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Symptom-level modelling unravels the shared genetic architecture of anxiety and depression

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Depression and anxiety are highly prevalent and comorbid psychiatric traits that cause considerable burden worldwide. Here we use factor analysis and genomic structural equation modelling to investigate the genetic factor structure underlying 28 items assessing depression, anxiety and neuroticism, a closely related personality trait. Symptoms of depression and anxiety loaded on two distinct, although highly genetically correlated factors, and neuroticism items were partitioned between them. We used this factor structure to conduct genome-wide association analyses on latent factors of depressive symptoms (89 independent variants, 61 genomic loci) and anxiety symptoms (102 variants, 73 loci) in the UK Biobank. Of these associated variants, 72% and 78%, respectively, replicated in an independent cohort of approximately 1.9 million individuals with self-reported diagnosis of depression and anxiety. We use these results to characterize shared and trait-specific genetic associations. Our findings provide insight into the genetic architecture of depression and anxiety and comorbidity between them.

Depression and anxiety are the two most prevalent psychiatric disorders and cause substantial disease burden, accounting for more than 10% of years lived with disability worldwide[1,2]. They are highly comorbid; around three-quarters of people with an anxiety disorder also meet diagnostic criteria for major depressive disorder[3]. Genetic factors have a substantial role in liability to these disorders, with heritability estimates between 30% and 40% for both depression and anxiety[4,5]. Twin and family studies suggest that their comorbidity is largely explained by shared genetic risk factors[6].

Neuroticism, characterized as the tendency to experience emotional negativity such as mood swings, sadness and worry[7,8], is a shared risk factor for depression and anxiety[9–12]. Genetic factors explain around 40% of variation in neuroticism[13], and these factors largely overlap with those that affect depression and anxiety[6,14–17]. Recent molecular genetic studies have uncovered extensive pleiotropy between these three traits[18–20], but little is known about their genetic overlap at a symptom-based level. Here, we investigate the genetic relationships between individual symptoms of anxiety, depression and neuroticism in order to elucidate their genetic overlap and gain insight into the biological mechanisms underlying comorbidity between anxiety and depression.

Genome-wide association studies (GWAS) have accelerated our progress in unravelling the genetic architecture of these psychiatric traits. The general observation is that complex traits are influenced by large numbers of genetic variants with small individual effect sizes (that is, high polygenicity) and consequently very large sample sizes are needed to detect them. Recent GWAS have identified more than 100 independent and robustly associated single nucleotide polymorphisms (SNPs) for depression and neuroticism[20–23]. By comparison, genetic studies of anxiety are still underpowered, with the largest studies to date having identified five genetic risk loci for lifetime anxiety disorder[18] and six loci for anxiety symptoms[24]. Although the full scope of risk conferring genetic loci remains to be discovered for these traits, bivariate genomic methods (for example, linkage disequilibrium score (LD score) regression[25]) have been used to obtain estimates of overall levels of genetic overlap. Consistent with family-based results, large SNP-based genetic correlations (r_g) have been reported across depression, anxiety and neuroticism[20,22] ($r_g > 0.70$). Moreover, pairwise comparison of genomic loci implicated in neuroticism and major depression found that a substantial portion (about 70%) of regions associated with major depression are also associated with neuroticism[26].

The extensive genetic overlap between depression, anxiety, and neuroticism may partly reflect overlap in item content and diagnostic criteria used to measure these traits[27]. Numerous scales of neuroticism include sub-scales or facets of both depression and anxiety (for example, NEO Personality Inventory-Revised (NEO-PI-R) and California Psychological Inventory Big Five), and many items within these scales closely resemble symptom measures of both depression and anxiety. For example, NEO-PI-R neuroticism items 'Sometimes I feel completely worthless' and 'I have sometimes experienced a deep sense of guilt or sinfulness' are very similar to the *Diagnostic and Statistical Manual of Mental Disorders* 5th edition (DSM-5) major depression symptom 'Feelings of worthlessness or excessive

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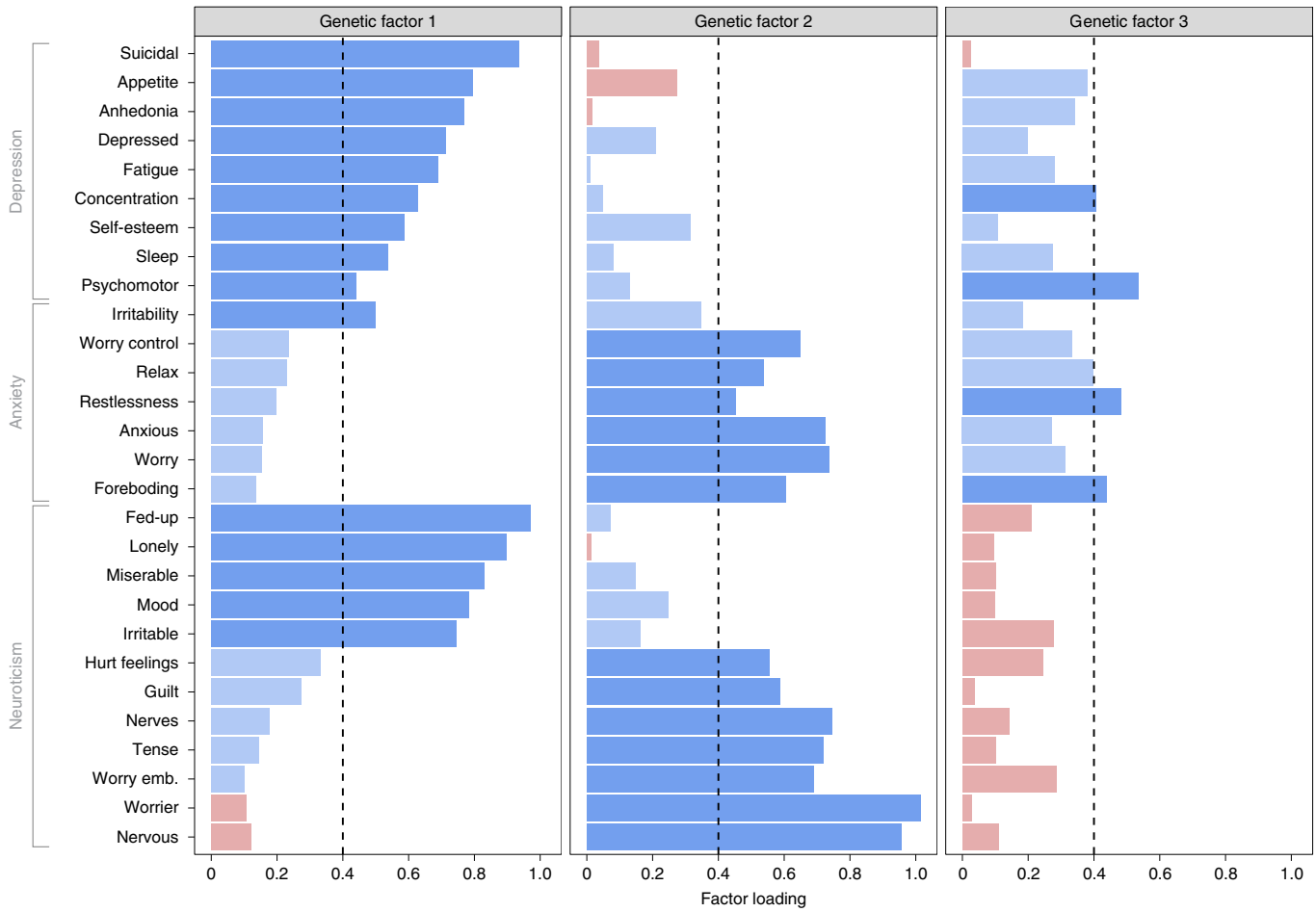

Fig. 1 | Genetic EFA of depression, anxiety and neuroticism. Standardized factor loadings from a genetic EFA of 28 items of depression, anxiety and neuroticism. Positive loadings are indicated in blue and negative loadings in red. Items with a standardized loading less than 0.4 are shown as transparent. Emb., embarrassment.

or inappropriate guilt'. Neuroticism is therefore not operationally distinct, which implies that by studying its underlying components, one could gain valuable insight into symptoms of anxiety and depression and their genetic influences. Indeed, hierarchical clustering of individual neuroticism items in the Eysenck Personality Questionnaire Revised-Short Form (EPQR-S)[28] revealed two genetic item clusters, termed 'depressed affect' and 'worry', displaying stronger genetic overlap with depression and anxiety, respectively[29].

