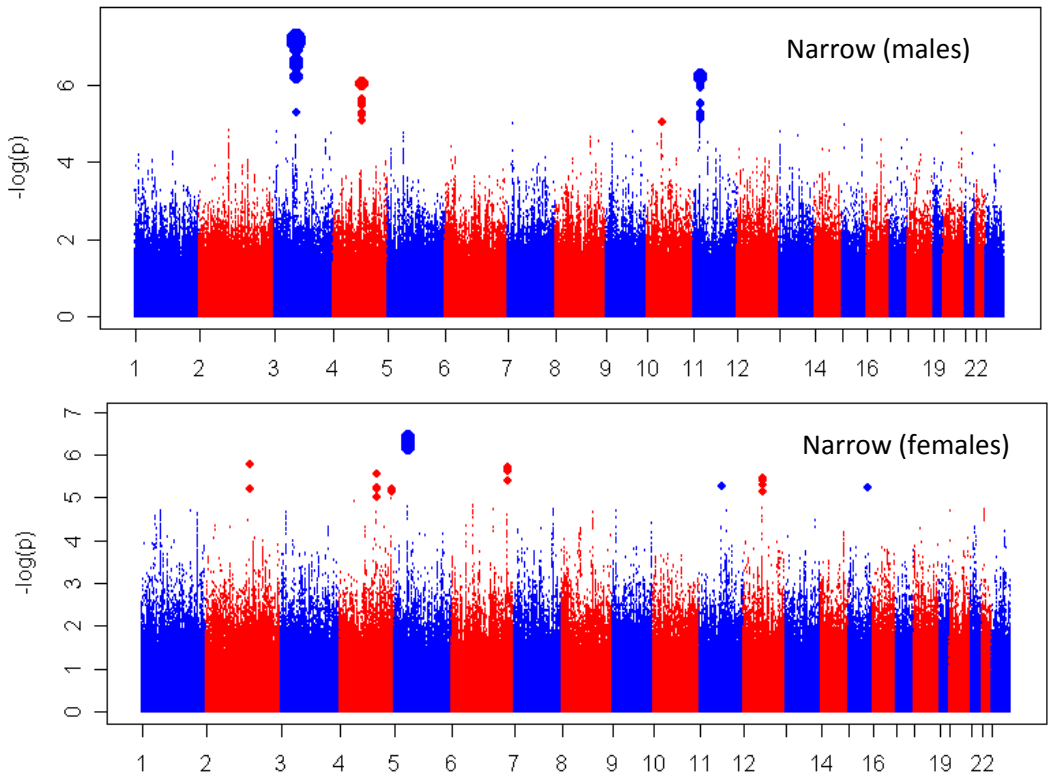
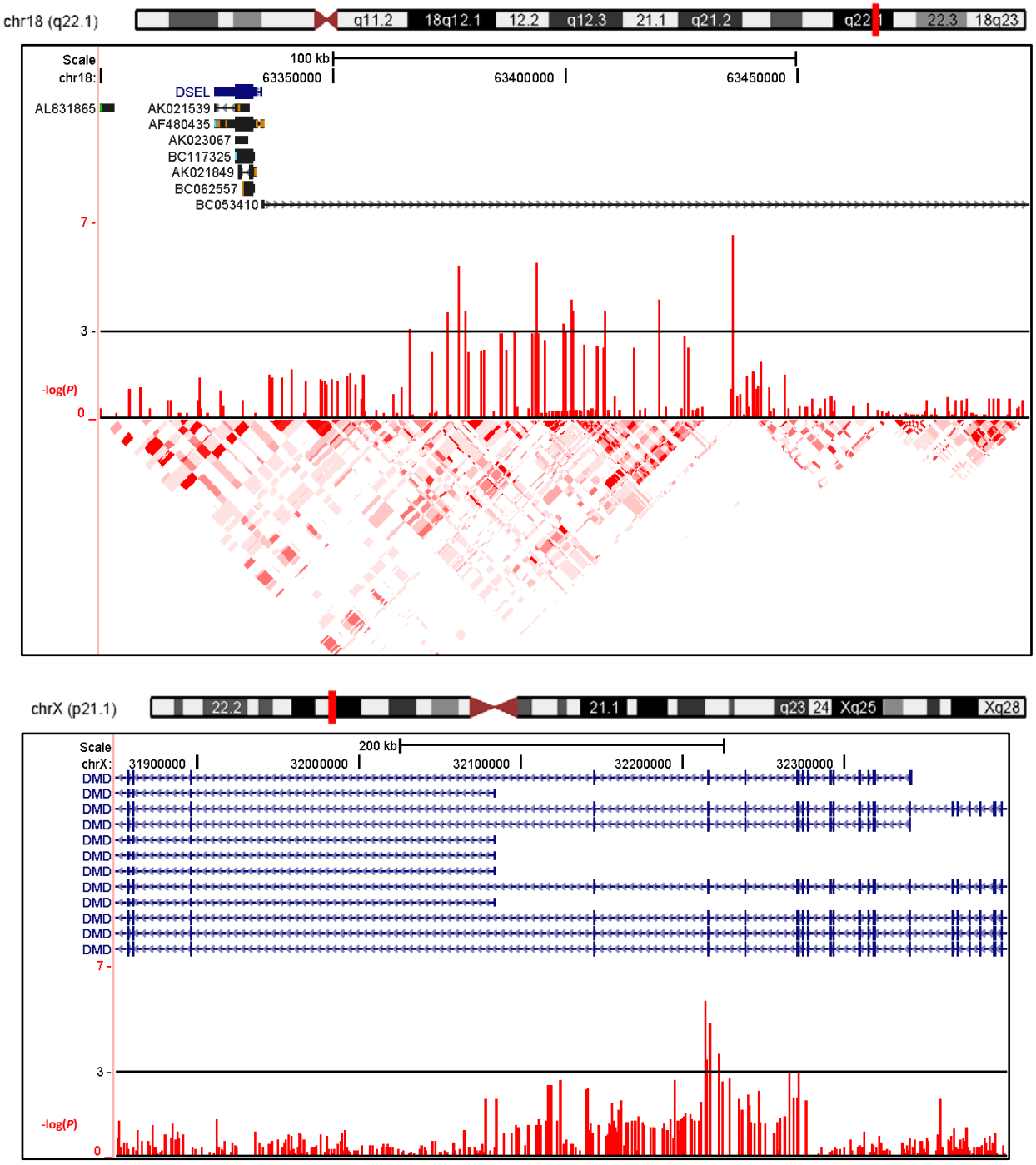
