Genetic analysis of the *PCSK9* locus in psychological, psychiatric, metabolic and cardiovascular traits in UK Biobank.

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Running headline: PCSK9 in mental and physical health

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Abstract

The association between severe mental illness (SMI) and cardiovascular and metabolic disease (CMD) is poorly understood. *PCSK9* is expressed in systems critical to both SMI and CMD and influences lipid homeostasis and brain function. We systematically investigated relationships between genetic variation within the *PCSK9* locus and risk for both CMD and SMI. UK Biobank recruited ~500,000 volunteers and assessed a wide range of SMI and CMD phenotypes. We used genetic data from white British ancestry individuals of UK Biobank. Genetic association analyses were conducted in PLINK, with statistical significance defined by the number of independent SNPs. Conditional analyses and linkage disequilibrium assessed the independence of SNPs and the presence of multiple signals. Two genetic risk scores of lipid-lowering alleles were calculated and used as proxies for putative lipid-lowering effects of *PCSK9*. *PCSK9* variants were associated with central adiposity, venous thrombosis embolism, systolic blood pressure, mood instability, and neuroticism (all $p < 1.16 \times 10^{-4}$). No secondary signals were identified. Conditional analyses and high linkage disequilibrium ($r^2 = 0.98$) indicated that mood instability and central obesity may share a genetic signal. Genetic risk scores suggested that the lipid-lowering effects of *PCSK9* may be causal for greater mood instability and higher neuroticism. This is the first study to implicate the *PCSK9* locus in mood-disorder symptoms and related traits, as well as the shared pathology of SMI and CMD. *PCSK9* effects on mood may occur via lipid-lowering mechanisms. Further work is needed to understand whether repurposing *PCSK9*-targeting therapies might improve SMI symptoms and prevent CMD.

Keywords: *PCSK9*, neuroticism, blood pressure, obesity, venous thromboembolism

Introduction

It has been long recognised that severe mental illness (SMI) is accompanied by an increased burden of cardiovascular and metabolic diseases (CMD) ¹⁻³. The direct cause of this comorbidity remains ambiguous; however, it is likely influenced by factors such as genetics, socioeconomic status, poor diet, sedentary lifestyle, substance abuse and side effects of psychiatric medication ⁴. Additionally, genetic studies have begun to provide evidence of shared mechanisms underlying mental and physical ill-health ⁵⁻⁸.

For many years CMD research has focused on *PCSK9* and its encoded protein (also *PCSK9*), because of its key role in lipid homeostasis. Specifically, *PCSK9* binds to the low-density lipoprotein (LDL) receptors (LDLR) and targets them for degradation ⁹. Lipid homeostasis is important for energy storage and release and is therefore central to metabolic processes. Inappropriate storage of lipids contributes to insulin resistance and systemic inflammation observed in CMD ^{9,10}. In particular, deposition of lipids in blood vessel walls is a key feature and risk marker of cardiovascular diseases including myocardial infarction and ischemic stroke. High levels of LDL have a causal relationship with atherosclerotic plaques ¹¹ and the importance of *PCSK9* in these processes is highlighted by the development of *PCSK9* inhibitors as a treatment of atherosclerotic disease.

PCSK9's association with SMI remains less established, however an association seems plausible due to its roles in brain functions ^{12,13,14} and the importance of insulin resistance in SMI ¹⁵. Under some circumstances, lipids can cross the blood brain barrier, and as well as influencing lipid regulation, *PCSK9* is expressed in areas with high proliferative ability. It has a positive correlation with increased post-mitotic neurones and decreased neuroepithelial cells. Additionally, it has been shown to promote or prevent apoptosis in many neuronal pathways. This emphasises the influence of *PCSK9* on neurogenesis and neuronal apoptosis. Finally, inhibition of *PCSK9* is associated with reduced neuroinflammation. Furthermore, *PCSK9* has a known association with plaque formation in Alzheimer's disease and is upregulated in alcohol use disorders or ischemic stroke ^{12,16}.

Given that the expression of PCSK9 is observed in the brain as well as the liver (Figure 1), and the known functions of PCSK9, we aimed to systematically investigate whether genetic variation in the *PCSK9* locus contributes to the shared genetic predisposition and underlying pathological mechanism of both CMD and SMI. Furthermore, we describe the genetic architecture in the *PCSK9* locus and identify mechanisms by which genetic variants in this region may influence biological traits. Finally, we evaluate whether the effects of *PCSK9* genetic variation were likely to act through LDLR-lowering pathways. If *PCSK9* variation contributes to SMI by the same mechanisms as its effect on CMD, there is the potential to repurpose CMD therapies for the improvement of SMI symptoms and CMD prevention in these high CMD-risk individuals.

Methods

UKB and Phenotypes

The UK Biobank (UKB) is a large dataset of 502,613 participants, which has generic ethical approval granted by the NHS National Research Ethics Service (approval letter dated 13 May 2016, Ref 16/NW/0274). This work was carried out using UKB application #6553 (PI Rona Strawbridge) and #17689 (PI Donald Lyall).

