Bivariate Genome-Wide Association Study of Depressive Symptoms With Type 2 Diabetes and Quantitative Glycemic Traits

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ABSTRACT

Objective: Shared genetic background may explain phenotypic associations between depression and Type 2 diabetes (T2D). We aimed to study, on a genome-wide level, if genetic correlation and pleiotropic loci exist between depressive symptoms and T2D or glycemic traits.

Methods: We estimated single-nucleotide polymorphism (SNP)-based heritability and analyzed genetic correlation between depressive symptoms and T2D and glycemic traits with the linkage disequilibrium score regression by combining summary statistics of previously conducted meta-analyses for depressive symptoms by CHARGE consortium (N = 51,258), T2D by DIAGRAM consortium (N = 34,840 patients and 114,981 controls), fasting glucose, fasting insulin, and homeostatic model assessment of β-cell function and insulin resistance by MAGIC consortium (N = 58,074). Finally, we investigated pleiotropic loci using a bivariate genome-wide association study approach with summary statistics from genome-wide association study meta-analyses and reported loci with genome-wide significant bivariate association p value (p < 5 × 10⁻⁸). Biological annotation and function of significant pleiotropic SNPs were assessed in several databases.

Results: The SNP-based heritability ranged from 0.04 to 0.10 in each individual trait. In the linkage disequilibrium score regression analyses, depressive symptoms showed no significant genetic correlation with T2D or glycemic traits (p > 0.37). However, we identified pleiotropic genetic variations for depressive symptoms and T2D (in the IGF2BP2, CDKAL1, CDKN2B-AS, and PLEKHA1 genes), and fasting glucose (in the MADD, CDKN2B-AS, PEX16, and MTNR1B genes).

Conclusions: We found no significant overall genetic correlations between depressive symptoms, T2D, or glycemic traits suggesting major differences in underlying biology of these traits. However, several potential pleiotropic loci were identified between depressive symptoms, T2D, and fasting glucose, suggesting that previously established phenotypic associations may be partly explained by genetic variation in these specific loci.

Key words: depression, meta-analysis, Type 2 diabetes, pleiotropy, GWAS.
T2D and another growing health problem (3), depression, show bidirectional relationship (4). Based on a meta-analysis involving 172,521 participants, individuals with T2D have a 24% increased risk of incident depression compared with people without diabetes (5). On the other hand, based on a meta-analysis involving 2,411,641 participants, individuals with depression have a 41% increased risk for developing any type of diabetes and 32% increased risk for developing T2D (6). Moreover, major depressive disorder (MDD) and subclinical depressive symptoms are associated with abnormal glucose metabolism, impaired insulin secretion, and insulin resistance (7,8). However, the underlying mechanisms linking these conditions are unknown. One possibility is that these moderately heritable (9,10) conditions share a common genetic basis. Studies examining this hypothesis are still scarce and show conflicting results. A recent twin study using Swedish and Danish population registries (N = 68,606 and N = 95,403, respectively) found a moderate but significant genetic correlation between depressive disorders and diabetes among females from both cohorts ranging between 0.18 and 0.23 and among Danish male twins (genetic correlation, 0.25) suggesting significant overall shared genome background between these disorders (11). Conversely, a recent single-nucleotide polymorphism (SNP)–based study using linkage disequilibrium score regression (LDSC) failed to show genetic correlations between MDD and T2D or quantitative glycemic traits (12). Similarly, a population-based study of 21,516 individuals showed no genetic overlap between MDD and T2D using polygenic risk score analysis, and Mendelian randomization in addition to LDSC (13).

Currently, there are no studies on genetic overlap between depressive symptoms and T2D or glycemic traits. There are several reasons why focusing on depressive symptoms rather than MDD is justified: most of the longitudinal studies investigating bidirectional associations between these traits use measures of depressive symptoms. Moreover, psychiatric patients may be reluctant to seek help or be suboptimally treated for their nonpsychiatric disorders (14). Finally, because depression in the population may be best characterized dimensionally as a continuum from depressive symptoms to subthreshold depression and finally to MDD (15), each step increasing adverse health influences (16,17), treating depression dimensionally rather than categorically could increase statistical power to detect phenotypic associations (18).

In addition to investigating overall genetic correlation between the traits, examining specific SNPs that associate with both conditions may increase our understanding in the common underlying biology of the traits (19). Methods to study these pleiotropic associations based on genome-wide meta-analyses summary data have become only recently available (20). For example, a recent study applied bivariate genome-wide association study (GWAS) analyses and identified several pleiotropic loci that associated with both C-reactive protein and lipids and suggested potential genetic interrelation between these traits (21).

