The PCLO gene and depressive disorders: replication in a population-based study

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Previous genome-wide association analysis revealed a new putative candidate gene for major depression: the PCLO gene. Replication in one population-based cohort did not yield genome-wide significance and further replication efforts in clinical studies were unsuccessful. We aimed to validate the association of single-nucleotide polymorphism (SNP) rs2522833 in the PCLO gene with depression in the Rotterdam Study, a prospective population-based cohort of elderly persons. In the Rotterdam Study, we identified 579 persons with a broad depression phenotype (depressive syndromes) of whom 178 cases with DSM-defined depressive disorders. The control group consisted of 912 persons free of depression during the follow-up period and in their histories. Logistic regression analysis showed an association between rs2522833 and depressive disorders ($P = 0.0025$). However, no association between the broader depressive syndrome group and this SNP was observed ($P = 0.20$). A meta-analysis combining all studies from the original publication and our study yielded a $P$-value of $2.16 \times 10^{-3}$ for the association between SNP rs2522833 and depressive disorders. However, as in the previous publication, high heterogeneity between studies was observed. Thus, a meta-analysis with the findings from three population-based studies was performed. This demonstrated a genome-wide significant $P$-value ($P = 1.93 \times 10^{-9}$). In conclusion, this study provides additional evidence for an association between PCLO and depressive disorders in a population-based study; no association with a broader syndromal phenotype was observed.

INTRODUCTION

Depression is a common mental illness characterized by persistent loss of interest or depressed mood. Familial aggregation of depression and its heritability of 31–42% suggest a genetic role in the aetiology of depression (1). Although many candidate genes have been tested, only six have been replicated (2,3). Genome-wide association studies might help to identify new candidate genes for depression. So far, two genome-wide studies on major depressive disorder were performed (4,5). One putative candidate gene, PCLO, was identified (4).

This finding was replicated in one population-based study. However, in a joint meta-analysis genome-wide significance was not reached and the result could not be replicated in five clinical populations (4). A recent joint reanalysis of 29 correlated single-nucleotide polymorphisms (SNPs) in the PCLO region in the original and the replication study supported the role of PCLO as a causal risk factor for major depression (6).

The goal of the current study was to validate the association of depression with the SNP rs2522833 in the PCLO gene, in the population-based Rotterdam Study (7). In addition, we report the result of meta-analyses summarizing our result and the original findings.

RESULTS

Study population

The current study was set in the Rotterdam Study, a prospective population-based cohort of elderly persons. The study
Population consisted of all genotyped participants (n = 5974) with valid depression data. SNP rs2522833 was successfully genotyped in 5968 persons. The genotype frequency for this SNP was in Hardy–Weinberg equilibrium (pHWE = 0.57).

Depression cases were ascertained by continuous monitoring of GP records, depression self-report and depression screens followed by clinical interviews during follow-up visits to the research center. We identified 579 persons with a depressive syndrome (minor depression by clinical interview, depression self-report with consult of a GP, other health specialist or anti-depressant treatment, diagnosis by GP) of whom 178 persons had a depressive disorder (145 major depression, 15 dysthymia and 18 depression not otherwise specified diagnosed by clinical interview or diagnosis by a psychiatrist). The control group consisted of 912 persons at low liability for a depressive disorder: they had no history of depressive disorders, syndromes and complaints; neither did they experience depression or depressive complaints during follow-up, and they scored in the lowest quartile for depression screening.

**PCLO SNP rs2522833 and depression**

The association of polymorphism rs2522833 with depressive disorder and syndromes was tested with logistic regression assuming an additive effect. Like Sullivan et al., we tested for the effect of the minor C allele. We first tested the association of SNP rs2522833 with the broad syndromal phenotype, also including self-reported depressions. No association of this syndromal phenotype with SNP rs2522833 was observed (P = 0.20). However, Sullivan et al. used a strict depression definition. Therefore, we restricted the case group to only those cases with Diagnostic and Statistical Manual of Mood Disorders (DSM)-defined depressive disorders. The association of depressive disorders with SNP rs2522833 was significant (P = 0.0025) (see Table 1). In addition, we further restricted the case group to those 145 cases with major depression, the case definition applied by Sullivan et al. This increased the strength of the association between depression and SNP rs2522833 [P = 0.0014, OR 1.50 (1.17–1.92)].

