Genome-wide analysis of over 106000 individuals identifies 9 neuroticism-associated loci

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Neuroticism is a personality trait of fundamental importance for psychological well-being and public health. It is strongly associated with major depressive disorder (MDD) and several other psychiatric conditions. Although neuroticism is heritable, attempts to identify the alleles involved in previous studies have been limited by relatively small sample sizes. Here we report a combined meta-analysis of genome-wide association study (GWAS) of neuroticism that includes 91 370 participants from the UK Biobank cohort, 6659 participants from the Generation Scotland: Scottish Family Health Study (GS:SFHS) and 8687 participants from a QIMR (Queensland Institute of Medical Research) Berghofner Medical Research Institute (QIMR) cohort. All participants were assessed using the same neuroticism instrument, the Eysenck Personality Questionnaire-Revised (EPQ-R-S) Short Form’s Neuroticism scale. We found a single-nucleotide polymorphism-based heritability estimate for neuroticism of ∼ 15% (i.e. = 0.7%). Meta-analysis identified nine novel loci associated with neuroticism. The strongest evidence for association was at a locus on chromosome 8 (P = 1.5 × 10−15) spanning 4 Mb and containing at least 36 genes. Other associated loci included interesting candidate genes on chromosome 1 (GRIK3 (glutamate receptor ionotropic kainate 3)), chromosome 4 (KLHL2 (Kelch-like protein 2)), chromosome 17 (CRHR1 (corticotropin-releasing hormone receptor 1) and MAPT (microtubule-associated protein Tau)) and on chromosome 18 (CEL4 (CUGBP elav-like family member 4)). We found no evidence for genetic differences in the common allelic architecture of neuroticism by sex. By comparing our findings with those of the Psychiatric Genetics Consortia, we identified a strong genetic correlation between neuroticism and MDD and a less strong but significant genetic correlation with schizophrenia, although not with bipolar disorder. Polygenic risk scores derived from the primary UK Biobank sample captured ∼ 1% of the variance in neuroticism in the GS:SFHS and QIMR samples, although most of the genome-wide significant alleles identified within a UK Biobank-only GWAS of neuroticism were not independently replicated within these cohorts. The identification of nine novel neuroticism-associated loci will drive forward future work on the neurobiology of neuroticism and related phenotypes.

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INTRODUCTION

Neuroticism is a dimension of personality that has been studied for ∼ 100 years, is present in most personality trait theories and questionnaires and is found in the lexicons of most human cultures.1 Individual differences in neuroticism are highly stable across the life course.2 Higher neuroticism is associated with considerable public health and economic costs,3 premature mortality4 and a range of negative emotional states and psychiatric disorders, including major depressive disorder (MDD), anxiety disorders, substance misuse disorders, personality disorders and schizophrenia.5–9 Thus, the study of neuroticism is not only important for understanding an important dimension of personality but may also illuminate the etiology of a range of psychiatric disorders.10,11

Eysenck12 suggested a biological basis for neuroticism over 50 years ago. Although the biological underpinnings of personality traits are not understood, genetic factors are clearly involved. Twin studies suggest that ∼ 40% of the trait variance for neuroticism is heritable,12–17 of which between 15 and 37% is explained by variation in common single-nucleotide polymorphisms (SNPs)12,18 and is potentially detectable using the genome-wide association

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GWAS and meta-analysis of neuroticism

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study (GWAS) paradigm. The clear links between neuroticism, psychopathology and other adverse health outcomes—and the implications for global health that would result from a better understanding of its mechanisms—provide a strong rationale for large-scale GWAS to identify its genetic architecture (and genetic aetiology).

