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Associations of *PER3* and *RORA* Circadian Gene Polymorphisms and Depressive Symptoms in Older Adults

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Abstract

Background—Depressive symptoms are common in older adults and associated with poor outcomes. While circadian genes have been implicated in depression, the relationship between circadian genes and depressive symptoms in older adults is unclear.

Methods—A cross-sectional genetic association study of 529 single nucleotide polymorphisms (SNPs) representing 30 candidate circadian genes was performed in two population-based cohorts: Osteoporotic Fractures in Men Study (MrOS, n=1270, age 76.58±5.61 years) and the Study of Osteoporotic Fractures (SOF) in women (n=1740, 84.05±3.53 years) and a meta-analysis was performed. Depressive symptoms were assessed with the Geriatric Depression Scale categorizing participants as having "none-few symptoms" (0-2), "some depressive symptoms" (>2<6), or "many depressive symptoms" (≥6).

Results—We found associations meeting multiple testing criteria for significance between the *PER3* intronic SNP rs12137927 and decreased odds of reporting "some depressive symptoms" in

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the SOF sample (OR 0.61, CI 0.48-0.78, df=1, Wald chi-square −4.04, p=0.000054) and the meta-analysis (OR 0.61, CI 0.48-0.78, z= −4.04, p=0.000054) and between the *PER3* intronic SNPs rs228644 (OR 0.74, CI 0.63-0.86, z= 3.82, p-value=0.00013) and rs228682 (OR 0.74, CI 0.86 0.63, z= 3.81, p-value=0.00014) and decreased odds of reporting "some depressive symptoms" in the meta-analysis compared to endorsing none-few depressive symptoms. The *RORA* intronic SNP rs11632098 was associated with greater odds of reporting "many depressive symptoms" (OR 2.16, CI 1.45-3.23, df=1, Wald chi-square 3.76, p=0.000168) in the men. In the meta analysis the association was attenuated and nominally significant (OR 1.63, CI 1.24-2.16, z=3.45, p=0.00056).

Conclusions—*PER3* and RORA may play important roles in the development of depressive symptoms in older adults.

Keywords

Depression; older adults; PER3; RORA; circadian; gene

Background

Depressive syndromes are common in older adults and represent a major public health concern. Major depressive disorder (MDD) is affects 3-10% of older adults in primary care settings (1-4) and "subthreshold" depressive syndromes (SubD, e.g. dysthymia, minor depression and subsyndromal depression) are even more common (9-24%) (3, 5-8). Both MDD and SubD are more prevalent in long-term care settings (9). Depression is a critical issue in this age group because of its association with adverse outcomes including functional impairment (10), medical illnesses (11), hospitalizations (12), and disability (13). In older adults first-line pharmacotherpies targeting monoamines often fail to adequately treat symptoms (14). A better understanding of the biological pathways contributing depression in older adults could lay the groundwork for the development of more effective treatment strategies such as novel interventions targeting other aspects of the underlying pathophysiology of depression. A better understanding of the role of contributing genes in the pathophysiology of depression could also help to identify subgroups of older adults at higher risk for depression who may be candidates for indicated prevention strategies.

Clinical and molecular studies support a role for chronobiological disturbances in the pathophysiology of depression (15-17). Circadian rhythm disturbances (e.g. disruptions in the daily cycles of body temperature, and rest and activity) occur in mood disorders (18, 19). Depression is associated with changes in protein expression in the superchiasmatic nucleus (SCN; the master circadian pacemaker) (20) and enzymes controlling catecholamine metabolism are regulated by circadian genes (21, 22). Associations between polymorphisms in the human circadian genes, *PER3 (23)*, *PER2* (24), *AANAT* (25), *CRY1* (26), *CRY2* (24), *NPAS2* (26), *ASMT* (27), *TIMELESS* (28), and *RORA* (29) and depression have been reported.

Aging is associated with changes in circadian rhythms (30-32). Few studies have investigated the relationship between chronobiological disturbances and depressive symptoms in older adults. We previously reported a cross-sectional graded association between desynchronization of circadian activity rhythms and greater levels of depressive

symptoms in a large cohort of community-dwelling older women, the Study of Osteoporotic Fractures (SOF)(33). This association was significant even for women endorsing sub-threshold levels of depressive symptoms. In this study, we examine the relationship between circadian gene polymorphisms and levels of depressive symptoms in the same cohort and a cohort of community-dwelling older men. We hypothesized that there would be associations between circadian gene polymorphisms and depressive symptom level, including subthreshold levels of depressive symptoms.

Methods

Participants

Data were collected from participants of two large, multi-center, cohort studies, SOF and the Osteoporotic Fractures in Men (MrOS) Study. The studies included participants who were ≥65 years old and excluded participants who required assistance from another person for ambulation or had undergone bilateral hip replacement. Details have been published(34). Data were collected with written informed consent and approval by review boards of participating institutions.

In SOF, the 9,704 older, primarily Caucasian women making up the original cohort were recruited from four locations (Baltimore, MD, the Monongahela Valley near Pittsburgh, PA; Minneapolis, MN; Portland, OR) between 1986 and 1988. Follow-up visits were conducted every two years. The current cross-sectional analyses focused on self-identified white women participating in SOF Visit 8 (2002-2004) which included assessment of circadian gene polymorphisms. There were 4,727 participants at Visit 8 (84% of active survivors). Of these women, 1731 self-identified white women who had DNA extracted, SNPs genotyped, and completed a Geriatric Depression Scale (GDS) were included in these analyses.

