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An Analysis of Two Genome-wide Association Meta-analyses Identifies a New Locus for Broad Depression Phenotype

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ABSTRACT

BACKGROUND: The genetics of depression has been explored in genome-wide association studies that focused on either major depressive disorder or depressive symptoms with mostly negative findings. A broad depression phenotype including both phenotypes has not been tested previously using a genome-wide association approach. We aimed to identify genetic polymorphisms significantly associated with a broad phenotype from depressive symptoms to major depressive disorder.

METHODS: We analyzed two prior studies of 70,017 participants of European ancestry from general and clinical populations in the discovery stage. We performed a replication meta-analysis of 28,328 participants. Single nucleotide polymorphism (SNP)-based heritability and genetic correlations were calculated using linkage disequilibrium score regression. Discovery and replication analyses were performed using a p-value-based meta-analysis. Lifetime major depressive disorder and depressive symptom scores were used as the outcome measures. **RESULTS:** The SNP-based heritability of major depressive disorder was 0.21 (SE 5 0.02), the SNP-based heritability of depressive symptoms was 0.04 (SE 5 0.01), and their genetic correlation was 1.001 (SE 5 0.2). We found one genome-wide significant locus related to the broad depression phenotype (rs9825823, chromosome 3: 61,082,153, p 5 8.2 3 10⁻⁹) located in an intron of the FHIT gene. We replicated this SNP in independent samples (p 5 .02) and the overall meta-analysis of the discovery and replication cohorts (1.0 3 10⁻⁹).

CONCLUSIONS: This large study identified a new locus for depression. Our results support a continuum between depressive symptoms and major depressive disorder. A phenotypically more

inclusive approach may help to achieve the large sample sizes needed to detect susceptibility loci for depression.

The etiology of depression—a worldwide leading cause of disability (1)—is not well understood. As indicated by family, twin, and adoption studies, genetic factors mediate part of vulnerability to major depressive disorder (MDD) with a modest heritability of around 40% (2). However, we understand little of the specific genetic architecture of MDD. Multiple genome-wide association studies (GWASs) for MDD have been published (3–10). The largest MDD GWAS was the mega-analysis by the MDD Working Group of the Psychiatric Genomics Consortium (PGC). In that study, more than 9000 MDD cases and 9500 control subjects were analyzed, but no association with MDD reached genome-wide significance (7). Recently, the CONVERGE (China, Oxford, and VCU Experimental Research on Genetic Epidemiology) consortium identified two genome-wide significant associations in 5303 Chinese women with severe and recurrent MDD (near the SIRT1 gene, $p = 5.2 \times 10^{-10}$, and in an intron of the LHPP gene, $p = 6.45 \times 10^{-12}$) (11). A GWAS of depressive symptoms (23%–29% heritability) (12,13) in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium in approximately 50,000 people from the general population found no genome-wide significant associations (14). Owing to the relatively small sample sizes, the previous GWASs of depressive disorders and depressive symptoms were arguably underpowered to detect small genetic effects (15,16).

Depression can be conceptualized along a continuum of severity from subthreshold or minor depression to MDD of varying severity (e.g., mild, moderate, severe) (17). Using a continuum approach may augment statistical power because sample size can be increased substantially and patients who fall into the gray area can be assessed. Several lines of evidence support a depression continuum. In longitudinal studies, there is an increased risk of MDD in patients with minor depression and subthreshold depression (18,19). Statistical studies of disorder classification (taxometric) suggested that severity of depression is continuously distributed and that there is no discontinuity in the latent structure of depression (19,20). Family studies report that relatives of probands with milder forms of depression have greater risk of MDD compared with relatives of probands without any mood disorders (21–24). A higher number of depressive symptoms is related to greater disability, worse quality of life, and higher mortality risk (18,25–29). MDD and continuous measures of depression are highly correlated, and severity of depressive symptoms along the continuum is linear (30,31).

The goal of the current study was to combine the results of the largest GWAS using categorical lifetime MDD and continuous measures of depression to identify genetic variants underlying the entire depression continuum.

