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## Identification of 15 genetic loci associated with risk of major depression in individuals of European descent

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### Abstract

Despite strong evidence supporting the heritability of Major Depressive Disorder, previous genome-wide studies were unable to identify risk loci among individuals of European descent. We used self-reported data from 75,607 individuals reporting clinical diagnosis of depression and 231,747 reporting no history of depression through 23andMe, and meta-analyzed these results with published MDD GWAS results. We identified five independent variants from four regions associated with self-report of clinical diagnosis or treatment for depression. Loci with  $p\text{val} < 1.0 \times 10^{-5}$  in the meta-analysis were further analyzed in a replication dataset (45,773 cases and 106,354 controls) from 23andMe. A total of 17 independent SNPs from 15 regions reached genome-wide significance after joint-analysis over all three datasets. Some of these loci were also implicated in GWAS of related psychiatric traits. These studies provide evidence for large-scale consumer genomic data as a powerful and efficient complement to traditional means of ascertainment for neuropsychiatric disease genomics.

### Keywords

genome-wide; depression; GWAS; single nucleotide polymorphism; SNP; MDD

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### Author Contributions

ARW, CLH, JRW conceived meta-analysis and statistical analysis. ARW, CLH, RHP oversaw dataset analysis and primary data interpretation. CLH designed and performed meta-analysis and further statistical analysis of the three datasets. XC provided statistical support and data visualization for the meta-analysis. MWN provided DEPICT functional annotation and LD score regression analyses. RHP, ARW, CLH wrote the manuscript. ARW, RHP, CLH, DAH, SAP, MWN provided data interpretation and revised the manuscript. JYT, DAH conceived and designed the 23andMe MDD GWAS. DAH and CT performed GWAS for 23andMe datasets and statistical support.

Major depressive disorder remains one of the most significant contributors to morbidity and mortality<sup>1–3</sup>. Efforts to develop novel interventions have been hindered by a limited understanding of the underlying neurobiology. Despite strong evidence of heritability<sup>4,5</sup>, efforts to clarify this biology through common or rare variant association studies have been unsuccessful, which has been attributed to the heterogeneity of disease and absence of a biological gold standard diagnosis. One recent study of a Han Chinese population identified two risk loci, in the *LHPP* gene and near the *SIRT1* gene, but neither was supported in European populations where the risk alleles are extremely rare<sup>6</sup>.

If one reasonable strategy adopted by that study is to develop more precise or refined phenotypes, another is to efficiently identify much larger cohorts for study despite less intensive phenotyping. This strategy has been validated in multiple non-psychiatric diseases, but not for psychiatric illness that is presumed to require more detailed interview. Here, we identified 75,607 individuals (62% female) who endorsed a prior clinical diagnosis of, or treatment for, major depression, and 231,747 (44% female) individuals reporting no clinical diagnosis of depression or treatment for depression. All subjects participated in the consumer genomics company 23andme's optional research initiative (for population socio-demographic features, see Table 1). These individuals were genotyped on one of four custom arrays containing genome-wide content and genotypes were imputed using the September 2013 release of 1000 Genomes Phase1 reference haplotypes. Research participants with > 97% European ancestry, excluding close relatives, were included in the GWAS analysis. The Manhattan Plot and Q-Q plot for the analysis are shown in Supplementary Figure 1a–b, p-values were adjusted for inflation using LD score regression (Supplementary Table 1).

## Novel major depression loci in a self-report population

From the discovery 23andMe dataset we identified two distinct regions containing SNPs with p-value <  $1 \times 10^{-8}$  and five additional loci with p-value <  $5 \times 10^{-8}$  (Supplementary Table 1) to be associated with self-report of depression. We have chosen to consider only the SNPs with pval <  $1 \times 10^{-8}$  to be genome-wide significant in this GWAS due to correction for 15 million SNPs in the 23andMe data. The most significant locus yielded an association at rs2806933 (adjusted p-value=  $8.53 \times 10^{-13}$ , OR= 0.955, 95% CI= 0.943–0.968 effect allele frequency in controls= 0.61) in a region spanning the 3' UTR for the olfactomedin-4 gene (*OLFM4*), not previously implicated in neuropsychiatric disease but known to be expressed in brain, including amygdala and medial temporal lobe<sup>7</sup>. The second, with peak association at rs768705 (p-value=  $2.91 \times 10^{-12}$ , OR= 1.051, 95% CI= 1.036–1.067, effect allele frequency in controls= 0.25), spans a locus containing the myocyte enhancer factor 2C (*MEF2C*) and transmembrane protein 161B (*TMEM161B*) genes. Variants in *MEF2C* has been previously associated with multiple CNS phenotypes including epilepsy and intellectual disability<sup>8,9</sup> and implicated in regulation of synaptic function<sup>10</sup>. *TMEM161B*, also brain-expressed, exhibits decreased levels of repressive dimethyl histone H3 Lys9/ Lys27 methylation in response to social isolation in a mouse model of depression<sup>11</sup>. While Schizophrenia and Alzheimer's Disease GWAS both identify the *MEF2C* region as a disease susceptibility locus, the peak schizophrenia and AD-associated SNPs are not in strong LD with the MDD SNP (Schizophrenia: rs181900<sup>12</sup>;  $r^2=0.001$  Alzheimer's disease: rs190982,  $r^2=0.016$ ). Using a population prevalence of 15% for MDD estimated by the PGC

Consortium<sup>13</sup>, we calculated heritability using LD score regression of  $h^2_{\text{liab}}=0.0528$  for this dataset. When using the 23andMe MDD observed population prevalence of 25%, this results in  $h^2_{\text{liab}}=0.0612$ .

Meta-analysis of the 23andMe dataset with the previously reported Psychiatric Genomics Consortium (PGC) meta-analysis of MDD, which encompassed 9,240 cases and 9,519 controls of European descent, is presented in Figure 1a–b (Supplementary Table 2). From the PGC cohort only 1.22 million SNPs overlapped with the 23andMe MDD data (no results were reported for X or Y chromosomes)<sup>14</sup> and only these SNPs were used for downstream analysis. As a result, several lead SNPs from the discovery 23andMe GWAS are absent including rs77741769 (*SPPL3-HNF1A*), rs144294997 (*N6AMTI*), rs1432639 (*NEGR1*), and rs67744457 (*EP300-L3MBTL2*). Each cohort was individually adjusted for inflation using LD score regression (as described in the Methods) and subsequently meta-analyzed using a standard fixed-effects, inverse-variance weighted approach<sup>15</sup>. Final results from the meta-analysis were further adjusted for the meta-analysis LD score regression intercept of 1.0025.

