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# Using Clinical Characteristics to Identify Which Patients With Major Depressive Disorder Have a Higher Genetic Load for Three Psychiatric Disorders

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## ABSTRACT

**BACKGROUND:** Limited successes of gene finding for major depressive disorder (MDD) may be partly due to phenotypic heterogeneity. We tested whether the genetic load for MDD, bipolar disorder, and schizophrenia (SCZ) is increased in phenotypically more homogenous MDD patients identified by specific clinical characteristics.

**METHODS:** Patients ($n$ = 1539) with a DSM-IV MDD diagnosis and control subjects ($n$ = 1792) were from two large cohort studies (Netherlands Study of Depression and Anxiety and Netherlands Twin Register). Genomic profile risk scores (GPRSs) for MDD, bipolar disorder, and SCZ were based on meta-analysis results of the Psychiatric Genomics Consortium. Regression analyses (adjusted for year of birth, sex, three principal components) examined the association between GPRSs with characteristics and GPRSs with MDD subgroups stratified according to the most relevant characteristics. The proportion of liability variance explained by GPRSs for each MDD subgroup was estimated.

**RESULTS:** GPRS-MDD explained 1.0% ($p = 4.19e^{-09}$) of MDD variance, and 1.5% ($p = 4.23e^{-09}$) for MDD endorsing nine DSM symptoms. GPRS–bipolar disorder explained 0.6% ($p = 2.97e^{-05}$) of MDD variance and 1.1% ($p = 1.30e^{-05}$) for MDD with age at onset <18 years. GPRS-SCZ explained 2.0% ($p = 6.15e^{-16}$) of MDD variance, 2.6% ($p = 2.88e^{-10}$) for MDD with higher symptom severity, and 2.3% ($p = 2.26e^{-13}$) for MDD endorsing nine DSM symptoms. An independent sample replicated the same pattern of stronger associations between cases with more DSM symptoms, as compared to overall MDD, and GPRS-SCZ.

**CONCLUSIONS:** MDD patients with early age at onset and higher symptom severity have an increased genetic risk for three major psychiatric disorders, suggesting that it is useful to create phenotypically more homogenous groups when searching for genes associated with MDD.

***Keywords:*** Clinical characteristics, Heterogeneity, Genetic load, Genetics, Major depressive disorder, Replication, Staging

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Major depressive disorder (MDD) has long been recognized as heritable (~37%) (1). However, today, the largest genome-wide association study (GWAS) in MDD by the Psychiatric Genomics Consortium (PGC) has failed to find significant associations with single genetic variants (single nucleotide polymorphisms [SNPs]) (2). One likely reason why it failed is that currently available sample sizes are underpowered to detect small genetic effects (3); studies have shown that a large proportion of MDD liability is due to joint polygenic effect of common SNPs with small effects scattered across the genome and shared with other psychiatric disorders such as bipolar disorder (BIP) and schizophrenia (SCZ) (3,4). A second reason may be the clinical heterogeneity of MDD: various patients with the same diagnosis will have experienced a differential illness course with variation in, for example, experienced number, duration, and severity of episodes (5). It has therefore been suggested that GWASs should be done in phenotypically more homogenous MDD patients (2,3). The Converge Consortium showed this by examining recurrent MDD cases in Chinese women (6). However, there might be other characteristics that could be selected to enhance the genetic signal. Based on family studies (7,8), it has been suggested that the highest genetic load will be found in the most severe MDD phenotype, such as patients with young age at onset (AaO), longer (chronic) duration of symptoms, higher severity of symptoms, and recurrent episodes (1,9,10). Moreover, clinical staging strategies using jointly different clinical characteristics to define stages of MDD progression (11–13) may also be applied.

To our knowledge, it has been barely examined whether genome-wide genomic profile risk scores (GPRSs) are associated

 
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with clinical depression characteristics that indicate a more severe MDD phenotype. One study in depression suggests that a higher GPRS increases an individual's susceptibility for experiencing chronically high levels of depressive symptoms (14).

The current study examines whether the genetic risk for MDD, BIP, and SCZ, estimated using GPRSs generated from PGC meta-analysis results (2,15,16), is increased in phenotypically more homogenous MDD subgroups of patients stratified by clinical characteristics reflecting a more severe MDD phenotype (younger AaO, longer duration of depressive symptoms, positive MDD family history, more DSM symptoms, higher severity of depressive symptoms, and the presence of recurring MDD episodes). In addition to single characteristics, we additionally stratify patients according to an established MDD clinical staging model reflecting MDD progression (12,13). Finally, we aim to replicate the main findings in an independent dataset (17).