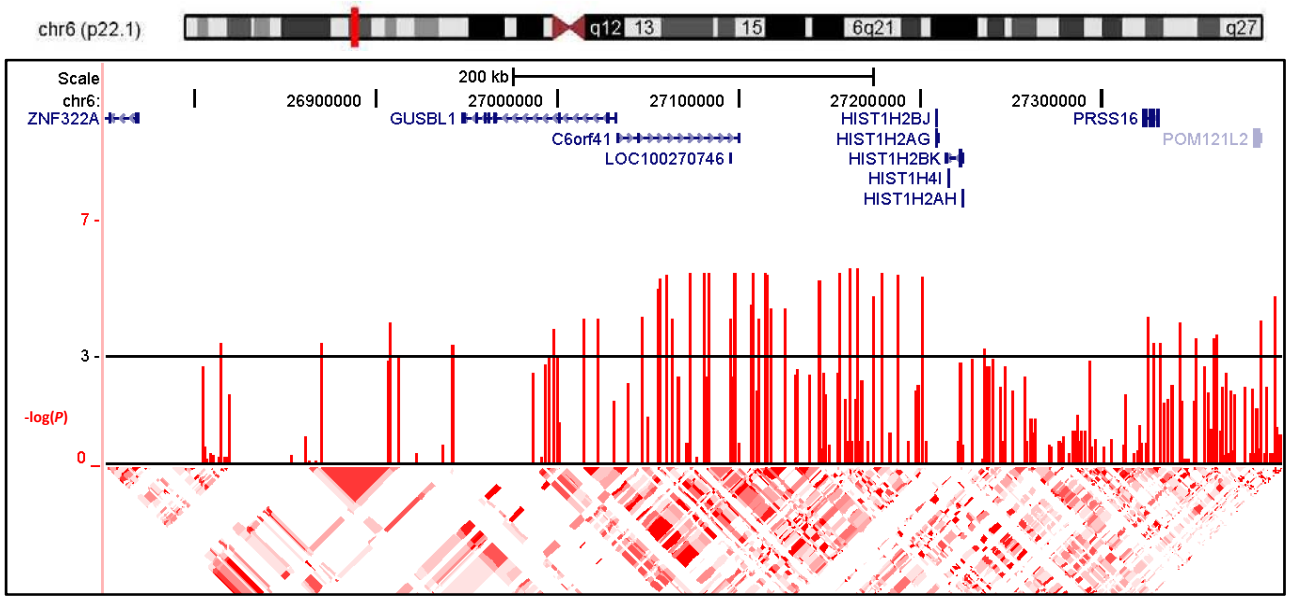