Item- or symptom-level genetic analyses enable investigation of the underlying genetic structure of a trait and have proved useful in disentangling genetic and phenotypic heterogeneity of neuroticism and depression[29,30]. In the present study, we extend this approach across multiple traits and investigate the genetic factor structure underlying 28 symptoms of depression, anxiety and neuroticism. We apply genomic structural equation modelling (genomic SEM)[31], a recently developed multivariate method that enables estimation of the joint genetic architecture of multiple complex traits based on summary statistics from GWAS. This enables genetic subtypes or combinations of genetically similar symptoms to be identified, leading to increased statistical power for the discovery of genetic loci and improved understanding of the comorbidity and genetic overlap across traits. We sought to answer three questions: (1) how do items used to measure neuroticism genetically relate to symptoms of depression and anxiety; (2) can we leverage genetic overlap with neuroticism to boost power for the discovery of genetic risk loci for anxiety and depressive symptoms; and (3) can we identify genetic associations that differentiate anxiety and depressive symptoms? First, we model the genetic factor structure across the three traits using item-level questionnaire data from the UK Biobank (anxiety and depressive symptoms, $n \approx 135{,}000$; neuroticism, $n \approx 400{,}000$). Then, we leverage this factor structure to identify genetic loci for latent factors of depressive symptoms and anxiety symptoms using genomic SEM. Finally, we identify genomic regions that are unique to or shared by depressive and anxiety symptoms to gain insight into the genetic architecture of these traits and the comorbidity between them.

Results

Factor analysis of symptoms of depression, anxiety and neuroticism. We explored genetic overlap between anxiety symptoms, depressive symptoms and neuroticism by modelling the genetic factor structure of items used to measure these traits. Item-level genome-wide association analyses were conducted individually on each of 28 items of neuroticism (12 items; EPQR-S), anxiety (7 items; 7-item Generalized Anxiety Disorder Scale (GAD-7)) and depression (9 items; 9-item Patient Health Questionnaire (PHQ-9)), in approximately 135,000 UK Biobank participants (Supplementary Table 1 shows item-specific sample sizes). LD score regression was used to calculate genetic correlations between all item pairs and an exploratory factor analysis (EFA) was then conducted on this genetic correlation matrix. A minimum average partial test suggested the optimal number of factors to extract is three (consistent with the eigenvalue-greater-than-one rule). Factor loadings of the three-factor model are presented in Fig. 1 and Supplementary Table 2. Depression items had high loadings (>0.4) on genetic factor 1 and

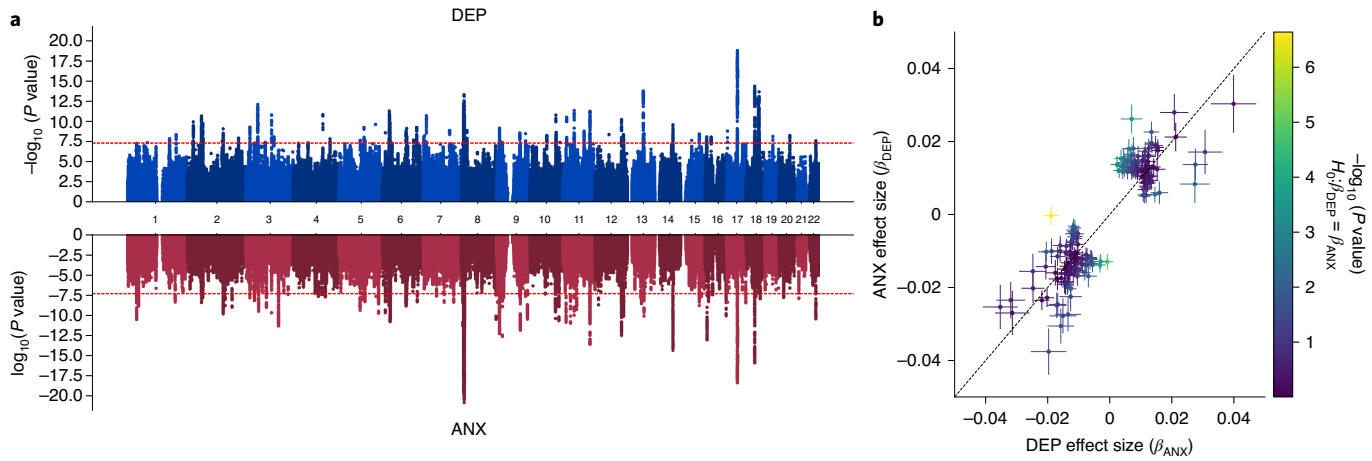

Fig. 2 | SNP-based associations of the DEP and ANX latent factors. a, Manhattan plots for GWAS of the DEP (top; blue) and ANX (bottom; red) latent factors. The red line denotes the *P*-value threshold for genome-wide significance ($P < 5 \times 10^{-8}$). **b**, Comparison of effect sizes for independent SNPs that reached genome-wide significance in the GWAS of either factor. Colour bar indicates the *P* value from the test of the null hypothesis (H_0) that the DEP and ANX SNP effect sizes are equal ($\beta_{DEP} = \beta_{ANX}$).

anxiety items had high loadings on genetic factor 2 (except for the item 'irritability', which loaded onto factor 1). Neuroticism items loaded highly on either factor 1 (5 items) or factor 2 (7 items), rather than forming a separ ate factor. Genetic factor 3 is characterized by relatively low loadings, which are positive for items of depression and anxiety, and negative for items of neuroticism. The items with the highest loadings on factor 3 are mostly somatic symptoms; therefore this factor may contain variance that separates a psychosomatic facet of depression and anxiety from neuroticism. Factor 3 is largely underpowered for further genetic analysis; we therefore restrict subsequent analyses to factor 1 and factor 2. In this paper, we refer to these two genetic latent factors as DEP (for depressive symptoms) and ANX (for anxiety symptoms). For comparison, we also conducted a phenotypic EFA, which revealed a factor structure consistent with the different measures (that is, distinct factors for neuroticism, anxiety symptoms and depressive symptoms; Supplementary Table 3).

We then submitted the genetic factor structure model to genomic SEM (retaining standardized loadings greater than 0.4; Supplementary Fig. 1) to assess its fit to the data (taking into account uncertainty in covariance estimates). The model provided adequate fit to the data (comparative fit index (CFI) = 0.890; standardized root mean squared residual (SRMR) = 0.087; factor loadings in Supplementary Table 4). As a first validation of the DEP and ANX latent factors that included the neuroticism items, we calculated genetic correlations between the latent factors and sum scores of depressive symptoms (PHQ-9; $n = 135{,}149$) and anxiety symptoms (GAD-7; $n = 135{,}747$). The genetic correlations between the PHQ-9 sum score and the DEP latent factor ($r_g = 0.94$, 95% confidence interval [0.87,1.01]), and the GAD-7 sum score and ANX latent factor ($r_g = 0.93$, 95% confidence interval [0.86,1.00]) were not significantly different from 1, suggesting that the DEP and ANX latent factors are good proxies for anxiety and depressive symptoms. The genetic correlation between the DEP and ANX factors was moderately high ($r_g = 0.80$, 95% confidence interval [0.77,0.83]) and was similar to the correlation between the PHQ-9 and GAD-7 sum scores ($r_g = 0.83$, 95% confidence interval [0.72,0.95]).

