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Research paper

PRKCDBP (CAVIN3) and CRY2 associate with major depressive disorder

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ABSTRACT

Background: Dysfunctions in the intrinsic clocks are suggested in patients with depressive disorders. The cryptochrome circadian clocks 1 and 2 (CRY1 and CRY2) proteins modulate circadian rhythms in a cell and influence emotional reactions and mood in an individual. The protein kinase C delta binding protein (PRKCDBP, or CAVIN3), similar to the serum deprivation response protein (SDPR, or CAVIN2), reduces metabolic stability of the PER2-CRY2 transcription factor complex that plays a role in the circadian rhythm synchronization. Our aim was to study SDPR, PRKCDBP, CRY1 and CRY2 genetic variants in depressive disorders.

Methods: The sample included 5910 Finnish individuals assessed with the Munich-Composite International Diagnostic Interview (M-CIDI) in year 2000. In year 2011, 3424 individuals were assessed again. After genotype quality control, there were 383 subjects with major depressive disorder, 166 with dysthymia, and 4154 healthy controls. A total of 48 single-nucleotide polymorphisms from SDPR, PRKCDBP, CRY1 and CRY2 genes were analyzed using logistic regression models controlling for age and gender.

Results: The earlier reported association of CRY2 variants with dysthymia was confirmed and extended to major depressive disorder (q < 0.05). In addition, novel associations of PRKCDBP rs1488864 with depressive disorders (q=0.02) and with major depressive disorder in specific (q=0.007) were found.

Limitations: The number of cases was moderate and coverage of PRKCDBP was limited.

Conclusions: CRY2 and PRKCDBP variants may be risk factors of major depressive disorder and provide information for diagnosis.

1. Introduction

Patients with depressive disorders often have not only sleep disturbances, but also abnormalities in their sleep-wakefulness cycle or rapid-eye-movement sleep that suggest there are dysfunctions in the intrinsic clocks generating and maintaining the circadian rhythms. Concerning the circadian rhythms, a strong repressor during the evening is irreplaceable to the morning phase in the normal circadian clock (Ukai-Tadenuma et al., 2008). The cryptochrome circadian clocks 1 and 2 (CRY1 and CRY2) are strong repressors, and their endogenous properties confer information as a function of time (Edwards et al., 2016). A delay in transcription of DNA elements from the CRY1 promoter and a further delay via clock-controlled DNA elements from the CRY1 intron are the steps that keep the amplitude of circadian clocks robust (Ukai-Tadenuma et al., 2011). Therefore, CRY1 and CRY2 are plausible targets of interest. Here, CRY2 has a key role, as it not only acts as a general repressor, but also opposes the actions of CRY1 and inhibits CRY1 from accessing to its DNA targets too early, possibly facilitated by PER proteins (Anand et al., 2013). Repression by CRY1 is like a homeostatic process with an autonomous and open-ended action that extends from circadian night into circadian day. It is the ratio rather than the absolute amounts of CRY proteins that determines the circadian period, and, e.g., if the CRY1-to-CRY2 ratio is greater than it is usually is, then the nuclear import rate of the PER-CRY complex slows down and the circadian period lengthens (Li et al., 2016). Further, it is only CRY2 however that can inhibit the activated forms of ARNTL, the key clock protein, while the CRY1 and PER proteins have no effect (Dardente et al., 2007). Intriguingly, as the levels of PER2 in the correct phase is far more critical for the circadian synchronization than PER2 levels as such, the dimerization of PER2 with CRY1 or with CRY2 may play a role in the pathogenesis (Chen et al., 2009). It is of note that the interval (alignment difference) tracks dawn and dusk, and that for the measurement of alignment the CRY1 and CRY2 proteins give a signal of the evening hours, whereas the PER1 and PER2 proteins give a signal of the morning hours in day-active animals, e.g. in sheep.
Healthy controls were free of depressive disorders, anxiety disorders (panic disorder w/o agoraphobia, generalized anxiety disorder, social phobia, and/or agoraphobia) and alcohol use disorders (abuse and/or dependence) in year 2000 and also in year 2011, if they took part in the follow-up. For depressive and anxiety disorders, the diagnoses were assessed for the past 12 months and for alcohol use disorders for the lifetime.