In MrOS, 5994 older men were recruited from six locations (Birmingham, AL; the Monongahela Valley near Pittsburgh, PA; Minneapolis, MN; Palo Alto, CA; San Diego, CA; and Portland, OR) between 2000 and 2002 (35, 36). The MrOS Sleep Study (2003-2005), recruited 3,135 MrOS participants at the time of their first two-year follow up (68%) and included assessment of circadian gene polymorphisms. Of the 2,859 men who did not participate, 344 had died, 36 had already stopped participating in MrOs, 332 were not invited because recruitment goals were met, 150 were ineligible and 1,997 refused. Among participants of the MrOS Sleep Study, 2,480 self-identified white men who had DNA extracted, SNPs genotyped, and completed a GDS were included in this cross-sectional analyses.

Directly genotyped circadian gene polymorphisms

A custom Illumina Golden Gate assay (Illumina, San Diego, CA, USA) designed to genotype polymorphisms in circadian genes was developed in a collaborative effort by investigators at the California Pacific Medical Center and the University of California San Diego as previously described(37). Candidate genes were selected based on a review of published human association studies and studies done in common model organisms (37).

TagSNPs were selected using Tagger (38) ($r^2 \geq 0.8$, minor allele frequency (MAF)≥0.01) with HapMap CEU Phase II (release 22) genotype data in the candidate gene regions including 10 kb upstream and downstream of transcript boundaries. Using these Tagger settings, 314 tagSNPs would have been selected from the 798 SNPs found within the 761 kb of genomic DNA that includes the RORA gene and 20 kb of flanking DNA. To reduce our RORA genotyping burden, the linkage disequilibrium (LD) threshold in Tagger was lowered to 0.6 and a maximum of 100 tagSNPs were selected for genotyping, resulting in 81% of the 798 RORA SNPs being captured at $r^2 \geq 0.6$. In total, 658 SNPs within the candidate gene regions were selected for genotyping. Genotypes were called using Beadstudio software. Genotype concordance rate was >0.99 (8% of MrOS samples and 3 SOF samples were plated in duplicate). Samples with <90% SNP call rate were excluded. SNPs with missing frequency >0.05 and MAF<0.01 were excluded. Autosomal SNPs with an HWE exact *P*-value $<8\times10^5$ (Bonferroni-corrected *P*-value for 658 SNPs) were excluded (39). Among the all-male MrOS samples, X-linked SNPs with heterozygous genotypes were excluded. Among the 658 genotyped SNPs, 529 in MrOS and 508 in SOF passed QC filters as previously described (37). To correct for residual population stratification in these self-identified European American cohorts, 195 independent, autosomal SNPs were used in multidimensional scaling analyses (MDS) as implemented in PLINK (40). The resulting first two MDS components were included as covariates in regression models.

Imputed circadian gene polymorphisms

Analysis of imputed circadian SNPs was used to provide additional information to fine map association signals detected in the analyses of directly genotyped SNPs. Imputation was performed using MACH v. 1.0.16 (http://www.sph.umich.edu/csg/abecasis/MaCH/). Phased haplotypes of 60 unrelated HapMap II CEU founders were used as the reference data (for autosomes: CEU_r22_ nr.b36_fwd.phased; http://hapmap.ncbi.nlm.nih.gov/downloads/phasing/2007-08_rel22/phased/ , for X chromosome and the pseudo-autosomal regions PAR1/PAR2: CEU_r21_nr_fwd_phased; http://hapmap.ncbi.nlm.nih.gov/downloads/phasing/2006-07_phaseII/phased/). Imputations were based on 537,371 genotyped SNPs in common with the reference data (G-C and A-T SNPs were excluded). To allow imputation with MACH for the haploid non-par regions of the male X chromosome, the phased haplotypes were duplicated. A two-step imputation approach was used: step 1 estimated per SNP error and per interval crossover rates at 50 iterations from a random sample of 200 genotyped European-American individuals, step 2 used these model parameters to estimate allele dosages and genotypes based on a maximum likelihood approach. A total of 488,335 SNPs were imputed, with 4446 high-confidence SNPs remaining after removal of SNPs in low LD (r2 < 0.3) with genotyped markers. Allelic imputation accuracy rates were estimated by masking 20% of the genotyped data and showed a <5% error rate. Adding SNPs not used for imputation purpose resulted in a total of 5076 genotyped and imputed SNPs available for association analyses. Of these, 93 imputed SNPs within the *PER3* gene region and 898 imputed SNPs within the *RORA* gene region were utilized in this study to fine map significant associations observed for directly genotyped SNPs in these genes.

Depressive Symptoms

Depressive symptoms were assessed using the Geriatric Depression Scale (GDS), a 15-item validated self-report questionnaire commonly used for assessment of depressive symptoms in older adults (41). Participants were categorized into three groups [0-2 (no/few depressive symptoms), >2 to<6 (some depressive symptoms), ≥6 (many depressive symptoms)] according to depressive symptoms reported at follow-up. To remain consistent with prior publications using this strategy (42), the GDS ≥6 group was referred to as "depressed." A standard cut-off of ≥6 on the GDS has been shown to have a sensitivity of 91% and specificity of 65% for diagnosis of a major depressive episode compared with the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (43).

Other Sample Characteristics

Demographic information was recorded at the baseline SOF or MrOS assessment. At SOF and MrOS follow-up visits including SOF Visit 8 and MrOS sleep study (MrOS Visit 2), participants completed questionnaires regarding health status, alcohol consumption, exercise, and medical history. Reported medical conditions included stroke, diabetes, Parkinson's disease, chronic obstructive pulmonary disease, congestive heart failure, myocardial infarction, thyroid disease, hypertension, and cancer. During a clinic interview participants were asked whether they had problems with 6 instrumental activities of daily living (IADLs). Height and weight were measured and BMI was calculated as weight in kilograms divided by height in meters squared. Medications taken daily or almost daily during the prior 30 days were recorded and categorized according to a computerized coding dictionary (44). Cognition was assessed by administration of the Modified Mini-Mental State Exam ($_3$MS)(45) in the MrOs cohort or the Mini-Mental State Exam (MMSE) (46) in the SOF cohort. This information is presented in Table 1.