Multivariate GWAS of anxiety and depressive symptoms. Having identified the genetic latent factor structure within the UK Biobank sample, our next step was to use this structure to identify genomic risk loci for the DEP and ANX latent factors. To maximize power in the multivariate GWAS, we expanded the neuroticism items to the full UK Biobank set (an additional approximately 270,000 individuals who completed the neuroticism questionnaire but not the depressive or anxiety symptoms questionnaires were included; for each neuroticism item, $n \approx 400{,}000$). We examined whether this changed the factor structure by fitting the EFA-derived model to the genetic covariance matrix of the full UK Biobank set. The EFA-derived factor structure retained adequate fit to the data (CFI = 0.893; SRMR = 0.088). Standardized factor loadings were concordant with loadings before expanding the sample size of neuroticism ($r = 0.95$, factor loadings in Supplementary Table 5), indicating that the differential sample size across items did not substantially affect the estimated model. The genetic correlation between the DEP and ANX factors remained the same ($r_g = 0.79$, 95% confidence interval [0.77,0.81]).

Multivariate GWAS were conducted by estimating the effects of individual SNPs on the DEP and ANX latent factors using genomic SEM. The GWAS of the DEP factor identified 7,677 genome-wide significant SNPs ($P < 5 \times 10^{-8}$), tagging 89 independent SNPs in 62 genomic risk loci (Fig. 2a and Supplementary Table 6). For the ANX factor, 11,163 SNPs reached genome-wide significance, tagging 102 independent SNPs in 73 loci (see Fig. 2a and Supplementary Table 7). LD score regression analyses indicate there was minimal uncontrolled inflation (prior to LD score adjustment) for either DEP (intercept = 1.017, standard error (s.e.) = 0.010) or ANX (intercept = 1.021, s.e. = 0.012). Effect sizes of independent significant SNPs showed high concordance between DEP and ANX (Fig. 2b and Supplementary Table 8). Three variants had significantly different effect sizes (after Bonferroni correction; $P < 2.76 \times 10^{-4}$). SNP rs613872 was associated with DEP only (DEP beta = −0.0189, ANX beta = −0.0003, $Z_{diff} = -5.17$, $P = 2.29 \times 10^{-7}$). Two SNPs were associated with ANX only: rs62250713 (DEP beta = −0.0008, ANX beta = −0.0129, $Z_{diff} = 4.30$, $P = 1.70 \times 10^{-5}$) and rs391957 (DEP beta = −0.0032, ANX beta = −0.0138, $Z_{diff} = 3.83$, $P = 1.29 \times 10^{-4}$).

We conducted a replication of the significant independent SNPs in a cohort of research participants from 23andMe with information on self-reported diagnosis of depression (634,037 cases; 1,308,690 controls) and anxiety (624,615 cases; 1,310,854 controls). For the DEP replication, 81 variants were tested (8 SNPs were unavailable or of insufficient quality in the 23andMe cohort). Of these variants, 58 were significant after Bonferroni correction ($\alpha = 0.05/81$; $P < 6.17 \times 10^{-4}$) and had the same direction of effect, and 40 reached

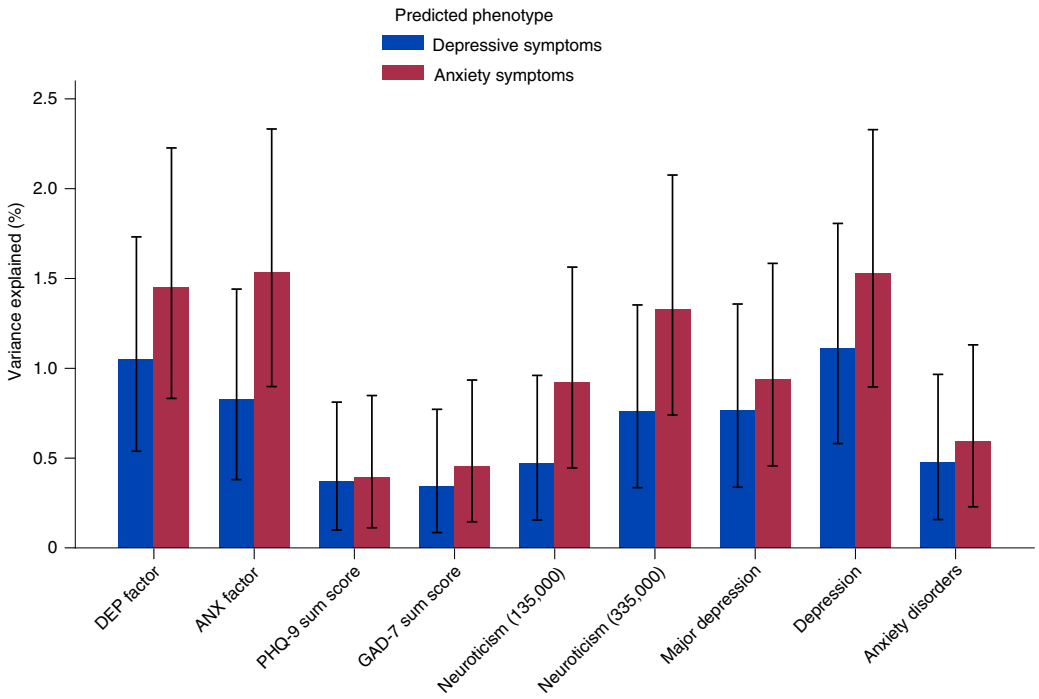

Fig. 3 | Polygenic risk prediction of depressive and anxiety symptoms. Amount of variance in depressive and anxiety symptoms explained by PRS for the DEP and ANX latent factors and a range of related phenotypes: PHQ-9 sum score ($n=135{,}149$), GAD-7 sum score ($n=135{,}747$), neuroticism ($n=136{,}212$ (UKB mental health questionnaire subset); $n=338{,}812$ (full UKB sample)), major depression[20] (QIMR and 23andMe cohorts excluded; $n=159{,}598$), depression[21] (QIMR and 23andMe cohorts excluded; $n=494{,}258$) and anxiety disorders ($n=114{,}019$). All PRS predictions were significant after Bonferroni correction for 18 tests ($\alpha=0.05/18$; $P<2.78\times10^{-3}$). Error bars represent 95% confidence intervals.

genome-wide significance ($P<5\times10^{-8}$). For ANX, 93 variants were tested for replication (9 SNPs were of insufficient quality in the 23andMe cohort), of which 73 were significant after Bonferroni correction ($\alpha=0.05/93$; $P<5.38\times10^{-4}$) and had the same direction of effect, and 39 reached genome-wide significance. For both traits, the number of SNPs reaching Bonferroni-adjusted and genome-wide significance levels in the replication sample was significantly higher than expected by chance (that is, under a null model), and higher than expected under a model assuming the true SNP effect sizes are their estimated effects corrected for the winner's curse (see Supplementary Table 9 and Supplementary Fig. 2).

MAGMA was used to conduct gene-based association tests and gene-set enrichment analyses. We identified 255 genes and 9 gene sets associated with DEP, and 325 genes and 21 gene sets associated with ANX (significant after Bonferroni correction; Supplementary Tables 10–13). There was substantial overlap with respect to enriched functional categories between the two traits (110 genes and 5 gene sets).

Polygenic risk prediction. To further validate the latent factors, we used polygenic risk scores (PRS) derived from the ANX and DEP summary statistics to predict both depressive and anxiety symptoms in an independent sample ($n=4{,}434$). PRS for DEP significantly predicted depressive symptoms ($P=2.69\times10^{-10}$), explaining 1.05% of variance (see Fig. 3). Similarly, PRS for ANX significantly predicted anxiety symptoms ($P=4.80\times10^{-14}$), explaining 1.53% of variance. For comparison, we also calculated PRS from PHQ-9 sum score, GAD-7 sum score, neuroticism, major depression[20], depression[21] and anxiety disorders[18]. The DEP and ANX latent factors explained a greater amount of variance than the sum scores (proportional increases in explained variance were 185% and 237%, respectively), indicating that polygenic prediction was improved by the combination of leveraging information from the neuroticism items and taking a more psychometrically informed approach (that is, factor analysis) to phenotype construction. Overall, specificity in polygenic prediction was low; PRS for depression phenotypes explained an equal or greater amount of variance in anxiety symptoms than depressive symptoms (Fig. 3 and Supplementary Table 14).