Figure S15b. Meta-analysis, Narrow males and Narrow females. GWAS test results (-$\log_{10}$[P-value])

Figure S16 (below)**.** Shown are annotated UCSC browser plots (and mRNAs for 18q22.1), for the best-supported regions in the **Narrow meta-analysis of All subjects** (males+females) (see Table 6 in the main text): 18q22.1 (strongest evidence for association); Xp21.1/DMD (3rd-best); and 6p22.1 (5th--best). The 2nd-best region (5p13.2) was supported only in females, as illustrated below. The 4th-best region (3p26.1) received stronger support in the Broad analysis, as illustrated in Figure 3 in the manuscript.



Note: DMD extends from chrX:31,042-729-33,267,647.
Evidence for association is only observed in the narrower region shown in the figure.



Note that the 6p22.1 peak region is the same LD block reported to be significantly associated with schizophrenia in a GWAS meta-analysis[3] that included the MGS sample (see text), using a superset of the GenRED and STAR*D controls used here; thus these depression and schizophrenia analyses were not independent. Note also that GUSBL1 (shown in the 6p22.1 plot) is a pseudogene (non-coding sequence).

Figure S17: Annotated UCSC browser plots for the regions with the strongest support for association in the **analyses of male subjects**: 11p15.1 (PLEKHA7) for Broad and 3p14.1 (no annotated genes or mRNAs) for Narrow analyses.