Statistical Analyses

Associations between directly genotyped circadian SNPs and depressive symptom levels were evaluated using multinomial logistic regression models to estimate odds ratios (OR) and 95% confidence intervals (CI) for falling into the "some depressive symptoms" or the "depressed" groups compared with the group with no or few depressive symptoms. Multinomial regression analysis was performed in each cohort separately, for all 520 circadian SNPs to identify gene regions of interest with significant SNP associations. To correct for multiple hypothesis testing in the presence of LD, the effective number of independent SNPs using our QC-filtered genotype data was estimated and a multiple-testing significance threshold of 1.7×10^{-4} was adopted.(47) Subsequently associations between imputed SNPs in the gene regions of interest and depressive symptom levels were evaluated using logistic regression models as described above. Results for all three modes of inheritance (additive, dominant, and recessive) are reported here for SNPs with significant (p-value <0.00017) or nominally significant (p-value<0.05) associations under the dominant inheritance mode. For the meta-analysis, results from the two cohorts were combined by fixed-effect meta-analysis using inverse variance weighting of effect estimates. *P*-values from the 2DF test in each cohort were combined by a Z-statistic based approach weighted by the square root of the sample size. Heterogeneity between studies was assessed using the I^2

statistic and the *P*-value from the Q-test. All SNPs presented had a p-value > 0.05 for heterogeneity. Association analysis was performed using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/), meta-analysis was performed using METAL,(48) and power calculations were performed using QUANTO (49). Two gene regions of interest were identified; *PER3* and *RORA* and regional association plots including imputed SNPs were generated using LocusZoom (http://csg.sph.umich.edu/locuszoom). Plots shown are for best-fit models (dominant mode of inheritance) for outcomes where significant associations were found ("some depressive symptoms" category for *PER3* region and "depressed" category for *RORA* region).

Results

Characteristics of the population

The MrOS sample was about 7.5 years younger, had a higher average BMI, and were more likely to report good or excellent health, less iADL impairment, fewer medical problems, and greater alcohol consumption (Table 1). Average scores on cognitive screening assessments (MrOS: mean (+/−standard deviation) $_3$MS score 93.14 (+/−5.54); SOF: mean MMSE score 27.97(+/−2.04) were in the normal range.

The average GDS score was in the "no or few depressive symptoms" range (1.76+/−2.14) in the MrOs group versus the "some depressive symptoms" range (2.74+/−2.86) in the SOF group. A greater proportion of the SOF sample were categorized as having "some depressive symptoms" (25.36% vs. 18.06%) or "depressed" (14.56% vs. 6.33%). The percentage of patients endorsing use of antidepressant medications was higher in the SOF sample (10.52% vs. 5.89%).

PER3 SNP Associations with Depressive Symptom Level

There were significant associations between several directly genotyped SNPs at the *PER3* locus and decreased odds of reporting "some depressive symptoms" in models testing a dominant mode of inheritance (Figure 1, Table 2). There was a significant association between the *PER3* intronic SNP rs12137927 and decreased odds of reporting "some depressive symptoms" in the SOF sample (OR_{DOM} 0.61, CI 0.48-0.78, df=1, Wald chi-square −4.04, p=0.000054) but not the MrOS sample. This association was also significant in the meta-analysis (OR_{DOM} 0.61, CI 0.48-0.78, z=4.04, p=0.000054). The PER3 intronic SNPs rs228644 (OR_{DOM} 0.74, CI 0.63-0.86, z=3.82, p-value=0.00013) and rs228682 (OR_{DOM} 0.74, CI 0.86-0.63, z=3.81 p-value=0.00014) were also both associated with decreased odds of endorsing "some depressive symptoms" in the meta-analysis. There were no significant associations between *PER3* SNPs and the "depressed" category for directly genotyped *PER3* SNPs.

A secondary analysis was performed examining associations between directly genotyped *PER3* SNPs and reporting "some or many" depressive symptoms (GDS>2) compared with "none or few" (GDS 0-2) depressive symptoms (Table 3). There was a significant association between rs13137927 and "some or many" depressive symptoms in the SOF sample (OR_{DOM} 0.67, CI 0.55-0.82, df=1, Wald chi-square −3.88, p-value=0.000104) but

not in the MrOS sample. This association was also significant in the meta-analysis (OR_{DOM} 0.76, CI 0.66 0.87, z=3.95, p-value=0.000078). There were nominal associations between rs228644 (OR_{DOM} 0.77, CI 0.63-0.93, df=1, Wald chi-square −2.71, p-value=0.0068) and rs228682 (OR_{DOM} 0.77, CI 0.64-0.94, df=1, Wald chi-square −2.71, p-value=0.009) and the "some or many" depressive symptoms category in the MrOS sample but not in the SOF sample. These associations were nominally significant in the meta-analysis (Table 3).

There were six imputed *PER3* SNPs that were associated with decreased odds (All OR_{DOM} 0.63, CI 0.50-0.80, df=1, Wald chi-square 3.78-3.83, p=0.00015) of "some depressive symptoms" in the SOF sample but not in the MrOS sample (Figure 1, Supplementary Table). In the meta-analysis these associations were attenuated and did not reach multiple testing criteria for significance. One imputed *PER3* SNP (rs11581279) was significantly associated with increased odds of falling into the "depressed" category under the additive mode of inheritance (OR_{ADD} 1.81, CI1.33-2.45, df=1, Wald chi-square 3.80, p-value=0.00014) while the association was nominally significant under the dominant mode of inheritance (OR_{DOM} 1.92, CI 1.33-2.77, df=1, Wald chi-square 3.49, p-value=0.00049). This association was attenuated and did not reach multiple testing criteria for significance in the meta-analysis. There were no significant associations for either directly genotyped or imputed *PER3* SNPs in models testing the recessive mode of inheritance.