Data obtained is provided by participants and collected by the National Health Service as part of their care and support. A more thorough detailed description of the UKB data set can be found elsewhere in open-access reports¹⁷⁻¹⁹.

To summarise, UKB data was collected from 22 assessment centres during 2006-2011. Individuals were largely aged between 40-69 at baseline. Trained clinical staff carried out physical examinations and touchscreen questionnaires to gather baseline measurements along with an individual and family medical history. A blood sample allowed biomarker analysis and DNA extraction for subsequent genome-wide genotyping. The genotyping of all individuals was conducted using two similar genotyping chips (BiLeve and Affymetrix)..

Quality control and genetic imputation used standard protocols and took place centrally. A physical examination by healthcare professionals collated data on the cardiovascular health of each volunteer. Firstly, an average blood pressure reading was calculated from two readings. This was then adjusted for the use of antihypertensive medications as per Ehret et al ²⁰. These values are recorded as SBPadj or DBPadj (systolic or diastolic blood pressure adjusted, respectively). Measurements of an anthropometric nature were recorded, this included weight, height and waist/hip circumferences. BMI (kg/m^2 , reflective of total obesity) and waist:hip ratio (reflective of central obesity) were calculated, and waist:hip ratio adjusted for BMI (WHRadjBMI, reflecting central obesity accounting for total obesity) was calculated to reflect central adiposity as per Shungin et al ²¹. Participants were identified (from self-reported medical history and medication data) to have type 2 diabetes as per Eastwood et al ²². Reported medical histories were confirmed by a follow-up interview with a nurse, this included history of venous thrombosis embolism (VTE), stroke, and ischaemic heart disease (ISH). Smoking was self-reported and ex-smokers were grouped with non-smokers (current smokers vs. non or former smokers).

Psychiatric traits were analysed by different questionnaires. Firstly, the 12 items of the Eysenck Personality Questionnaire-Revised Short form were used to assess neuroticism. During the baseline assessment, the frequency of lack of enthusiasm in the last two weeks was recorded to assess individual's anhedonia (data field 2060). Individuals responding "not at all" were considered controls, whilst those responding "several days", "more than half of the days" or "nearly every day" were considered cases. Risk-taking was assessed by a self-reported question asking, "Would you describe yourself as someone who takes risks?" (data field 2040) and mood instability by the question "Does your mood often go up and down?" (data field 1920). Individuals responding "yes" were considered cases, and those responding "no" were considered as controls. Any individual responding "prefer not to answer", or "don't know" was excluded from the analysis.

A follow up online mental health questionnaire (collected 2016-2017) was used to clarify the lifetime prevalence of SMI^{23,24} and related traits. This included generalised anxiety disorder (Life GAD), major depressive disorder (Life MDD), bipolar disorder (Life BD), or presence of an addiction^{23,24}.

SNP selection

The *PCSK9* locus is here defined as the *PCSK9* gene (coding region) ± 250 kb (UCSC GRCh37/hg19, Chromosome 1:55,505,221-55,530,525 $\pm$ flanking regions). Initially all genetic variants within the locus were identified. Genetic variants with minor allele frequencies of $>1\%$ were retained in analyses. Discovery analyses included only unrelated individuals of white British ancestry (N=402,820, SNP N=2,037). Secondary analyses was conducted in unrelated individuals of white European ancestry (N=50,510, SNP N=2,017), south Asian ancestry (N=7,726, SNP N= 2,167), African-Caribbean ancestry (N=7,644, SNP N= 3,386) and mixed ancestry (N=10,447, SNP N=2,532).

Statistical analyses

Minitab 19.2 was used to generate descriptive statistics for the cohort. For genetic analyses, an additive model was assumed and Plink 1.07²⁵ was used with logistic or linear regression being used for binary or continuous variables respectively. All continuous variables were assessed for normality prior to analyses. All analyses were adjusted for age, sex, eight genetic principal components and genotyping chip, except WHRadjBMI where these covariates are included in its construction. Treatment with lipid-lowering and anti-hypertensive medications was also included as a covariate for analysis of ISH and stroke. The number of independent loci was determined using independent pairwise filtering using default parameters on PLINK 1.07, and Bonferroni correction was then applied for multiple testing to define the threshold p-value required for statistical significance. The threshold for SNPs to be significantly associated with a trait in discovery analyses was p-value $< 1.16 \times 10^{-4}$. The same process was used to determine significance levels in secondary analyses,

with p-value for significance being set at 1.28×10^{-4} for South Asian, 4.69×10^{-5} for African-Caribbean, 1.20×10^{-5} for white European and 8.85×10^{-5} for mixed ancestry individuals.