METHODS AND MATERIALS

Study Design and Samples

This study was a collaboration between investigators on the PGC MDD and CHARGE genome-wide association meta-analyses (GWAMA). In the discovery phase, we aggregated two GWAMAs published in 2013 (7,14). Basic descriptive features and phenotype definitions of the contributing samples are provided in Supplemental Table S1. The mega-analysis of MDD consisted of nine studies of 9240 cases meeting international criteria for lifetime MDD and 9519 healthy control subjects. The CHARGE meta-analysis of depressive symptoms included 22 cohorts and comprised 51,258 persons. Each cohort contributing to the GWAMA of the PGC and CHARGE was distinct. In the replication analyses, 16 case-control studies with DSM-IV MDD (6718 cases and 13,453 control subjects) were

included along with 8157 subjects from the general population with assessment of depressive symptoms. All subjects were of European ancestry. Institutional review boards approved all studies, and all participants provided written informed consent.

Phenotype Characteristics

In the PGC GWAMA, MDD was established with structured clinical interviews (e.g., Clinical Interview Schedule–Revised, Diagnostic Interview for Genetic Studies, and Structured Clinical Interview for DSM-IV). All clinical evaluations were made by experienced clinicians or interviewers. Most cases were ascertained from clinical sources. Control subjects were screened in most of the studies to require the absence of MDD and were recruited from the general population. Full details about the PGC samples can be found in the previous publication (7). In the CHARGE GWAMA, depressive symptoms were assessed with validated questionnaires. Measures include the Center for Epidemiological Studies–Depression scale, Geriatric Depression Scale, Patient Health Questionnaire-9, and Beck Depression Inventory-II, mostly assessing depressive symptoms during previous weeks rather than lifetime MDD (14). Persons with schizophrenia, bipolar disorder, or dementia were excluded. Persons aged 40 years or older with genotype data and depressive symptom scores were included.

The 16 MDD case-control replication samples were part of an expanded but unpublished PGC MDD analysis. MDD was diagnosed with interviews. In the depressive symptom replication cohort, the Health and Retirement Study, the 8-item Center for Epidemiological Studies–Depression scale was applied. Respondents were excluded if they were under 40 years of age or displayed evidence of cognitive impairment.

Genotyping and Imputation

In the PGC samples (Supplemental Table S1), individual genotypes were assembled, processed through a central quality control pipeline, and imputed using the CEU (Central Europe) and TSI (Toscani in Italy) HapMap3 reference panels. Quality control procedures were extensive (7). In the CHARGE cohorts, genotype quality control and imputation were conducted in each study separately. The imputation reference was the HapMap2 CEU panel (14). In the MDD replication cohorts (Supplemental Table S3), imputation was performed using IMPUTE2 or SHAPEIT (chunk size of 3 Mb and default parameters). The imputation reference set consisted of 2186 phased haplotypes from the 1000 Genomes Project. In the Health and Retirement Study, imputation was performed using the HapMap2 CEU reference panel.

Statistical Analyses

Linkage disequilibrium score regression was used to compute the single nucleotide polymorphism (SNP)-based heritability and the genetic correlation using the 1000 Genomes CEU reference panel (32).

In the PGC GWAMA, a logistic regression analysis was used to test the association between MDD and imputed SNP dosages under an additive model and adjusting for study indicators and five principal components (7). In the CHARGE GWAMA, a linear regression analysis was applied to test the association of depressive symptom score on imputed SNP dosages in the contributing studies adjusting for age and sex. Analyses were adjusted for principal components for most, but not all, cohorts in the CHARGE GWAMA. A p-value-based meta-analysis was applied in the CHARGE GWAMA (14). Effect size estimates were based on a dichotomous outcome in the PGC and on a continuous outcome in the CHARGE GWAMA. To combine these effect estimates, a p-value-based meta-analysis weighted by sample size with METAL (<http://www.sph.umich.edu/csg/abecasis/metal/>) was used.

This method allows different weights for each study and takes into account the direction of effect at each SNP (33). To specify the direction of the effect, the PGC used the logistic regression coefficient beta and the CHARGE used z scores. Weights were based on the number of MDD cases in the PGC study (n = 9240), and the number of individuals in the CHARGE with clinically significant depressive symptoms (n = 5976) using population-specific cutoff scores of the questionnaires was considered for weighting. To test whether the results are affected by different sample size weightings, equal weights per study, or no weight as suggested by Stouffer et al. (34), we carried out a series of sensitivity analyses.