RORA SNP Associations with Depressive Symptom Level

In the MrOS sample, the directly genotyped *RORA* intronic SNP rs11632098 was associated with greater odds of falling into the "depressed" category (Table 2; OR_{DOM} 2.16, CI 1.45-3.23,df=1, Wald chi-square 3.76, p=0.000168). This association was not significant in the SOF cohort (Supplemental Table) and was attenuated and nominally significant (OR_{DOM} 1.63, CI 1.24-2.16, z=3.45, p=0.00056) in the meta-analysis. There were also nominally significant associations between the intronic SNP rs10519084 and the "depressed" category (odds ratios 0.66-0.73, df=1, Wald chi-square −2.66 to −2.39, p-values 0.0035 0.024) in the SOF and MrOS samples and in the meta-analysis in models testing the additive and dominant mode of inheritance.

Sixteen imputed *RORA* SNPs were significantly associated with the "depressed" category and 2 were nominally associated with the "depressed" category in the MrOS cohort only (Supplementary Table). There were no significant associations between imputed *RORA* SNPs and depressive symptom level in the SOF cohort.

Discussion

These analyses identified associations between SNPs in two circadian genes, *PER3* and *RORA*, and levels depressive symptoms in two large cohorts of older adults. These data add to a growing body of evidence implicating chronobiological pathways e in the pathophysiology of depression (50, 51). Because older adults are particularly vulnerable to depression risk factors (e.g. medical comorbidities, cerebrovascular changes, dementia, and decreased socialization), the etiology of depression in late-life is often multi-factorial. None-the-less, our data suggests that circadian genes continue to exert an effect on mood in late-life.

We identified a significant association between one directly genotyped *PER3* SNP and 0.61-fold decreased odds of reporting “some depressive symptoms” in the SOF group and nominally significant associations between two additional directly genotyped SNPs and 0.71-0.77 fold decreased odds of endorsing “some depressive symptoms” in both SOF and MrOS samples. These findings are supported by the meta-analysis in which the associations between all three SNPs and the “some depressive symptoms” category met multiple testing criteria for significance and the additional significant associations identified in the imputated *PER3* SNP set. No significant association was found between these PER3 SNPs and the “depressed” category. One possible explanation for this is that these particular PER3 gene polymorphisms influence milder forms of depression. However, it is notable that the “depressed” category included fewer women. It is therefore possible that the power to detect such associations in models testing associations between SNPs and the “depressed” outcome” was insufficient. In a secondary analysis, similar associations were found between the directly genotyped *PER3* SNPs and “some to many” depressive symptoms (GDS>2). Thus, PER3 variants may be associated with decreased risk for depressive symptoms, including subthreshold levels of depressive symptoms, in older adults.

The period family is a core component of the molecular biological clock machinery and PER3 plays a role in sleep-wake cycle and circadian phenotypes (52) (53-55). The literature regarding *PER3* gene variants and mood disorders is mixed. Studies have reported significant associations between *PER3* variants and mood disorder characteristics such as age of onset, response to pharmacotherapy, and circadian mood oscillations (23, 56, 57). Other studies found only suggestive associations (58, 59), or no significant associations (26). Associations between circadian gene polymorphisms and subthreshold levels of depressive symptoms have not previously been reported in older adults. In this age group SubD is twice as common as MDD (3, 5-7) and associated with adverse outcomes including functional impairment (60), disability (61), physical decline (62), and progression to MDD (5). Attention is warranted to increasing knowledge about SubD and to developing treatment interventions.

We identified one directly genotyped *RORA* SNP and 16 imputed *RORA* SNPS that were significantly associated with 2.16-2.34 fold increased odds being categorized “depressed” in the men but not in the women. There was also an association for one directly genotyped *RORA* SNP and 0.66-0.68 fold decreased odds of falling into the “depressed” category in both cohorts and in the meta-analysis. However the later association did not meet multiple testing criteria for significance.

RORA belongs to the NR1 subfamily of nuclear hormone receptors. RORA is involved in regulation of circadian rhythms, neurodevelopment, neuroprotection, and regulation of steroid hormones. Disruption of any of these processes could potentially contribute to the pathophysiology of depression. These results are consistent with other studies linking *RORA* with depression-related variables including diagnoses (24), depression-associated personality traits (29), and response to citalopram (63). In contrast, a recent GWAS analysis found no associations reaching significance at the genome-wide multiple testing level between *RORA* SNPs and MDD or dysthymia (64). Other studies found associations between *RORA* variants and bipolar disorder (65), post-traumatic stress disorder (66),

attention deficit hyperactivity disorder (67), and autism *(68).* Accordingly, it been suggested that polymorphisms in RORA may increase non-specific vulnerability to mental illness. We found associations between RORA polymorphisms and depressive symptoms in the older male, but not the older female, cohort. The data raise the possibility that RORA variants could contribute to the development of depressive symptoms particularly in older men. However, the analyses presented here were not designed to examine gender differences and gender is confounded by study (i.e. SOF vs. MrOS). Another candidate gene study in which an association between depression with early morning wakening and an interaction between SNPs in *TIMELESS* and *RORA* was observed in males but not females (28). These observations could be explained, in part, by the fact that *RORA* is a hormone-dependent transcription factor that is differentially regulated by male and female sex hormones (69). Future studies could be designed specifically to determine whether associations can be replicated in other cohorts and whether RORA's role is gender specific.