## METHODS AND MATERIALS

### Sample

The sample consisted of 3331 unrelated participants (median year of birth 1967, range 1926–1994) of North European ancestry from the NESDA (Netherlands Study of Depression and Anxiety) ($n$ = 1851) and from the NTR (Netherlands Twin Register) ($n$ = 1480). The methodology of both NESDA and NTR and their biobank projects have been extensively described elsewhere (18–20). The genetic sample selection is identical to the one used by Milaneschi *et al.* (21).

In short, NESDA is an ongoing longitudinal study into the onset and course of depressive and anxiety disorders. At baseline (2004–2006), 2981 adults between 18 and 65 years of age were recruited from community (19%), general practice (54%), and specialized mental health care (27%) settings to represent the entire developmental spectrum of both disorders, including healthy control subjects. After baseline assessment, 2-, 4-, and 6-year follow-up assessments were performed.

NTR has collected longitudinal data on Dutch twin families involving nearly 40,000 adult participants. The ethical review boards of contributing universities approved both studies and all participants signed informed consent.

**MDD Diagnoses.** The present study consisted of 1539 cases with a lifetime diagnosis of MDD (history of an MDD episode during any of their interviews) and 1792 control subjects. All cases were drawn from NESDA. The presence of MDD was assessed with the DSM-IV Composite International Diagnostic Interview (CIDI) version 2.1 (22) administered by specially trained research staff at baseline or one of the three follow-up assessments. From NESDA, we selected healthy control subjects ($n$ = 312), who were participants without lifetime MDD or anxiety disorder.

From NTR, the majority of control subjects ($n$ = 1480) were drawn and were participants who had no report of MDD and a low factor score based on a multivariate analyses of depressive complaints, anxiety, neuroticism, and somatic anxiety (23,24).

**Clinical Characteristics.** For MDD cases (all from NESDA), several clinical characteristics were assessed. AaO was ascertained via CIDI interview. Duration of depressive symptoms was examined with the Life-Chart (25) and expressed as the percentage of ~10 years (~4 years before baseline + ~6 years of follow-up) spent with depressive symptoms. Presence (yes or no) of a first-degree family member with depression was assessed with the family-tree method (26). Two different measures indexed depression severity: the highest number of DSM symptoms ever endorsed during an MDD episode extracted from the CIDI (range 5–9), and the average score on 4 measures (at each assessment) of the Inventory of Depressive Symptoms (IDS) (27). Recurring MDD episodes (yes or no) was extracted from the CIDI. Finally, we applied a clinical staging algorithm (12,13,28) (Supplement and Supplemental Figure S1), combining different clinical characteristics. Cases were assigned to one of three stages: stage 2 ($n$ = 303) first episode; stage 3 ($n$ = 631) recurrent/relapse episode; stage 4 ($n$ = 605) chronic, an episode lasting longer than 2 years as indicated by the CIDI at baseline, or the Life-Chart during follow-up.

### Genotyping and Genetic Relationship Matrix

Blood sample collection and DNA extraction methods have been previously described (18). Autosomal SNPs were genotyped on the Human Genome-Wide SNP Array 6.0 (Affymetrix, Santa Clara, CA) in three separate batches. Quality control (QC) steps have been previously described (29,30). Primary analyses included 497,347 SNPs. Additional stringent QC was performed to build a genetic-relationship matrix (GRM) to reduce the possibility that estimates from GRM-based analyses could be inflated by artifacts. The remaining 435,579 SNPs were used to build the GRM using GCTAv.1.24.1 (31). The QC steps are described in the Supplement.

### Genomic Profile Risk Scores

As previously described (21) (more detail is available in the Supplement), results from the PGC were used to derive GPRSs for MDD (2), BIP (15), and SCZ (16). Eight sets of scored alleles were selected based on significance thresholds (Pt) (<.0001, <.001, <.005, <.01, <.05, <.1, <.5, <1) of the discovery samples associations. GPRSs were calculated as the number of scored alleles weighted by effect sizes (log-OR) from the discovery statistics (number of SNPs included for each Pt, see Supplemental Table S2). Because the GPRS construction method is based on linkage disequilibrium (LD) pruning and $p$ thresholding, it may limit their predicting accuracy by discarding information on LD structure (32). Additionally, we derived GPRSs using the LDpred approach using LD information from a reference panel (32). Both GPRS thresholds and LDpred were standardized to a mean of 0 and standard deviation of 1 to aid interpretation of results.

### Statistical Analyses

Differences in demographics between MDD cases and control subjects were examined using Mann-Whitney $U$ test for continuous and chi-square test for categorical variables.