Here, we report findings from a study that applied a bivariate genome-wide association analysis using summary statistics from three relatively large univariate GWAS for depressive symptoms and glycemic traits (22–24). In these studies, Dupuis and colleagues (23) identified 25 independent loci and conducted follow-up analysis leading to 16 loci that associated with fasting glucose/homeostatic model assessment of β-cell function (HOMA-β) and two that associated with fasting insulin/homeostatic model assessment of insulin resistance (HOMA-IR), and Morris and colleagues (24) found 10 new loci that associated with T2D. For depressive symptoms, one SNP in the 5q21 region reached genome-wide significance (22). In sum, we first tested in large genome-wide meta-analyses data sets (22–24) the hypothesis that a positive genetic correlation between depressive symptoms and T2D or glycemic traits exists. Second, in the same data sets, we also explored if there are potential pleiotropic SNPs that are associated with both depressive symptoms and T2D and the glycemic traits. Because of the vast number of tests in genome-wide analyses and our limited understanding on the etiology of these traits, we did not place specific hypothesis on SNPs and their direction.

METHODS

GWAS Summary Statistics

Our analyses are based on previously published univariate GWAS summary statistics. Data on T2D were contributed by DIAGRAM investigators and include 34,840 cases with T2D and 114,981 controls without T2D (www.diagram-consortium.org, accessed October 10, 2015)(24). T2D diagnosis in DIAGRAM was based on several different criteria including self-reported T2D, physician’s diagnosis, registry data, and oral glucose tolerance test applying World Health Organization criteria (25). Data on glycemic traits were contributed by MAGIC investigators and downloaded from www.magicinvestigators.org (accessed November 26, 2015). The quantitative glycemic traits in MAGIC included fasting insulin, fasting glucose, HOMA-β [20 × fasting plasma insulin/(fasting plasma glucose − 3.5)] for assessment of β-cell function, and HOMA-IR (fasting plasma insulin × fasting plasma glucose/22.5) for estimation of the degree of insulin resistance (26) involving up to 46,186 participants without diabetes from up to 21 cohorts (23). Data on depressive symptoms were contributed by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Depression Working Group investigators and include 51,258 individuals (22). Most cohorts used the Center for

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Epidemiological Studies Depression Scale 10-, 11-, or 20-item versions (27) to assess depressive symptoms, but some cohorts used the Geriatric Depression Scale, Patient Health Questionnaire, Maastricht Questionnaire, or Beck Depression Inventory-II.

**Statistical Analyses**

To estimate genetic correlation of depressive symptoms with T2D and quantitative glycemic traits, we combined results from univariate GWAS meta-analyses using the LDSC tool (28) according to the manual with default options. This command line tool is for estimating heritability and genetic correlation from GWAS summary statistics that relies on the fact that the GWAS effect size estimate for a given SNP incorporates the effects of all SNPs in linkage disequilibrium with that SNP. LDSC is not biased by sample overlap and has been described in detail elsewhere (12).

To simultaneously analyze two outcome variables (phenotypes) at once, we performed bivariate (or multivariate with more than two phenotypes at once) GWAS analyses. Such bivariate analysis by joint association analysis of two traits in a GWAS allows us to identify potential pleiotropic genetic effects and offers several advantages over analyzing each trait in a separate GWAS. To identify potential pleiotropic SNPs associated with both depressive symptoms and each of the T2D and quantitative glycemic traits, we performed bivariate GWAS analyses using empirical-weighted linear-combined test statistics (eLC) (29) with aggregate statistics of all SNPs from univariate GWAS meta-analyses as an approximation.

**RESULTS**

**Heritability Estimates and Genetic Correlation Between the Traits**

Based on the LDSC analysis, we found that SNP-based heritability estimates were 0.09 [0.07, 0.12] for T2D, 0.10 [0.06, 0.15] for fasting glucose, 0.07 [0.05, 0.10] for fasting insulin, 0.07 [0.05, 0.09] for HOMA-β, 0.05 [0.03, 0.07] for HOMA-IR, and 0.04 [0.01, 0.07] for depressive symptoms. There were no significant SNP-based genetic correlations between depressive symptoms and T2D and quantitative glycemic traits; however, all correlations were negative (Table 1). T2D and all quantitative glycemic traits showed significant SNP-based genetic correlations, except for T2D and HOMA-β (Table S1, Supplemental Digital Content 1, http://links.lww.com/PSYMED/A435).