The mechanism by which alterations in the *PER3* and *RORA* genes could impact depressive symptoms remains unclear. The "phase-shift hypothesis" posits that depression is caused by a misalignment of circadian phase compared to the sleep-wake cycle (70). We previously reported a cross sectional association between less robust circadian rest-activity rhythms but no association between circadian rest-activity rhythm timing and depressive symptoms in SOF (33). Further, no significant associations were found between the *PER3* and *RORA* SNPs reported here and circadian rest-activity rhythm characteristics in the SOF or MrOS cohorts (37). Therefore the identified gene polymorphisms may impact depression through alternate mechanisms. For example, the "neuroinflammatory hypothesis" of depression argues that inflammatory cytokines, elevated in the setting of stress or illness can contribute to depression through a combination of several mechanisms: 1) influencing regulation of neurotransmitter (especially monoamine) metabolism in the brain, 2) promoting deleterious effects on cells in the brain including decreased neurotrophic support, decreased neurogenesis, and induction of apoptosis in microglia, and dysregulation of glial/neuronal interactions with chronic inflammation (71). Both RORA and Per3 may play roles in down regulation of pro-inflammatory cytokines such as IL-6. More investigation is required to determine how these genes could mediate their effects on mood.

Two strengths of this study are the inclusion of models testing for associations with the "some depressive symptoms" category which is likely to represent subthreshold depression and the use of two large cohorts of participants who weren't selected on the basis of mood symptoms or circadian characteristics. Our study avoids many pitfalls commonly associated with earlier candidate gene studies (72). The Clock gene pathway, from which the candidate genes were selected, is well established and we systematically surveyed common genetic variation in our set of candidate genes rather than choosing individual variants to test within genes. Multiple test correction was applied using modern techniques that take LD into account. Population stratification was accounted for by restricting the analyses to self-identified Caucasian participants and adjusting for genetic ancestry using components from multidimensional scaling analyses. One possible disadvantage of the candidate gene approach is that genetic associations not represented in our candidate gene pool would not be detected.

A limitation of the study is that the sample size for the “depressed” category was smaller reducing the power to detect significant associations in models including that category as an outcome. Therefore the lack of identified associations between PER3 SNPs and the “depressed” category could represent a false-negative result. Furthermore, the sample size was not adequate to determine whether circadian SNPs were associated with increased or decreased odds of falling into the “depressed” versus the “some depressive symptoms” category. Because each study enrolled only women or men, it was not possible to evaluate gender differences in associations between SNPs and depressive symptoms. Another limitation of the study is the use of a questionnaire rather than a diagnostic interview to assess depressive symptoms. The cut-off of GDS total score ≥6 has been validated in comparison to a diagnosis of MDD according DSM-IV criteria but the score range used for the “some depressive symptom” category has not been validated compared with DSM diagnoses (e.g. minor depression). The analysis was restricted to older Caucasian participants and results may not be generalizable to other populations.

In summary *PER3* and *RORA* may play important roles in the pathophysiology of depression in older adults.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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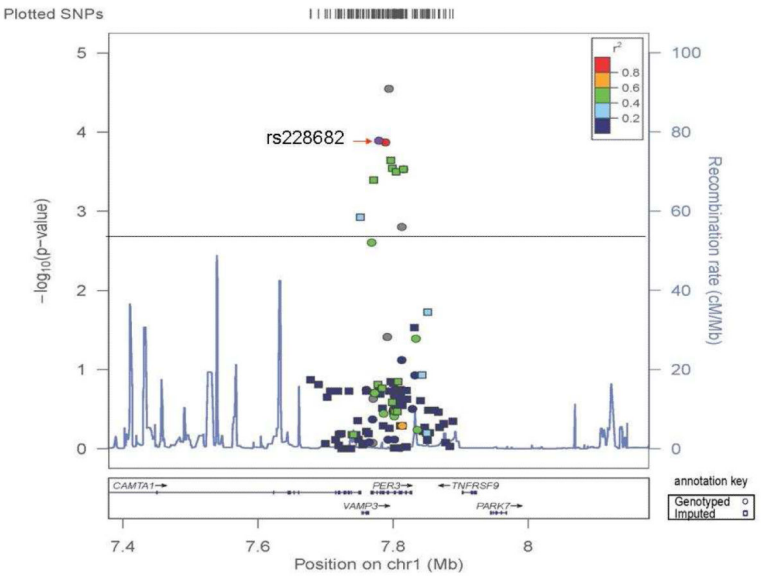

FIGURE 1. A regional association plot is shown for directly genotyped and imputed *PER3* SNPs and the "some depressive symptoms" category

Data shown is for models testing the dominant mode of inheritance (i.e. the best-fit model) in the meta-analysis. Circles represent directly genotyped SNPs and squares represent imputed SNPs. SNP: single nucleotide polymorphism, Chr: chromosome.

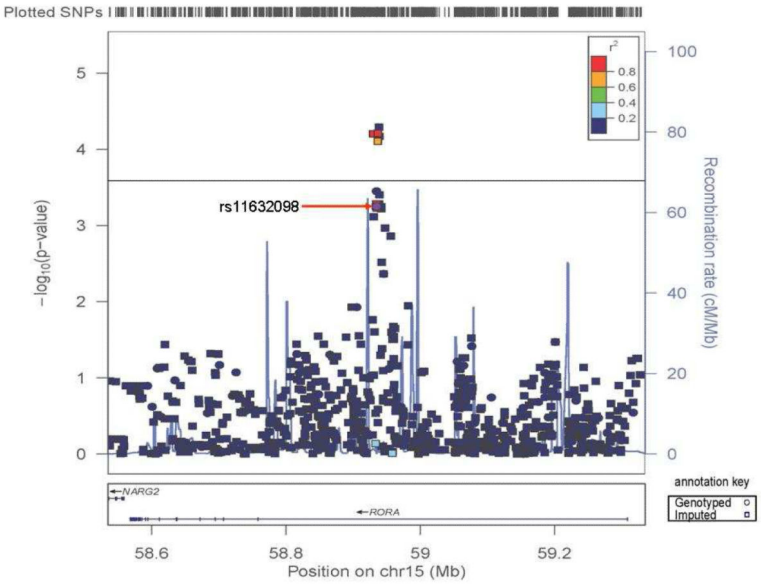

FIGURE 2. A regional association plot is shown for directly genotyped and imputed *RORA* SNPs and the "depressed" category

Data shown is for models testing the dominant mode of inheritance (i.e. the best-fit model) in the meta-analysis. Circles represent directly genotyped SNPs and squares represent imputed SNPs. SNP: single nucleotide polymorphism, Chr: chromosome.