First, focusing on MDD cases ($n$ = 1539), we regressed genetic risk (GPRS thresholds and LDpred) over clinical characteristics of MDD (AaO, duration of symptoms, family

**Table 1. Descriptions of Control Subjects and MDD Cases ($n$ = 3331), Characteristics of MDD Cases ($n$ = 1539)**

| | Control Subjects ($n$ = 1792) | MDD Cases ($n$ = 1539) |
|---|---|---|
| Demographics | | |
| Year of birth[a] | 1972 (1958–1979) | 1962 (1952–1973) |
| Female[a] | 61.0 (1094) | 68.0 (1047) |
| Characteristics | | |
| Age at onset, years | | 26.0 (18.0–38.0) |
| Duration mean over 10 years (%) | | 21.0 (6.62–46.5) |
| Family history (yes) | | 75.7 (1165) |
| Number of DSM symptoms highest ever | | 8.00 (7.00–9.00) |
| Severity of symptom (IDS) average score | | 21.7 ± 11.6 |
| Recurring MDD | | |
| First (no) | | 29.4 (452) |
| Recurrent (yes) | | 70.0 (1078) |
| Stage of MDD | | |
| Stage 2 (first episode) | | 19.7 (303) |
| Stage 3 (recurrent episode) | | 41.0 (631) |
| Stage 4 (chronic) | | 39.3 (605) |

Values are median (interquartile range), mean ± SD, or percentage ($n$).
IDS, Inventory of Depressive Symptoms; MDD, major depressive disorder.
[a]$p$ value < .001.

history, number of DSM symptoms, severity of symptoms, recurring episodes, stages) using linear regression analyses. To discard spurious correlations, we applied a strategy combining permutation-based empirical $p$ values of GPRSs of the same characteristics and false discovery rate (FDR) across main clinical characteristics for each GPRS (Supplement). Only the GPRS-characteristic pairs showing the most consistent (higher number of significant tests across GPRSs) profile of associations were selected for further analyses.

Thus, MDD cases were stratified in subgroups of similar dimensions (based on distribution quantiles for continuous characteristics) according to each clinical characteristic selected in the previous step. The associations between GPRSs and MDD (subgroups) were estimated with (multivariate) logistic regressions with control subjects as reference.

Next, the proportion of variance explained by GPRSs on the liability scale for MDD (subgroups) was estimated using the $R^2$ coefficient proposed by Lee *et al.* (33), which is directly comparable with heritability and is robust against ascertainment bias. Linear transformation on the liability scale was based on prevalence ($K$) of 0.18 for MDD [Dutch lifetime prevalence (34)]; $K$s for subgroups were empirically derived by dividing the prevalence for MDD by the number of subgroups.

Finally, the total variance in liability explained by the joint effect of all SNPs (SNP heritability, $h^2$ SNP) for specific subsets of MDD selected according to clinical characteristics was estimated using genomic relationship matrix restricted maximum likelihood analyses (35). The $h^2$ SNP is estimated in a linear mixed model in which the measure of genetic similarity (based on the GRM) is included as a random effect to predict the phenotype. Furthermore, the genetic covariance between specific subsets of MDD selected according to clinical characteristics and the traits on which the risk scores were trained was estimated using the AVENGEME package (36) utilizing the results from GPRS analyses (applied settings in Supplemental Table S4).

All analyses were adjusted for year of birth, sex, and three ancestry informative principal components to take possible population stratification into account (30). Analyses, were performed with SPSS (v. 20.0, IBM Corp., Armonk, NY), R (v. 3.2.3, R Project for Statistical Computing), and GCTAv.1.24.1 (31). Nominal significance was set at $p < .05$, using two-tailed tests.

## Replication Sample

One main finding was replicated in RADIANT-UK, an independent cohort (37), from which we selected 1602 MDD cases with a lifetime MDD diagnosis and 1390 control subjects who were screened for absence of any psychiatric disorder. MDD presence was assessed with the Schedules for Clinical Assessment in Neuropsychiatry interview (38).

Imputed (HapMap3) genotype data of RADIANT-UK were processed according to QC steps described in detail in a previous publication by our group (39). GPRSs-SCZ were prepared on 76,201 independent SNPs (see Supplement and Supplemental Table S6).

RADIANT-UK analyses were adjusted for age at interview, sex, and 10 principal components (17).

## RESULTS

MDD cases ($n$ = 1539) were older and more often female than were control subjects ($n$ = 1792) (Table 1). Of the MDD cases, 70.5% had recurrent episodes. Chronic episodes (stage 4, lasting longer than 2 years) were experienced by 33% of those with a first and >40% of those with a recurrent episode.