Genetic correlations with other complex traits. We estimated genetic correlations between the ANX and DEP latent factors and a range of human complex traits (see Fig. 4 and Supplementary Table 15). While patterns of correlations were similar in magnitude and direction across most of the traits, some traits showed differential genetic overlap with DEP and ANX. Smoking-related phenotypes (initiation, age of initiation, cigarettes per day and cessation) genetically correlated with DEP ($|r_g|>0.27$), but not with ANX. Genetic overlap with socio-economic traits (Townsend deprivation index, household income and educational attainment) was consistently larger for DEP than ANX. Conversely, ANX showed stronger overlap with obsessive compulsive disorder, anorexia nervosa and schizophrenia. The overall pattern of correlations with external traits was highly similar to the PHQ-9 and GAD-7 sum scores (Supplementary Fig. 3).

Shared and trait-specific genetic associations. We sought to identify trait-specific genomic regions by conducting a pairwise analysis of the DEP and ANX GWAS summary statistics in order to characterize regions as pleiotropic or as uniquely associated with either DEP or ANX. We used gwas-pw[32] to estimate the posterior probability that a given genomic region is associated with (1) DEP only, (2) ANX only, (3) both DEP and ANX, and (4) both DEP and ANX but via separate causal variants. Out of the 1,703 tested regions, 123 (7%) had a posterior probability greater than 0.5 of containing a causal variant for at least one of the two traits. Of these regions, 10 were uniquely associated with DEP, 20 were uniquely associated

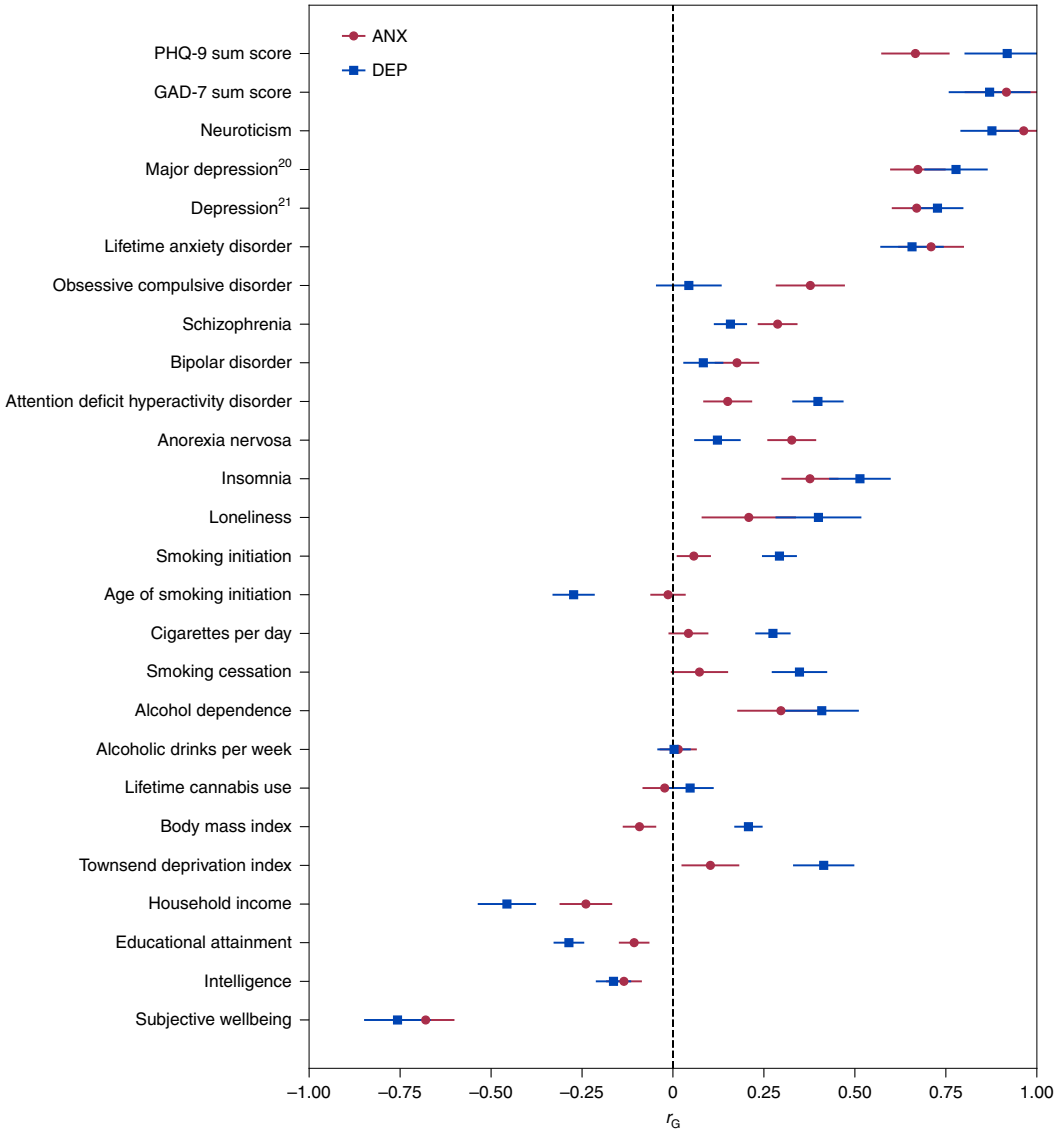

Fig. 4 | Genetic correlations with other complex traits. Genetic correlations between the DEP and ANX latent factors and 26 psychiatric, substance use and socio-economic complex traits. Error bars represent 95% confidence intervals.

with ANX, 71 were associated with both DEP and ANX, and 22 were associated with both traits but via separate variants (Fig. 5a and Supplementary Table 16).

Next, we conducted gene-based association tests separately for regions that were specific to DEP, specific to ANX, or shared. We identified 26 genes significantly associated with DEP-specific regions, 47 genes associated with ANX-specific regions, and 144 associated with shared regions (Supplementary Tables 17–19). To further identify genes for trait-specific and shared regions we also mapped SNPs (that reached genome-wide significance in the GWAS) to genes on the basis of proximity, expression quantitative trait loci (eQTL) and chromatin interactions. These three strategies mapped 49 genes to DEP-specific regions, 74 genes to ANX-specific regions and 470 genes to shared regions (Supplementary Tables 20–22). The total number of genes identified (across all methods) was 63, 102 and 509 genes for DEP-specific, ANX-specific and shared regions, respectively (Fig. 5b).

Using all genes linked to trait-specific and shared regions (excluding the major histocompatibility complex (MHC) region), we conducted gene-set enrichment analysis against gene sets defined by traits in the National Human Genome Research Institute (NHGRI)–European Bioinformatics Institute (EBI) GWAS catalogue[33]. Genes prioritized for DEP-specific regions were significantly enriched in a gene set for hypertriglyceridaemia ($P=1.99\times10^{-7}$). Genes mapped to ANX-specific regions showed enrichment in multiple gene sets (Supplementary Table 23), including schizophrenia ($P=1.22\times10^{-11}$), autism spectrum disorder or schizophrenia ($P=1.18\times10^{-25}$), response to cognitive behavioural therapy in major depressive disorder ($P=1.39\times10^{-13}$), and multiple gene sets related to blood pressure: mean arterial pressure ($P=4.60\times10^{-7}$), systolic blood pressure ($P=2.95\times10^{-7}$), pulse pressure ($P=2.67\times10^{-7}$) and hypertension ($P=1.10\times10^{-5}$). Genes prioritized for shared regions were significantly enriched in 46 gene sets, including autism spectrum disorder or schizophrenia ($P=3.56\times10^{-59}$), blood protein levels ($P=7.05\times10^{-11}$), sarcoidosis ($P=1.09\times10^{-13}$), systolic blood pressure ($P=7.97\times10^{-4}$) and lung cancer ($P=5.99\times10^{-8}$).