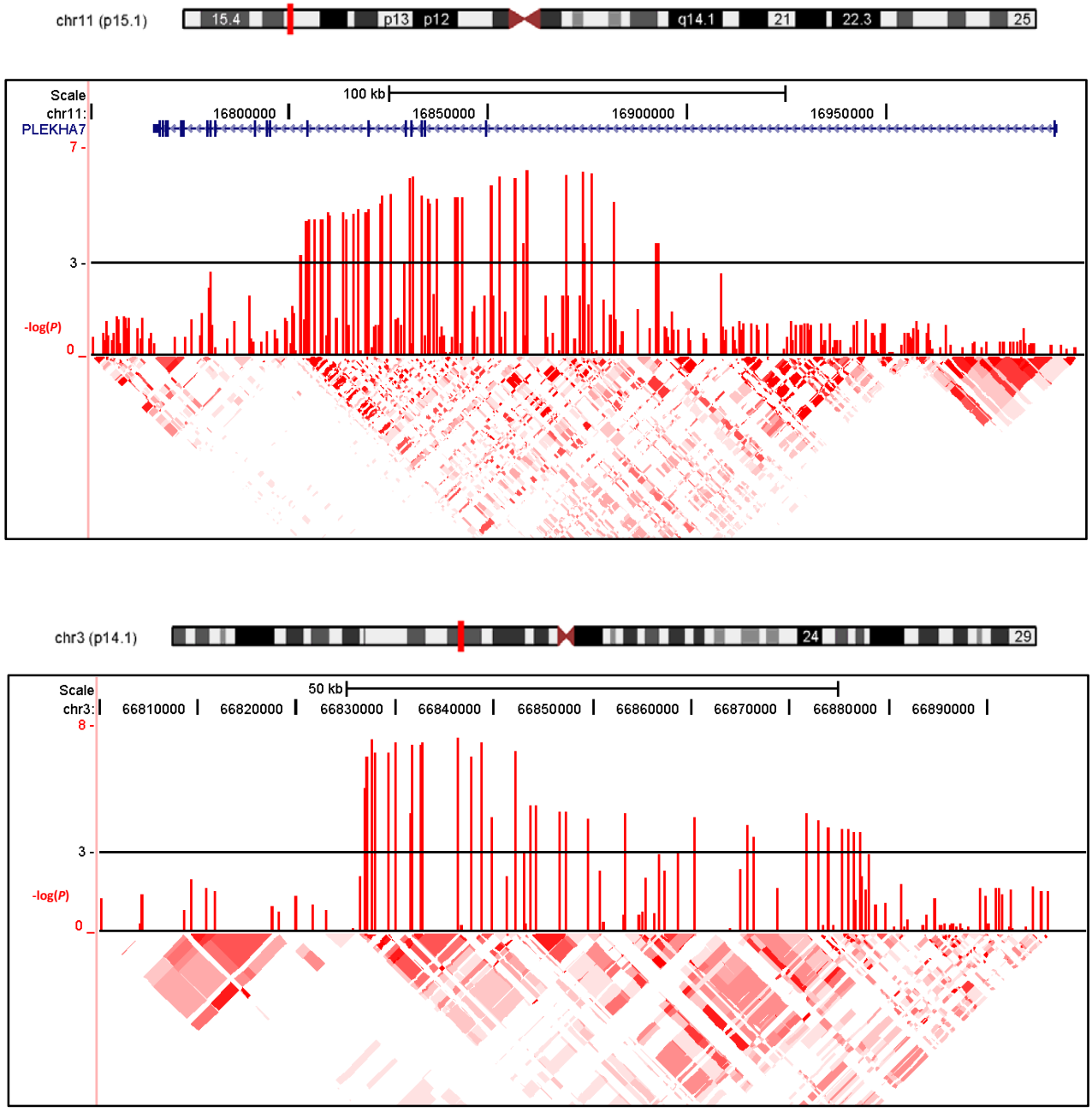
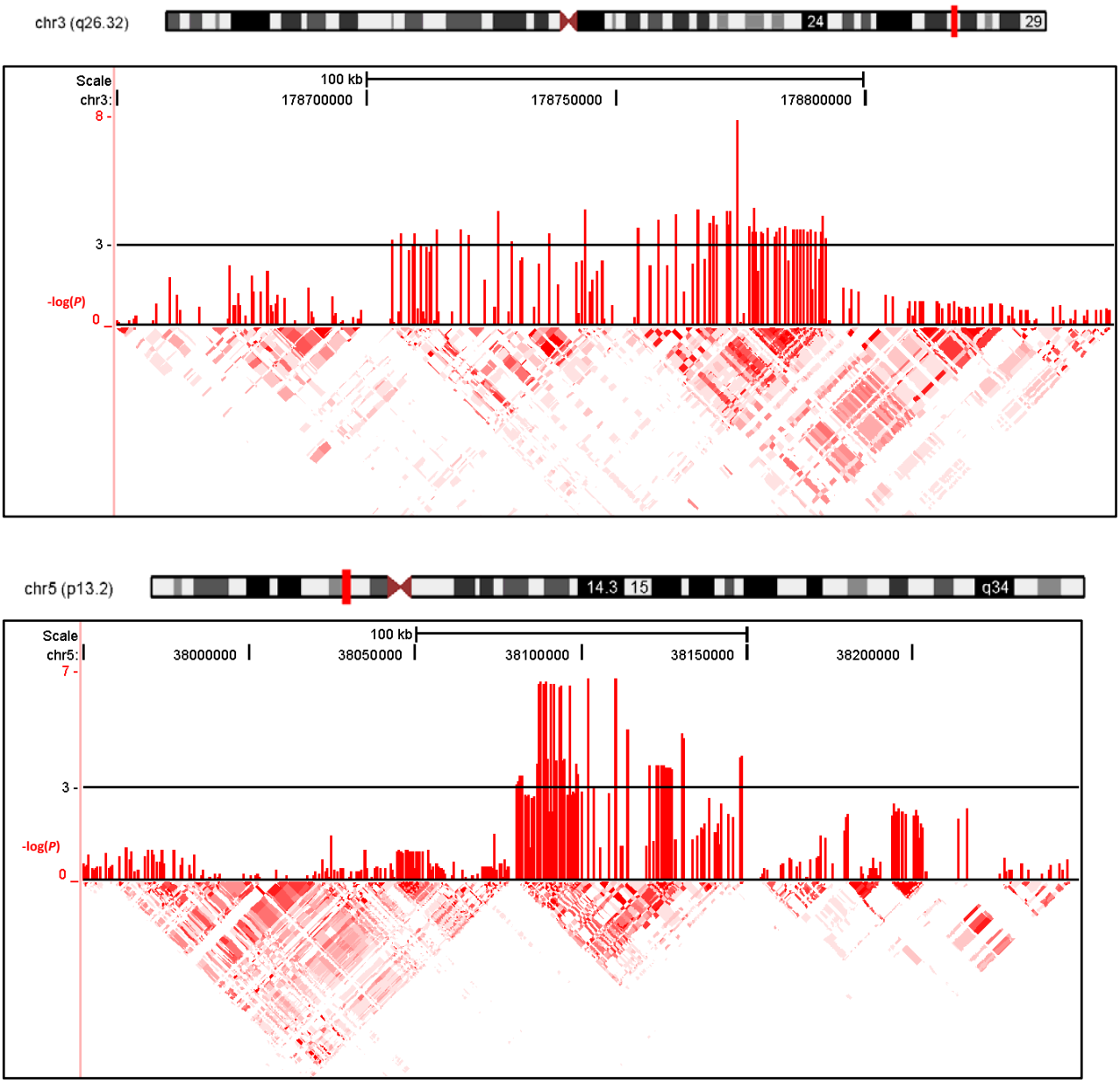