To date, individual GWASs of neuroticism have been limited by modest sample sizes and have delivered equivocal findings. Large meta-analyses of GWASs have also delivered modest findings. The recent Genetics of Personality Consortium (GPC) meta-analysis of neuroticism, which included 73 447 individuals from 29 discovery cohorts plus a replication cohort, identified only one genome-wide significant associated locus, at MAGI1 on chromosome 3 (\(P = 2.38 \times 10^{-8}\)). Within two of the cohorts in this GPC study, common genetic variants explained \(\sim 15\%\) of the variance in neuroticism.\(^\text{18}\)

In our study, seeking additional associated loci, we have conducted a meta-analysis that included GWAS results from the UK Biobank cohort, the Generation Scotland: Scottish Family Health Study (GS:SFHS) cohort\(^\text{20}\) and the QIMR (Queensland Institute of Medical Research) Berghofer Medical Research Institute Study in Adults (QIMR) cohort.\(^\text{2,14}\) The UK Biobank is the largest single GWAS sample of neuroticism to date and probably the most homogeneous in terms of ascertainment strategy and assessment methodology. In addition, we evaluated the genetic relationship between neuroticism and three major psychiatric phenotypes for which there are large, publically accessible GWAS data sets: MDD, schizophrenia and bipolar disorder (BD). Finally, we have compared our findings with those from the GPC meta-analytic GWAS of neuroticism,\(^\text{18}\) as well as the CONVERGE consortium for MDD.\(^\text{21}\)

MATERIALS AND METHODS

Sample

UK Biobank is a large prospective cohort of more than 502 000 residents of the United Kingdom, aged between 40 and 69 years.\(^\text{22}\) The aim of UK Biobank aim is to study the genetic, environmental, medication and lifestyle factors that cause or prevent disease in middle and older age. Recruitment occurred over a 4-year period from 2006 to 2010. Baseline assessments included social, cognitive, personality (the trait of neuroticism), lifestyle and physical health measures. For the present study, we used the first genetic data release (June 2015) based on approximately one-third of UK Biobank participants. Aiming to maximise homogeneity, we restricted the sample to those who reported being of white UK ancestry and for whom neuroticism phenotype data were available (\(n = 91\,370\)).

We also made use of data provided by investigators from the GS:SFHS\(^\text{20}\) and QIMR cohorts\(^\text{2,13,14}\) to conduct a meta-analysis based on samples for which we could readily access individual genotypes and which were assessed using the same measure of neuroticism. The GS:SFHS sample comprised 7196 individuals and the QIMR sample comprised 8687 individuals. Individuals (\(n = 537\)) who had participated in both UK Biobank and GS:SFHS were removed from the GS:SFHS sample based on relatedness checking using the genetic data.

Note that we were unable to incorporate the published data from the GPC as the neuroticism measure used in that study was derived from an item response theory analysis (prohibiting inverse variance-weighted meta-analysis due to the differences in variance and heterogeneity of the measure). In addition, there was no information on the sample size for each SNP (prohibiting sample size-weighted meta-analysis) and the majority of participants in the QIMR cohort were included within the GPC meta-analysis.

This study obtained informed consent from all participants and was conducted under generic approval from the National Health Service (NHS) National Research Ethics Service (approval letter dated 17 June 2011, Ref 11/NW/0382) and under UK Biobank approvals for application 6553 ‘Genome-wide association studies of mental health’ (principal investigator Daniel Smith) and 4844 ‘Stratifying Resilience and Depression Longitudinally’ (principal investigator Andrew McIntosh).

Neuroticism phenotype

Neuroticism was assessed in all three cohorts (UK Biobank, GS:SFHS and QIMR) using the 12 items of the neuroticism scale from the Eysenck Personality Questionnaire-Revised Short Form (EPQ-R-S)\(^\text{23}\) (Supplementary Table S1). Respondents answered ‘yes’ (score 1) or ‘no’ (score 0) to each of the questions, giving a total neuroticism score for each respondent of between 0 and 12. This short scale has a reliability of more than 0.8 (ref. 23) and high concurrent validity; for example, in a sample of 207 older people EPQ-R-S scores correlated 0.85 with the neuroticism score from the NEO-Five Factor Inventory, the scale most widely used internationally.\(^\text{24,25}\)