We selected the genome-wide significant SNPs in two loci from the discovery stage for replication. After analyzing these data, we performed a p-value-based meta-analysis combining all replication samples. Furthermore, we analyzed the results of the discovery and all replication samples weighting for number of cases.

RESULTS

In the discovery stage, we performed a GWAMA in 70,017 participants of European ancestry by combining the PGC MDD (7) and CHARGE GWAMA (14). We applied a linkage disequilibrium score regression to the summary statistics from each study to compute the SNP-based heritabilities and the genetic correlation. As reported previously (35), the SNP-based liability scale heritability of MDD was 0.2 (SE = 0.02) for 20% of prevalence. The lambda was 1.1 and the regression intercept was 1.0 (SE = 0.01). The SNP-based heritability of depressive symptoms was 0.04 (SE = 0.01). The lambda was 1.1 and the regression intercept was 1.0 (SE = 0.01). The SNP-based heritability of the broad depression phenotype was 0.3 (SE = 0.04). MDD and depressive symptoms showed significant coheritability (1.001, SE = 0.2, z score = 4.6, p = 4.6 × 10⁻⁶). This result supports the contention of a continuum between depressive symptoms and MDD. However, the genetic correlation should be interpreted carefully because linkage disequilibrium regression is quite sensitive to environmental confounding and, like twin studies, often lacks precision. In addition, different evaluation methods of the depression phenotypes might cause different genetic correlation estimates that cannot easily be compared.

We conducted a meta-analysis of the PGC MDD and the CHARGE depressive symptoms GWAMA using a weighted, p-value-based meta-analysis. The results are summarized in Figure 1 and Supplemental Figures S1 to S3. The combined meta-analysis was conducted for 918,921 SNPs. Two loci were genome-wide significant: an SNP in an intron of the FHIT gene (rs9825823, chromosome 3: 61,082,153, p = 8.2 × 10⁻⁹) and an SNP in an intron of PLEK2 (rs9323497, chr14: 67,873,128, p = 3.3 × 10⁻⁸) (Table 1). All SNPs with a p value of association, $\leq 3 \times 10^{-5}$ are presented in Supplemental Table S2. Using different weights or Stouffer's unweighted method had only slight effects on the results (data not shown). Supplemental Figures S4 and S5 show forest plots for two SNPs shown in Table 1.

Table 2 presents the replication analyses and the meta-analysis of discovery and replication results. One of the genome-wide significant variants within the FHIT gene (rs9825823) was associated with the depression continuum in the replication cohorts (z score = 2.4, p = .02). The result of the final meta-analysis of discovery and replication samples also indicated a positive replication as indexed by a lower p value (z score = 6.1, p = 1.0 × 10⁻⁹). This SNP had a positive association with depressive symptoms in the CHARGE study (p = 5.5 × 10⁻⁴), and a similar pattern was observed in the PGC study (p = 4.1 × 10⁻⁶). The SNP in an intron of PLEK2 (rs9323497) was not related to the depression continuum significantly (z score = 0.2, p = .90).

We performed an additional replication analysis of our two genome-wide significant SNPs using the publicly available data of the recently published GWAMA of depressive disorders in a sample of Chinese women (the CONVERGE study) (11). In CONVERGE, rs9825823 (odds ratio 5 1.01, $p = .12$) and rs9323497 (odds ratio 5 0.97, $p = .0002$, with a different direction of association than in our discovery sample) were not related to depression at the genome-wide significance level, although the latter reached nominal significance. However, in the joint meta-analysis of the Health and Retirement Study, the PGC MDD study, and CONVERGE study, we found that the association between rs9825823 and the depression continuum (z score 5 2.85, $p = .004$) was slightly stronger than our initial replication analysis. When these replication and discovery samples were combined, the association with our top hit also became stronger (analysis without the CONVERGE data: z score 5 6.1, $p = 1.3 \times 10^{-9}$; analysis with the CONVERGE data: z score 5 6.2, $p = 6.8 \times 10^{-10}$). Results of additional replication analyses are given in Supplemental Table S4.