From the original 23andMe lead SNPs, only the *N6AMTI* locus is not represented in the meta-analysis results at a p-value less than  $5 \times 10^{-6}$  due to absence of the lead 23andMe SNP in the meta-dataset as well as an absence of significant secondary signals in the region. SNPs in the *OLFM4*, *TMEM161B-MEF2C* (two independent SNPs), *MEIS2-TMCO5A*, and *NEGR1* regions reached genome-wide significance in the meta-analysis ( $p < 5 \times 10^{-8}$ , correcting for 1.22 million SNPs) (Supplementary Table 2). Regional association plots are shown for these regions in Figure 2. Heritability for the meta-analysis was estimated at  $h^2_{\text{liab}}=0.059$  and 0.069, for 15 and 25% prevalence.

## Replication of 15 loci associated with major depression

We assessed the ability of the top signals ( $p\text{-val} < 1.0 \times 10^{-5}$ ) from the meta-analysis to replicate in a separate cohort of 45,773 cases and 106,354 controls from 23andMe (Table 1). All individuals in the replication dataset were independent from subjects included in the discovery 23andMe dataset and had similar characteristics for sex and age distributions. The replication cohort provided additional support for three of the five genome-wide significant SNPs in the *TMEM161B-MEF2C* (2 SNPs) and the *NEGR1* locus. In a joint-analysis of the discovery 23andMe dataset, PGC, and the 23andMe replication dataset, a total of 15 independent loci (17 SNPs) reached genome-wide significance ( $p\text{-val} < 5 \times 10^{-8}$ ) (Table 2). Of the remaining 46 SNPs with a p-value less than  $1 \times 10^{-5}$  in the meta-analysis of the 23andMe Discovery dataset and 23andMe, 41 had a consistent direction of effect between the meta-analysis and replication cohort (p-values across all analyses including joint-analysis are shown in Supplementary Table 2 for SNPs that reached a pval < than  $1 \times 10^{-5}$  in the meta-analysis).

To explore the biological implications of our findings we used DEPICT to derive tissue enrichment, gene-set enrichment, and gene predictions (Supplementary Table 3) for SNPs with a p-value less than  $1 \times 10^{-5}$  in the meta-analysis. While identification of the functional variant or gene is not straightforward many of the top associations in our dataset appear in or

near transcription factors with known CNS developmental functions (for additional gene predictions from DEPICT and functional annotation for each region see Supplementary Table 4). Gene-set enrichment analysis prioritized the *MEIS2* subnetwork ( $p$ -value =  $2.30 \times 10^{-6}$ ). *MEIS2* is a TALE homeodomain transcription factor known to function in development. While most studies implicate *MEIS2* in peripheral tissue development, recent studies have shown a role for *MEIS2* regulated pathways in neurogenesis through interactions with Pax6, as well as interactions with Pax3 and Pax7<sup>16,17</sup>. Notably, our analysis identified significant associations with MDD in the *MEIS2*, *PAX6*, and *PAX5* regions ( $p$ -value =  $2.04 \times 10^{-8}$ ,  $3.94 \times 10^{-7}$ , and  $2.59 \times 10^{-5}$  in the 23andMe Discovery dataset). Tissue enrichment analysis showed an over-representation of central nervous system, with 12 of the 19 nominally associated tissues being from different brain regions (with a Nervous System as a second level MeSH term). Although these associations did not pass multiple-testing correction, the top results from our MDD GWAS are enriched for CNS expression and transcriptional function important for CNS development or neurogenesis. Further functional annotations of predicted genomic/molecular function, brain tissue or monocyte eQTLs, gene predictions for each region using DEPICT<sup>18</sup>, and disease associations using publicly available GWAS datasets and the OMIM database are presented in Supplementary Table 4 for all 17 SNPs reaching genome-wide significance in the joint-analysis (Table 2).

After distance (300 kb) and LD pruning ( $r^2 > 0.1$ ), three regions had multiple SNPs with  $p$ -values less than  $1.0 \times 10^{-6}$  in the meta-analysis results. We tested SNPs in each of these regions for independence in the replication dataset using Wald and likelihood ratio tests. We conducted this analysis in the replication dataset to avoid SNP selection bias from the original findings. By conditioning on each SNP within the models at each locus we find two SNPs in each of the *TMEM161B-MEF2C* and *NEGR1* regions are likely independent (rs10514299 and rs454214, rs11209948 and rs2422321, respectively), with the variance in the region being explained best by both SNPs, while most of the variance in the *MLFI* region is explained by rs1656369 alone (with no additional significance provided by inclusion of rs4645169) (Supplementary Table 5).

## Validity of the self-report phenotype for major depression

As the PGC cohort is substantially smaller than the 23andMe single cohort, power in the PGC MDD GWAS to detect the effect sizes for the two genome-wide significant loci observed in the preliminary 23andMe GWAS was less than 0.6 at a nominal level of significance ( $p < 0.05$  uncorrected), and the analogous power to replicate the remaining 23andMe loci in PGC declined thereafter<sup>19</sup>. However, the probability of PGC showing the same direction of effect in 23andMe exceeded 90% for each of the top ten independent 23andMe loci that were also evaluated in PGC (corresponds to all overlapping peak-pruned 23andMe loci with unadjusted  $p < 1.0 \times 10^{-7}$  in 23andMe). We therefore conducted a sign-test examining concordance between PGC effect direction and the 23andMe effect direction for the top overlapping 23andMe peak loci. Nine of the top 10 loci matched sign, (Fisher's exact test  $p = 0.033$ ). The test continued to deviate significantly from chance at a range of thresholds, suggesting consistent signal between the PGC results and 23andMe. For the 82 independent SNPs with nominal  $p$ -values less than  $1 \times 10^{-5}$  in 23andMe, the  $p$ -value for the sign test was  $p = 2 \times 10^{-6}$  with the odds ratio for a sign match being 10.6 (95% CI = 3.5–37.1).

Furthermore, the effect sizes for the top independent 23andMe loci are correlated with the effect sizes of those SNPs in PGC (removing loci with MAF < 5% to avoid highly variable values). This correlation peaks at the 39<sup>th</sup> peak 23andMe locus with 68% correlation ( $p=2.5\times 10^{-9}$ ). Additionally, we calculated the genetic correlation between the two datasets using LD score regression<sup>20</sup> and found the two major depression datasets were highly and positively correlated ( $r_g=0.725$ ,  $SE=0.093$ ,  $p=7.05\times 10^{-15}$ ).