Genotyping and imputation

In June 2015, UK Biobank released the first set of genotyped data for 152 729 UK Biobank participants. Approximately 67% of this sample was genotyped using the Affymetrix UK Biobank Axiom array (Santa Clara, CA, USA) and the remaining 33% were genotyped using the Affymetrix UK BILEVE Axiom array. These arrays have over 95% content in common. Only autosomal data were available under the current data release. Data were pre-imputed by UK Biobank as fully described in the UK Biobank interim release documentation.\(^\text{26}\) Briefly, after removing genotyped SNPs that were outliers or were multiallelic or of low frequency (minor allele frequency (MAF) < 1%), phasing was performed using a modified version of SHAPEIT2 and imputation was carried out using IMPUTE2 algorithms, as implemented in a C++ platform for computational efficiency.\(^\text{27,28}\) Imputation was based upon a merged reference panel of 87 696 888 biallelic variants on 12 570 haplotypes constituted from the 1000 Genomes Phase 3 and UK10K haplotype panels.\(^\text{29}\) Variants with MAF < 0.001% were excluded from the imputed marker set. Stringent quality control before release was applied by the Wellcome Trust Centre for Human Genetics, as described in UK Biobank documentation.\(^\text{30}\)

Statistical analysis

Quality control and association analyses. Before all analyses, further quality control measures were applied. Individuals were removed based on UK Biobank genomic analysis exclusions (Biobank Data Dictionary item #22010), relatedness (#22012: genetic relatedness factor; a random member of each pair of individuals with KING-estimated kinship coefficient > 0.0442 was removed), gender mismatch (#22001: genetic sex), ancestry (#22006: ethnic grouping; principal component (PC) analysis identified probable Caucasians within those individuals who were self-identified as British and other individuals were removed from the analysis) and quality control failure in the UK BILEVE study (#22050: UK BILEVE Affymetrix quality control for samples and #22051: UK BILEVE genotype quality control for samples). A sample of 112 031 individuals remained for further analyses. Of these, 91 370 had neuroticism scores. Genotype data were further filtered by removal of SNPs with Hardy–Weinberg equilibrium \(P < 10^{-6}\), with MAF < 0.01, with imputation quality score < 0.4 and with data on < 95% of the sample after excluding genotype calls made with < 90% posterior probability, after which 8 268 322 variants were retained.

Association analysis was conducted using linear regression under a model of additive allelic effects with sex, age, array and the first 8 PCs (Biobank Data Dictionary items #22009.01 to
Heritability, polygenicity and cross-sample genetic correlation. Univariate GCTA-GREML analyses were used to estimate the proportion of variance explained by all common SNPs for the neuroticism phenotype. We additionally applied linkage disequilibrium score regression (LDSR) to the summary statistics to estimate SNP heritability ($h^2_{SNP}$) and to evaluate whether inflation in the test statistics is the result of polygenicity or of poor control of biases such as population stratification. Genetic correlations between neuroticism scores in the three cohorts (UK Biobank, QIMR and GS:SFHS) were tested, and genetic correlations between neuroticism, schizophrenia, BD and MDD were evaluated in the UK Biobank sample using LDSR, a process that corrects for potential population structure within the sample, and the hidden population structure within the sample, and the genetic PCs were included to control for polygenicity or of poor control of biases such as population stratification. Genetic correlations between neuroticism scores in the three cohorts (UK Biobank, QIMR and GS:SFHS) were tested, and genetic correlations between neuroticism, schizophrenia, BD and MDD were evaluated in the UK Biobank sample using LDSR, a process that corrects for potential population overlap without relying on the availability of individual genotypes. For the psychiatric phenotypes, we used GWAS summary statistics provided by the Psychiatric Genomics Consortium (http://www.med.yale.edu/pgc/).