## Clinical Characteristics and GPRS

Within the MDD cases ($n$ = 1539), the regression analyses showed consistent patterns of associations for five GPRS-characteristic relationships (Supplemental Table S1): high GPRS-MDD with increased number of DSM symptoms (four significant, three of which had FDR $q < 0.10$; top: Pt < .05, β = .063, SE = 0.026, empirical $p$ = .014); high GPRS-BIP with earlier AaO (five significant, 3 of which had FDR $q < 0.10$; top: Pt < .005, β = − .115, SE = 0.031, empirical $p = 2e^{-04}$); and high GPRS-SCZ with higher IDS scores (five significant, four of which had FDR $q < 0.10$; top: Pt < .01, β = .089, SE = 0.025, empirical $p = 3.74e^{-04}$). Both high GPRS-BIP and high GPRS-SCZ were also associated with number of DSM symptoms (GPRS-BIP: three significant, one of which had FDR $q < 0.10$; top: Pt < .05, β = 0.065, SE = 0.026, empirical $p$ = .012; GPRS-SCZ: three significant, two of which had FDR $q < 0.10$; top: LDpred, β = .064, SE = 0.026, empirical $p$ = .013). These five GPRS-characteristic pairs were carried forward in subsequent analyses.

Family history, duration of symptoms, recurring episodes, and MDD stages showed no consistent associations with GPRSs.

**Subgroup Analyses.** MDD cases were stratified in subgroups of approximately similar dimensions according to AaO quartiles (Q1 >37 years [$n$ = 392], Q2 26–37 years [$n$ = 380], Q3 18–25 years [$n$ = 398], Q4 <18 years [$n$ = 360]), number of DSM symptoms (DSM-5/6 [$n$ = 244], DSM-7 [$n$ = 302], DSM-8 [$n$ = 442], DSM-9 [$n$ = 499]), and IDS score quartiles (IDS < 13 [$n$ = 384], IDS 13–20.25 [$n$ = 385], >20.25–29 [$n$ = 387], and IDS > 29 [$n$ = 377]).

Figure 1 depicts the proportion of variance explained by GPRSs on the liability scale for MDD (subgroups); $p$ values are from (multinomial) logistic regression (full results in Supplemental Table S3). GPRS-MDD explained maximal 1.0% of liability variance for overall MDD and 1.5% for MDD endorsing nine DSM symptoms. GPRS-BIP explained maximal 0.6% for overall MDD, 1.1% for MDD with AaO <18 years, and 0.7% for MDD endorsing nine DSM symptoms. GPRS-SCZ explained maximal 2.0% for overall MDD, 2.6% for MDD with IDS score > 29, and 2.3% for MDD endorsing nine DSM symptoms.

Analyses were repeated collapsing the two subgroups with the highest explained variance for each characteristic: GPRS-MDD explained maximal 1.1% of liability variance for MDD endorsing ≥8 DSM symptoms (DSM-high, $n$ = 941); GPRS-BIP maximal 0.8% for MDD with an onset <26 years (AaO-young, $n$ = 758), and maximal 0.9% for DSM-high; and GPRS-SCZ maximal 2.7% for MDD with IDS scores > 20.25 (IDS-high, $n$ = 764), and 2.2% for MDD DSM-high.

Previous analyses were repeated after the inclusion of 590 control subjects selected with less stringent criteria (see Supplement and Supplemental Figure S3): results were unchanged, suggesting that different selection criteria for control subjects do not impact on the association with GPRSs.

**SNP Heritability of MDD Subgroups and Genetic Covariance With Psychiatric Traits.** We estimated $h^2$ SNP for the subgroups of MDD AaO-young, DSM-high, and IDS-high, allowing us to focus on approximately one-half of the cases. SNP heritability could not be reliably estimated for AaO-young ($K$ = 0.09; estimate = 0.208, SE = 0.15, $p = 7.95e^{-2}$). This may suggest that the drop in sample size (one-half) was not balanced by an increased genetic homogeneity of this subgroup. Indeed, considering the results depicted in Figure 1, an increased genetic signal may be expected especially at very early AaO, which would not allow us to retain a substantial sample size for genomic relationship matrix restricted maximum likelihood analyses. Genomic relationship matrix restricted maximum likelihood analyses showed that $h^2$ SNP estimates were 0.44 for DSM-high ($K$ = 0.12; SE = 0.14, $p = 8.24e^{-4}$) and 0.48 for IDS-high ($K$ = 0.09; SE = 0.15, $p = 5.52e^{-4}$), although all with large standard errors due to restricted sample sizes. Estimates for DSM-high and IDS-high were suggestively higher than the estimate for MDD overall previously reported in same sample (estimate = 0.31; SE = 0.13; $p$ = .006) (21), although with overlapping confidence boundaries. The genetic covariance with bipolar disorder was 0.16 (95% confidence interval, 0.11–0.22) when focusing on cases with AaO-young. The genetic covariance with schizophrenia was 0.11 (95% confidence interval, 0.09–0.13) when focusing on cases with IDS-high, and 0.12 (95% confidence interval, 0.10–0.14) in cases with DSM-high.