## Associations of lead SNPs with related phenotypes

To investigate the polygenic nature of this trait, we generated a genetic risk score from 17 SNPs (Supplementary Table 6) with  $p$ -values <  $5\times 10^{-8}$  in the joint-analysis (Discovery 23andMe, PGC, and replication 23andMe) and tested for association of the weighted MDD GRS with reporting of related phenotypes, medication use, and age-at-onset (Table 3) in the combined discovery and replication cohort, adjusting for depression case/control status. The GRS was significantly associated ( $FDR < 0.05$ ) with each of these phenotypes. Importantly, the MDD GRS significantly associated with an earlier-age-of-onset in cases (effect= -1.49 years per unit of log odds, standard error= 0.37,  $p$ -val=  $6.1\times 10^{-5}$ ).

The independent effect of each GRS SNP on this set of related phenotypes is presented in Supplementary Table 7. Importantly, while rs12552 in the OLFM4 region was not strongly supported in the replication dataset, this SNP is associated with increased reporting of panic attacks, use of medication to treat mental health problems, prescription sleep aids, and pain medication, BMI greater than 27, earlier age-of-onset of MDD, and commensurately associated with lower continuous age of onset. Individually, rs12552 and rs4543289 had the largest effect on age-at-onset, with a total of five SNPs having nominal significance ( $p$ -val < 0.05).

## Sex effects

Due to known sex-disparities in the presentation of depression, incidence rate, and the suggestion of differences in underlying biology, we tested for sex-specific effects on our top SNPs as well as genotype-sex interaction for each SNP in the 23andMe discovery cohort (Supplementary Table 8). In the discovery cohort, four SNPs had nominal  $P<0.05$  but none survived a multiple testing correction. No results reached nominal  $P<0.05$  in the replication cohort. Our GWAS results thus provide no support for gender differences in genetic predisposition to depression.

## Cohort Characteristics

We further validated the novel self-report phenotype by assessing expected characteristics of medication use, comorbid symptoms, and risk factors commonly seen in MDD within the 23andMe self-report cohort (Supplementary Table 9). Reporting of anxiety, panic attacks, insomnia were significantly increased ( $p$ -val <  $5.0\times 10^{-243}$  for all traits tested) among subjects reporting depression as well as a BMI greater than 27 (i.e. overweight) and a BMI greater than 30 (i.e. obese). Reporting of current SSRI use, medication for mental health problems, prescription sleep aids, and pain medication were also increased with the highest

odds ratio for any trait tested being for SSRIs and psychotropic use (13.35 and 44.83, respectively), further supporting the validity of the phenotype ascertainment. Cohort characteristics were also tested separately in males and females with no evidence of sex-specific differences (Supplementary Table 10).

Studies have shown a degree of shared genetic liability for different psychiatric disorders, likely a result of multiple factors including genetic pleiotropy, diagnostic overlap, comorbid disease, or disease progression. To initially assess shared genetic risk across psychiatric disorders we present p-values across five psychiatric traits (Schizophrenia, Bipolar Disorder, Neuroticism, Depressive symptoms, and Subjective Well-Being) for SNPs with p-values less than  $1 \times 10^{-5}$  in the MDD meta-analysis (Supplementary Table 11)<sup>21</sup>. The MDD SNPs showed the highest degree of overlap (smallest p-values) in the Schizophrenia dataset, followed by Neuroticism, with less replication in the Bipolar, Depressive Symptoms, and Subjective Well-being phenotypes. Schizophrenia and Bipolar GWAS are from the publicly available PGC datasets<sup>12,22</sup> while corresponding p-values for Neuroticism, Depressive Symptoms, and Subjective Well-being were provided by the bior The lack of correlation with SSGAC depressive symptoms self-report data may arise from the latter considering only depressive symptoms experienced during the previous two weeks-versus lifetime major depression in the primary cohorts. Conversely, the trait measure of neuroticism has previously been show to overlap with major depression, consistent with our results.

In order to rigorously assess genetic correlation of the MDD GWAS with other neuropsychiatric disease, we utilized available GWAS from PGC, including Bipolar Disorder and three Schizophrenia GWAS (different versions of the Schizophrenia PGC datasets), as well as neurodegenerative disease GWAS to test pairwise genetic correlation with the 23andMe MDD GWAS dataset using LD score regression. Due to the use of overlapping controls in the PGC datasets we did not use the results of the meta-analysis between 23andMe and PGC. The highest correlation with the primary 23andMe GWAS was observed for the PGC2 Schizophrenia GWAS ( $r = 0.282$ ,  $SE = 0.03$ ,  $p\text{-val} = 2.18 \times 10^{-21}$ ) followed by Bipolar Disorder and the additional Schizophrenia GWAS (Table 4); however, we observed little to no correlation for the Parkinson's disease and Alzheimer's disease datasets. Additionally, we checked for correlation between 23andMe MDD and a trait with no known epidemiological correlation to depression (LDL Cholesterol) and observed no genetic correlation between the two traits.

## Discussion

In this study we present a complementary approach to collecting large-scale genotypic data on major depression. By utilizing the self-report data on major depression from 23andMe, we were able to identify SNPs at a genome-wide level of significance associated with risk for depression in a cohort of European descent. Through a meta-analysis of the 23andMe data with PGC MDD GWAS and a joint-analysis with an independent 23andMe replication cohort, we identify 17 independent SNPs significantly associated with diagnosis of major depression. Through tissue and geneset enrichment analysis utilizing DEPICT, we find that these SNPs are predicted to be enriched in genes expressed in the CNS and function in transcriptional regulation related to neurodevelopment. We find no robust evidence for sex-

specificity among our top results but this study combined both genders and only adjusted for sex as a covariate, and was therefore not structured to identify sex-specific loci. This would ideally be done through a sex-stratified GWAS.

Although the variance explained by these SNPs is small, we find that our cohorts identified by self-report of major depression are highly genetically correlated with cohorts identified by clinical interview, a result further corroborated by significant sign-test and effect size matching between the top 23andMe SNPs (nominal  $p$ -val  $< 1 \times 10^{-5}$ ) and their counterparts in PGC in self-report and clinical-interview datasets. To better understand the phenotypic characteristics of the 23andMe self-report subjects, we assessed reporting of medication use and comorbidities and found that all tested characteristics were significantly increased in the subjects reporting Depression, similar to what is seen in clinically ascertained subjects. Notably, many of the most significant SNPs show evidence of pleiotropy when examined in other clinically ascertained psychiatric disorders with the smallest  $p$ -values among individual SNPs seen for MDD SNPs in the PGC Schizophrenia and neuroticism datasets. This finding is unsurprising given the pleiotropy reported by other GWAS and cross-psychiatric analyses<sup>13</sup>, and lends further support to the relevance of a self-report phenotype to clinical disease.