Polygenic risk score analyses in the QIMR and GS:SFHS samples. In the QIMR sample (N = 8687 individuals), polygenic risk scores for neuroticism (PRS-N) based on the summary statistics from the UK Biobank GWAS were computed with PLINK 1.90 (version 3 September 2015, https://www.cog-genomics.org/plink2/), for $P$-value thresholds ($P_1$ 0.01, 0.05, 0.1, 0.5 and 1), following the procedure described by Wray et al. All subjects had GWAS data imputed to 1000G v.3 (http://csg.sph.umich.edu/abecasis/MaCH/download/). Only SNPs with a MAF $\geq$ 0.01 and imputation quality $r^2 \geq 0.6$ were used in the calculation of the PRS-N. Genotypes were LD pruned using clumping to obtain SNPs in approximate linkage equilibrium with an $r^2 < 0.1$ within a 10 000 bp window. As QIMR participants were related, predictions were calculated using GCTA (Genome-wide Complex Trait Analysis, version 1.22), using the following linear mixed model: $EPQ-N = \text{intercept} + b_0 \times \text{covariates} + b_2 \times g + e$ with $g \sim N(0, \text{GRM})$, where $EPQ$ is neuroticism measured by EPQ (standardised sum score); covariates are age, sex, imputation chip, 10 genetic PCs and the standardised PRS ($P_1$ 0.01, 0.05, 0.1, 0.5 or 1); $e$ is error; and GRM is genetic relationship matrix. $P$-values were calculated using the t-statistic on the basis of the $\beta$ and s.e. from the GCTA output. Variance explained by the PRS was calculated using: $\text{var}(x) = \beta^2/\text{var}(x)$, where $x$ is the PRS, $b$ is the estimate of the fixed effect from GCTA and $y$ is the phenotype.

In the GS:SFHS sample, PRS-N based on the UK Biobank neuroticism GWAS results were created using PRSice from observed genotypes in 7196 individuals. SNPs with a MAF $< 0.01$ were removed before creating PRS-N. Genotypes were LD pruned using clumping to obtain SNPs in linkage equilibrium with an $r^2 < 0.25$ within a 200-kb window. As above, five PRS-N were created containing SNPs according to the significance of their association with the phenotype, with $P_1$ of 0.01, 0.05, 0.1, 0.5 and 1 (all SNPs). Linear regression models were used to examine the associations between the PRS-N and neuroticism score in GS, adjusting for age at measurement, sex and the first 10 genetic PCs to adjust for population stratification. The false discovery rate method was used to correct for multiple testing across the PRS-N at all five thresholds.

Meta-analysis. Inverse variance-weighted meta-analysis of UK Biobank, GS:SFHS and QIMR was performed, restricted to variants present in all three samples, using the METAL package (http://www.sph.umich.edu/csg/abecasis/Metal). Data were available across all 3 studies for 7 207 648 of the original 8 268 322 variants from the UK Biobank analysis. The total sample size included in the meta-analysis was $N = 106 716$ (UK Biobank $N = 91 370$; GS:SFHS $N = 6659$; and QIMR $N = 8687$).

RESULTS
Neuroticism phenotype within UK Biobank and sociodemographic characteristics
Sociodemographic details of the 91 370 UK Biobank participants used in this analysis, as well as the full UK Biobank cohort, are provided in Table 1 and the distributions of neuroticism scores for males and females in our sample are provided in Figure 1. The proportion of the UK Biobank neuroticism GWAS sample holding a degree was 31.4%, and the mean age of leaving full-time education for those without a degree was 16.5 years. Those in the full UK Biobank sample who responded to the neuroticism questions tended to be better educated than those who did not (33.4% had an undergraduate degree versus 27.7% in nonresponders). As expected, mean neuroticism scores were lower for men than for women (men mean EPQ-R-S = 3.58, s.d. = 3.19; women mean EPQ-R-S = 4.58, s.d. = 3.26; $P = 0.001$). PC analysis of the 12 EPQ-R-S items showed that all items loaded highly on a single component, and the internal consistency (Cronbach’s $\alpha$) coefficient was 0.84 (Supplementary Table S2). Analysis of the entire UK Biobank sample (N with data = 401 695) gave very similar results (Supplementary Table S2), suggesting the subsample analysed here is representative of the whole UK Biobank cohort.