**Potential Pleiotropic Loci**

We found several SNPs showing potential pleiotropic effects between depressive symptoms and T2D and fasting glucose (Fig. 1, Tables S2 and S3, Fig. S1, Supplemental Digital Content 1, http://links.lww.com/PSYMED/A435). Bivariate GWAS analysis for depressive symptoms and T2D implicated two intronic SNPs in the insulin-like growth factor 2 mRNA binding protein 2 (IGF2BP2; chr 3; rs6769511; bivariate \( p \) value = 3.32 \( \times 10^{-11} \)) and CDK5 regulatory subunit associated protein I-like 1 (CDKL1; chr 6; rs10946398; bivariate \( p \) value = 4.49 \( \times 10^{-22} \)) genes and two intergenic SNPs close to the CDKN2B antisense RNA 1 (CDKN2B-AS; chr 9; rs10965250; bivariate \( p \) value = 6.18 \( \times 10^{-7} \)) and pleckstrin homology domain-containing family A member 1 (PLEKH1; chr 10; rs10510110; bivariate \( p \) value = 1.83 \( \times 10^{-5} \)) gene (Fig. 1A, Table S2, Fig. S1, Table 1).
Supplemental Digital Content 1, http://links.lww.com/PSYMED/A435). Of these SNPs, rs6769511 in \textit{IGFBP2} and rs10965250 in \textit{CDKN2B-AS} showed an association in the same direction with depression and T2D, whereas for rs10946398 in \textit{CDKAL1} and rs10510110 in \textit{PLEKHA1}, the effect was opposite. All these SNPs were associated with significant alterations in expression of several genes in brain tissues in the BRAINEAC database, as well as rs6769511 and rs10510110 with expression in other tissues in the GTEx database (Table S4, Supplemental Digital Content 1, http://links.lww.com/PSYMED/A435).

**FIGURE 1.** Manhattan plots showing bivariate \( p \) values against genomic positions for associations between depressive symptoms and T2D (A), fasting glucose (B), and fasting insulin (C). Red line is indicating bivariate genome-wide significance \([-\log_{10}(5 \times 10^{-8})]\). All variants here have univariate \( p \) values of less than.05. GWAS = genome-wide association study; T2D = Type 2 diabetes. Color image is available only in online version (www.psychosomaticmedicine.org).
Bivariate GWAS analysis for depressive symptoms and fasting glucose implicated intronic SNPs in the MAP kinase-activating death domain protein (MADD; chr11; rs11039183; bivariate \( p \) value = \( 4.40 \times 10^{-12} \)) and peroxisomal biogenesis factor 16 (PEX16; chr 11; rs11038708; bivariate \( p \) value = \( 1.27 \times 10^{-8} \)) genes and two intergenic SNPs near CDKN2B-AS (chr 9; rs7020996; bivariate \( p \) value = \( 2.08 \times 10^{-8} \)) and melatonin receptor 1B (MTNR1B; chr 11; rs6483221; bivariate \( p \) value = \( 2.99 \times 10^{-8} \)) (Figs. 1B and 2-4; Table S3, Supplemental Digital Content 1, http://links.lww.com/PSYMED/A435). The associations of rs11039183 (MADD) and of rs7020996 (CDKN2B-AS) with depression and glucose levels were in the same direction, whereas for rs11038708 and rs10510110 in PEX16 and MTNR1B, the effect was opposite. Of these four SNPs, rs11039183 was associated with expression of several genes in the brain and other tissues in the BRAINEAC and GTEx databases (Table S4, Supplemental Digital Content 1, http://links.lww.com/PSYMED/A435).

Bivariate GWAS analyses for depressive symptoms and a) fasting insulin (Fig. 1), b) HOMA-β (Fig. S2, Supplemental Digital Content 1, http://links.lww.com/PSYMED/A435), and

**FIGURE 2.** Regional visualization for rs7020996 of univariate and bivariate GWAS results for depressive symptoms and fasting glucose. GWAS = genome-wide association study.
c) HOMA-IR (Fig. S2, Supplemental Digital Content 1, http://links.lww.com/PSYMED/A435) did not result in any genome-wide significance.