Lead SNP analyses

Two sets of conditional analysis were run using each lead SNPs as an independent variable. This firstly assessed the presence of multiple signals for each trait and secondly, independence of the lead SNPs for each trait. LocusZoom²⁶ was used to generate regional plots. Cross trait observations of the data produced by the analysis of each lead SNP assessed any directional effects or overlapping pathways. Haploview²⁷ allowed us to visualise the linkage disequilibrium (LD) between the lead SNPs; this complemented the conditional analyses. LD is the non-random co-inheritance of alleles at two or more loci.

PCSK9 polygenic scores

Three studies have highlighted genetic variants with functional effects such that they can be used to proxy lifelong reduction in LDL levels^{14,28,29}. Schmidt et al used four SNPs in a polygenic score (PGS), whilst Ference used slightly different criteria to select seven SNPs. Lyall et al used six of the seven Ference SNPs and noted that results were unchanged when individual SNPs were removed, therefore this PGS contains very similar, but less, information compared to the Ference SNPs. Using these variants, two PGS were constructed, using four variants (PGS4²⁸) or seven variants (PGS7²⁹). The PGS was generated by summing the lipid-lowering alleles for each individual. Only individuals with complete genotyping for the four of seven SNPs were included.

Follow-up analyses

The GTEx portal (<https://www.gtexportal.org/home/>³⁰, accessed 2021-08-26) was used to explore the locations of *PCSK9* gene expression and genotype-specific gene expression patterns. LDExpress was used to explore genotype-specific gene expression patterns of proxies for the lead SNPs (<https://ldlink.nci.nih.gov/?tab=ldexpress>³¹, accessed 2021-08-26). The NHGRI-EBI Catalog of Human Genome-Wide Association Studies (GWAS Catalog,

<https://www.ebi.ac.uk/gwas/>, accessed 2021-08-17), was used to identify traits that have been previously associated with *PCSK9* in GWAS studies. Ensembl Variant Effect Predictor (VEP, <https://www.ensembl.org/info/docs/tools/vep/index.html>, ³²) was used to explore the predicted functional effects of a SNP on the gene product, with SIFT and PolyPhen being used to assess tolerance. The Human Protein Atlas (<https://www.proteinatlas.org/>, ³³) was used to investigate the levels of PCSK9 protein expression.

Results

Table 1 provides the cohort characteristics. The average age of participants was 56.9 years, with 20.6% being treated with lipid-lowering medication and as expected from the age and relatively healthy population ³⁴, the rates of ISH and stroke were low (6.1% and 2.1% respectively).

PCSK9 locus and cardiometabolic traits

Significant associations are summarised in Table 2 and Figure 2. One genetic variant was associated with SBP_{adj} (rs2647282-A, Beta(se) -0.196 (0.048), $p=4.20 \times 10^{-5}$, Figure 2A), eight with WHR_{adj}BMI (lead rs7543163-C, 0.001 (0.000), $p=9.21 \times 10^{-6}$, Figure 2B) and five with VTE (lead rs746952505-CA, OR (95% confidence interval) 1.07 (1.04-1.11), $p=3.74 \times 10^{-5}$). As the lead SNP for VTE is not available in LocusZoom, the second significant variant was used for plotting and also considered in conditional analyses (rs12071742-A, 1.09 (1.04-1.13), $p=8.89 \times 10^{-5}$, Figure 2C). One variant was associated significantly with stroke (rs7266260-G, 0.86 (0.80-0.93), $p=9.56 \times 10^{-5}$, Figure 2D). Conditional analyses (including the lead variant as a covariate in the analyses) were conducted in order to identify any secondary signals. These analyses demonstrated that there was only one signal for SBP (Stable 1, SFig 1A-B), WHR_{adj}BMI (Stable 2, SFig 2A-B), VTE (Stable3, SFig 3A-B), irrespective of which VTE SNP was conditioned on and stroke (Stable 4, SFig 4A-B). No associations were observed for ISH.

207 *PCSK9 locus and psychiatric and related traits*

208 When considering psychiatric and psychological traits (Table 2 and Figure 2), 30 significant
209 associations were identified for mood instability (lead rs1120514-C, 1.02 (1.01-1.03),
210 $p=2.68 \times 10^{-5}$, Figure 2E) and 39 were observed for neuroticism score (lead rs12069079-G,
211 0.45 (0.010), $p=4.53 \times 10^{-6}$, Figure 2F). As was observed for the cardiometabolic traits,
212 conditional analyses rendered the associations non-significant for mood instability (Stable 5,
213 SFig 5A-B) and neuroticism score (Stable 6, SFig 6A-B).

214 *Independence of signals*

215 Conditional analyses, visual inspection and LD were used to investigate whether signals for
216 the five significant traits overlap or are distinct. Effect sizes varied less than p-values with the
217 fluctuations in sample size, therefore these, rather than p values were considered. No
218 distance measure was used to assess independence.