We were unable to replicate the genome-wide significant loci identified in the recent CONVERGE study<sup>6</sup> although we identified modest associations in each region (*LHPP*, rs145655839, minimum  $p$ -val= 0.0024 out of 6,204 SNPs in the region, and *SIRT1*, rs187810158, minimum  $p$ -val=0.0102 out of 5,111 SNPs in the region). This is unsurprising given that our study looked for genetic determinants of susceptibility in both males and females of European descent, and likely represented a very different population structure than that of the CONVERGE study of Han Chinese women.

Taken together, our results indicate the utility of complementary strategy to intensive phenotyping for identifying common variant associations with phenotypically heterogeneous neuropsychiatric diseases. The inter-rater reliability of lifetime MDD diagnosis even with structured interview is modest, with a kappa of 0.32–0.57<sup>23,24</sup>; conversely, the reliance on treatment-seeking patients in the present analysis rather than volunteers responding to advertisements lends additional face validity to the phenotype<sup>25</sup>. The finding in other large-scale analyses that cohorts ascertained based on treatment rather than structured interview yield similar associations<sup>12</sup>, and that such phenotypes are consistent with structured interview<sup>26</sup>, adds confidence to the validity of this approach<sup>12</sup>. In light of the massive impact of such disorders on health worldwide, any approach that can help elucidate pathophysiology merits consideration. The finding that a locus previously linked to other neuropsychiatric disease increases MDD risk also adds to a burgeoning literature indicating the pleiotropy of such risk genes.

## Methods

### Data Access

The full GWAS summary statistics for the 23andMe Discovery dataset will be made available through 23andMe to qualified researchers under an agreement with 23andMe that

protects the privacy of the 23andMe participants. Please contact David A. Hinds (dhinds@23andme.com) for more information and to apply to access the data. Information for the most significant 10,000 SNPs from the discovery 23andme GWAS are included in Supplementary Table 12.

### Population and Study Design

Participants were part of the customer base of 23andMe, Inc., a consumer genetics company. This cohort has been described in detail elsewhere<sup>27,28</sup>. Participants provided informed consent and participated in the research online. The protocol was approved by an external AAHRPP accredited IRB, Ethical and Independent Review Services. The discovery cohort was selected from participant data available in January 2015, and the replication cohort was selected in January 2016 from additional data available at that time.

### Genotyping, Quality Control, and Imputation

DNA extraction and genotyping were performed on saliva samples by National Genetics Institute (NGI), a CLIA licensed clinical laboratory and a subsidiary of Laboratory Corporation of America. Samples were genotyped on one of four genotyping platforms. The V1 and V2 platforms were variants of the Illumina HumanHap550+ BeadChip, including about 25,000 custom SNPs selected by 23andMe. The V3 platform was based on the Illumina OmniExpress+ BeadChip, with custom content to improve the overlap with the V2 array. The V4 platform use most recently is a fully custom array, including a lower redundancy subset of V2 and V3 SNPs with additional coverage of lower-frequency coding variation. The platforms contained 586,916; 584,942; 1,008,948; and 570,000 SNPs, respectively. Samples that failed to reach 98.5% call rate were re-analyzed. Individuals whose analyses failed repeatedly were re-contacted by 23andMe customer service to provide additional samples, as is done for all 23andMe customers.

Participant genotype data were imputed against the September 2013 release of 1000 Genomes Phase1 reference haplotypes, phased with ShapeIt<sup>29</sup>. We phased using an internally developed phasing tool, Finch, which implements the Beagle haplotype graph-based phasing algorithm<sup>30</sup>, modified to separate the haplotype graph construction and phasing steps. Finch extends the Beagle model to accommodate genotyping error and recombination, to handle cases where there are no consistent paths through the haplotype graph for the individual being phased. We constructed haplotype graphs for European samples on each 23andMe genotyping platform from a representative sample of genotyped individuals, and then performed out-of-sample phasing of all genotyped individuals against the appropriate graph.

In preparation for imputation, we split phased chromosomes into segments of no more than 10,000 genotyped SNPs, with overlaps of 200 SNPs. We excluded SNPs with Hardy-Weinberg equilibrium  $P < 10^{-20}$ , call rate  $< 95\%$ , or with large allele frequency discrepancies compared to European 1000 Genomes reference data. Frequency discrepancies were identified by computing a  $2 \times 2$  table of allele counts for European 1000 Genomes samples and 2000 randomly sampled 23andMe customers with European ancestry, and identifying SNPs with a chi squared  $P < 10^{-15}$ . We imputed each phased segment against all-ethnicity



1000 Genomes haplotypes (excluding monomorphic and singleton sites) using Minimac2<sup>31</sup>, using 5 rounds and 200 states for parameter estimation.

For the X chromosome, we built separate haplotype graphs for the non-pseudoautosomal region and each pseudoautosomal region, and these regions were phased separately. We then imputed males and females together using Minimac2, as with the autosomes, treating males as homozygous pseudo-diploids for the non-pseudoautosomal region.

### Ancestry Determination

We restricted the analysis to include individuals who have >97% European ancestry, as determined through an analysis of local ancestry<sup>32</sup>. Briefly, our algorithm first partitions phased genomic data into short windows of about 100 SNPs. Within each window, we use a support vector machine (SVM) to classify individual haplotypes into one of 31 reference populations. The SVM classifications are then fed into a hidden Markov model (HMM) that accounts for switch errors and incorrect assignments, and gives probabilities for each reference population in each window. Finally, we used simulated admixed individuals to recalibrate the HMM probabilities so that the reported assignments are consistent with the simulated admixture proportions. The reference population data is derived from public datasets (the Human Genome Diversity Project, HapMap, and 1000 Genomes), as well as 23andMe customers who have reported having four grandparents from the same country.

A maximal set of unrelated individuals was chosen for each analysis using a segmental identity-by-descent (IBD) estimation algorithm<sup>33</sup>. Individuals were defined as related if they shared more than 700 cM IBD, including regions where the two individuals share either one or both genomic segments identical-by-descent. This level of relatedness (roughly 20% of the genome) corresponds approximately to the minimal expected sharing between first cousins in an outbred population. When constructing the replication cohort, we identified unrelated individuals who were also unrelated to all individuals used in the discovery analysis.

We used principal component analysis (PCA) to characterize residual population structure in the subset of 23andMe participants with European ancestry. We computed principal components using 82,654 SNPs that were genotyped on all 23andMe array designs, with Hardy-Weinberg  $P > 1e-40$ , minor allele frequency  $> 0.01$ , call rate  $> 99\%$ , and excluding regions of extended long range linkage disequilibrium. We used the ARPACK library<sup>34</sup> to compute principal components using data for 519,914 individuals across all array designs; additional individuals were then projected onto this set of eigenvectors.