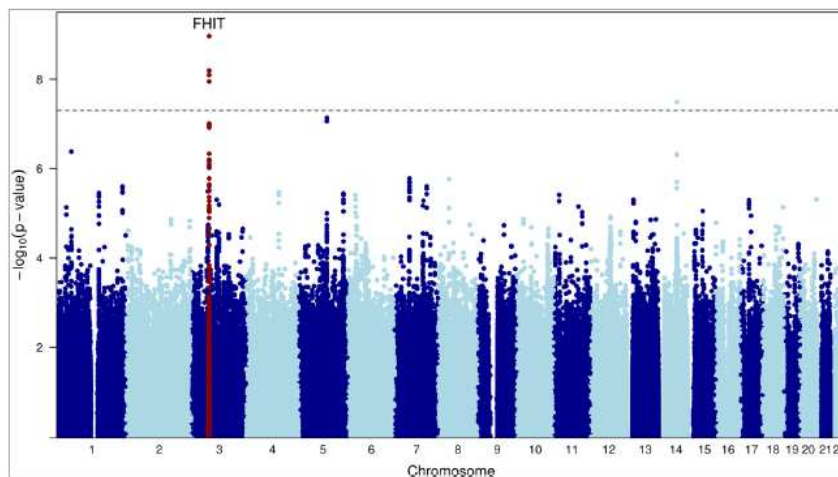


Figure 1. Manhattan plot. The x axis represents the chromosomal position for each SNP, and the y axis represents the $-\log_{10} p$ value for association with depression.

DISCUSSION

We report the results of a combined GWAMA of the depression continuum including MDD (18,759 cases and control subjects) and depressive symptoms (51,258 participants). In the discovery stage, we found genome-wide significant associations in the FHIT and PLEK2 genes. One SNP in the intron of the FHIT gene showed a significant association in the combined analysis of discovery and replication samples of MDD and depressive symptoms samples, and it exceeded a genome-wide significance threshold.

The significant locus (rs9825823, chr3: 61,082,153) maps to the intronic region of the FHIT gene, a tumor suppressor protein implicated in several cancers (36). FHIT is expressed in multiple brain regions (amygdala, anterior cingulate cortex, caudate nucleus, prefrontal cortex, hippocampus, and hypothalamus; <http://www.gtexportal.org/home/gene/FHIT>). It plays an important role in oxidative stress and level of DNA damage (37), biological processes implicated in MDD (38,39).

FHIT is a circadian clock modifier gene (40) and has been related to daytime sleepiness (41), which may be salient to the etiology of depression.

In a GWAS of recurrent, early-onset MDD, three SNPs located in the FHIT gene were among the strongest associations in the overall and sex-stratified analyses (8), although none had genome-wide significance. Genetic variants located in FHIT have been reported in genetic studies of anxiety (42),

autism (43), mental stress (44), comorbid depressive syndromes and alcohol dependence (45), citalopram-induced side effects (46), and a latent class analysis of MDD symptoms (7), but none has met genome-wide significance.

Table 2. Replication Analyses and Final Meta-analysis of Discovery and Replication Stages

| SNP | Chr:BP | Allele | PGC Replication (N = 20,171) | | HRS Replication (N = 8157) | | Overall Replication (N = 28,328) | | Meta-analysis of Discovery and Two Replication Samples (N = 98,345) | | | |
|-----------|---------------|--------|---------------------------------|-----|-------------------------------|-----|-------------------------------------|------|--|---------------------|---------|----------------------|
| | | | Direct ^a | p | Direct | p | Direct ^b | p | MAF | Direct ^c | z Score | p |
| rs9825823 | 3:61,082,153 | T/C | +++--+++++ | .04 | + | .20 | ++ | 0.02 | 0.46 | +++ | 6.10 | 1.0×10^{-9} |
| rs9323497 | 14:67,873,128 | T/C | +++--+++++ | .80 | - | .80 | +- | 0.9 | 0.05 | --- | 4.61 | 4.0×10^{-6} |

Allele: minor/major on the + strand.
 BP, base pair; Chr, chromosome; Direct, direction; HRS, Health and Retirement Study; MAF, minor allele frequency (GRCh37/hg19); PGC, Psychiatric Genomics Consortium; SNP, single nucleotide polymorphism.
^aOrder of the studies: Cognitive Function and Mood Study (CoFaMS), Psychiatric Arm of the Population-based CoLaus (PsyCoLaus) Study, MDD2000–Edinburgh cases, GENPOD/NEWMEDS, Depression Genes and Networks (DGN), Genetics of Recurrent Early-Onset Depression Phase II (GenRED2), Harvard i2b2, Janssen, Max Planck Institute of Psychiatry (MPIP) Munich Antidepressant Response Signature (MARS) Study, OMNlex, Queensland Institute of Medical Research (QIMR)–COEX, RADIANT Irish cases, RADIANT U.S. cases, RADIANT Denmark cases, Roche Study, Study of Health in Pomerania (SHIP)–TREND, TwinGene.
^bOrder of the studies: PGC Replication, Health and Retirement Study.
^cOrder of the studies: PGC Replication, Health and Retirement Study, combined meta-analysis of discovery samples.