219 The effect size of rs2647282 on SBPadj demonstrated little change when conditioning on the
220 lead SNPs of the other traits (STable 1, SFig 1 C-G). This is consistent with the low LD with
221 other other lead SNPs (max $r^2=0.21$, Figure 3). The effects of WHRadjBMI SNPs were
222 unchanged when conditioning on the lead SNPs for SBP, VTE, stroke or neuroticism score
223 (STable 2, SFig 2C-E, G) but the association was rendered null by conditioning on the lead
224 SNP for mood instability (SFig3 F). This is consistent with the very low LD ($r^2=0.4$, Figure 3)
225 with other lead SNPs, but almost complete LD with the mood instability lead SNP ($r^2=0.98$,
226 Figure 3). It is worth noting that WHRadjBMI-increasing allele of the lead SNP also had a
227 mood instability-increasing effect (1.02 (1.01-1.03) $p=1.50 \times 10^{-4}$). The VTE signal appears to
228 be distinct from the other signals, as conditioning on the other lead SNPs had little or no
229 impact (STable 3, SFig 3C-G), which is consistent with visual inspection of the regional plots
230 and with very low LD with the other signals (max LD $r^2=0.02$, Figure 3). Similarly, the stroke
231 signal appears independent from the other signals, with conditional analyses having minimal
232 effect (STable 4, SFig 4C-G) and very low LD with any other signal (max $r^2=0.05$).

Visual inspection suggests that the signals for mood instability and neuroticism partially overlap (STable 5-6, SFigs 5-6), which is consistent with the results of the conditional analyses. However, the mood instability question is a component of the neuroticism score, therefore the conditional analyses likely reflect the phenotypic relationship between these two variables, rather than a shared genetic signal, as the LD between the lead SNPs is very low (LD $r^2=0.04$, Figure 3). Again, it is worth noting that the mood instability-increasing allele also had a WHRadjBMI-increasing effect (0.001 (0.0001), $p=1.25 \times 10^{-5}$).

PCSK9-functional SNPs

PCSK9 has multiple functions, of which the lipid-lowering functions are best understood. In order to explore whether lipid-lowering (or other) functions of PCSK9 might explain the associations observed, we tested two lipid-lowering PGS for impact on our traits of interest (PGS4 as per ²⁸ and PGS7 as per ²⁹). The individual effects of each SNP in the PGS on the traits of interest are presented in Stable 7. Only rs10888897 had significant effects on WHRadjBMI (T, 0.001 (0.000), $p=3.75 \times 10^{-5}$), and a non-significant effect on mood instability (T, 1.02 (1.01-1.03) $p=0.0003$), which is consistent with the high LD between this SNP and the lead SNPs for these traits (Figure 3). The non-significant effect on neuroticism again likely reflects phenotypic similarity of these traits, as the LD with the lead neuroticism trait is low. PGS4 and PGS7 showed consistent null effects on SBP, WHRadjBMI, VTE and stroke ($p>0.139$, STable 8), suggesting that that effects of the *PCSK9* locus influences these traits are independent of lipid-lowering. For VTE and stroke, this is consistent with previous observations in animal models ³⁵, where *PCSK9* has been shown to influence thrombosis. For mood instability, PGS7 but not PGS4 had nominally significant ($p<0.05$) effects on mood instability and both PGS4 and PGS7 had nominally significant ($p<0.05$) effects on neuroticism, where increased genetic predisposition to lower lipids was associated with increased risk of mood instability and neuroticism score, suggesting that lipid-lowering effects of the *PCSK9* locus might contribute to these psychological traits. Whilst rs10888897 is in high LD with the lead SNPs for mood instability ($r^2=0.95$, Figure 3), it is possible that this

signal is driving the PRS association with mood instability. The same is not true for the associations of the PGS with neuroticism, as PGS4 contains only SNPs with minimal LD (max $r^2=0.01$, Figure 3) with the lead SNP for neuroticism (and max $r^2=0.12$ with the lead SNP for mood instability).

Secondary analyses

Secondary analyses of additional ancestry groups were restricted to phenotypes that were significant in the discovery analyses and sufficient data was available. No SNPs met the thresholds for significance in any analyses (neuroticism, mood instability, SBP_{adj} or WHR_{adj}BMI in all groups or VTE in white European ancestry). Lead SNPs for secondary analyses (reaching $p<1\times10^{-3}$) are presented in STable 9 and a comparison of effect directions of the lead SNPs from the discovery analysis, across ancestry groups is provided in STable 10. Overall, the lack of significant findings in non-white British ancestry individuals is likely due to underpowered analyses, which limits the interpretation of consistency.

LD between the lead SNPs in the non-white British ancestry individuals confirmed the conclusion that the signals for WHR_{adj}BMI and mood instability overlap (also with one lipid-lowering variant), but the other signals are independent.