Figure S18. Shown are annotated UCSC browser plots for the regions with the strongest support for association in the analyses of **female** subjects: 3q26.32 (no annotated genes) for Broad and 5p13.2 (no annotated genes) for Narrow analyses. See Tables 5 and 6 in the main text.



c. Intensity (cluster) plots. Please see the separate supplementary file, SNP_intensity_cluster_plots.txt, for cluster plots of the SNPs yielding the best results, or their best tags. That file includes **Tables S9-S10**, and **Figures S19-S21**.

d. Case-control allele frequencies

Table S11: Case-control allele frequencies for best GenRED results (All subjects)

Band	SNP	BP	Alleles	R^2	Case	Cont	OR	P
18q22.1[1]	rs17077540	63436259	A/**G**	0.86	0.152	0.105	1.61	1.83E-07
21q21.2	rs2828520	24064990	A/**G**	1	0.365	0.306	1.35	4.33E-07
5p13.2[2]	rs270545	38087350	A/**G**	1	0.754	0.689	1.37	1.03E-06
1p13.3	rs6537837	1.1E+08	C/**T**	0.99	0.225	0.167	1.43	1.31E-06
2p23.2	rs882632	29134265	C/**T**	1	0.356	0.287	1.34	2.41E-06
3p14.2	rs10514718	61388854	**C**/G	0.82	0.965	0.940	2.12	3.96E-06
1p13.3	rs12049330	1.1E+08	**G**/T	0.95	0.194	0.143	1.44	5.87E-06
7p15.3	rs17144465	21470952	A/**G**	1	0.066	0.036	1.82	5.97E-06
13q21.33[3]	rs9572423	69744204	A/**G**	0.96	0.913	0.875	1.54	9.25E-06
1p33	rs1167264	49705632	**C**/T	0.99	0.251	0.204	1.35	9.51E-06
11p15.4	rs2898938	3788833	G/**T**	1	0.768	0.716	1.34	9.60E-06
11p15.5	rs11024034	2746739	**C**/T	0.99	0.126	0.091	1.45	9.64E-06

Table S12: Case-control allele frequencies for best Meta-analysis results (All subjects)