Table 1. Sociodemographic characteristics in UK Biobank

|                           | Full UK Biobank sample (N = 502 665) | UK Biobank neuroticism GWAS sample (N = 91 370) |
|---------------------------|-------------------------------------|-----------------------------------------------|
| Age in years, mean (s.d.) | 56.5 (8.1)                          | 56.7 (7.9)                                     |
| Age range (years)         | 37–73                               | 40–73                                         |
| Female, N (%)             | 273 472 (54.4)                       | 47 196 (51.7)                                 |
| Neuroticism score, mean (s.d.) | 4.12 (3.3)                        | 4.10 (3.3)                                    |
| Undergraduate degree, N (%) | 162 026 (32.2)                  | 28 727 (31.4)                                 |
| Age when left full-time education (for those without an undergraduate degree), mean (s.d.) | 16.4 (3.5)                  | 16.5 (2.8)                                    |

Abbreviation: GWAS, genome-wide association study.
Meta-analysis of UK Biobank, GS:SFHS and QIMR samples

In the combined data set, we obtained genome-wide significance for 9 independent loci: on chromosome 1 (two loci), chromosome 3, chromosome 4, chromosome 8, chromosome 9 (two loci), chromosome 17 and chromosome 18 (Figure 2 and Tables 2a and b).

Full details are provided in Tables 2a and b, and the associated regions are depicted graphically as region plots in Supplementary Figures S3a–i. Candidate genes of particular note mapping to the associated loci include: the glutamatergic kainate receptor GRIK3 (Supplementary Figure S3a); CELF4, which regulates excitatory neurotransmission (Supplementary Figure S3i); and CRHR1, encoding corticotropin-releasing hormone receptor 1 (Supplementary Figure S3h), a protein that is central to the stress response. Associated loci are considered in greater detail within the discussion.

Genome-wide association results in UK Biobank

Genome-wide association results from the UK Biobank cohort are summarised in Supplementary Materials: Supplementary Figure S1 (QQ plot); Supplementary Figure S2 (Manhattan plot); and Supplementary Table S3 (genome-wide significant loci associated with neuroticism).

Overall, the GWAS data showed modest deviation in the test statistics compared with the null ($\lambda_{GC} = 1.152$); this was negligible in the context of sample size ($\lambda_{GC1000} = 1.003$) (Supplementary Figure S1). LDSR suggested that deviation from the null was due to a polygenic architecture in which $h^2_{SNP}$ accounted for ~14% of the population variance in neuroticism (liability scale $h^2_{SNP} = 0.136$ (s.e. 0.0153)), rather than inflation due to unconstrained population structure (LD regression intercept = 0.982 (s.e. 0.014)). Estimates of heritability using GCTA were similar to those using LD score regression ($R^2 = 0.156$, s.e. = 0.0074).

We observed a total of 8 independent loci exhibiting genome-wide significant associations with neuroticism (Supplementary Figure S2 and Supplementary Table S3) with the strongest evidence for association coming from a locus on chromosome 8 ($P = 1.02 \times 10^{-15}$) at which there is an extensive LD block spanning 4 Mb (attributable to an inversion polymorphism that has suppressed recombination) containing at least 36 genes. Similar findings to those from the UK Biobank data set in a GWAS primarily assessing the genetics of well-being have also recently been posted in a non-peer-reviewed format.