Table 1

Characteristics of the population

Key characteristics of the SOF and MrOS samples are shown. SOF: Study of Osteoporotic Fractures; MrOS Osteoporotic Fractures in Men Study.

Characteristic	MrOS	SOF	p-value (df)
Participants included in analysis	2480	1731	
^ **Geriatric Depression Scale total score, mean(±SD)**	1.76 +/− 2.14	2.74 +/− 2.86	<0.0001 (2)
No or few depressive symptoms (0-2), n(%)	1875 (75.6)	1040 (60.08)	<0.0001
Some depressive symptoms (>2 - <6), n(%)	448 (18.06)	439 (25.36)	<0.0001
Many depressive symptoms (≥6), n(%)	157 (6.33)	252 (14.56)	<0.0001
‡ **Age (years), mean(±SD)**	76.58 (±5.61)	84.04 (±3.51)	<0.0001 (4209)
‡ **Body mass index (kg/m^2)**	27.25 (±3.79)	26.72 (±4.81)	0.0003 (4209)
^ **Self-reported health status excellent or good, n(%)**	2166 (87.34)	1268 (73.25)	<0.0001 (1)
^ **Greater than 12 years education, n(%)**	1390 (56.05)	649 (37.49)	<0.0001 (1)
^ **Current smoker, n(%)**	46 (1.85)	46 (2.64)	0.0796 (1)
† **Average number alcoholic beverages per week, mean(±SD)**	1.93 (±1.72)	0.92 (±2.66)	<0.0001
^ **Current antidepressant use, n(%)**	146 (5.89)	163 (10.53)	<0.0001 (1)
† **Instrumental activities of daily living, mean(±SD)**	0.36 (±0.83)	3.09 (±4.04)	<0.0001
† **Number medical conditions, mean (±SD)**	1.07 (±0.99)	1.5 (±1.17)	<0.0001

^ Compared using Chi-squared tests

‡ Compared using t-tests

† Compared using non parametric Mann-Whitney tests

Table 2

Genetic Association of Circadian SNPs and Depressive Symptom Level

Associations between directly genotyped circadian SNPs and different levels of depressive symptoms are shown. Odds ratios (OR) and 95% confidence intervals (CI) are given for each depressive symptom level category compared with the GDS 0-2 group. MAF is the weighted average between MAFs for the SOF and MrOS cohorts. The p-values for associations passing multiple testing criteria for significance (p-value <0.00017) are bolded and underlined. Nominally significant p-values (≥0.00017 but ≤0.05) are underlined. Non-significant p-values > 0.05 are in normal font; MAF: Minor allele frequency; SOF: Study of Osteoporotic Fractures; MrOS Osteoporotic Fractures in Men Study.

Directly Genotyped SNPs							**Some Depressive Symptoms (2>GDS<6)**			**Depressed (GDS≥6)**		
SNP	**Gene**	**Location**	**Alleles**	**MAF**	**Mode**		**MrOS (n=448)**	**SOF (n=439)**	***Meta (n=887)**	**MrOS (n=157)**	**SOF (n=252)**	**Meta (n=409)**
rs12137927	***PER3***	**Intron**	**C/T**	**0.219**	Add	OD (95%CI)	0.86 (0.72-1.03)	0.69 (0.57-0.85)	0.69 (0.56-0.84)	0.98 (0.74-1.29)	0.85 (0.67-1.09)	1.1 (0.92-1.33)
						Test statistic (df)	−1.59(1df)	−3.5(1df)	1.43	−0.15(1df)	−1.29(1df)	1.06
						P-value	0.111	0.0004624	0.1541	0.8787	0.1984	0.2882
					Dom	OD (95%CI)	0.80 (0.65-1)	**0.61 (0.48-0.78)**	**0.61 (0.48-0.78)**	0.98 (0.7-1.36)	0.78 (0.58-1.04)	0.86 (0.69-1.06)
						Test statistic (df)	−1.99(1df)	**−4.04(1df)**	**4.04**	−0.14(1df)	−1.69(1df)	1.36
						P-value	0.04619	**0.00005423**	**0.00005405**	0.8921	0.09096	0.1726
					Rec	OD (95%CI)	1.03 (0.65-1.62)	0.91 (0.53-1.58)	1.1 (0.63-1.89)	0.96 (0.46-2.02)	1.13 (0.6-2.14)	0.95 (0.59-1.54)
						Test statistic (df)	0.11(1df)	−0.33(1df)	0.12	−0.11(1df)	0.37(1df)	0.21
						P-value	0.9122	0.7439	0.9012	0.9143	0.7112	0.8334
					2DF	Test statistic (df)	−1.43(2df)	−2.21(2df)	3.68	0.02(2df)	−1.42(2df)	0.07
						P-value	0.7945	0.3394	0.00023	0.9004	0.9676	0.9422
rs228644	***PER3***	**Intron**	**G/A**	**0.406**	Add	OD (95%CI)	0.81 (0.7-0.94)	0.9 (0.76-1.06)	0.85 (0.76-0.95)	0.95 (0.75-1.2)	1.18 (0.96-1.45)	1.07 (0.92-1.25)
						Test statistic (df)	−2.7(1df)	−1.24(1df)	2.85	−0.44(1df)	1.55(1df)	0.88
						P-value	0.006372	0.214	0.004369	0.6575	0.1217	0.3813
					Dom	OD (95%CI)	0.71 (0.57-0.88)	0.77 (0.61-0.97)	**0.74 (0.63-0.86)**	0.98 (0.69-1.39)	1.22 (0.9-1.66)	1.11 (0.88-1.39)
						Test statistic (df)	−3.16(1df)	−2.2(1df)	**3.82**	−0.11(1df)	1.26(1df)	0.87
						P-value	0.001593	0.02753	**0.0001346**	0.9102	0.207	0.3842
					Rec	OD (95%CI)	0.85 (0.65-1.13)	1.08 (0.8-1.47)	0.95 (0.78-1.17)	0.85 (0.55-1.34)	1.26 (0.88-1.81)	1.08 (0.81-1.43)