						Case-control allele freqs and ORs for each dataset										
			Imputation R^2			-----GAIN-----			-----GenRED-----			-----STAR*D-----			Meta-analysis	
Band	SNP	A1/2	GAIN	GR	S*D	Case	Cont	OR	Case	Cont	OR	Case	Cont	OR	OR	P
Broad phenotype																
8p21.3	rs1106634	**A**/G	0.70	1	0.99	0.156	0.137	1.27	0.143	0.124	1.27	0.156	0.122	1.346	1.295	**6.78E-07**
7p15.3†	rs17144465	A/**G**	1	1	0.46	0.045	0.032	1.44	0.066	0.036	1.82	0.048	0.041	1.325	1.561	**7.68E-07**
3p26.1	rs9870680	C/**T**	0.85	1	1	0.484	0.443	1.23	0.478	0.433	1.22	0.457	0.433	1.104	1.188	**1.11E-06**
7q32.3	rs10265216	**A**/T	1	0.99	0.80	0.327	0.284	1.23	0.314	0.292	1.14	0.31	0.286	1.161	1.190	**3.02E-06**
3q26.32†	rs644695	**A**/G	0.74	0.67	0.56	0.91	0.877	1.61	0.889	0.874	1.26	0.884	0.885	0.998	1.354	**4.46E-06**
2p14†	rs724568	A/**C**	1	1	0.99	0.4	0.358	1.2	0.368	0.355	1.09	0.395	0.357	1.191	1.171	**5.32E-06**
10p11.23	rs1612122	A/**T**	0.96	0.95	0.92	0.514	0.482	1.15	0.522	0.481	1.2	0.558	0.524	1.266	1.230	**6.11E-06**
Xq21.33	rs5990417	**T**/C	0.94	0.87	0.55	0.859	0.83	1.2	0.859	0.825	1.25	0.853	0.827	1.751	1.411	**6.52E-06**
6p25.1	rs2326810	C/**G**	0.93	0.86	0.75	0.949	0.939	1.21	0.942	0.921	1.48	0.942	0.916	1.177	1.169	**6.98E-06**
Narrow phenotype																
18q22.1	rs17077540	A/**G**	0.33	0.86	0.65	0.105	0.098	1.25	0.152	0.105	1.61	0.115	0.099	1.302	1.481	**6.04E-07**
5p13.2†	rs270592	A/**G**	1	0.99	0.99	0.608	0.573	1.17	0.639	0.572	1.32	0.6	0.571	1.15	1.223	**1.88E-06**
Xp21.1	rs2405829	**A**/G	0.96	0.96	0.96	0.624	0.557	1.28	0.577	0.537	1.13	0.596	0.54	1.19	1.180	**3.84E-06**
6p22.1	rs6930508	C/**T**	0.99	1	1	0.604	0.553	1.24	0.605	0.564	1.18	0.612	0.564	1.231	1.213	**4.73E-06**
10p11.23	rs1612122	A/**T**	0.96	0.95	0.92	0.535	0.482	1.28	0.522	0.481	1.2	0.556	0.524	1.176	1.218	**5.39E-06**
3p26.1	rs9870680	C/**T**	0.85	1	1	0.493	0.443	1.29	0.478	0.433	1.22	0.46	0.43	1.134	1.211	**5.56E-06**
3q25.1	rs1456139	A/**G**	1	0.84	0.84	0.646	0.608	1.18	0.656	0.615	1.24	0.659	0.613	1.242	1.219	**7.92E-06**
19p13.11	rs7249956	A/**C**	0.89	0.90	0.73	0.813	0.778	1.27	0.813	0.773	1.26	0.792	0.761	1.316	1.277	**8.11E-06**
12q23.3	rs1895943	A/**C**	0.99	1	0.80	0.055	0.042	1.35	0.067	0.041	1.6	0.058	0.04	1.602	1.519	**8.70E-06**
6q22.1	rs1855625	G/**T**	0.998	1	0.996	0.723	0.699	1.11	0.733	0.684	1.28	0.73	0.68	1.279	1.226	**9.34E-06**
1p32.2	rs6694643	A/**T**	0.99	1	0.89	0.165	0.117	1.51	0.145	0.123	1.21	0.132	0.114	1.189	1.294	**9.50E-06**