Supplementary Figure 2a shows the proportion of variance explained by each principal component, and Supplementary Figure 2b shows the proportion of each component's variance that is explained by country of ancestry, for a set of individuals reporting four grandparents from a single country. The first 5 PCs are largely explained by geographic ancestry, while higher order PCs are not.

## GWAS and Meta-analysis

In the GWAS and replication analysis, we computed association test results by logistic regression assuming additive allelic effects. For tests using imputed data, we use the imputed dosages rather than best-guess genotypes. We included covariates for age, gender, and the top 5 principal components to account for residual population structure. While we could justify the choice of 5 PCs based on the preceding ancestry analysis, we actually chose to use 5 based on computational considerations, and others have noted this to be a reasonable choice<sup>35</sup>.

For quality control of genotyped GWAS results, we removed SNPs that were only genotyped on our “V1” and/or “V2” platforms due to small sample size, and SNPs on chrM or chrY because many of these are not genotyped reliably. Using trio data, we flagged SNPs that failed a test for parent-offspring transmission; specifically, we regressed the child’s allele count against the mean parental allele count and flagged SNPs with fitted  $\beta < 0.6$  and  $P < 10^{-20}$  for a test of  $\beta < 1$ . We removed SNPs with a Hardy-Weinberg  $P < 10^{-20}$  in Europeans; or a call rate of  $< 90\%$ . We also tested genotyped SNPs for genotype date effects, and removed SNPs with  $P < 10^{-50}$  by ANOVA of SNP genotypes against a factor dividing genotyping date into 20 roughly equal-sized buckets. For imputed GWAS results, we removed SNPs with average  $r^2 < 0.5$  or minimum  $r^2 < 0.3$  in any imputation batch, as well as SNPs that had strong evidence of an imputation batch effect. The batch effect test is an F test from an ANOVA of the SNP dosages against a factor representing imputation batch; we removed results with  $P < 10^{-50}$ . Prior to GWAS, we identified, for each SNP, the largest subset of the data passing these criteria, based on their original genotyping platform – either v2+v3+v4, v3+v4, v3, or v4 only – and computed association test results for whatever was the largest passing set. After quality control, the 23andMe discovery GWAS included results for 13,474,321 imputed variants, and 60,949 genotyped variants that did not have imputed results passing our filters, for a total of 13,535,270 variants. Of these, 15,774 test results could not be computed due to logistic regression fitting problems, leaving 13,519,496 tests. HWE and batch-effect pvalues are presented in Supplementary Table 13.

Results from 23andMe were adjusted for variance inflation by multiplying the variance (i.e. square of the standard error) of each genetic effect estimate by the intercept of 1.0598 as calculated by LD score regression<sup>20</sup>. Meta-analysis with PGC was conducted by inverse-variance fixed effects meta-analysis on overlapping SNPs after adjusting the standard errors of each individual analysis for its own lambda (LD score regression intercept in PGC was 1.0243). Final results from the meta-analysis were further adjusted for the overall LD score regression intercept of 1.0025 (for more details on LD score regression methods see section on LD score regression).

## LD score regression

We calculated LD scores (LD Score (LDSC) version 1.0.0) as previously described using the European 1000 Genomes reference panel (phase 3 version 5a) with a minor allele frequency cutoff for SNP inclusion greater than 5%. GWAS summary statistics data was collected from the following resources: Psychiatric Genomics Consortium (MDD, Bipolar Disorder, SCZ1, SCZ1+SWE, SCZ2), the International Genomics of Alzheimer’s Project (IGAP AD), the

International Parkinson Disease Genomics Consortium (IPDGC PD), and the Global Lipids Genetics Consortium (GLGC LDL). GWAS data was harmonized using the `munge_sumstats.py` function, (using the SNP list derived from LD score calculation) and genomic inflation control intercepts were calculated for the 23andMe MDD data, PGC MDD data, and PGC+23andMe meta-analysis data using the `ldsc.py` function (using all default settings and options). Additionally, we calculated liability heritability estimates for the meta-analysis using the same function, with a population prevalence estimation of 15%, and 25% as previously described<sup>13</sup>. Finally, we calculated the cross-trait regression between 23andMe MDD GWAS and the PGC datasets, the IGAP data, the IPDGC data, and the GLGC data.

### Trait Ascertainment

Subjects with depression were identified through self-report in web-based surveys. A total of six survey data sources were used to compose the depression phenotype:

1. (Your Medical History survey: 2009–2013) *“Have you ever been diagnosed by a doctor with any of the following psychiatric conditions?”* (options for Depression: Yes, No, I don’t know)
2. (Research Snippet: 2010–2014) *“Have you ever been diagnosed with clinical depression?”* (answers: Yes, No, I’m not sure)
3. (Health Intake survey, unbranched: 2014–2015) *“Have you ever been diagnosed with or treated for any of the following conditions?”* (options for Depression: Yes, No, I’m not sure)
4. (Health Intake survey, branched: 2013)
  - 4a *“Have you ever been diagnosed or treated for any of the following conditions?”* (“A mental health or psychiatric condition”: Yes, No, I’m not sure)
  - 4b *“What mental health problems have you had? Please check all that apply”* (check box: Depression)
5. (Health Profile survey: 2015–2016)
  - 5a *“Have you ever been diagnosed with or treated for any of the following conditions? Anxiety, Attention deficit disorders, Bipolar disorder / manic depression, Depression, Eating disorder (such as anorexia or bulimia)”* (answers: Yes, No, I’m not sure)
  - 5b *“Have you ever been diagnosed with or treated for depression?”* (answers: Yes, No, I’m not sure)
6. (Health Followup survey: 2014–2015) *“In the last 2 years, have you been newly diagnosed with or started treatment for any of the following conditions?”* (options for Depression: Yes, No, I’m not sure)

Sources 1, 3, 4, and 5 represent four different iterations of a general medical history survey, administered over successive time periods from 2009 to 2016. Source 2 used a different

mechanism for presenting individual questions to participants outside of the context of a formal survey. Source 6 was a survey administered to a subset of participants at least a year after they had completed one of the Health Intake surveys.

Sources 1 to 5 were combined by keeping the first non-missing response among these sources for each participant, evaluated in the specified order (the “coalesced response”). We then incorporated responses to source 6, by defining cases as the union of cases from the coalesced response and cases from source 6; and defining controls as individuals who were controls for either and cases for neither of these.

For the branched data sources 4 and 5, participants were first asked a screening question (4a or 5a), and if they answered affirmatively, were asked a specific follow-up question (4b or 5b). Cases were defined as positive responses to the follow-up question, and controls were the union of ‘no’ responses to either the screening or follow-up questions.