Follow-up analyses

PCSK9 expression was reported by the Human Protein Atlas to be detected as mRNA and protein, in both the liver and brain. mRNA expression of *PCSK9* mRNA in systems essential to cardiometabolic and psychiatric pathology is striking (Figure 1A). The expression of two of other genes in this locus is worth noting: firstly, *DHCR24* (Figure 1B) which is predominantly expressed in the adrenal gland and spinal cord and *USP24* (Figure 1C) which showed ubiquitous expression. When considering the effects of SNPs on gene expression, the lead SBP_{adj} SNP demonstrated genotype-specific gene expression patterns were observed in heart, nerve and muscle tissues, but for *USP24*, not *PCSK9* (Table 3). No other lead SNP demonstrated effects on *PCSK9* mRNA expression. For proxies for the WHR_{adj}BMI lead

SNP, genotype-specific gene-expression was observed for *DHCR24* in brain tissue, *USP24* in heart tissue and *PCSK9* in brain and adipose tissues, where the WHRadjBMI-increasing allele was associated with decreased expression in the brain and increased expression in adipose tissue. The opposite direction of effects of one SNP in the brain vs adipose tissue is curious and may suggest that the balance of *PCSK9* and therefore lipids, between the brain and adipose tissues contributes to mood instability and central fat accumulation (as indicated by WHRadjBMI).

Of the SNPs significant for any trait, VEP highlighted rs487230 (significant for mood instability and neuroticism score) as a tolerated/benign missense variant. This SNP has genotype-specific effects on *USP24*, in many tissues, where the mood instability/neuroticism-increasing allele was associated with reduced levels of *USP24*. No other trait-associated SNPs were predicted to have functional effects.

The GWAS catalogue was used to explore previous robust associations with the *PCSK9* locus. STable 11 summarises the previous findings. The trait-associated SNPs in this study have not previously been robustly associated with any trait. Nevertheless, there was an abundance of alternative *PCSK9* SNPs to be associated with cardiometabolic pathology, specifically lipid, lipoprotein and PCSK9 levels, use or response to lipid-lowering medication and cardiovascular disease (including abdominal aortic aneurysm, myocardial infarction and coronary artery disease). All reports in the GWAS catalog coincide with our results as it highlights an abundance of *PCSK9* SNPs can be associated with features crucial to cardiometabolic disease development. There were no reported associations of *PCSK9* SNPs with VTE or psychiatric traits in GWAS. (STable 11)

Discussion

We systematically assessed potential associations of *PCSK9* SNPs with traits of relevance to psychiatric and cardiometabolic disease within the large UK Biobank population cohort. We identified associations between *PCSK9* SNPs and four cardiometabolic traits: VTE, stroke,

SBP_{adj} and WHR_{adj}BMI. In addition, we identified novel associations of the *PCSK9* locus with traits of relevance to psychiatric disorders, namely mood instability and neuroticism score. We demonstrated that the effects of this locus on CMD traits (SBP, WHR_{adj}BMI, stroke and VTE) were independent of *PCSK9* lipid-lowering functions, but the effects on SMI traits likely act through lipid-lowering mechanisms. Finally, we present evidence for regulation of *PCSK9*, *DHCR24* and *USP24* expression levels as the mechanism by which these genetic variants act.

This study was motivated by the high level of *PCSK9* expression in the brain in addition to liver (reported by GTEx) along with its known roles in brain function and cardiovascular processes. The associations with SBP_{adj} and WHR_{adj}BMI were not surprising given the relationship of obesity and blood pressure to lipids and cardiovascular disease, however, it is unusual that SBP_{adj} was significantly associated with this locus but not DBP_{adj}. The association of the *PCSK9* locus with VTE was unexpected and requires further exploration. Lipid accumulation and inflammation, key components of arterial vascular diseases are generally considered less relevant to VTE, where the coagulation cascade appears to be key. However, in experimental animals, *PCSK9* knockout resulted in reduced platelet activation³⁵, with the authors also highlighting the role of the LDLR family in the clearance of clotting factor VIII, thereby providing an additional explanation for non-lipid lowering pathways by which *PCSK9* might impact on VTE as well as stroke. To our knowledge this is the first study to report an effect of the *PCSK9* locus on psychological phenotypes (mood instability and neuroticism score) of relevance to psychiatric traits. The association of the lipid-lowering PGS with these traits provides evidence that this effect is indeed acting through lipid-lowering mechanisms. That genetically reduced lipids appear to increase the risk of mood instability and neuroticism is intriguing.

A key finding of this study is the almost complete LD reported between mood instability and WHR_{adj}BMI, which poses further questions: does mood instability subsequently influence central fat accumulation (through diet and exercise preferences for example), or does this

genetic signal have multiple effects on different genes and in different tissues (pleiotropy), or is there one mechanism common to these two very different traits? The gene expression evidence of the minor allele (associated with increased WHRadjBMI) both increasing *PCSK9* levels in adipose tissue and decreasing *PCSK9* levels in brain tissue suggests that looking at one system in isolation might not be as informative as the balance between the systems.