Shown in each table are results from GenRED GWAS Table2 and meta-analysis Tables 5 and 6 for All Subjects with case and control allele frequencies (some columns have been omitted for clarity). The allele frequencies and ORs are for the bolded allele. These details are provided to give the reader a sense of the kinds of frequency differences that are detected here. We have avoided providing exact frequencies for large numbers of SNPs because of issues of subject identifiability. [1] Full data are available

under controlled access procedures from the NIMH and dbGAP repositories. Note that for a small proportion of SNPs (here, rs1612122 is the only example), the control frequencies for GenRED and STAR*D differ more than expected, despite the fact that the control groups are identical. This occurs for some imputed SNPs because the control genotypes have been imputed with the SNPs available for the case group for each analysis -- ~ 260K SNPs for STAR*D vs. ~ 670K for GenRED. The reasons for the size of the discrepancy require further study, but this phenomenon was not observed for the best findings in the candidate genes that have been highlighted in the text.

e. Gene symbols, descriptions and functions

Table S13: Gene names and functions for genes listed in GenRED GWAS Table 2

Symbol	Description	Function
AGBL4	ATP/GTP binding protein-like 4	Processing of tubulin
AMPD2	adenosine monophosphate deaminase 2	Energy metabolism
ATXN7L2	ataxin 7-like 2	Potential transcription factor
C2orf71	hypothetical protein LOC388939	Hypothetical protein
CSMD1	CUB and Sushi multiple domains 1	Regulator of complement and inflammation in CNS
CYB561D1	cytochrome b-561 domain containing 1	Binds 2 heme groups non-covalently (By similarity)
DNM3	dynamin 3	Actin membrane budding
DSEL	dermatan sulfate epimerase-like	(DSE: D-glucuronic acid metabolism; tumor rejection)
DYNC2H1	dynein, cytoplasmic, heavy polypeptide 2	Intracelleular transport
FAM179A	hypothetical protein LOC165186	Hypothetical protein
FHIT	fragile histidine triad gene	Purine metabolism; FRA3B fragile site
FRAG1	FGF receptor activating protein 1	Mediates DNA damage-induced apoptosis
GDNF	glial cell derived neurotrophic factor	Survival and differentiation of dopaminergic neurons
GNAI3	guanine nucleotide binding protein (G protein)	Transmembrane signaling
GNAT2	guanine nucleotide binding protein, alpha	Visual system protein (transducin)
KCNQ1	potassium voltage-gated channel, KQT-like	Voltage-gated potassium channel
KIAA0859	CGI-01 protein	Methyltransferase
MYOC	Myocilin	Primarily found in uveal meshwork (visual)
NUP98	nucleoporin 98kD	Bidirectional transport across nucleoporin complex
PTPRG	protein tyrosine phosphatase, receptor type G	Stimulatory G protein (K^+ channels)
SP4	Sp4 transcription factor	Transcriptional activator; binds to estrogen 1 receptor
STIM1	stromal interaction molecule 1 precursor	Mediation of Ca^{++} influx following Ca^{++} depletion (ER)
SYPL2	mitsugumin 29	Sarcoplasmic reticulum membrane communication
VAMP4	vesicle-associated membrane protein 4	Maturation of secretory granules

Shown are gene symbol, description and function of genes mentioned in Table 2 (genes with SNPs with $P < 10^{-5}$ within the gene, , or within 50 kb upstream or downstream).

Table S14: Gene names and functions for genes listed in Meta-Analysis GWAS Tables 5 and 6