Stratification by sex in UK Biobank

Neuroticism scores are in general higher in women than in men and it has been postulated that neuroticism may play a stronger aetiologic role in MDD in women than in men, potentially explaining the greater prevalence of depressive and anxiety disorders in women. This suggests the possibility of sex-related genetic heterogeneity. We therefore conducted secondary analyses looking for sex-specific neuroticism loci in women ($N = 47,196$) and men ($N = 44,174$) respectively. To minimise heterogeneity, this analysis was restricted to the UK Biobank samples. SNP heritability (measured by LDSR) for each sex was comparable (female $h^2_{SNP} = 0.149$ (s.e. = 0.0169); male $h^2_{SNP} = 0.135$ (s.e. = 0.0237)), and was highly correlated between the sexes (genetic correlation = 0.911 (s.e. = 0.07); $P = 0.21$). In both sexes separately, the chromosome 8 locus was associated at genome-wide significance but no other single locus attained significance.

Overall, we found no evidence for genetic differences in the common allelic architecture of neuroticism by sex.
Genetic correlation of neuroticism with MDD, schizophrenia and BD

LDSR showed strong genetic correlation between neuroticism and MDD (genetic correlation = 0.64, s.e. = 0.071, P = 3.31 × 10−19) and a smaller, but significant, correlation between neuroticism and schizophrenia (genetic correlation = 0.22, s.e. = 0.05, P = 1.96 × 10−05) (Table 3). We found no significant overlap between neuroticism and BD (genetic correlation = 0.07, s.e. = 0.05, P = 0.15). Similar results based solely on the UK Biobank data set have been reported recently in a non-peer-reviewed format.49

Genetic correlations of neuroticism between UK Biobank, GS:SFHS and QIMR samples

The LDSR-calculated genetic correlation for neuroticism between the three samples was strong: between UK Biobank and GS:SFHS the genetic correlation was 0.91 (s.e. = 0.15, P = 4.04 × 10−09); between UK Biobank and QIMR the genetic correlation was 0.74 (s.e. = 0.14, P = 2.49 × 10−07); and between GS: SFHS and QIMR the genetic correlation was 1.16 (s.e. = 0.35, P = 0.0009). Note that the true maximum for a genetic correlation is bounded by 1. That the LD score estimate is greater than this reflects the imprecision in the estimate as indicated by the large s.e., in the context of which we interpret this as evidence for high but imprecisely estimated genetic correlation between the two samples.

PRS analysis for neuroticism in GS:SFHS and QIMR samples

Table 4 shows the results of PRS analysis (based on the UK Biobank-only GWAS) within the GS:SFHS and QIMR samples. At all thresholds tested, PRS-N predicted neuroticism, although the amount of variance explained was small (at ~1%).

Comparison with findings from GPC meta-analysis

In contrast to the finding of the GPC meta-analysis, we did not identify a genome-wide significant association close to MAGI1 within 3p14.121 Neither of these index SNPs was associated with neuroticism within the UK Biobank sample (for rs35855737) did show a trend for association (β = 0.035, s.e. = 0.02, P = 0.07; two tailed).

Comparison with findings from the CONVERGE consortium study of MDD

The recently published CONVERGE consortium study of Chinese women with recurrent and melancholic MDD identified two loci contributing to risk of MDD on chromosome 10: one near the SIRT1 gene (rs12415800; P = 2.53 × 10−10) and the other in an intron of the LHPP gene (rs35936514; P = 6.45 × 10−13).19 Neither of these index SNPs was associated with neuroticism within the UK Biobank sample (for rs12415800 β = −0.107, s.e. = 0.066, P = 0.1036, freq A = 0.013; and for rs35936514 β = 0.021, s.e. = 0.0378, P = 0.5832, freq T = 0.041).
DISCUSSION

The identification of nine independent loci showing genome-wide significant associations with neuroticism within our combined meta-analysis represents a significant advance. In contrast, a recent meta-analysis of neuroticism conducted by the GPC \((n = 73,447)\) identified only a single genome-wide significant locus.18 There are several possible explanations for this difference. All three of the cohorts in our study used the same 12-item neuroticism assessment instrument (the EPQ-R), whereas the GPC study used multiple different neuroticism assessment instruments (the EPQ-R-S), whereas the GPC study used multiple different neuroticism assessment instruments (the EPQ-R-S). Furthermore, the UK Biobank cohort is by far the largest sample ever studied for neuroticism genetics and all of the participants were of white British ethnicity, minimising population stratification and also addressing potential problems with cultural variation in the interpretation of neuroticism questionnaire items. In addition, quality control steps in the UK Biobank sample were performed in a single centre in a consistent way.