Discussion

Recent studies have revealed substantial genetic correlations between depression, anxiety and neuroticism[20–22,24]. The extensive

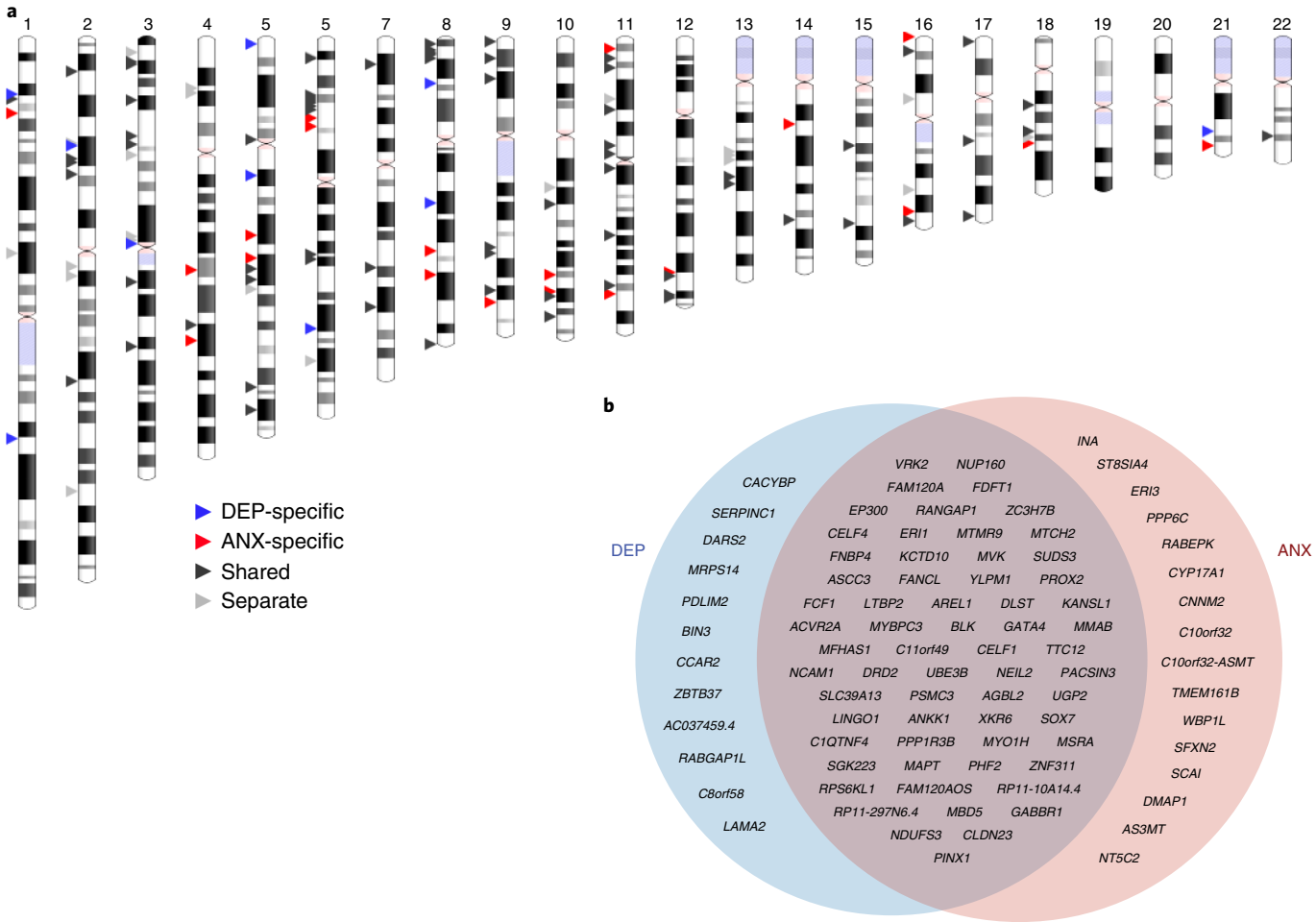

Fig. 5 | Shared and trait-specific genetic associations of depressive and anxiety symptoms. a, Genomic regions that are specific to DEP (blue arrowheads), specific to ANX (red arrowheads), shared (dark grey arrowheads), or associated with both traits via separate variants (light grey arrowheads). **b**, Prioritized genes for shared and trait-specific regions. Genes presented reached significance (after Bonferroni correction) in the gene-based test and were identified by at least two additional methods (position, eQTL or chromatin interaction; see Supplementary Tables 17–22 for all mapped genes). *C10orf32* is also known as *BORCS7*.

genetic overlap partly reflects the overlap in items used to measure these traits, which motivated us to explore the genetic factor structure underlying 28 symptoms of depression, anxiety and neuroticism. Leveraging the underlying factor structure, we conducted GWAS on latent factors of depressive symptoms (89 independent genome-wide significant variants) and anxiety symptoms (102 variants) using data from over 400,000 individuals in the UK Biobank. More than 70% of associated variants replicate in an independent cohort of self-reported diagnosis of depression and anxiety. We also characterize shared and trait-specific genetic associations and report on gene-set analyses targeted at understanding shared and trait-specific aetiology.

Modelling the genetic factor structure of symptoms of depression, anxiety and neuroticism revealed two key findings. First, symptoms of anxiety and depression loaded onto different factors, although the genetic correlation between the factors was high. This implies that while symptoms of depression and anxiety are closely related, symptoms are genetically more similar to symptoms within the same disorder than to symptoms between disorders. This observation is consistent with a phenotypic-level network analysis of the PHQ-9 and GAD-7 items within a psychiatric sample[34], which found that symptom connections were higher within each disorder than between disorders. The large degree of genetic overlap between symptoms of anxiety and depression is in agreement with twin-based symptom-level analyses[35], although our results suggest some specificity of common genetic risk factors. Second, neuroticism items loaded highly onto either the DEP or ANX factors rather than forming their own factor, suggesting that at a genetic level neuroticism is not itself a distinct construct, and probably encapsulates (sub-clinical) symptoms of both depression and anxiety. The partitioning of neuroticism items among two distinct factors is in line with the results of a hierarchical clustering analysis of the genetic correlation matrix derived from these neuroticism items when analysed in isolation[29].

While the separation of anxiety and depressive symptoms into distinct factors was consistent across genetic and phenotypic factor analyses, the pattern of factor loadings for neuroticism showed a key difference. Phenotypically, neuroticism items loaded onto two separate factors that were distinct from anxiety and depressive symptoms. This difference between the phenotypic and genetic factor structure suggests that neuroticism items more strongly correlate with anxiety and depressive symptoms at a genetic level than a phenotypic level. The discrepancy could potentially reflect a larger impact of correlated measurement error in the phenotypic analysis[36]. Measurement error would be expected to correlate to a larger extent between measures that are more similar (for example, PHQ-9 and GAD-7 items) than measures that are less similar (for example, PHQ-9 and neuroticism items)—the neuroticism questionnaire is

structured and scaled differently to the PHQ-9 and GAD-7 and was administered at a different time and place. This observation highlights the importance of investigating the underlying genetic structure of psychological traits, as phenotypic correlations may not be representative of the aetiological overlap across items[37].

It is important to note that we implemented a particular model (that is, a correlated factors model) that enabled us to explore the genetic overlap between anxiety and depression and to address a set of relevant research questions. Although this model provided adequate fit to the data, it is not necessarily the optimal representation of the genetic structure of the internalizing psychiatric symptoms. For example, a hierarchical model, with a general internalizing factor and sub-factors of anxiety and depressive symptoms, is an alternative representation of the structure of the internalizing items[38,39]. Indeed, the large correlations between genetic factors in our model are in line with this hierarchical model.

By leveraging item-level genetic overlap with neuroticism, we substantially increased statistical power to identify genomic risk loci for depressive and anxiety symptoms. We identify 62 loci for depressive symptoms, of which 33 overlap with a previous GWAS of depressive symptoms or major depression[20–22,40–44] and 29 are novel depression loci. For anxiety symptoms, we identify 73 loci (a substantial increase from previous studies which have found 6 loci for anxiety disorders[18,19] and 6 loci for anxiety symptoms[24]). Two of the 73 loci overlap with a previous GWAS of anxiety disorders[18], and 71 loci are novel for anxiety. Unexpectedly, many of the DEP (49 out of 62) and ANX (63 out of 73) loci have previously been associated with neuroticism or sub-clusters of neuroticism[22,23,29,45].