Several methodological aspects should be discussed. First, we evaluated the depression continuum by combining cases from clinical populations diagnosed with MDD and participants from the general population who had been assessed for depressive symptoms. Such an inclusive approach may increase heterogeneity of the phenotype especially because lifetime MDD was evaluated, whereas depressive symptoms indicate past weeks only. If anything, such an approach would cause an underestimation of the effects because less information on depressive symptoms was obtained. However, the advantages of a large sample can outweigh the disadvantages of a less precisely defined phenotype. This has been observed in the GWAS of educational attainment that was successfully used as a proxy for intelligence (47). Our additional replication analysis showed that increasing the sample size yielded a stronger association of the top hit with the depression continuum. It is complex to calculate statistical power of the current analysis because quantitative and qualitative measures were combined. In the current study, a genetic association with the depression continuum may reflect an effect on broad depressive phenotypes but could also be accounted for by an association with low levels of general well-being (12%–18% heritability) that co-occur with depressive symptoms (48). Second, we used a p-value-based meta-analysis because effect estimates could not be directly evaluated in a straight-forward manner. Third, the heterogeneity of the imputation methods used in the PGC and CHARGE discovery samples might reduce the statistical power. However, different imputation references did not change the results in the published PGC MDD study (7).

In conclusion, in this large GWAMA of a broad depression phenotype, we detected a locus associated with depression in clinical and general population samples. Our results suggest the importance of a broader depression phenotype to identify genetic variants underlying depression. Large samples with different depression phenotypes may also help to disentangle the genetic background of different forms of depression.

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REFERENCES

1. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. (2012): Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: A systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 380:2197–2223.
2. Sullivan PF, Neale MC, Kendler KS (2000): Genetic epidemiology of major depression: Review and meta-analysis. *Am J Psychiatry* 157:1552–1562.
3. Kohli MA, Lucae S, Saemann PG, Schmidt MV, Demirkan A, Hek K, et al. (2011): The neuronal transporter gene SLC6A15 confers risk to major depression. *Neuron* 70:252–265.
4. Lewis CM, Ng MY, Butler AW, Cohen-Woods S, Uher R, Pirlo K, et al. (2010): Genome-wide association study of major recurrent depression in the U.K. population. *Am J Psychiatry* 167:949–957.
5. Muglia P, Tozzi F, Galwey NW, Francks C, Upmanyu R, Kong XQ, et al. (2010): Genome-wide association study of recurrent major depressive disorder in two European case–control cohorts. *Mol Psychiatry* 15:589–601.