The most significant associated locus on chromosome 8, which was independently associated at genome-wide significance for both men and women, spans a 4-Mb region of extended LD (the result of an inversion polymorphism) containing at least 36 genes (Tables 2a and b, and Supplementary Figure S3e). The extended LD at this locus means that identifying the specific genes responsible for the association is likely to prove challenging. As an initial attempt to resolve the signal, we queried the index SNP (rs12682352) at the BRAINeAC (http://www.braineac.org/) brain expression quantitative trait locus resource. This identified ERI1 as the only protein coding gene within the locus whose expression was associated with the index SNP in brain, but only nominally so \((P = 0.019)\) and not at a level that would reliably point to this gene as likely explaining the association.

The locus on chromosome 17 (rs111433752 at 43.8 Mb; Supplementary Figure S3h) similarly maps to an inversion polymorphism spanning multiple genes and therefore we cannot attribute the association to any particular gene. As with the locus on chromosome 8, inspection of expression quantitative trait loci in the region in BRAINeAC did not help to resolve the signal. Nevertheless, this locus contains a notable candidate gene, CRHR1, encoding corticotropin-releasing hormone receptor 1. In the presence of corticotropin-releasing hormone, CRHR1 triggers the downstream release of the stress response-regulating hormone cortisol. CRHR1 is therefore a key link in the hypothalamic–pituitary–adrenal pathway that mediates the body’s response to stress and that is abnormal in severe depression.53 CRHR1 per se has also been shown to be involved in anxiety-related behaviours in mice and has also been genetically associated with panic disorder in humans.50

Another potential candidate gene within the extended region of genome-wide significant association at the chromosome 17 locus is MAPT, which encodes the microtubule-associated protein Tau. There is evidence that Tau is present in the postsynaptic compartment of many neurons,51 and MAPT knockout in mice leads to defects in hippocampal long-term depression,52 as well as mild network-level alterations in brain function.53 The strongest candidate gene at one of the other loci, CELF4 on chromosome 18 at \(\sim 35\) Mb, encodes an RNA-binding protein known to participate in a major switch in neuron function.54 CELF4 is expressed predominantly in glutamatergic neurons, and recent studies suggest it has a central role in regulating excitatory neurotransmission by modulating the stability and/or translation of a range of target mRNAs.55

The finding of an association with a locus on chromosome 1 \((rs490647)\), which includes the glutamatergic kainate receptor GRIK3, is of considerable interest given that abnormalities of the glutamate system are implicated in the pathophysiology of MDD.55–60 Furthermore, a recent glutamate receptor gene expression study in a large cohort of post-mortem subjects, including some individuals with MDD who had completed suicide, found GRIK3 to be the strongest predictor of suicide.43

On chromosome 4, rs62353264 lies a short distance upstream of KLHL2 that encodes a BTB-Kelch-like protein. KLHL2 is an actin-binding protein and has also been reported to be part of a complex that ubiquitinates NPTX1, the neuronal pentraxin receptor, among other targets. Expression of KLHL2 has been reported to be enriched in brain, and it is localised to cytoplasm and processes of neurons and astrocytes, being found at sites of ruffles and other actin network-containing membrane outgrowths.62,63 The associated region at this locus is short \((\sim 150\) kb), and although several other genes lie within 500 kb of the peak association at this locus, none is as promising a candidate as KLHL2.
The associated region in chromosome 9p23 at ~11.2–11.7 Mb contains no protein-coding genes; the nearest gene on the telomeric side, with its 5'-end located ~650 kb from the associated region, is PTPRD. This gene encodes a receptor-type protein tyrosine phosphatase known to be expressed in brain and with an organising role at a variety of synapses, including those that play a role in synaptic plasticity. PTPRD is also known to harbour variation associated with restless legs syndrome. This is a credible candidate but particular caution is required given the distance between the associated locus and this gene.