The large overlap with previously known neuroticism loci, raises the question of whether the genetic latent factors are merely sub-facets of neuroticism rather than representing genetic factors of anxiety and depressive symptoms. The findings of multiple analyses support the validity of the latent factors representing depression and anxiety. First, genetic correlations between the latent factors and the PHQ-9 and GAD-7 sum scores (that is, between the DEP factor and the sum score of the nine depressive symptoms, and between the ANX factor and the sum score of the seven anxiety symptoms) were very high and not significantly different from one. These correlations persisted when incorporating approximately 270,000 additional individuals with neuroticism information only into the latent factors. Second, a substantial proportion (around 72% for DEP and around 78% for ANX) of genome-wide significant variants replicated in a large, independent cohort of self-reported diagnosis of anxiety and depression. Third, PRS analyses showed that the latent factors significantly predicted depressive and anxiety symptoms in a second independent sample.

The current findings are notable, as we provide evidence that genomic loci linked to neuroticism also have a role in individual differences in depression and anxiety. In other words, GWAS of neuroticism are tapping into the same construct as sub-clinical anxiety and depressive symptoms. The identification of a large number of replicable genomic loci for anxiety and depressive symptoms unlocks the possibility of leveraging statistical genetic approaches (for example, Mendelian randomization or drug repositioning) that were not possible with previous anxiety GWAS. We implemented one such approach, a regional pairwise analysis, in order to disentangle the shared genetic architecture of anxiety and depression and identify trait-specific genetic loci.

Our results indicate that depression-specific genomic regions are linked to hypertriglyceridemia. This is consistent with several studies that have found an association between depression and triglyceride levels[46–49], and previous GWAS of depression have reported significant genetic correlations with triglycerides[20,21] ($r_g = 0.14$). This suggests that depression may contain a larger metabolic component than anxiety. Conversely, genes mapped to anxiety-specific regions were enriched in gene sets related to multiple blood pressure phenotypes. While perhaps unsurprising given increased blood pressure is a direct physiological effect of the stress response, anxiety has also been linked to increased risk of hypertension[50]. Interestingly, genes unique to ANX were also enriched in a gene set linked to response to cognitive behavioural therapy in major depression[51], suggesting that the presence of comorbid anxiety symptoms may influence treatment response for depression. Indeed, comorbid anxiety and major depressive disorders are associated with higher symptom severity and impairment, disorder persistence and reduced response rates[52–56]. There was also significant enrichment of ANX-specific regions in schizophrenia gene sets, consistent with a larger genetic correlation of schizophrenia with the ANX factor than with the DEP factor. Anxiety symptoms are highly prevalent in patients with schizophrenia[57] and are associated with the positive symptom domain of schizophrenia[58].

Genetic correlations with other complex traits were largely concordant in direction and magnitude across DEP and ANX. Smoking-related phenotypes, however, were genetically correlated with depression but not with anxiety. Observational studies on the association of smoking with anxiety and depression are largely mixed with regards to the direction of effect[59]. We find moderate genetic correlations between DEP and smoking initiation, cigarettes per day, cessation (positive) and age of initiation (negative). Genetic correlations were not significant between any smoking phenotype and ANX, suggesting a stronger relationship between smoking behaviour and depression.

It is well established that depression and anxiety share a substantial amount of genetic liability. Our results provide additional evidence for this notion, with a high genetic correlation between the DEP and ANX factors ($r_g = 0.80$), consistent with the genetic overlap between major depression and anxiety disorders in previous studies ($r_g = 0.75$–0.80). Further, the amount of polygenic overlap (that is, the fraction of genetic variants associated with both traits) was considerable, with 71 out of 123 genomic regions containing a causal variant shared between traits. We note that this is likely an underestimate of the proportion of shared genetic effects, as the correction for sample overlap in pairwise GWAS is conservative and some truly shared genetic effects may be corrected out[32]. PRS derived from the ANX and DEP latent factors significantly predicted anxiety (1.53% variance) and depressive symptoms (1.05% of variance), representing an increase in polygenic prediction of an anxiety phenotype (previous studies explained up to 0.5% of variance[18,24]), but less predictive compared to previous studies of depression (up to 3.2% of variance[21]). Given the high genetic overlap and substantial comorbidity between depression and anxiety[3,53], it is unsurprising that there was very little differentiation in polygenic prediction of depressive and anxiety symptoms. This was not specific to the latent factors; poor specificity was also seen with PRS derived from clinical depression and anxiety phenotypes[18,20].

The findings of the present study should be interpreted in light of some key limitations. The DEP and ANX latent factors represent depressive and anxiety symptoms within a population-based cohort. Although these factors had relatively high genetic correlations with clinical phenotypes, they probably do not capture the entire spectrum of genetic effects on major depression and anxiety disorders[60–62]. For example, the episodic nature of major depression or the persistence of generalized anxiety are not well captured by symptom questionnaires. Second, by leveraging neuroticism items in the extended UK Biobank sample, we increased statistical power to identify variants for symptoms that overlap with depression and anxiety (predominately 'psychological' symptoms). However, the somatic, motor, and neurovegetative symptoms characteristic of major depression and anxiety disorders are not well represented in neuroticism, and consequently not within our phenotypes. Third, while we excluded genes within the MHC region from the shared and trait-specific gene-set enrichment analyses, we cannot rule out

that other gene-dense regions may be driving some of the associations. These enrichment findings should therefore be viewed as preliminary.

More than a decade of molecular genetic studies have confirmed the presence of widespread pleiotropy across psychiatric disorders[63–65]. The substantial sharing of genetic risk factors challenges the utility of analysing discrete diagnostic categories of psychopathology defined by current classification systems (such as DSM-5) in the discovery of genetic risk loci and prediction of disease. As a result, there has been recent interest in alternative psychiatric phenotyping approaches[66]. Symptom-level analyses are one such approach that may prove useful in advancing our understanding of the genetic aetiology of psychopathology, by allowing the discovery of symptom-specific genetic associations and the identification of genetic subtypes and trans-diagnostic factors of genetic liability. We show that analysing genetically homogeneous combinations of symptoms across traits can increase statistical power to identify loci and elucidate comorbidity and genetic overlap between psychiatric phenotypes.

Methods

Ethical regulations. The UK Biobank study was approved by the NHS National Research Ethics Service (reference 11/NW/0382) and all participants provided written informed consent to participate. The Queensland Institute of Medical Research (QIMR) Adult Twin Study was approved by the QIMR Human Research Ethics Committee and all participants provided informed consent. All individuals from the 23andMe research cohort included in the replication analyses provided informed consent and participated in the research online, under a protocol approved by the external Association for the Accreditation of Human Research Protection Programs-accredited Institutional Review Board, Ethical and Independent Review Services (http://www.eandireview.com).

UK Biobank. Data for the main analyses came from the UK Biobank, a major health data resource containing phenotypic information on a wide range of health-related measures and characteristics in more than 500,000 participants from the United Kingdom general population[67]. Participants were excluded from the present study on the basis of ancestry, relatedness and withdrawn consent. Participants were included if they were of white British ancestry, identified through self-reported ethnicity and ancestral principal components. Participants who self-reported as not white British, but for whom the first two genetic principal components indicated them to be genetically similar to those of white British ancestry were also included in order to maximize sample size[68].

Depressive and anxiety symptoms. Depressive symptoms were assessed with the PHQ-9[69], and anxiety symptoms with GAD-7[70]. More than 150,000 participants completed the PHQ-9 and GAD-7 as part of a UK Biobank mental health follow-up questionnaire[71] administered online in 2016. Each item assesses the frequency of a particular symptom over the past two weeks, rated on a four-point ordinal scale: (0) not at all, (1) several days, (2) more than half the days, or (3) nearly every day. The ordinal scale of measurement of these items complicates interpretation of SNP-based heritability h^2_{SNP} estimates. We have previously shown[30] that analysing the PHQ-9 items on a continuous scale (that is, assuming the ordinal-scale items are continuous) systematically reduced estimates of h^2_{SNP}. As h^2_{SNP} estimates are used in genomic SEM, we transformed each item into a binary phenotype in order to produce interpretable and unbiased h^2_{SNP} estimates (genetic correlations between the ordinal-scale items and binary items were all >0.95; median $r_g = 0.98$). Items were dichotomized such that an item was considered to be endorsed if the item score was ≥1 (several days, more than half the days, or nearly every day), and not endorsed if the score was 0 (not at all). A cut-off score of 1 was used to represent presence vs. absence of the symptom, and to maximize the number of participants who endorsed an item and hence statistical power.