Directly Genotyped SNPs							Some Depressive Symptoms (2>GDS<6)			Depressed (GDS≥6)		
SNP	**Gene**	**Location**	**Alleles**	**MAF**	**Mode**		**MrOS (n=448)**	**SOF (n=439)**	***Meta (n=887)**	**MrOS (n=157)**	**SOF (n=252)**	**Meta (n=409)**
						Test statistic (df)	−1.04(1df)	0.52(1df)	0.41	−0.67(1df)	1.25(1df)	0.55
						P-value	0.2982	0.6021	0.6837	0.5052	0.2117	0.584
					2DF	Test statistic (df)	−1.57(2df)	−2.24(2df)	2.97	0.52(2df)	−0.07(2df)	0.53
						P-value	0.02885	0.5515	0.003	0.5835	0.1241	0.5967
rs228682	***PER3***	**Intron**	**T/C**	**0.407**	Add	OD (95%CI)	0.81 (0.7-0.94)	0.9 (0.76-1.06)	0.85 (0.76-0.95)	0.97 (0.77-1.23)	1.18 (0.96-1.46)	0.92 (0.79-1.08)
						Test statistic (df)	−2.66(1df)	−1.24(1df)	2.84	−0.34(1df)	1.61(1df)	0.98
						P-value	0.006536	0.2138	0.004443	0.7306	0.1082	0.3253
					Dom	OD (95%CI)	0.71 (0.57-0.88)	0.77 (0.61-0.97)	**0.74 (0.86-0.63)**	1.01 (0.71-1.43)	1.23 (0.9-1.67)	0.89 (0.7-1.12)
						Test statistic (df)	−3.16(1df)	−2.22(1df)	**3.81**	−0.11(1df)	1.31(1df)	0.91
						P-value	0.001779	0.02631	**0.0001414**	0.9102	0.1895	0.3636
					Rec	OD (95%CI)	0.85 (0.64-1.13)	1.09 (0.8-1.47)	1.05 (0.85-1.29)	0.89 (0.57-1.38)	1.27 (0.88-1.83)	0.91 (0.69-1.2)
						Test statistic (df)	−0.97(1df)	0.54(1df)	0.34	−0.49(1df)	1.29(1df)	0.69
						P-value	0.3336	0.5858	0.7347	0.6265	0.1963	0.493
					2DF	Test statistic (df)	−1.64(2df)	−2.28(2df)	2.93	0.35(2df)	−0.06(2df)	0.67
						P-value	0.03285	0.5587	0.0034	0.6797	0.1107	0.502
rs10519084	***RORA***	**Intron**	**A/G**	0.2832	Add	OD (95%CI)	1.06 (0.90-1.25)	1.03 (0.86-1.22)	1.04 (0.93-1.78)	0.73 (0.55-0.96)	0.73 (0.58-0.93)	0.73 (0.61-0.87)
						Test statistic (df)	0.71(1df)	0.28(1df)	0.70	−2.26(1df)	−2.61(1df)	3.46
						P-value	0.48	0.7797	0.4826	0.024	0.0091	0.00055
					Dom	OD (95%CI)	1.10 (0.89-1.35)	1.00 (0.80-1.25)	1.05 (0.90-1.22)	0.68 (0.51-0.90)	0.66 (0.47-0.93)	0.67 (0.54-0.84)
						Test statistic (df)	0.86(1df)	0(1df)	0.62	−2.39(1df)	−2.66(1df)	3.58
						P-value	0.3923	0.9974	0.5333	0.01687	0.017	0.00035
					Rec	OD (95%CI)	1.02 (0.69-1.49)	1.14 (0.77-1.68)	1.07 (0.82-1.41)	0.73 (0.37-1.42)	0.71 (0.40-1.24)	0.72 (0.46-1.10)
						Test statistic (df)	0.08(1df)	0.64(1df)	0.51	−0.93(1df)	−1.21(1df)	1.53
						P-value	0.9342	0.5213	0.6124	0.3528	0.2255	0.1272
					2DF	Test statistic (df)	0.52(2df)	−0.61(2df)	0.12 (1df)	−0.71(2df)	−0.58(2df)	2.14