**DISCUSSION**

We first showed that SNP-based heritability estimate for depressive symptoms was 4% in the 51,258 individuals of the CHARGE consortium GWA meta-analyses. As shown earlier (12), SNP-based heritability estimates for T2D and quantitative glycemic traits were between 5% and 10% in the GWA meta-analyses of the DIAGRAM and MAGIC consortia. The heritability estimates are low compared with heritability estimates from Nordic twin study for depression (40%–52%) and T2D (72%–73%) (11). However, heritability estimates based on family or twin data may show inflated values due to biases related to study designs, such as assumption of size of common environmental factors (30). At the same time, heritability estimates based on SNPs may be deflated for several reasons: we may not be able to capture relevant genomic variation with the current genome-wide genotyping arrays, and we may lack power in our original GWA analyses. Consequently, our results on the heritability of depressive symptoms and T2D and quantitative glycemic traits are in line with the

**FIGURE 3.** Regional visualization for rs11039183 of univariate and bivariate GWAS results for depressive symptoms and fasting glucose. GWAS = genome-wide association study.
missing heritability gap between the SNP-based studies and twin studies.

Next, we tested, to our knowledge for the first time with GWA data, whether there are genetic correlations between depressive symptoms and T2D or quantitative glycemic traits. These correlations might explain the well-established phenotypic relationship between depression and T2D and quantitative glycemic traits (4,7,31–33). Contrary to our hypothesis, we did not find a significant overall genetic correlation between depressive symptoms and T2D or with quantitative glycemic traits by using data from more than 2.3 million SNPs, suggesting that the contribution of genetic variation to the phenotypic association is relatively small or nonexisting.

Our results are in line with previous studies suggesting lack of significant genetic overlap between MDD and T2D and glycemic traits. A recent SNP-based genetic correlation study applying GWAS meta-analyses data using LDSC showed a nonsignificant overall genetic correlation between MDD and T2D, fasting glucose, fasting insulin, HOMA-β, or HOMA-IR (12). Another recent population-based study found no genetic overlap between

FIGURE 4. Regional visualization for rs6483221 of univariate and bivariate GWAS results for depressive symptoms and fasting glucose. GWAS = genome-wide association study.
MDD and T2D using several different methods including LDSC (13). These studies rely on available summary statistic data of contemporary GWA studies, and their ability to detect genetic correlations is determined by the ability of the original GWA studies to detect genetic associations. Thus, future studies on genetic correlation between depressive symptoms and T2D or glycemic traits will benefit from GWA meta-analyses with even larger samples providing increased power to detect genetic correlations. Moreover, also twin studies have pointed toward the role of nongenetic factors explaining the link between depression and T2D. Mezuk and colleagues (34) showed in twins that unique environmental factors contribute significantly to the association between MDD and T2D in a study of 37,043 twins. Moreover, another recent twin study concluded that environmental factors unique to the individual but common to both depressive disorders and T2D, for example, psychosocial stress, contribute to their co-occurrence in males (11).

Although major part of the link between depression and T2D or glycemic traits may be due to nongenetic factors, some proportion may still be explained by genetic overlap, as suggested by a recent twin study showing moderate, but significant, genetic correlation between MDD and T2D in two Nordic samples, especially in females (11). Despite our limited understanding of the etiology of depression, previous research suggests that common biological pathways for both disorders may relate to functions of the hypothalamic–pituitary–adrenocortical axis, autonomic nervous system, cytokine-mediated inflammatory response, or aberrations in circadian rhythms (35,36). Moreover, it has been proposed that the reciprocal relationship between depressive symptoms and T2D may be mediated by behavioral risk factors that are partially affected by genetic makeup, such as eating habits and life-style choices (37).