Neuroticism. Neuroticism was measured using the 12-item EPQR-S[28], with each item assessed on a dichotomous scale (yes or no). The questionnaire was administered to the entire UK Biobank cohort (~500,000 participants).

Genome-wide association analyses. GWAS analyses of the 28 individual items (9 depression items, 7 anxiety items, 12 neuroticism items) were conducted via logistic regression in PLINK v2.00a[72]. If two individuals in the sample were related (pi-hat >0.2) one individual was removed (preferentially from the control set if the related individuals were in both case and control sets). GWAS analyses of the PHQ-9, GAD-7, and EPQR-S sum scores were conducted via linear regression. Analyses were limited to autosomal SNPs with high imputation quality score (INFO score ≥0.80) and a minor allele frequency of 1% or higher, resulting in 9,417,325 SNPs being tested for association. Age, sex, genotyping array and 20 principal components were included as covariates.

Factor analyses. We first explored the factor structure across the 28 items of neuroticism, anxiety and depression by conducting phenotypic and genetic EFA. The phenotypic EFA was conducted on the tetrachoric correlation matrix between all items ($n = 125{,}650$). The genetic EFA was based on the genetic correlation matrix, using only participants who completed the UK Biobank mental health questionnaire (n range 132,602–137,461; item-specific sample sizes in Supplementary Table 1). As participants who completed the UK Biobank mental health questionnaire differ significantly from the entire UK Biobank cohort (for example, higher educational attainment, higher socio-economic status and lower rates of smoking)[71], we restricted the genetic EFA to a subset for neuroticism to ensure these systematic differences did not bias the EFA. Cross-trait LD score regression was used to estimate genetic correlations between each of the 28 items. These estimates are not biased by sample overlap[25]. The R package psych was used to conduct the EFAs, with an ordinary least squares extraction method and oblimin rotation method. Two procedures were used to decide on the optimal number of factors to extract: a minimum average partial test[73] (the lowest average squared partial correlation indicates the number of factors to extract) and the eigenvalue-greater-than-one rule[74] (factors with an eigenvalue above 1 are extracted).

The genetic factor model identified in the EFA (retaining factor loadings >0.4) was subsequently carried forward in a confirmatory factor analysis in genomic SEM[31]. This was done to assess the fit of the factor model to the data while taking into account uncertainty in covariance estimates, and to allow the estimation of genetic correlations between latent factors and external traits (that is, PHQ-9 and GAD-7 sum scores). The default diagonally weighted least squares estimator was used. SNP-based heritability estimates (diagonal of the genetic covariance matrix) were converted to the liability scale, where the population prevalence of the items was estimated from the UK Biobank sample (population prevalence = sample prevalence; Supplementary Table 1).

Multivariate genome-wide association analyses. GWAS of the latent factors of anxiety and depressive symptoms were conducted in genomic SEM. All summary statistics were standardized with respect to the variance in the phenotype (that is, STDY) using the sumstats function in genomic SEM. The factor structure identified in the genetic EFA was specified as the model. SNPs tested for association in the univariate item-level GWAS and also contained in the 1000 genomes phase 3 reference sample (with minor allele frequency (MAF) >0.01) were included, resulting in the analysis of 7,746,079 SNPs. The conservative option in genomic SEM to correct for genomic inflation by multiplying standard errors by the LD score intercept was used. We applied the conventional genome-wide significance threshold of $P < 5 \times 10^{-8}$. The results were annotated using FUMA[75]. Significant SNPs were clumped into blocks high in linkage disequilibrium (the non-random association of alleles at a specific locus) using a threshold of $r^2 < 0.10$ (correlation between allele frequencies of two SNPs). Genomic risk loci were identified by merging independent SNPs if $r^2 \geq 0.10$ and their linkage disequilibrium blocks were physically close to each other at a distance of 1,000 kb.

23andMe replication cohort. In the 23andMe replication analysis, case–control status was determined by self-reported depression (634,037 cases; 1,308,690 controls) or self-reported anxiety (624,615 cases; 1,310,854 controls) from samples of European ancestry (close relatives removed) in the 23andMe research cohort. The self-reported phenotype of depression was defined as cases if samples have ever been diagnosed with depression, or controls if samples have never been diagnosed with depression; the self-reported phenotype of anxiety was defined as cases if samples have ever been diagnosed with anxiety, or controls if samples have never been diagnosed with anxiety. Association analyses were conducted by 23andMe; a logistic regression assuming an additive model for allelic effects was used with adjustment for age, sex, indicator variables to represent the genotyping platforms and the first five genotype principal components. The summary statistics were provided for independent genome-wide significant SNPs in the depression and anxiety latent factors GWAS. Association results were adjusted for inflation by scaling the association test statistics by the LD score intercept (depression, 1.326 (s.e. = 0.015); anxiety, 1.308 (s.e. = 0.011)). In the replication analysis of self-reported depression, 8 SNPs were unavailable or of insufficient quality in the replication sample—thus 81 variants were tested for replication. In the replication analysis of self-reported anxiety, 9 SNPs were of insufficient quality or unavailable in the replication samples—thus 93 variants were tested for replication.

To benchmark the observed replication record against the expected replication record, we used the approach described in Okbay et al.[76]. First, the degree of replication of lead SNPs was compared with the expected degree of replication by chance (under a null hypothesis that each of the lead SNP effects are null in both discovery and replication samples). A two-tailed binomial test was used to compare the observed and expected number of SNPs that have concordant directions of effect, reached significance at a Bonferroni-corrected level, and a genome-wide level. Second, the degree of replication was compared with the expected degree of replication assuming that the true underlying SNP effect sizes are their estimated

effects corrected for the winner's curse (taking into account the discovery GWAS results, and the discovery and replication sample sizes). The discovery GWAS SNP effect sizes were corrected for winner's curse using false-discovery rate (FDR) inverse quantile transformation[77]. We used the method described in Okbay et al. (supplementary section 1.8.3) to estimate the expected number of SNPs with concordant signs, and the expected number of SNPs meeting significance thresholds.

Polygenic risk prediction. The target sample consisted of an adult cohort ($n = 4{,}434$) from the QIMR Adult Twin Study. Depressive and anxiety symptoms were assessed by the Delusions–Symptoms–States Inventory: Anxiety and Depression Scales[78], which consists of seven anxiety and seven depression items. Each item assesses the degree of distress due to a particular symptom, rated on a four-point ordinal scale: (0) none, (1) a little, (2) a lot, or (3) unbearably. Additional details of the cohort and assessment procedures have been reported elsewhere[15].

In total, nine PRS were created, using SNP weights from: DEP latent factor (UK Biobank), ANX latent factor (UK Biobank), PHQ-9 sum score (UK Biobank; $n = 135{,}149$), GAD-7 sum score (UK Biobank; $n = 135{,}747$), neuroticism (UK Biobank - MHQ subset; N = 136,212), neuroticism (UK Biobank; N = 338,812), major depression (ref. [20], with QIMR and 23andMe cohorts excluded; $n = 159{,}598$), depression (ref. [21], with QIMR and 23andMe cohorts excluded; $n = 494{,}258$), and anxiety disorders (ref. [18]; $n = 114{,}019$). We used SBayesR[79] to account for the correlation in effect sizes arising from linkage disequilibrium. In brief, SBayesR implements Bayesian multiple regression to jointly analyse all SNPs and account for linkage disequilibrium between SNPs. As recommended, a shrunk matrix derived from ~3 million SNPs (MAF > 0.01) on 50,000 participants from the UK Biobank was used as the linkage disequilibrium correlation matrix. All other input parameters were the SBayesR default. PRS were calculated in PLINK v1.90[80].