As a result of the staging of the discovery and replication analyses, the discovery cohort did not include any responses from source 5, and the replication cohort consisted almost entirely of responses from sources 3 or 5.

In survey sources 1,3,4, and 5, we also asked for an age of first diagnosis of depression. This data was provided by a majority of participants, including 74% of cases in the discovery cohort and 85% of cases in the replication cohort.

We used Cohen’s Kappa to assess agreement across responses for sources 1 to 5, taking advantage of participants who had responded to more than one of the survey data sources (Table 14).

Agreement was good in most comparisons ( $\kappa > 0.7$ ), but was somewhat worse for comparisons with branched source 4 ( $\kappa$  between 0.5 and 0.7). Source 4 systematically under-called cases compared to the other sources, apparently due to the wording of the screening question. This tendency is partially mitigated in the logic for the combined phenotype, where we preferentially use responses to sources 1 to 3 if available.

The logic for composing the depression phenotype in this way was based on several considerations. For most participants (>95%), we have either just one response, or the available responses are all in agreement, so a deeper analysis of the mismatch data was unlikely to substantially affect downstream results. Our strategy of selecting one response per participant without regard for their other responses also seemed least likely to introduce bias in classification of participants who provided multiple responses.

## Secondary Phenotypes

A set of common co-morbidities of depression were defined based on responses to single questions, as follows:

- Anxiety (Health Intake survey, unbranched, 2014–2015): “Have you ever been diagnosed with or treated for anxiety?” (Yes, No, I don’t know)

- Panic attacks (Health Intake survey, unbranched, 2014–2015): “Have you ever been diagnosed with or treated for panic attacks?” (Yes, No, I don’t know)
- Insomnia (Research Snippet, 2013–2016): “Do you routinely have trouble getting to sleep at night?” (Yes, No, I don’t know)
- Taking an SSRI (Research Snippet, 2013–2016): “Are you currently taking an SSRI (selective serotonin reuptake inhibitor) for any reason?” (options: Yes, No, I don’t know)
- Ever taken medication for a mental health condition; prescription sleep aids; or prescription pain medication (Health Intake survey, unbranched, 2014–2015): “*Have you ever taken these medications?*” “*Medications to treat depression or anxiety or another mental health condition*”, “*Prescription sleep aids*”, “*Prescription pain medications*” (checkbox for each category)

Overweight and obesity were defined based on BMI (>27, >30), computed from self-reported height and weight, which were collected using fill-in forms in multiple survey contexts.

### Associations with secondary phenotypes and age-of-onset

We computed genetic risk scores based on the 17 SNPs with p-values < 5e-8 in the joint analysis of 23andMe discovery, PGC, and 23andMe replication results, as a linear combination of independent single-SNP effect sizes estimated from that joint-analysis (Supplementary Table 2). We tested each secondary phenotype for association with these scores in the combined 23andMe discovery and replication cohorts; we tested for effects on age-of-onset in depression cases only (Table 3). For age-of-onset, we defined “early onset” as onset before age 30, and fit this binary outcome by logistic regression; we also fit a model for continuous age-of-onset using linear regression. In all these tests, we included covariates for age, gender, five PCs, and depression case/control status. In this way, we were testing for residual association not explained by depression status, and thus these associations are independent of the data that was used to identify these 17 variants. Separately, we tested each of the 17 SNPs individually for association with this same set of phenotypes, including the same covariates (Supplementary Table 7).

### DEPICT Functional Analysis

We utilized DEPICT<sup>18</sup> to determine the most likely causal gene at each of the depression-associated loci, and to assess reconstituted gene sets enriched for and tissues highly expressing those genes. The reconstituted gene sets used in the analysis are derived from publicly available gene set annotations, which are then integrated with 77,840 gene expression arrays<sup>36</sup> to predict which other genes are likely to be part of these gene sets.

For the analysis, we selected SNPs significantly associated to depression at  $p < 1 \times 10^{-5}$ . After clumping those SNPs using 500 kb flanking regions and an LD cutoff threshold  $r^2 > 0.1$ , 63 independent SNP signals were identified from 816 top variants. These 63 top SNPs were further merged into 59 non-overlapping loci containing 157 genes, which were then