Symbol	Description	Function
ARRB1	arrestin beta 1	Regulates beta-adrenergic receptor function; densensitize G-protein-coupled receptors
ATP6V1B2	vacuolar H+ATPase B2	See text
C14orf109	hypothetical protein LOC26175	
C19orf50	hypothetical protein LOC79036	
C19orf60	hypothetical protein LOC55049	
C8A	complement component 8, alpha polypeptide	Constituent of the membrane attack complex
C8B	complement component 8, beta polypeptide	Constituent of the membrane attack complex
CLRN1	clarin 1	Usher's syndrome IIIa; development of inner ear and retina
CRLF1	cytokine receptor-like factor 1	May be involved in immune and nervous system development
DMD	Dystrophin	Muscular dystrophies; cytoskeletal protein in muscle fibers
DSEL	dermatan sulfate epimerase-like	DSE: D-glucuronic acid metabolism; tumor rejection)
EID3	E1A-like inhibitor of differentiation 3	Repressor of nuclear receptor-dependent transcription; possibly interferes with CREBBP-dependent coactivation
FAM19A2	family with sequence similarity 19 (chemokine)	Thought to be brain-specific cytokine
GALNT13	UDP-N-acetyl-alpha-D-galactosamine:polypeptide	Oligosaccharide biosynthesis; possible role in synthesis of Tn antigen in neurons
GDNF	glial cell derived neurotrophic factor	Survival and differentiation of dopaminergic neurons
GRM7	glutamate receptor, metabotropic 7	See text
GUSBL1	glucuronidase, beta-like 1	Spinal muscular atrophy candidate gene 3-like 2
HS3ST5	heparan sulfate (glucosamine),heparan sulfate	Biosynthesis of anticoagulant heparan sulfate; generates binding site for Herpes simpliex virus-1
IGSF9B	immunoglobulin superfamily, member 9B	
INTU	inturned planar cell polarity effector homolog (Drosophila)	Formerly: PDZ domain containing 6
ITPK1	inositol 1,3,4-triphosphate 5/6 kinase	Phosphorylates inositol polyphosphates; modulates TNF-alpha-induced apoptosis
KLHL25	BTB/POZ KELCH domain protein	Kelch-like 25 (Drosophila)
KLHL26	kelch-like 26	Kelch-like 26 (Drosophila)
KSP37	kidney specific protein of 37 kda	
LY86	MD-1, RP105-associated	Mediates immune response to bacterial lipopolysaccharide and cytokine production
LZTS1	leucine zipper, putative tumor suppressor 1	Regulation of cell growth
MOAP1	modulator of apoptosis 1	Required for death receptor-dependent apoptosis.
PLEKHA7	pleckstrin homology domain containing, family A	See text
PLXDC2	plexin domain containing 2 precursor	May play a role in tumor angiogenesis
PRDM4	PR domain containing 4	May function as a transcription factor involved in cell differentiation
SLC18A1	solute carrier family 18 (vesicular monoamine)	Acts to accumulate cytosolic monoamines into vesicles
SP4	Sp4 transcription factor	Transcriptional activator; binds to estrogen 1 receptor*
TMEM59L	transmembrane protein 59-like	Brain-specific predicted type-I membrane glycoprotein
TXNRD1	thioredoxin reductase 1	Induces actin and tubulin polymerization, mediates interferon-beta-induced cell death
UBA52	ubiquitin and ribosomal protein L40 precursor	proteasomal degradation; maintenance of chromatin structure; regulation of gene expression; stress response, ribosome biogenesis; DNA repair

Table S15: Gene names and descriptions for genes listed in STAR*D GWAS Table 4

Symbol	Description
AK125573	(mRNA)
ANKRD46	ankyrin repeat domain 46
C21orf94	chromosome 21 open reading frame 94
C8orf71	chromosome 8 open reading frame 71
CDH20	cadherin 20, type 2 preproprotein
COX8A	cytochrome c oxidase subunit 8A
CSMD3	CUB and Sushi multiple domains 3 isoform 1
CTNND2	catenin (cadherin-associated protein), delta 2
GVIN1	GTPase, very large interferon inducible 1
HLX	H2.0-like homeo box 1
HPCAL1	hippocalcin-like 1
NLGN4X	X-linked neuroligin 4
PABPC1	poly(A) binding protein, cytoplasmic 1
RAB30	RAB30, member RAS oncogene family
RPRM	reprimo, TP53 dependant G2 arrest mediator
RUFY4	RUN and FYVE domain containing 4
SCIN	Scinderin
SLC5A12	solute carrier family 5 (sodium/glucose
SRD5A2	3-oxo-5 alpha-steroid 4-dehydrogenase 2
SVIP	small VCP/p97-interacting protein
TMX3	thioredoxin-related transmembrane protein 3

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