## Replication: GPRS-SCZ and MDD With High Number of DSM Symptoms

We used RADIANT-UK to replicate our finding on increased GPRS-SCZ in cases with a high number of DSM symptoms (DSM-high, endorsing eight or nine symptoms). We selected this association as the benchmark for several reasons: 1) DSM symptoms were available in both cohorts (IDS only in NESDA) and had a similar distribution (see Supplemental Figure S4); 2) GPRS-SCZ explained a higher proportion of liability variance for DSM-high than did GPRS-MDD (Figure 1); 3) RADIANT-UK had no overlapping samples with PGC-SCZ discovery (16), whereas it shared samples with PGC-MDD and PGC-BIP discovery sets (2,15). The association between GPRS-BIP and young MDD onset is replicated in a paper by Power *et al.* (40) based on all the contributing PGC cohorts (including NESDA and RADIANT-UK) and therefore was not considered further here.

Polygenic scores analyses in NESDA predicting DSM-high (941 MDD cases vs. 1792 control subjects) had ≥80% power [estimated using the AVENGEME package (36), see parameter settings in Supplemental Table S5] to detect a significant (α = 0.05) association for GPRS-SCZ with Pts equal to or higher than <0.01, with an expected $R^2$ range of 0.3% to 1.8%. In RADIANT-UK, the power to detect the same significant association with 878 MDD cases and 1390 control subjects was ≥80% for GPRS-SCZ with Pts equal to or higher than <0.05, with an expected $R^2$ range of 0.8% to 1.4% (see parameter settings in Supplemental Table S8).

RADIANT-UK MDD cases ($n$ = 1602) were older (mean 46.4 years) than control subjects ($n$ = 1390, mean 41.8 years) and more often female (70.6% vs. 60.2%). A total of 1462 MDD cases had information on the number of DSM symptoms (median 8.00, range 5–9) experienced. GPRS-SCZ explained