assessed using the DEPICT algorithm for gene set and tissue enrichment<sup>18</sup>. Results shown in Supplementary Table 3 are not corrected for multiple testing.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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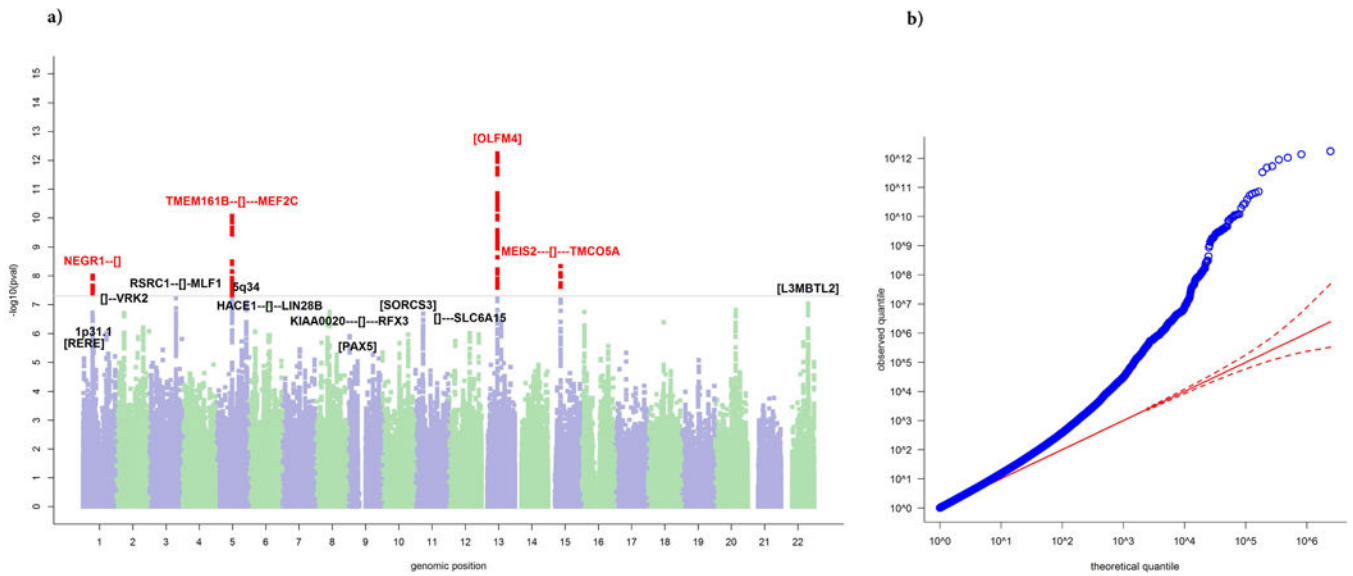
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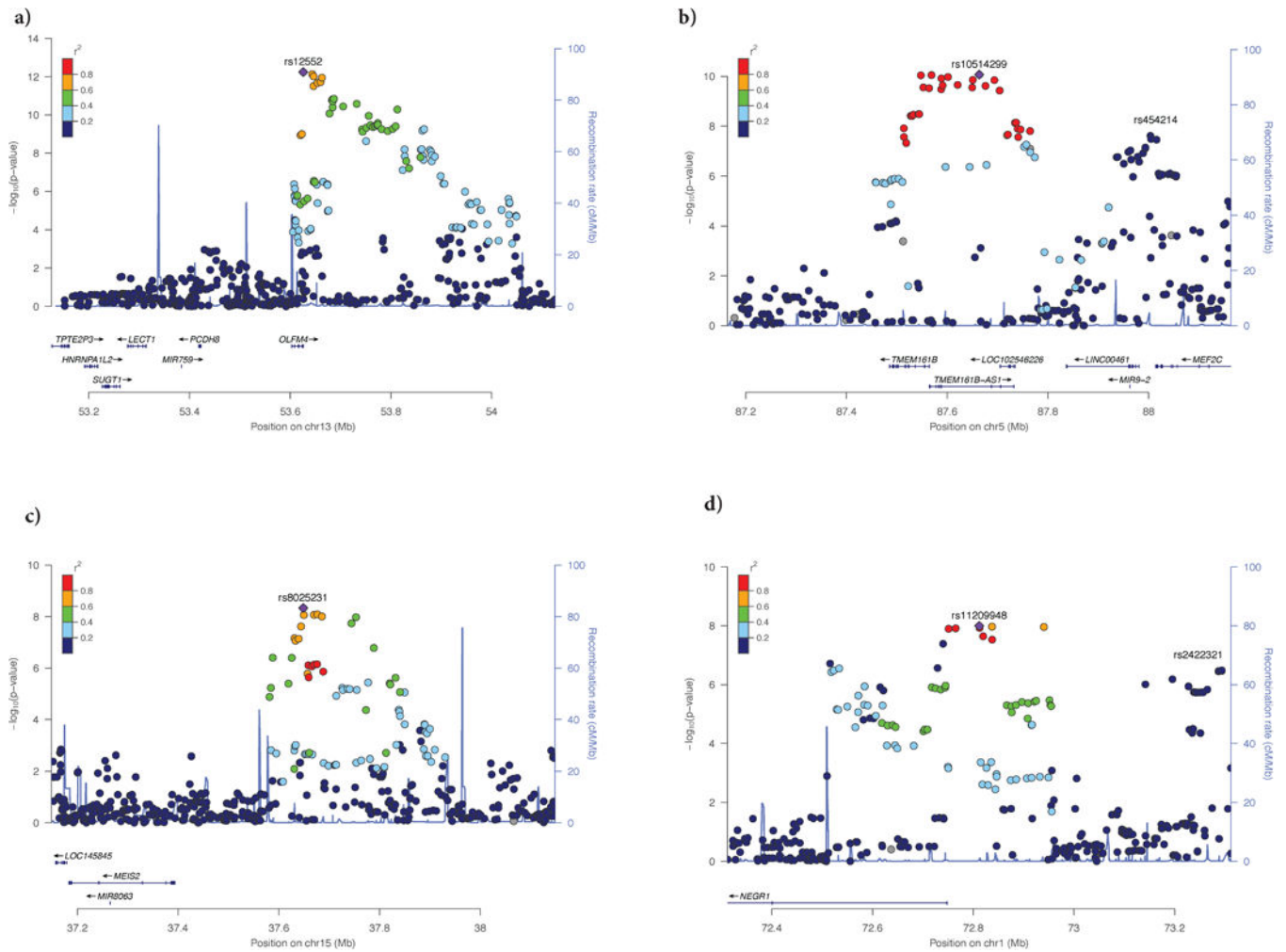
**URL list**

- Psychiatric Genomics Consortium (MDD, Bipolar Disorder, SCZ1, SCZ1+SWE, SCZ2 - <https://www.med.unc.edu/pgc>)
- International Genomics of Alzheimer's Project (IGAP AD - [http://www.pasteur-lille.fr/en/recherche/u744/igap/igap\\_download.php](http://www.pasteur-lille.fr/en/recherche/u744/igap/igap_download.php))
- International Parkinson Disease Genomics Consortium (IPDGC PD – [www.pdgene.org](http://www.pdgene.org))
- Global Lipids Genetics Consortium (GLGC LDL - <http://csg.sph.umich.edu/abecasis/public/lipids2013/>).
- Social Science Genetics Association Consortium (SSGAC). The SSGAC phenotypes come from meta-analysis of self-report questionnaires and publicly available datasets (Subjective Well-Being, Neuroticism, Depressive Symptoms- <http://biorxiv.org/content/early/2014/10/18/010512>)



**Figure 1.**

Discovery phase meta-analysis of 23andMe self-report ascertainment of major depression (75,607 cases and 231,747 controls) and PGC MDD (9,240 cases and 9,519 controls). a) Manhattan plot of Discovery phase 23andMe GWAS. LD score regression calculated intercept was used for inflation correction. The threshold for genome-wide significance ( $p < 5 \times 10^{-8}$ ) is indicated by the purple line. Red dots represent SNPs with p-values smaller than the genome-wide significant threshold. Regions labeled in black denote loci that reached genome-wide significance in the join-analysis. b) Q-Q plot for the 23andMe MDD GWAS.



**Figure 2.** Regional association plots for genome-wide significant regions and secondary independent signals identified in each region. a) *OLFM4* locus (rs12552), b) *TMEM161B-MEF2C* (rs10514299), c) *MEIS2-TMCO5A* locus (rs8025231), and d) *NEGR1* locus (rs11209948). Secondary signals in *TMEM161B-MEF2C* and *NEGR1* (rs454214, rs2422321 respectively) are shown. Purple diamonds represent smallest p-value for each locus.