To get better insight into the possible biological processes and shared genomic background linking depressive symptoms and glycemic traits, we next identified potential pleiotropic loci between the traits of interest. We observed potential pleiotropic loci for depressive symptoms and T2D in the IGF2BP2 and CDKAL1 genes and near the CDKN2B-AS and PLEKHA1 genes. Variants in the CDKAL1 and PLEKHA1 showed associations in the opposite direction with depressive symptoms and T2D. However, such findings are not uncommon: rs12720356 in the TMY2 is associated with both Crohn's disease and psoriasis, yet the G allele increases risk for Crohn's disease and decreases risk for psoriasis (38,39). Similarly, Sirota and colleagues (40) show cross-autoimmune diseases that opposite effects are frequent. These results address the fact that biological processes underlying phenotypic correlation may operate in both directions. Moreover, depression is a heterogeneous disorder and it is possible that some subtypes of depression show associations with genomic variation opposite to the others: McCaffrey and colleagues (41) recently tried to replicate the most significant signals in the study by He et al. (22) and found that of the eight top SNPs, three associated nominally with depressive symptoms in the opposite direction as in the original study in a sample of overweight/obese subjects with T2D. Of identified variants, CDKAL1 and PLEKHA1 have been implicated not only in T2D and metabolic traits (9,42) but also in neurodevelopmental traits. IGF2BP2 variants have been implicated in schizophrenia (43) and CDKN2B-AS variants in Parkinson's and Alzheimer's diseases, albeit showing only modest association with cognitive function (44). In the same study, no significant associations were found between CDKAL1 variants and Parkinson's and Alzheimer's diseases (44). However, CDKAL1 was 1 of the 226 genes associated with bipolar disorder in a gene-based meta-analyses of four studies (45), and in another study, CDKAL1 harbored an SNP with potential association with externalizing symptoms in bipolar disorder (rs17622252; p = 4.39 × 10^{-6}) (46). Interestingly, the minor allele of our top SNP rs10510110, an intergenic variant near PLEKHA1, was associated with down-regulated FGFR2 expression in substantia nigra. FGFR2 abnormalities have been linked with aberrant glia cell function (47), and MDD patients have shown down-regulated FGFR2 in several brain regions (48). Moreover, an SNP in FGFR2 has been implicated in bipolar disorder (49). Although associated with neurodevelopmental traits, these intronic variants in the IGF2BP2 and CDKAL1 genes and intergenic variants near the CDKN2B-AS and PLEKHA1 genes are novel in relation to depressive symptoms. Interestingly, all the potentially pleiotropic variants that associated with depressive symptoms and T2D altered an expression of genes in various regions of the brain. Future research should focus on those associations and the biological relevance of those SNPs on depression.

For depressive symptoms and fasting glucose, we identified potential pleiotropic loci in the MADD and in PEX16 genes as well as close to the CDKN2B-AS and MTNR1B genes, all novel in relation to depressive symptoms. Importantly, variants in the PEX16 and MTNR1B showed associations in the opposite direction with depressive symptoms and fasting glucose. MADD has been associated with fasting glucose (23), insulin (50), and high-density lipoprotein values (51), but its variation also influences apoptosis and it plays an essential role in Ca^{2+}-dependent neurotransmitter release (52). Recent studies reported up-regulation of PEX16 in patients with chronic fatigue syndrome (CFS) (53,54), and one study showed that allelic distribution of rs3802758 in PEX16 differed between those with CFS and healthy controls (55). This is interesting because CFS often show symptoms typical to depression such as lack of energy and sleep problems (56). Genetic variation in the PEX16 gene has also been associated with fatty acid oxidation (57). The nearest gene to rs6483221 is melatonin receptor 1B gene (MTNR1B). Genetic variation in the MTNR1B gene has been associated not only with T2D-related outcomes (58) but also with sleep (59) and recurrent MDD (60). A possibility that variation in the MTNR1B gene could affect both depression and T2D through effects on circadian regulation also exists (61,62).

Together, these data could suggest that genetic variation in the IGF2BP2, CDKAL1, CDKN2B-AS, MADD, PEX16, and MTNR1B genes may be associated with both metabolic traits and symptoms of depression. Variation in the PLEKHA1 gene could modulate expression of the FGFR2 gene, which, in turn, could influence the development of neurodevelopmental diseases. The direction of the association, however, can vary and risk SNPs associated with T2D can show protection against depressive symptoms as seen for SNPs in the IGF2BP2 and in CDKN2B-AS genes. A similar situation was observed for the obesity-associated rs9939609 A-variant in the FTO gene, which has also been associated with reduced risk of MDD (63).

There are some limitations of our study. First, heterogeneity of the phenotypes may alleviate our ability to detect genomic risk loci. In addition, combining results from meta-analyses misses several SNPs, although after quality control, the number of SNPs remained between 2,351,566 and 2,390,179, which should provide adequate genomic coverage. We lacked data sets for replication, and we were not able to test these differences separately in men and
women. Future studies should replicate our findings in larger data sets with higher genotypic resolution. Moreover, these analyses should be run separately in males and females. Not only may the factors that influence the association between depression and T2D or glycemic traits differ, but also women may be more vulnerable to comorbid depression and T2D (64). Despite these limitations, we showed that in the currently existing GWAS data sets, no overall genetic relation exists between depressive symptoms and glycemic traits. However, we were able to identify several SNPs that showed potential pleiotropy by being associated both with depressive symptoms and T2D and glycemic traits. Understanding of the comorbidity may help in classifying biologically more homogenous subtypes of depression and T2D.

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REFERENCES


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