Whilst the non-white British ancestry results were largely uninformative (most likely due to limited power), the consistent LD patterns across ancestry groups supports the overlap of signals for mood instability and central fat accumulation, and the independence of the other signals (SFig7).

Whilst the *PCSK9* expression data provides evidence for a direct role in CMD and SMI, the roles of *DHCR24* and *USP24* should also be considered: *DCH24* has been shown to have a neuroprotective role during inflammation³⁶ with loss of *DCH24* expression being observed in Alzheimer's disease^{36,37} and the subsequent reduction in autophagy reportedly resulting in increased accumulation of tau protein³⁷. Therefore, the proxy SNP whereby the WHRadjBMI-increasing allele was associated with reduced *DHCR24* levels in the brain could suggest that increased central adiposity is associated with loss of neuroprotection. *USP24* is implicated in Parkinson's disease, where reduced expression levels are associated with increased autophagy³⁸. The gene expression data presented here indicate a role for *USP24* in heart, nerve, and muscle tissue, but not adipose or brain tissues.

The multiple functions of *PCSK9* could contribute to the overlapping pathology of CMD and SMI at a gene level. Nevertheless, it is important to consider the environmental influences on this comorbidity pairing. It is plausible that *PCSK9* contributes to SMI and CMD as it underlies one pathology, which alters an individual's behaviour increasing their risk for another pathology. For example, disrupted systemic lipid levels caused by reduced/increased *PCSK9* might result in cardiometabolic disease, which could then alter the behaviours of an individual, thereby increasing the risk for SMI development. Furthermore, SMI could be caused by disrupted *PCSK9* function in the brain, and this SMI could influence the lifestyle

choices and behaviours of an individual resulting in an increased risk for cardiometabolic disease. Figure 4 shows the potential mechanisms and pathways by which PCSK9 could contribute to SMI and CMD.

Strengths and limitations

SMI proves an ongoing burden in public health due to its costly effects on society and individuals and CMD worsens the morbidity and mortality of SMI. Therefore, a strength of this study is its relevance to current medical practice. This is the first study to systematically explore the genetic association of the *PCSK9* locus with both SMI and CMD, and highlights candidate genes and tissues for further investigation.

The large sample size and consistent phenotyping in the UKB is a key strength of this study, providing reliable and accurate results, and limiting the effects of outliers or confounding variables. Bonferroni correction, although somewhat conservative, was implemented to limit the risk of a type 1 error. PGS testing broadened our results as it allowed us to observe if the CMD or SMI traits identified were associated with a proxy lifelong reduction in LDL levels.

The sampling methods used by the UKB to assess psychiatric traits could provide inconsistent results. As both mood instability and risk taking used single self-reported questions, this meant the results were very subjective to an individual's self-view. Individuals with SMI may not regard their behaviour as 'risky' or recognize their mood to fluctuate. In addition, this study was limited to individuals of white British ancestry, to minimise genetic heterogeneity, thus the results might not be generalisable to the general population.

Furthermore, UKB is known to be biased towards the wealthy healthy population^{34,39}. Many factors contribute to this selection bias including the burden of physical or mental health that an individual may experience. Therefore, UKB likely underestimates the true population's prevalence of disease. Despite this, the relationship and associations identified in this cohort could still be generalisable to the wider European ancestry population. Further exploration of these findings in additional ancestry groups would be of great value.

Conclusion

We identified genetic associations between the *PCSK9* locus and SBPsdj, WHRadjBMI, VTE, stroke, mood instability and neuroticism score. A key finding was that the WHRadjBMI and mood instability SNPs represent the same genetic signal (indicated by LD $r^2 = 0.98$), therefore this is the first study to implicate *PCSK9* locus in the potentially shared pathology of CMD and SMI. This finding is further emphasized by the lipid-lowering PGS demonstrating a nominal association with neuroticism score and mood instability. Further analysis should consider the temporal patterns and relationships of *PCSK9*, alongside *DCHR24*, to further understand the pathology linking CMD and SMI. The results of this study address a gap in the field and hopefully inspire and pave the way for future investigations. Likewise, further exploration of this locus in a more diverse cohort would help identify and clarify the evidence of *PCSK9* being associated with CMD and SMI.

Data availability

All UK Biobank data (raw, coding and results) are available to approved researchers after application to the UK Biobank study (<https://www.ukbiobank.ac.uk/>). Coding and summary level results are available upon request (Dr Rona J Strawbridge, rona.strawbridge@glasgow.ac.uk).

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Author contributions

Analyses and original draft: RH. Data management and curation: BC, NG, DML, AA, JW. Resources: JPP, DJS. Study conception, supervision and original draft: RJS. Draft review and editing: all authors.

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Ethical Approval

The UK Biobank study has generic ethical approval granted by the NHS National Research Ethics Service (approval letter dated 13 May 2016, Ref 16/NW/0274) and under UK Biobank applications #6553 (PI Rona Strawbridge) and #17689 (PI Donald Lyall).