**Table 1**

Cohort Demographics for the primary and replication 23andMe datasets

	Discovery		Replication	
	MDD	Controls	MDD	Controls
<b>Total (n=)</b>	75607	231747	45773	106354
<b>Age, counts</b>				
<b>under 30</b>	12.1%	11.6%	13.8%	13.4%
<b>30–45</b>	29.9%	27.5%	29.8%	25.4%
<b>45–60</b>	28.8%	27.2%	29.6%	27.7%
<b>60+</b>	29.3%	33.7%	26.7%	33.3%
<b>Sex, count</b>				
<b>Male</b>	38.0%	56.2%	33.8%	52.6%
<b>Female</b>	62.0%	43.8%	66.2%	47.4%

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**Table 2**

Summary statistics for 17 SNPs reaching genome-wide significance ( $p_{\text{val}} < 5 \times 10^{-8}$ ) in the joint analysis (23andMe Discovery, PGC, and 23andMe replication dataset). Corresponding p-values, effect, and standard error shown for each phase of analysis: 23andMe MDD (Discovery dataset), PGC MDD, meta-analysis (23andMe Discovery + PGC), 23andMe Replication (23andMe Replication cohort), and joint analysis. Effect and standard error are unadjusted. P-values are adjusted for 23andMe Discovery, PGC, and meta-analysis

rs ID	Gene Context	23andMe	PGC	Meta	Replication*	Joint*	sign-match
rs10514299	TMEM161B—[]—MEF2C	4.35E-12	5.73E-01	8.50E-11	1.15E-04	9.99E-16	Y
rs1518395	[]—VRK2	1.45E-07	5.83E-01	2.01E-07	1.50E-05	4.32E-12	Y
rs2179744	[L3MBTL2]	4.34E-08	7.74E-01	9.24E-08	7.26E-04	6.03E-11	Y
rs11209948	NEGR1—[]	4.41E-08	8.57E-02	1.01E-08	9.39E-04	8.38E-11	Y
rs454214	TMEM161B—[]—MEF2C	6.28E-08	1.62E-01	2.42E-08	6.39E-03	1.09E-09	Y
rs301806	[RERE]	3.72E-06	8.68E-01	7.28E-06	2.52E-04	1.90E-09	Y
rs1475120	HACE1—[]—LIN28B	2.32E-06	2.29E-01	1.14E-06	9.27E-04	4.17E-09	Y
rs10786831	[SORCS3]	1.75E-06	2.98E-01	1.09E-06	2.33E-03	8.11E-09	Y
rs12552	[OLFM4]	1.23E-12	1.57E-01	5.74E-13	8.70E-01	8.16E-09	N
rs6476606	[PAX5]	2.59E-05	1.52E-01	9.30E-06	1.94E-04	1.20E-08	Y
rs8025231	MEIS2—[]—TMCO5A	2.04E-08	8.49E-02	4.66E-09	7.50E-02	1.23E-08	Y
rs12065553	[]	8.53E-07	8.67E-01	2.88E-06	6.79E-03	1.32E-08	Y
rs16563569	RSRC1—[]—MLF1	8.19E-08	3.22E-01	6.05E-08	3.56E-02	1.34E-08	Y
rs4543289	[]	1.19E-06	1.15E-02	8.23E-08	5.26E-03	1.36E-08	Y
rs2125716	[]—SLC6A15	4.33E-07	8.78E-01	9.58E-07	2.24E-02	3.05E-08	Y
rs2422321	NEGR1—[]	5.12E-06	6.91E-03	3.28E-07	3.13E-03	3.18E-08	Y
rs7044150	KIAA0020—[]—RFX3	3.97E-07	9.64E-01	1.24E-06	3.05E-02	4.31E-08	Y

\* meta-analysis and replication not adjusted for inflation in joint analysis

Adjustments were done using LD score regression calculated intercepts. Sign-match is shown for matching of direction of effect between the meta-analysis and 23andMe replication datasets. All alleles are on the (+) or forward genomic strand. All effects are reported for the 2nd allele when listed in alphabetical order. Text representation of SNP location in relation to other genes in the region is shown. The SNP location is denoted by []. If the SNP occurs between genes then the distance from those genes are denoted by dashes (-), with '\*' = <10kb, '\*-' = <100kb, '\*---' = <1000kb. HG19 release of UCSC was used for mapping. EAF= effect allele frequency for controls. Results for GWAS results are peak-pruned by distance (300 kb) and LD ( $r^2 > 0.1$ )

**Table 3**

MDD gene risk score association with secondary phenotypes. Gene risk score explained in Supplementary Table 6. MDD age-at-onset associations were conducted in subjects with MDD. All other trait associations were conducted in cases and controls and adjusted for case/control status from the general 23andMe research community.

Phenotype	N	effect (SE)	pvalue	FDR
Early-onset	94891	0.283 (0.095)	2.90E-03	3.20E-03
Age-of-onset	94891	-1.49 (0.372)	6.10E-05	8.40E-05
Anxiety	250528	0.323 (0.061)	1.00E-07	2.50E-07
Panic attacks	247167	0.319 (0.072)	9.80E-06	1.50E-05
Insomnia	248576	0.272 (0.051)	1.10E-07	2.50E-07
Taking an SSRI	52698	0.448 (0.162)	5.50E-03	5.50E-03
Medication for mental health	349287	0.421 (0.057)	1.40E-13	1.50E-12
Prescription sleep aid	350119	0.184 (0.05)	2.70E-04	3.20E-04
Prescription pain medication	346989	0.236 (0.041)	5.60E-09	3.10E-08
Overweight (BMI>27)	401552	0.212 (0.038)	3.00E-08	1.10E-07
Obesity (BMI>30)	401552	0.216 (0.045)	1.50E-06	2.70E-06

**Table 4**

Cross-trait genetic correlation with 23andMe MDD (LD score regression). The observed heritability score for the 23andMe discovery cohort is  $h^2 = 0.038$ . Genetic correlation of the 23andMe Discovery MDD dataset with related psychiatric disorders (PGC MDD, PGC SZ1, PGC SCZ1+SWE, PGC SCZ2, PGC Bipolar Disorder), non-psychiatric neurological disorders (IGAP AD, IPDGC PD (2012)), and non-psychiatric and non-neurological GWAS are shown (GLGC LDL).  $r_g$  = genetic correlation

phenotype	$r_g$ (se)	nominal p-value	cohort observed $h^2$	significant after Bonferroni correction
<b>PGC MDD</b>	<b>0.725 (0.093)</b>	<b>7.05E-15</b>	0.128	*
PGC SCZ1	0.23 (0.042)	4.028E-08	0.543	*
PGC SCZ1+SWE	0.261 (0.036)	8.132E-13	0.411	*
PGC SCZ2	0.282 (0.03)	2.182E-21	0.371	*
PGC Bipolar Disorder	0.264 (0.049)	7.446E-08	0.350	*
IGAP AD	-0.069 (0.071)	0.3331	0.039	ns
IPDGC PD (2012)	0.185 (0.091)	0.04123	0.200	ns
GLGC LDL	0.056 (0.031)	0.072	0.191	ns