In addition to identifying genome-wide significant loci, our study contributes further to understanding the genetic architecture of neuroticism and its relationship to other disorders. Our SNP-based heritability estimate for neuroticism was \( \hat{h}^2 = 0.15 \), as estimated using GCTA, and only slightly lower using LDSR. This is consistent with the estimates reported by the GPC in the two homogeneous subsets of the data they tested, and considerably greater than some earlier reports of ~6%. Despite differences in the distribution of neuroticism by sex, SNP-based heritability was similar for both men and women and the genetic correlation between sexes was not significantly different from 1, suggesting a similar common variant architecture for both, and that differences in trait scores between the sexes are likely to result from structural variants, rare alleles and/or environmental exposures.

PRS analysis of neuroticism within the GSS:SFHS and QIMR samples supported the expected highly polygenic architecture of neuroticism; despite the large discovery UK Biobank sample—but consistent with the modest number of GWS findings identified in this large sample—extremely weakly associated alleles at relaxed association thresholds (for example, \( P_r \) up to at least 0.5) contributed to the variance captured by the signal.

Consistent with current practice, we regard the meta-analysis results as the primary outputs of this study. However, it is notable that although the results of the polygenic risk score analyses show that an "en masse" alleles that associate with neuroticism in UK Biobank tend to do the same in those with higher neuroticism within GSS:SFHS and QIMR, this is not evident for the loci attaining genome-wide significance. It should be noted that most of the associated alleles identified from the UK Biobank GWAS were not independently replicated within the GSS:SFHS and QIMR cohorts, nor within the large Genetics of Personality Consortium meta-analysis. Of the eight loci that were genome-wide significant in the UK Biobank data set, only five were significant within the meta-analysis. With the exception of the loci on chromosome 17, none of these were replicated across the GSS:SFHS and QIMR samples, and the most significantly associated locus, that on chromosome 8, is not significant in either sample (Supplementary Table S4). The large standard errors for the estimates of effect sizes in GSS:SFHS and QIMR are consistent with low power of these population samples to detect loci (with the effect sizes seen in complex traits), and with the fact that fully independent replication (or refutation) will require much larger samples.

By comparing the overall association analysis results in our study with those from the Psychiatric Genomics Consortia, we identified a strong genetic correlation between neuroticism and MDD (0.64), and a weaker but still significant genetic correlation with schizophrenia (0.22), although not with BD. These findings are line with evidence suggesting that neuroticism and MDD—as well as, to a lesser extent, neuroticism and schizophrenia—share genetic risk factors in common. However, the present findings do not distinguish between a direct causal link between neuroticism and those other disorders and pleiotropy, whereby a proportion of risk alleles that influence neuroticism also exert an effect on the clinical diagnoses. Nevertheless, our findings suggest neuroticism as a potentially fruitful measure for efforts to drive future work on the neurobiology of a personality trait that has fundamental importance to human health and well-being.

CONCLUSION
Overall, our findings confirm a polygenic basis for neuroticism and substantial shared genetic architecture between neuroticism and MDD, and to a lesser extent with schizophrenia, though not with BD disorder. The identification of nine new loci associated with neuroticism represents a significant advance in this field and will drive future work on the neurobiology of a personality trait that has fundamental importance to human health and well-being.

CONFLICT OF INTEREST
JPP is a member of the UK Biobank Scientific Advisory Board and UD and DJP were participants in UK Biobank. The other authors declare no conflict of interest.

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