Competing Interests

The authors have no competing interests to declare.

Figure Legends

Figure 1: Gene Expression of A) *PCSK9* B) *DHCR24* and C) *USP24*, by organ (GTEx analysis release V8). Expression values are shown in transcription per million (TPM) calculated from a gene model with isoforms collapsed to a single gene. Box plots are shown as median and 25th and 7th percentiles; points are displayed as outliers if they are above or below 1.5 times the interquartile range.

Figure 2: Regional plots of main results in white British ancestry individuals. A) SBP_{adj}, B) WHR_{adj}BMI, C) VTE, D) stroke, E) mood instability and F) neuroticism score. Locuszoom regional plots of each significant SNP identified for a trait in the UKB. SNPs are aligned on the X-axis by their position on chromosome 9, and by their association with the trait on the Y-axis (P-values are on a $-\log_{10}$). Significance was set at $p < 1.16 \times 10^{-4}$ or $-\log_{10} P > 3.93$ (approximated by the red horizontal line). SNPs are colour coded by their estimates of pairwise LD (r^2) with lead SNP.

Figure 3: Haploview LD plot showing the lead SNPs in the *PCSK9* locus associated with cardiometabolic or psychiatric traits, and the SNPs included in PGS4 (*) and PGS7 (#). Each box provides estimated statistics of % frequency of coinheritance (r^2 values). Darker shaded boxes represent stronger LD (r^2 colours).

Figure 4: Proposed pathways of how genetic variation in the *PCSK9* locus might influence cardiovascular and metabolic traits.

Table 1: Characteristics of the UK Biobank cohort

	White British	White European	South Asian	African-Caribbean	Mixed ancestry
N (% male)	402,820 (46.0)	50,510 (43.8)	7,726 (53.9)	7,644 (43.0)	10,447 (42.8)
Age (years)	56.9 (8.0)	55.6 (8.1)	53.4 (8.5)	51.9 (8.1)	52.4 (8.1)
BMI (KG/m2)	27.4 (4.8)	27.2 (4.9)	27.3 (4.5)	29.5 (5.4)	27.0(4.9)
WHR	0.87 (0.09)	0.9 (0.09)	0.9 (0.09)	0.9 (0.1)	0.9(0.1)
SBP (mmHg)	138 (19)	135.5 (18.5)	134.9 (18.6)	137.8 (18.7)	133.4 (18.6)
DBP (mmHg)	82 (10)	81.5 (10.2)	82.5 (10.2)	84.5 (10.7)	81.9 (10.5)
SBP* (mmHg)	141 (21)	138.2 (20.5)	139.1 (21.4)	142.5 (21.7)	136.4(21.0)
DBP* (mmHg)	84 (11)	83.3 (11.3)	85.2 (11.5)	87.7 (12.4)	83.9 (11.9)
Current smoking	40,618 (10.1)	6597 (13.1)	687 (8.9)	952 (12.5)	1,437 (13.8)
Hypertension	20,5846 (53.6)	22,787 (45.1)	3,832 (49.6)	4,321 (56.5)	4,523(43.3)
Anti-hypertensive medication	83,951 (21.0)	9,235(18.3)	2,082 (26.9)	2,340 (30.6)	2,048(19.6)
Lipid-lowering medication	70,184 (20.6)	8,042 (15.9)	2,049 (26.5)	1,212 (15.9)	1,807(17.3)
PGS4	1.39 (0.97)				
PGS7	3.88 (1.52)				
ISH	18383 (6.1)	2135 (4.2)	575 (7.4)	264 (3.5)	414 (4.0)
Stroke	6,150 (2.1)	708 (1.4)	128 (1.7)	118 (1.5)	94 (0.9)
VTE	10,444 (3.7)	1,239 (2.5)	131 (1.7)	185 (2.4)	189 (1.8)
T2D	17,420 (4.3)	2,121 (4.2)	1,288 (16.7)	812 (10.6)	865 (8.3)
Anhedonia	78,879 (19.6)	11,255 (22.3)	2,813 (36.4)	2,389 (31.3)	3,336 (31.9)
Mood instability	177,994 (45.2)	22,089 (43.7)	2,778 (48.9)	3,724 (48.7)	4,754 (45.5)
neuroticism score	1.65 (5.90)	1.39 (6.11)	-0.73 (7.06)	-0.25 (6.44)	-0.28 (6.79)
Risk-taking	98,435 (25.3)	16,240 (32.2)	2,688 (34.8)	2,986 (39.1)	3705 (35.5)
Mental Health questionnaire	130370 (32.4)	16,751 (33.2)	1,043 (13.5)	1,104 (14.4)	2,277 (21.8)
Addiction**	7,471 (5.7)	1,280 (2.5)	46 (0.6)	64 (0.8)	167 (1.6)
GAD**	9,096 (10.1)	1,282 (2.5)	74 (1.0)	54 (0.7)	167 (1.6)
BD**	1,873 (1.5)	324 (0.6)	24(0.3)	24 (0.3)	51 (0.5)
MDD**	30,875 (28.1)	4,186 (8.3)	196 (2.5)	203 (2.7)	539 (5.2)