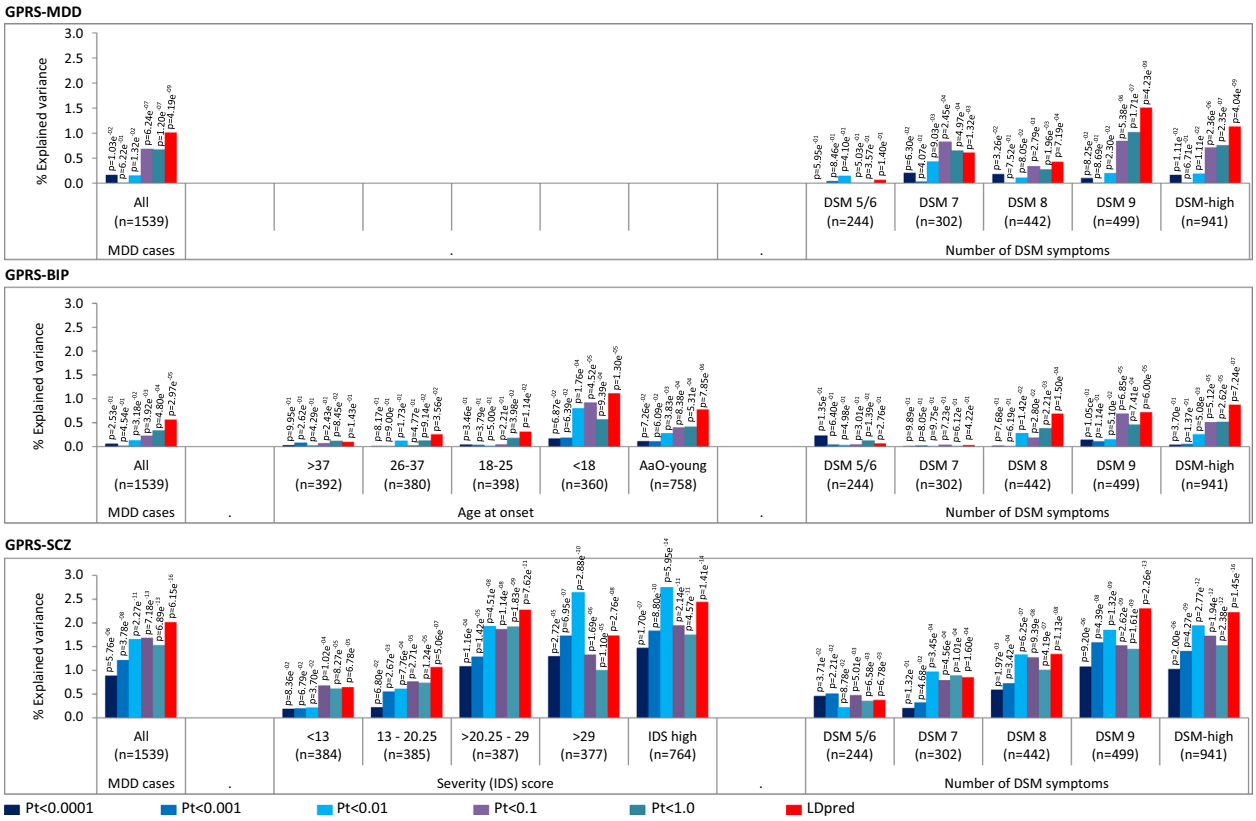


**Figure 1.** NESDA (Netherlands Study of Depression and Anxiety), percentage of explained variance for major depressive disorder (MDD) status (all MDD cases or MDD subgroups of DSM/Inventory of Depressive Symptoms [IDS]/age at onset [AaO] or those with DSM-high/IDS-high/AaO-young) vs. control subjects. Explained variance assuming a liability threshold model and $K = 0.18$ (MDD), $K = 0.18/4$ (DSM, IDS, and AaO quartiles), $K = 0.18/2$ (DSM-high, IDS-high, AaO-young). The $p$ values are from binary logistic regression (MDD-all, DSM-high, IDS-high, AaO-young) and multinomial (subgroups) logistic regression (reference = control subjects, $n = 1792$), which are adjusted for year of birth, sex, and three principal components. BIP, bipolar disorder; GPRS, genomic profile risk score; Pt, significance threshold; SCZ, schizophrenia.

maximal 0.9% of liability variance for overall MDD and 1.1% for DSM-high ($n = 878$) (Figure 2, Supplemental Table S7).

Pooled data analyses of the odds ratios derived from logistic regression analyses comparing the GPRS-SCZ in MDD overall versus control subjects and in MDD DSM-high versus control subjects both in NESDA and RADIANT-UK showed that the odds for DSM-high versus control subjects were higher than the odds for MDD overall versus control subjects (Figure 3, Supplemental Table S9).

## DISCUSSION

The current study examined whether the genetic risk for MDD, BIP, and SCZ is increased in phenotypically more homogenous MDD subgroups of patients stratified by clinical characteristics reflecting a more severe MDD phenotype and stratified by clinical MDD stages reflecting progression of MDD. The present findings showed that MDD cases with a younger AaO have a higher genetic load for BIP, and those with severe depression, as indexed by repeated measure of depressive symptoms, had higher genetic risk for SCZ. Moreover, cases with a high number of endorsed DSM symptoms showed also higher genetic risk for all major psychiatric disorders considered.

Differential association between polygenic scores for different psychiatric disorders and MDD-specific clinical characteristics indicate that these features may be able to identify specific subgroups of depressed patients who are genetically more similar to the discovery traits (Supplemental Figure S2). Indeed, polygenic score for bipolar explained 0.6% of liability variance for MDD and 1.1% when focusing on cases with early AaO. Similarly, polygenic score for SCZ explained 2.0% of liability variance for MDD and 2.7% when focusing on cases with high severity of symptoms. Association of polygenic scores of a trait (e.g., GPRS-MDD) on subgroups of the same trait (e.g., MDD characteristic groups) is often more difficult to interpret, especially when the index characteristics and its distribution are unknown in the discovery data (35). However, in this case, some interpretation is more plausible, as the number of DSM symptoms, associated with polygenic score of MDD, may clearly represent a proxy for disease severity. A further noticeable finding is that GPRS-SCZ scores explained the highest proportion of variance in MDD liability, higher than GPRS-MDD scores. This higher explanatory power is attributable to the larger training set for GPRS-SCZ (41); in a previous paper, we calculated that if we were to have the same size of training set for depression GPRS-MDD

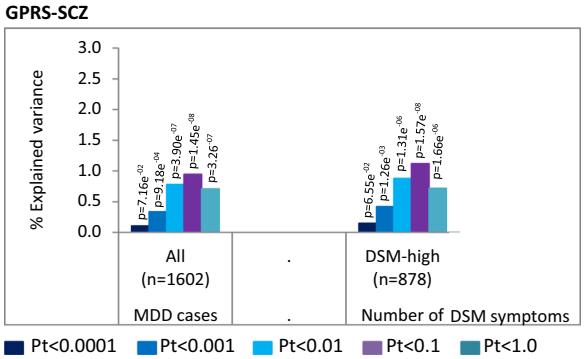


**Figure 2.** RADIANT-UK, percentage of explained variance for major depressive disorder (MDD) status (all MDD cases or those with DSM-high) vs. control subjects. Explained variance assuming a liability threshold model and $K = 0.18$ (MDD), $K = 0.18/2$ (DSM-high). The $p$ values are from binary (MDD-all, DSM-high) logistic regression (reference = control subjects, $n = 1390$), which was adjusted for age, sex, and 10 principal components. GPRS, genomic profile risk score; Pt, significance threshold; SCZ, schizophrenia.

as the one used for GPRS-SCZ, we would have, at least, similar variance explained (21).