Like Sullivan et al., we examined other SNPs in the gene region. The Illumina array holds 73 SNPs in the PCLO gene with minor allele frequency above 5% (base 82 225 378 to 82 630 133 on chromosome 7). Thirty-seven SNPs (51%) had a significant association (P < 0.05) with DSM-IV depressive disorders (the association of each SNP in the PCLO region with depressive disorders is listed in Supplementary Material, Table S1). After adjusting for SNP rs2522833, only two SNPs remained independently and significantly associated with depression (results of this analysis for all SNPs in the PCLO region can be found in Supplementary Material, Table S1).

**Meta-analysis**

In addition, we performed a Z-score-based meta-analysis weighted by effective sample size. Combining results from all seven studies (NESDA-NTR, QIMR, Max-Planken Institute Psychiatry, West Germany, STAR-D, U Edinburgh and DeCC) from the publication of Sullivan et al., with results of the present study, yielded a P-value of 2.16 × 10⁻³. However, Sullivan et al. indicated high inter-study heterogeneity of the case groups. They showed in a principal component analysis that the Australian QIMR study was most similar to the original sample. The QIMR was the only study that, like the original sample and our study, ascertained their cases from the population. Extending the post hoc analyses from Sullivan et al., we thus performed a second meta-analysis. For this analysis, we included only the results of studies with population-based ascertainment of cases. The result of the original cohort (NESDA-NTR, Z = 5.01), a population-based replication cohort (QIMR, Z = 2.20) and the Z-score for the association with depressive disorders observed in our study (Z = 3.01) were included. This resulted in a genome-wide significant P-value of 1.93 × 10⁻⁷. Meta-analyzing the five clinical studies only, yielded a P-value of 0.39 for the association between SNP rs2522833 and major depressive disorders. The effect was in the opposite direction of the effect in the population-based studies.

We formally evaluated heterogeneity between studies by calculating Cochran’s Q and I². Combining all eight studies, 79% of all variation could be explained by heterogeneity Q = 32.90, df = 7, P < 0.0001, I² = 0.79), compared with only 31% when including the three population-based studies (Q = 2.90, df = 2, P = 0.23, I² = 0.31). The five clinical studies were most alike (Q = 2.92, df = 4, P = 0.57, I² = 0).

**DISCUSSION**

Candidate gene studies on major depression so far, have not been very successful (3,8). However, our results suggest that the PCLO gene may qualify as a new and replicated candidate gene for major depression. The PCLO protein (piccolo) is part of a pathway that was not investigated in any of the more than 100 candidate gene studies reviewed by Lopez-Leon et al. (3). This underlines the importance of further and larger genome-wide significant P-value of 1.93 × 10⁻⁷. Meta-analyzing the five clinical studies only, yielded a P-value of 0.39 for the association between SNP rs2522833 and major depressive disorders. The effect was in the opposite direction of the effect in the population-based studies.

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wide association studies to identify more putative genes and disease mechanisms (5).

Our results also highlight the importance of two features of successful replication that are particularly challenging for large-scale psychiatric genetic research, namely standardized clinical phenotype assessment and similar setting. Both have been pointed out previously as important criteria for positive replication (9), besides the existence of a real effect.

The semi-structured interview used in the Rotterdam Study, combining clinical expertise with standardized assessment, reduced misclassification of depressive disorders. With this approach, we successfully replicated the finding of Sullivan et al. Including syndromal depression more than doubled the case group, but syndromal depression was ascertained less rigorously and replication was unsuccessful.

Furthermore, it is important to note that Sullivan et al. confirmed their finding only in the one population-based sample (the Australian QIMR) and not in any of five clinical samples. Like Sullivan et al., we observed high heterogeneity between studies, which was substantially reduced when analyzing only the clinical or population-based samples. This suggests that it is more appropriate and informative to meta-analyze the three population-based studies and the five clinically based studies separately than to combine the results of the eight studies. In the population-based Rotterdam Study, we replicated the result of Sullivan et al. Combined with the original results from population-based studies, a genome-wide significant association between depressive disorders and SNP rs2522833 was observed. The meta-analysis of clinical studies did not show an association between major depressive disorders and this polymorphism. Sullivan et al. argued that cases, but not controls, were different between clinical and population-based samples. Case ascertainment from clinical settings may introduce confounding, for example by co-morbidity or by population stratification as was already discussed by Sullivan et al. In addition, selection bias or referral filters may explain case differences, for example cases from clinical studies are more often recurrent and typically have an earlier onset than cases from the population-based studies (4). Hence, the characteristics of depression observed in clinical studies and population-based studies may not be very similar, and may, in part, have a different genetic susceptibility.