Directly Genotyped SNPs							Some Depressive Symptoms (2>GDS<6)			Depressed (GDS≥6)		
SNP	**Gene**	**Location**	**Alleles**	**MAF**	**Mode**		**MrOS (n=448)**	**SOF (n=439)**	***Meta (n=887)**	**MrOS (n=157)**	**SOF (n=252)**	**Meta (n=409)**
						P-value	0.7655	0.5752	0.9079	0.1728	0.08452	0.0323
rs11632098	***RORA***	**Intron**	**T/C**	0.0708	Add	OD (95%CI)	1.05 (0.77-1.42)	1.07 (0.80-1.43)	1.06 (0.86-1.31)	1.93 (1.34-2.80)	1.27 (0.89-1.80)	1.55 (1.20-2.00)
						Test statistic (df)	0.47(1df)	0.32(1df)	0.56	3.49(1df)	1.31(1df)	3.35
						P-value	0.6368	0.7518	0.5744	0.00049	0.19	0.00081
					Dom	OD (95%CI)	1.06 (0.77-1.45)	1.10 (0.80-1.52)	1.08 (0.86-1.35)	**2.16 (1.45-3.23)**	1.26 (0.86-1.85)	1.63 (1.24-2.16)
						Test statistic (df)	0.36(1df)	0.59(1df)	0.67	**3.76(1df)**	1.17(1df)	**3.45**
						P-value	0.7223	0.5539	0.5058	**0.000168**	0.2426	**0.00056**
					Rec	OD (95%CI)	0.32 (0.04-2.58)	1.46 (0.45-4.75)	1.01 (0.36-2.82)	0.94 (0.12-7.63)	1.96 (0.52-7.39)	1.59 (0.52-4.87)
						Test statistic (df)	0.63(1df)	−1.07(1df)	0.02	−0.05(1df)	0.99(1df)	0.81
						P-value	0.5308	0.287	0.9848	0.957	0.3228	0.4208
					2DF	Test statistic (df)	−0.46(2df)	1.26(2df)	0.16	1.34(2df)	−0.39(2df)	0.58
						P-value	0.5257	0.2946	0.8747	0.9399	0.3049	0.5614

*Test statistic: Wald test for SOF and MrOs analyses; Z-statistic based approach weighted by the square root of the sample size for meta-analysis

Table 3
Genetic Association of *PER3* SNPs and Depressive Symptoms GDS ≥2

Associations between directly genotyped Per3 SNPs and geriatric depression scale (GDS) score ≥2 are shown. Odds ratios (OR) and 95% confidence intervals (CI) are given for having depressive symptoms compared with the GDS 0-2 group. MAF is the weighted average between MAFs for the SOF and MrOS cohorts. The p-values for associations passing multiple testing criteria for significance (p-value <0.00017) are bolded and underlined. Nominally significant p-values (≥0.00017 but ≤0.05) are underlined. Non-significant p-values > 0.05 are in normal font; MAF: Minor allele frequency; SOF: Study of Osteoporotic Fractures; MrOS Osteoporotic Fractures in Men Study.

Directly Genotyped SNPs						**Combined Subthreshold/Depressed (GDS≥2)**		
SNP	**Location**	**Alleles**	**MAF**		**Mode**	**MrOS (n=605)**	**SOF (n=691)**	**Meta (n=1296)**
rs12137927	**Intron**	**C/T**	**0.224**	Add	OD (95%CI)	0.89 (0.76-1.04)	0.75 (0.63-0.89)	0.83 (0.73-0.93)
					Test statistic (df)	−1.45(1df)	−3.26(1df)	3.27
					P-value	0.1478	0.001119	0.001075
				Dom	OD (95%CI)	0.84 (0.70-1.018)	**0.67 (0.54-0.82)**	**0.75 (0.66-0.87)**
					Test statistic (df)	−1.78(1df)	**−3.88(1df)**	**3.95**
					P-value	0.07564	**0.000104**	**0.00007769**
				Rec	OD (95%CI)	1.01 (0.67-1.51)	0.99 (0.62-1.57)	1.001 (0.73-1.36)
					Test statistic (df)	0.03(1df)	−0.05(1df)	0.01
					P-value	0.9747	0.962	0.9949
				2DF	Test statistic (df)	−1.19(2df)	−2.39(1df)	0.67
					P-value	0.769	0.4903	0.5043
rs228644	**Intron**	**G/A**	**0.412**	Add	OD (95%CI)	0.84 (0.74-0.96)	0.99 (0.86-1.143)	0.91 (0.83-1.00)
					Test statistic (df)	−2.49(1df)	−0.11(1df)	1.91
					P-value	0.01266	0.909	0.056
				Dom	OD (95%CI)	0.76 (0.63-0.93)	0.90 (0.73-1.10)	0.82(0.72-0.95)
					Test statistic (df)	−2.71(1df)	−1.04(1df)	2.69
					P-value	0.00683	0.2998	0.007235
				Rec	OD (95%CI)	0.86(0.67-1.10)	1.16(0.89-1.50)	0.99(0.83-1.18)
					Test statistic (df)	−1.22(1df)	1.1(1df)	0.15
					P-value	0.2229	0.2694	0.8782
				2DF	Test statistic (df)	−1.05(2df)	−1.84(2df)	1.86
					P-value	0.03261	0.7345	0.0632
rs228682	**Intron**	**T/C**	**0.412**	Add	OD (95%CI)	0.85(0.74-0.97)	0.99(0.86-1.15)	0.91(0.83-1.007)
					Test statistic (df)	−2.41(1df)	−0.09(1df)	1.83
					P-value	0.01583	0.9323	0.068
				Dom	OD (95%CI)	0.77 (0.63-0.93)	0.90(0.73-1.10)	0.83(0.72-0.95)

Directly Genotyped SNPs						Combined Subthreshold/Depressed (GDS≥2)		
SNP	**Location**	**Alleles**	**MAF**		**Mode**	**MrOS (n=605)**	**SOF (n=691)**	**Meta (n=1296)**
					Test statistic (df)	−2.71(1df)	−1.02(1df)	2.68
					P-value	0.00683	0.3059	0.007425
				Rec	OD (95%CI)	0.87(0.68-1.12)	1.164(0.90-1.51)	0.99(0.83-1.20)
					Test statistic (df)	−1.08(1df)	1.14(1df)	0.09
					P-value	0.2821	0.2532	0.93
				2DF	Test statistic (df)	−1.19(2df)	−1.86(2df)	1.80
					P-value	0.04204	0.7087	0.07187

*Test statistic: Wald test for SOF and MrOs analyses; Z-statistic based approach weighted by the square root of the sample size for meta-analysis