For each set of PRS we tested for an association with sum scores of both anxiety symptoms and depressive symptoms in the target sample using a linear mixed effects model regression to adjust for sample relatedness. This was performed with the tool genomic restricted maximum likelihood (GCTA-GREML). This approach uses the genetic relatedness matrix as a random effect in order to model relatedness within the target sample. While GREML is typically used to estimate the heritability of a trait, it can be used to account for relatedness while testing for the association of fixed effects (in this case, the PRS). To this end, we include the PRS as a fixed effect along with age, sex, age × sex, sex^2, 10 genetic ancestry principal components and genotype imputation batch. Variance explained was estimated from the PRS fixed effect size using the formula:

$$R^2 = \left(\frac{\beta}{\sigma_{\text{pheno}}}\sigma_{\text{PRS}}\right)^2$$

Where β represents the PRS fixed effect size estimate, σ_{pheno} is the standard deviation of the phenotype (sum score) and σ_{PRS} is the standard deviation of the PRS. This approach has been used previously to deal with relatedness in PRS target samples[81,82], and is similar to the linear mixed modelling approach used in GWAS to adjust for cryptic relatedness[83]. Statistical significance was corrected for multiple testing using a Bonferroni correction procedure ($P < 0.0028$). As sensitivity analyses, we also estimated the amount of variance explained by PRS in log-transformed and inverse normal-transformed sum scores of depressive and anxiety symptoms (presented in Supplementary Table 14).

Gene-based tests and gene-set analysis. MAGMA v1.07[84] was used to conduct gene-based and gene-set analyses on the summary statistics of the DEP and ANX latent factors. The gene-based analysis tested 18,756 protein-coding genes for association. A Bonferroni-corrected significance threshold was applied ($P < 2.67 \times 10^{-6}$). The gene-set analysis tested 7,250 gene sets for association with DEP and ANX factors. A Bonferroni-corrected significance threshold was applied ($P < 6.90 \times 10^{-6}$).

Pairwise analysis of GWAS summary statistics. The pairwise GWAS analysis was implemented using gwas-pw[32]. First, the genome is split into 1,703 approximately independent regions[85]. Then the posterior probability of each of the following models is calculated: (1) the region is associated with DEP only, (2) the region is associated with ANX only, (3) the region is associated with both DEP and ANX, and (4) there are separate associations for ANX and DEP within that region. To account for sample overlap across the two traits, gwas-pw requires the correlation between effect sizes in the two traits in non-associated regions. We used fgwas[86] to calculate the posterior probability of association (PPA) for each region with both traits. We then calculated the correlation in effect sizes for SNPs in regions with a PPA <0.2 for both ANX and DEP. All other input parameters were the gwas-pw default. Given one of the models has a posterior probability >0.5, we report the model with the highest posterior probability. Results are presented in the form of an ideogram, created in the Complex-Traits Genetics Virtual Lab[87].

Gene mapping of trait-specific or shared regions. First, MAGMA was used to conduct gene-based tests separately on genomic regions reported to be associated with only DEP (120 protein-coding genes tested; Bonferroni-corrected significance threshold, $P < 4.17 \times 10^{-4}$), regions associated with only ANX (390 protein-coding genes tested; Bonferroni-corrected significance threshold, $P < 1.57 \times 10^{-4}$), and regions associated with both ANX and DEP (1,038 protein-coding genes tested; Bonferroni-corrected significance threshold, $P < 4.82 \times 10^{-5}$).

Three additional methods implemented in FUMA were used to map SNPs in trait-specific or shared regions (GWAS $P < 5 \times 10^{-8}$) to genes. (1) Positional mapping: SNPs are mapped to genes based on proximity (within a 10-kb window). (2) eQTL mapping: SNPs are mapped to a gene if they have a significant (FDR <0.05) association with the expression level of that gene. We used eQTL information from GTEx v8 (ref. [88]), the CommonMind Consortium[89], and BRAINEAC[90]. (3) Chromatin interaction mapping: genes are mapped if there is a significant (FDR $< 1 \times 10^{-6}$) chromatin interaction between a genomic region (within a genomic risk locus) and promoter regions of genes 250 bp upstream and 500 bp downstream of the transcription start site. Hi-C sequence data was used to identify chromatin interactions from 23 human tissue and cell types[91].

All prioritized genes for trait-specific and shared regions were used to conduct gene-set enrichment analyses (hypergeometric test performed in FUMA) against gene sets defined by traits in the NHGRI-EBI GWAS catalogue[33]. We note that this test does not account for regional gene density, and therefore we excluded genes within the gene-dense MHC region from this analysis. Multiple testing was corrected for with a Benjamini–Hochberg FDR of 0.05.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All GWAS summary statistics generated from UK Biobank data are available from the authors upon request. Individual-level data for UK Biobank participants are available to eligible researchers through the UK Biobank (www.biobank.ac.uk). Access to 23andMe data is available upon request to 23andMe (further information is available from https://research.23andme.com/collaborate/).

Code availability

Code used to conduct analyses presented in this manuscript is available from the authors upon reasonable request.

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Author contributions

J.G.T. and E.M.D. conceived and directed the study. J.G.T. performed most of the statistical and bioinformatics analyses with the UK Biobank data, with support from A.I.C., A.D.G., Z.F.G., J.A., J.-S.O. and E.M.D. W.W., S.S. and the 23andMe Research Team conducted the replication analyses in the 23andMe cohort. A.I.C. conducted the polygenic risk prediction analyses, with support from J.G.T. N.G.M. collected and contributed data from the QIMR Adult Twin Study. Z.F.G., E.M.B., S.M., N.G.M., S.E.M., C.M.M. and E.M.D. provided methodological and psychiatric expertise. J.G.T. and E.M.D. wrote the manuscript, with all authors providing comments and suggestions.

Competing interests

W.W., S.S. and members of the 23andMe Research Team are employees of 23andMe Inc. The other authors declare no competing interests.

Additional information

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Software and code

Policy information about availability of computer code

Data collection	No software was used for data collection
Data analysis	R (3.5.1) PLINK (2.00a) LD Score Regression (1.0.1) Genomic structural equation modelling (1.0.1) FUMA (online platform: https://fuma.ctglab.nl) PLINK (1.90) GCTB (2.0) GCTA (1.92) MAGMA (1.07) gwas-pw (0.21)

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All GWAS summary statistics generated from UK Biobank data are available from the authors upon request. Individual level data for UK Biobank participants are available to eligible researchers through the UK Biobank (www.biobank.ac.uk). Access to 23andMe data is available upon request to 23andMe, Inc. (further information is available from https://research.23andme.com/collaborate/).

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to predetermine sample sizes. We included all participants in the UK Biobank with symptom-level data on anxiety, depression or neuroticism available (and not excluded due to criteria detailed below) in order to maximize sample size.
Data exclusions	Participants were excluded based on ancestry, relatedness (to avoid bias due to population stratification and cryptic relatedness) and withdrawn consent. Participants were excluded if they were not deemed to be of white British ancestry, identified through self-reported ethnicity and ancestral principal components. If two individuals in the sample were related (pi-hat > 0.2) one individual was removed.
Replication	We conducted a replication association analysis of SNPs that reached genome-wide significance in the DEP and ANX GWAS in a large, independent cohort (~1.9 million individuals).
Randomization	Participants were allocated to either case or control groups based on self-reported measures of anxiety, depression and neuroticism.
Blinding	N/A

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Methods

n/a	Involved in the study
☒	☐ ChIP-seq
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Policy information about studies involving human research participants

Population characteristics	The UK Biobank is a population-based cohort (mean age = 56.52, SD = 8.09; 54% female). The QIMR Adult Twin Study is a population-based cohort (mean age = 41.29, SD = 12.8; 61% female)
Recruitment	The UK Biobank recruited over 500,000 people aged 40-69 between 2006 and 2010 at 22 recruitment centres across the UK. For the QIMR Adult Twin Study, a questionnaire was mailed out to all twins over 18 years old enrolled in the Australian Twin Registry

Ethics oversight	The UK Biobank study was approved by the NHS National Research Ethics Service (ref. 11/NW/0382). The QIMR Adult Twin Study was approved by the Queensland Institute of Medical Research Human Research Ethics Committee. 23andMe data collection was conducted under a protocol approved by the external AAHRPP-accredited IRB, Ethical & Independent Review Services (http://www.eandireview.com)

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