Where: WHR, waist: hip ratio; *, adjusted for use of anti-hypertensive medication, as per Ehret et al; PGS4, polygenic score including 4 SNPs as per Schmidt et al; PGS7, polygenicscore including 7 SNPs as per Ference et al; ISH, ischemic heart disease; VTE, venous thromboembolism; T2D, type 2 diabetes; ** of those who ocmpleted the metnal health questionnaire. Continuous variables are presented as mean (se), binary variables are presented as N (%).

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Table 2: Lead SNPs in White British ancestry subset

Phenotype	CHR	SNP	BP	A1	A2	MAF	N	BETA	SE	OR	L95	U95	P	N SNPs
SBP _{adj}	1	rs2647282	55724437	A	C	0.36	343,109	-0.196	0.048				4.20E-05	1
WHR _{adj} BMI	1	rs7543163	55515481	C	T	0.39	400,588	0.001	0.000				9.21E-06	8
VTE (Lead)	1	rs746952505	55750180	CA	C	0.24	264,073			1.07	1.04	1.11	3.74E-05	5
VTE (Proxy)	1	rs12071742	55747825	A	G	0.12	277,385			1.09	1.04	1.13	8.89E-05	
Stroke	1	rs72662600	55732403	G			339,008			0.86	0.80	0.93	9.56E-05	1
Mood	1	rs11206514	55516004	C	A	0.39	377,209			1.02	1.01	1.03	2.68E-05	30
Neuroticism Score	1	rs12069079	55652791	G	A	0.20	326,396	0.045	0.010				4.53E-06	48

Where: A1, effect or minor allele; A2, non-effect allele; MAF, effect or minor allele frequency; N SNPs, N SNPs meeting the threshold for significance; rs746952505 is not available in locuszoom, so for plots rs12071742 is presented as the lead. LD r^2 between these 2 SNPs=0.46.

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Table 3: Genotype-specific gene expression of trait-associated SNPs or proxies (LD R2>=0.50)

Trait	Lead SNP	Proxy SNP	Position	R2	D'	Gene	Tissue	EAF	Effect	Pvalue
SBP	rs2647282		55724437			<i>MROH7</i>	Esophagus- Mucosa	A=0.324	-0.119	1.11E-04
						<i>USP24</i>	Heart- Atrial Appendage	A=0.324	-0.139	3.08E-06
						<i>USP24</i>	Muscle- Skeletal	A=0.324	-0.080	9.75E-06
						<i>USP24</i>	Nerve- Tibial	A=0.324	-0.093	1.43E-04
						<i>USP24</i>	Skin- Sun Exposed (Lower leg)	A=0.324	-0.115	5.28E-07
						<i>USP24</i>	Skin-Not Sun Exposed (Suprapubic)	A=0.324	-0.185	2.44E-11
WHRadjBMI	rs7543163	rs11206513	55507649	0.915	1.000	<i>DHCR24</i>	Brain- Cortex	T=0.544	0.129	5.06E-05
		rs11206514	55516004	1.000	1.000	<i>DHCR24</i>	Brain- Cortex	A=0.566	0.128	4.83E-05
		rs11436234	55511623	0.893	0.977	<i>DHCR24</i>	Brain- Cortex	TC=0.549	0.136	2.47E-05
		rs7530425	55511471	0.915	1.000	<i>DHCR24</i>	Brain- Cortex	T=0.544	0.136	2.47E-05
		rs10888896	55509213	0.579	1.000	<i>PCSK9</i>	Adipose- Visceral (Omentum)	C=0.692	-0.277	2.66E-05
		rs10888898	55516508	0.978	1.000	<i>PCSK9</i>	Skin-Not Sun Exposed (Suprapubic)	G=0.56	-0.138	9.28E-05
		rs2479410	55505861	0.619	0.814	<i>PCSK9</i>	Brain- Cerebellum	A=0.418	-0.369	9.61E-05
						<i>PCSK9</i>	Skin-Not Sun Exposed (Suprapubic)	A=0.418	0.162	1.73E-05
		rs644000	55511995	0.694	0.902	<i>PCSK9</i>	Skin-Not Sun Exposed (Suprapubic)	G=0.396	0.152	4.58E-05
		rs6681159	55507882	0.579	1.000	<i>PCSK9</i>	Adipose- Visceral (Omentum)	T=0.692	-0.255	5.79E-05
		rs7543163	55515481	1.000	1.000	<i>USP24</i>	Heart- Atrial Appendage	T=0.566	0.116	8.22E-05

Where: proxies are defined as SNPS in at least moderate LD (LD R2>=0.50)

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Figure 3

A)