The hypothesis that phenotypically more homogenous MDD cases as stratified by characteristics reflecting a more severe MDD phenotype (young AaO, recurrent, chronic) have an increased genetic load is among other things based on the finding that some characteristics predict a familial risk on major depression. The risk of depression has consistently been shown to be higher in family members of probands with early-onset recurrent MDD than in family members of late-onset single episode (1,7). Our finding that GPRS-BIP is significantly higher in a young-onset MDD versus older-onset MDD could suggest that patients with an early onset of depression may have a higher genetic risk to develop bipolar disorder later in life. This is in line with literature showing that the AaO for BIP is generally younger than the AaO for MDD (7), and that an early age at depression onset is a risk factor for developing bipolar disorder later on (42–44). One other study on MDD patients showed that early AaO (<18 years) was associated with a higher genetic bipolar load (45). Besides AaO, we found that higher number of DSM symptoms and higher severity of depressive symptoms are also associated with an increased genetic risk, especially GPRS-SCZ. It could be that a higher genetic risk for SCZ might cause more severe MDD. It is known that severe forms of MDD often present with psychotic symptoms (46,47). To our knowledge, one other study (14) has examined the association between depression severity and genetic load, and it found that the mean number of depressive symptoms was associated with genetic risk. That study (14), however, only included older adults (>50 years), GPRSs were based on same trait examined, and no official depression diagnosis was made. In a larger sample than the current one, it would be interesting to examine whether this association between high number or severity of symptoms and increased genetic SCZ risk is driven by specific symptoms that are particularly relevant to psychosis.

We found no associations between genetic load and duration of symptoms, family history of depression, recurring MDD episodes, and MDD stage. An explanation for our negative results on family history and GPRS could be that family history is important for the onset of MDD, but in persons with MDD a higher genetic load exists regardless of their family history. In addition, our measurement of family history may not have been sensitive enough to distinguish only the most severe family cases, as reported family history was quite high. Finally, familial aggregation may be considered a rather broad index for genetic risk (which may include also the effect of all kinds of genetic influences and the shared environment). In the current study, we used GPRSs, which rely only on the additive effect of common variants, while we considered only the additive genetic risk arising from common variants. A reason why we did not find a genetic load difference between first and recurrent episodes may be that patients with a first episode will develop a recurrent episode in the future and therefore will be phenotypically the same as those that have already a recurrent episode. In addition, quite a large proportion of our first-episode patients had already a chronic episode. Finally, there was no significant difference in genetic load across the staging model of MDD either (see Supplement and Supplemental Table S1).

Overall, the present results suggest that subgroups of MDD patients selected according to specific clinical characteristics may be genetically more homogenous. For instance, estimates of the proportions of variance explained by common genetic variants on the liability for MDD cases with severe symptoms (48%) and high number of endorsed DSM symptoms (44%) were suggestively higher than the estimate obtained for overall MDD in the same sample (31%) (21). Nevertheless, these results require replication in larger samples, as the limited sample size determined substantial uncertainty around the estimates. These genetically more homogenous subphenotypes could be applied to large gene-discovery studies to boost the power to detect variants associated with MDD. However, detailed data on the clinical characteristics may not be available in all cohorts participating in large collaborative genetic studies. In this case, a simpler strategy based on data likely available in the majority of studies may still represent a viable option to harmonize subphenotypes across cohorts. In the present study, MDD with severe symptoms (as indexed by repeated measures of the IDS) showed the highest $h^2$ SNP and therefore may have represented the best potential candidate subphenotype. However, not all MDD genetic studies necessarily include longitudinal measurements by the same scale. In replication analyses in RADIANT-UK, not including IDS assessments, we focused on the available data of the number of endorsed DSM symptoms. RADIANT-UK showed the same pattern of a stronger association, as compared to overall MDD, between cases with high number of DSM symptoms and genetic risk score for SCZ. Our results showed that selection of cases with an early AaO might represent another option to stratify MDD patients. This is underlined by the findings of a study based on the larger PGC-MDD data pool that found that GPRS-BIP is associated with an earlier age at MDD onset (40).

Our results suggest that focusing on phenotypically more homogenous MDD subgroups of patients, as stratified according to characteristics reflecting a more severe MDD phenotype, might be a solution to find SNPs and genes associated