In conclusion, within a population-based study, we validated the association between depressive disorder and the PCLO gene. Further research is required to elucidate the mechanism of PCLO function in depression.

**MATERIALS AND METHODS**

The study was embedded in the Rotterdam Study, a prospective population-based cohort of persons over 55 years. Ascertainment of depressive disorders and syndromes was described previously (10). Briefly, during follow-up visits, participants were screened for depression with the Center for Epidemiologic Studies Depression Scale (CES-D). Screen-positive persons (CES-D score ≥16) were invited for a semi-structured interview with the Present State Examination (PSE) by a clinician to diagnose current depression status. In addition, GP records and specialist letters were surveilled actively for the occurrence of depression. Furthermore, physicians conducted repeated interviews assessing self-reported history of depression and incidence of depression during the interval period between interviews.

DSM-defined depressive disorders were diagnosed only if assessed by a psychiatrist in routine care or with the PSE (major depression or dysthymia). Depressive syndromes comprised self-reported depression with consultation of a health professional, minor depression diagnosed with PSE and depression recorded by a GP or physician.

Genotype data on SNP rs2522833 was available from the Infinium II HumanHap550K Genotyping BeadChip® version 3 (Illumina) in 5968 persons from a total of 5974 persons with genotype data (genotyping and quality control previously described) (11). Genotyping was performed blind to case–control status. The minor allele frequency (MAF) of SNP rs2522833 in controls (0.43) matched HapMap CEU frequency (0.43) and the MAF in the controls of Sullivan et al. (0.43). We therefore did not further verify genotyping of SNP rs2522833 in an independent assay.

Of the 5968 successfully genotyped persons, 524 persons died before depression screening and 747 persons did not participate in depression screening. Five persons with bipolar disorder were also excluded from the analyses. Of the remainder, 178 persons were diagnosed with DSM-defined depression during follow-up (145 major depressions, 18 dysthymias and 15 depressions not otherwise specified), and 401 persons had a depressive syndrome. Past depression before baseline was not used to define a case, as this information is largely based on retrospective self-report and recall over a long period is not very reliable.

In the first analysis, DSM-defined depressive disorders and depressive syndromes were combined, in a second analysis only DSM-defined depressions were used as cases. In a third analysis, the case group was further restricted to major depression only. The control group for both analyses consisted of 912 persons at low liability of depression. We excluded persons from the control group with a self-reported history of depression (symptoms and syndromes, n = 695) or clinically relevant depressive complaints during follow-up (n = 714) and those without information on history of depression (n = 368). To mirror the control group of Sullivan et al., we also excluded persons based on CES-D scores. We included only persons scoring in the lowest 25% at depression screening. As 35% of the participants scored 0, we excluded the 1424 persons scoring above 0.

As mentioned above, we also excluded persons with bipolar disorder (n = 5) and neither cases nor controls had a diagnosis of schizophrenia. In contrast, a comorbid anxiety disorder which was assessed only during the last interview was no exclusion criterion. Again, this definition of the study population was in line with the report of Sullivan et al. Of the persons still alive and participating in the last interview 25 of 701 controls (3.6%) and 38 of 106 cases with depression (35.8%) had a prevalent anxiety disorder.

Genotype data on SNP rs2522833 was analyzed with logistic regression in SPSS. PLINK v.1.06 (12) was used to run a logistic regression analysis on all SNPs with a minor allele frequency greater than 5% in the PCLO region (base 82 225 378).
using logistic regression parameters to determine Cochran’s $Q$ and $I^2$.

SUPPLEMENTARY MATERIAL
Supplementary Material is available at HMG online.

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Conflict of Interest statement. None declared.

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