6. Rietschel M, Mattheisen M, Frank J, Treutlein J, Degenhardt F, Breuer R, et al. (2010): Genome-wide association-, replication-, and neuro-imaging study implicates HOMER1 in the etiology of major depression. *Biol Psychiatry* 68:578–585.
7. Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, Ripke S, Wray NR, Lewis CM, Hamilton SP, Weissman MM, et al. (2013): A mega-analysis of genome-wide association studies for major depressive disorder. *Mol Psychiatry* 18:497–511.
8. Shi J, Potash JB, Knowles JA, Weissman MM, Coryell W, Scheftner WA, et al. (2011): Genome-wide association study of recurrent early-onset major depressive disorder. *Mol Psychiatry* 16:193–201.
9. Shyn SI, Shi J, Kraft JB, Potash JB, Knowles JA, Weissman MM, et al. (2011): Novel loci for major depression identified by genome-wide association study of Sequenced Treatment Alternatives to Relieve Depression and meta-analysis of three studies. *Mol Psychiatry* 16: 202–215.
10. Sullivan PF, de Geus EJ, Willemsen G, James MR, Smit JH, Zandbelt T, et al. (2009): Genome-wide association for major depressive disorder: A possible role for the presynaptic protein piccolo. *Mol Psychiatry* 14:359–375.
11. CONVERGE consortium (2015): Sparse whole-genome sequencing identifies two loci for major depressive disorder. *Nature* 523:588–591.
12. Jansson M, Gatz M, Berg S, Johansson B, Malmberg B, McClearn GE, et al. (2004): Gender differences in heritability of depressive symptoms in the elderly. *Psychol Med* 34:471–479.
13. Johnson W, McGue M, Gaist D, Vaupel JW, Christensen K (2002): Frequency and heritability of depression symptomatology in the second half of life: Evidence from Danish twins over 45. *Psychol Med* 32:1175–1185.
14. Hek K, Demirkan A, Lahti J, Terracciano A, Teumer A, Cornelis MC, et al. (2013): A genome-wide association study of depressive symptoms. *Biol Psychiatry* 73:667–678.
15. Wray NR, Pergadia ML, Blackwood DH, Penninx BW, Gordon SD, Nyholt DR, et al. (2012): Genome-wide association study of major depressive disorder: New results, meta-analysis, and lessons learned. *Mol Psychiatry* 17:36–48.
16. Levinson DF, Mostafavi S, Milaneschi Y, Rivera M, Ripke S, Wray NR, et al. (2014): Genetic studies of major depressive disorder: Why are there no genome-wide association study findings and what can we do about it? *Biol Psychiatry* 76:510–512.
17. Lobo DM, Agius M (2012): The mental illness spectrum. *Psychiatria Danubina* 24(suppl 1):S157–S160.
18. Cuijpers P, de Graaf R, van Dorsselaer S (2004): Minor depression: Risk profiles, functional disability, health care use, and risk of developing major depression. *J Affect Disord* 79:71–79.
19. Cuijpers P, Smit F (2004): Subthreshold depression as a risk indicator for major depressive disorder: A systematic review of prospective studies. *Acta Psychiatrica Scandinavica* 109:325–331.
20. Prisciandaro JJ, Roberts JE (2009): A comparison of the predictive abilities of dimensional and categorical models of unipolar depression in the National Comorbidity Survey. *Psychol Med* 39:1087–1096.

21. Lewinsohn PM, Klein DN, Durbin EC, Seeley JR, Rohde P (2003): Family study of subthreshold depressive symptoms: Risk factor for MDD? *J Affect Disord* 77:149–157.
22. Remick RA, Sadovnick AD, Lam RW, Zis AP, Yee IM (1996): Major depression, minor depression, and double depression: Are they distinct clinical entities? *Am J Med Genet* 67:347–353.
23. Kessler RC, Zhao S, Blazer DG, Swartz M (1997): Prevalence, correlates, and course of minor depression and major depression in the National Comorbidity Survey. *J Affect Disord* 45:19–30.
24. Chen LS, Eaton WW, Gallo JJ, Nestadt G, Crum RM (2000): Empirical examination of current depression categories in a population-based study: Symptoms, course, and risk factors. *Am J Psychiatry* 157: 573–580.
25. Cuijpers P, Vogelzangs N, Twisk J, Kleiboer A, Li J, Penninx BW (2013): Differential mortality rates in major and subthreshold depression: Meta-analysis of studies that measured both. *Br J Psychiatry* 202:22–27.
26. Ayuso-Mateos JL, Nuevo R, Verdes E, Naidoo N, Chatterji S (2010): From depressive symptoms to depressive disorders: The relevance of thresholds. *Br J Psychiatry* 196:365–371.
27. Sakashita C, Slade T, Andrews G (2007): Empirical investigation of two assumptions in the diagnosis of DSM-IV major depressive episode. *Aust N Z J Psychiatry* 41:17–23.
28. de Graaf LE, Huibers MJ, Cuijpers P, Arntz A (2010): Minor and major depression in the general population: Does dysfunctional thinking play a role? *Compr Psychiatry* 51:266–274.
29. Backenstrass M, Frank A, Joest K, Hingmann S, Mundt C, Kronmuller KT (2006): A comparative study of nonspecific depressive symptoms and minor depression regarding functional impairment and associated characteristics in primary care. *Compr Psychiatry* 47:35–41.
30. Radloff LS (1977): The CES-D Scale: A self-report depression scale for research in the general population. *Appl Psychol Meas* 1:385.
31. Rakofsky JJ, Schettler PJ, Kinkead BL, Frank E, Judd LL, Kupfer DJ, et al. (2013): The prevalence and severity of depressive symptoms along the spectrum of unipolar depressive disorders: A post hoc analysis. *J Clin Psychiatry* 74:1084–1091.
32. Bulik-Sullivan BK, Loh PR, Finucane HK, Ripke S, Yang J, Schizophrenia Working Group of the Psychiatric Genomics Consortium, et al. (2015): LD score regression distinguishes
33. Willer CJ, Li Y, Abecasis GR (2010): METAL: Fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 26: 2190–2191.
34. Stouffer S, Suchman E, DeVinnery L, Star S, Williams R (1949): *The American Soldier*, vol. 1: Adjustment During Army Life. Princeton, NJ: Princeton University Press, 56.
35. Cross-Disorder Group of the Psychiatric Genomics Consortium, Lee SH, Ripke S, Neale BM, Faraone SV, Purcell SM, et al. (2013): Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nat Genet* 45:984–994.
36. Wali A (2010): FHIT: Doubts are clear now. *Scientific World J* 10: 1142–1151.
37. Karras JR, Paisie CA, Huebner K (2014): Replicative stress and the FHIT gene: Roles in tumor suppression, genome stability and prevention of carcinogenesis. *Cancers* 6:1208–1219.