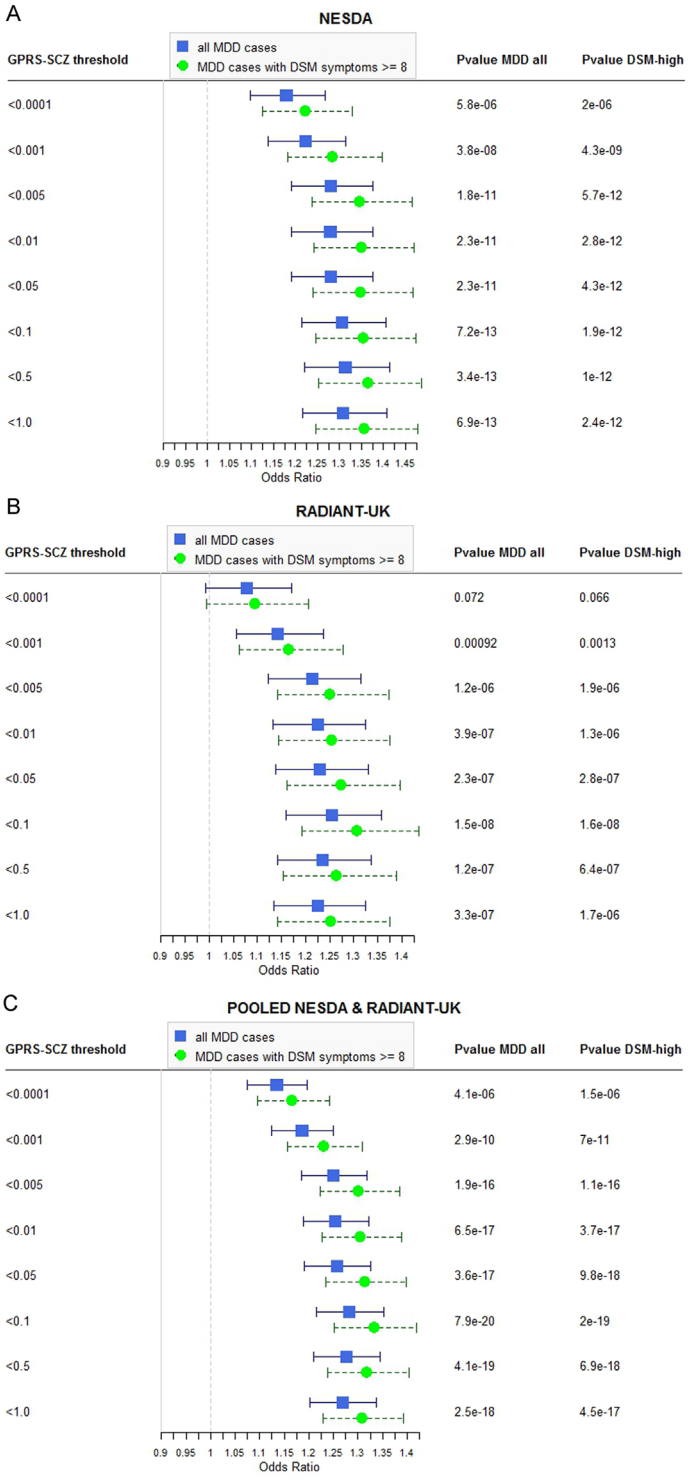


**Figure 3.** NESDA (Netherlands Study of Depression and Anxiety), RADIANT-UK, pooled, comparing odds ratios of major depressive disorder (MDD) all cases vs. DSM-high cases, reference are control subjects. **(A)** NESDA MDD cases ($n$ = 1539) vs. MDD cases DSM symptoms high ($n$ = 941); reference are control subjects ($n$ = 1792). **(B)** RADIANT-UK MDD cases ($n$ = 1602) vs. MDD cases DSM symptoms high ($n$ = 878); reference are control subjects ($n$ = 1390). **(C)** Pooled NESDA and RADIANT-UK MDD cases ($n$ = 3141) vs. MDD cases DSM symptoms high ($n$ = 1819); control subjects ($n$ = 3182). GPRS, genomic profile risk score. SCZ, schizophrenia.

with MDD. This was recently supported by a GWAS that found two genetic-loci significantly contributing to the risk of MDD in a homogenous subgroup of Chinese women with recurrent MDD (6). Besides standard clinical characteristics (recurrence, AaO) to create phenotypically more homogenous MDD subgroups that are genetically more identical, there is evidence that subgroups based on symptom subtype (melancholic vs. atypical MDD) (21) or postpartum depression (48) might also be a possibility to identify genetically more homogenous MDD groups. Moreover, it could be useful to

focus on subgroups exposed to a certain environmental factor when studying the genetic effect on MDD (29).

The core strengths of our study are the large number of participants with available genetic data; that participants are well characterized in terms of clinical MDD characteristics; and that the study represents different developmental stages of MDD. Moreover, we used GPRSs based on large international consortia, and we additionally built GPRSs with the new LDpred approach, which is suggested to increase predictive accuracy above commonly used methods for GPRSs (32).

In conclusion, the present study showed that the genetic risk for three major psychiatric disorders is increased in persons with phenotypically more homogenous MDD according to characteristics reflecting a more severe MDD phenotype. Our results showed that MDD patients with an early AaO, high number of DSM symptoms, and moderate to severe symptoms across years have the highest genetic risk. Our results suggest that in genetic studies for depression, in conjunction with a continuous effort in increasing sample sizes, it may be useful to create more homogenous subgroups based on those phenotypical characteristics in search for genes associated with MDD.

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