38. Jorgensen A, Krogh J, Miskowiak K, Bolwig TG, Kessing LV, Fink-Jensen A, et al. (2013): Systemic oxidatively generated DNA/RNA damage in clinical depression: Associations to symptom severity and response to electroconvulsive therapy. *J Affect Disord* 149: 355–362.
39. Lopresti AL, Maker GL, Hood SD, Drummond PD (2014): A review of peripheral biomarkers in major depression: The potential of inflammatory and oxidative stress biomarkers. *Prog Neuropsychopharmacol Biol Psychiatry* 48:102–111.
40. Byrne EM, Heath AC, Madden PA, Pergadia ML, Hickie IB, Montgomery GW, et al. (2014): Testing the role of circadian genes in conferring risk for psychiatric disorders. *Am J Med Genet B Neuro-psychiatr Genet* 165B:254–260.
41. Gottlieb DJ, O'Connor GT, Wilk JB (2007): Genome-wide association of sleep and circadian phenotypes. *BMC Med Genet* 8(suppl 1):S9.
42. Luciano M, Huffman JE, Arias-Vasquez A, Vinkhuyzen AA, Middeldorp CM, Giegling I, et al. (2012): Genome-wide association uncovers shared genetic effects among personality traits and mood states. *Am J Med Genet B Neuropsychiatr Genet* 159B:684–695.
43. Tsang KM, Croen LA, Torres AR, Kharrazi M, Delorenze GN, Windham GC, et al. (2013): A genome-wide survey of transgenerational genetic effects in autism. *PLoS One* 8:e76978.
44. Nikpay M, Seda O, Tremblay J, Petrovich M, Gaudet D, Kotchen TA, et al. (2012): Genetic mapping of habitual substance use, obesity-related traits, responses to mental and physical stress, and heart rate and blood pressure measurements reveals shared genes that are overrepresented in the neural synapse. *Hypertens Res* 35:585–591.
45. Edwards AC, Aliev F, Bierut LJ, Bucholz KK, Edenberg H, Hesselbrock V, et al. (2012): Genome-wide association study of comorbid depressive syndrome and alcohol dependence. *Psychiatr Genet* 22:31–41.
46. Adkins DE, Clark SL, Aberg K, Hettema JM, Bukszar J, McClay JL, et al. (2012): Genome-wide pharmacogenomic study of citalopram-induced side effects in STAR*D. *Transl Psychiatry* 2:e129.
47. Rietveld CA, Medland SE, Derringer J, Yang J, Esko T, Martin NW, et al. (2013): GWAS of 126,559 individuals identifies genetic variants associated with educational attainment. *Science* 340:1467–1471.
48. Rietveld CA, Cesarini D, Benjamin DJ, Koellinger PD, De Neve JE, Tiemeier H, et al. (2013): Molecular genetics and subjective well-being. *Proc Natl Acad Sci USA* 110:9692–9697.