2.3. Gene and SNP selection

Selection of SNPs in SDPR, PRKCDBP, CRY1 and CRY2 was based on HapMap phase 3 data (http://www.hapmap.org/), and tagging was done using the Tagger program in the Haplovie 4.1 software (Barrett et al., 2005). The linkage disequilibrium (LD) within the gene and within 10 kb of their 5’ and 3’ flanking regions, that is, 122 kb for CRY1 (chr12: 107 375–107 497 kb, GRCh37/hg19 assembly), 56 kb for CRY2 (chr11: 45 859–45 915 kb), 22 kb for PRKCDBP (chr11: 6329–6351 kb) and 33 kb for SDPR (chr2: 192 689–192 722), was used to select tag-SNPs capturing most of the genetic variation. The aim was to capture all the SNPs having a minor allele frequency (MAF) of > 5% in the European population (CEU and TSI) in the Hapmap database. The pair-wise r² was set to ≥0.9 in order to select a tag-SNP among the SNPs that were in high LD. Ten out of 21 CRY1, 10 out of 34 CRY2 and 3 out of 14 SDPR SNPs fulfilled the criterion and were all successfully included in the genotyping multiplexes. Of the 12 out of 19 PRKCDBP SNPs fulfilling the criterion, 8 were successfully included. In addition to the aforementioned tag-SNPs, 20 potentially functional CRY1 (12) and CRY2 (8) variants were selected using Pupasuite,21 Variowatch,22 database of SNPs affecting miR Regulation (dbSMR)23 and microRNA SNP24 databases, and were included in the study. Supplementary Table S1 presents all the 53 SNPs that were successfully genotyped in this study.

2.4. Genotyping

Genomic DNA was isolated from whole blood according to standard procedures. The SNPs were genotyped at the Institute for Molecular Medicine Finland (FIMM), Technology Centre, University of Helsinki, using the MassARRAY iPLEX method (Sequenom, San Diego, CA, USA) (Jurinke et al., 2002), with excellent success (>95%) and accuracy (100%) rates (Lafermo et al., 2006). For quality control purposes, positive (CEPH) and negative water controls were included in each 384-plate. Genotyping was performed blind to phenotypic information.

314 of 5910 individuals were excluded due to a high missing genotype rate (i.e. > 0.1). The total genotype rate in the remaining individuals was 0.997. Three SNPs turned out to be non-polymorphic (CRY2 rs35488012, rs117531403 and rs76545099), and two SNPs were removed because their minor allele frequency was <0.01 (CRY2 rs3747548, CRY1 rs7294758). Finally, there were 5596 individuals and 48 SNPs for the statistical analyses.

2.5. Statistical analyses

SNP and haplotype statistical analyses were performed using logistic regression and additive genetic model controlling for age and gender (unadjusted models in Supplementary Table S3 online) with PLINK software version 1.07 (Purcell et al., 2007). Only haplotypes with more than 5% frequency are reported. In order to account for multiple comparisons, false discovery rate (FDR) q-values (Storey, 2003) were computed to correct for the SNPs and phenotypes analyzed using stats package in R software version 3.0.0 (R Core Team, 2015).

3. Results

General characteristics of the participants are shown in Table 1, and...
the genotype frequencies, minor allele frequencies and Hardy-Weinberg equilibrium (HWE) p-values in the whole sample are reported in Supplementary Table S2 online (please, see Supplementary Tables S2–S4 online for the genotype counts of major depressive disorder, dysthymia and depressive disorders, respectively). All the single-nucleotide polymorphisms (SNPs) were in HWE (P > 0.01).

Table 2 displays the significant (q < 0.05) SNP association results of the adjusted model. In brief, one _PRKDCBP_ SNP (rs1488864) associated both with major depressive disorder (OR=1.6, 95% CI=1.2–2.0, p=0.0002, q=0.007) and with depressive disorders (OR=1.5, 95% CI=1.2–1.8, p=0.0009, q=0.02). Seven _CRY2_ SNPs associated with dysthymia, major depressive disorder, or depressive disorders. All the SNP association results are shown in Supplementary Tables S5 and S6 online (adjusted and unadjusted models, respectively).

The haplotype blocks formed are shown in Table 3, the significant (q < 0.05) adjusted haplotype associations in Table 4, and all haplotype associations in Supplementary Table S7 online. _PRKDCBP_ haplotype _ATT1_ (rs1488864|rs2947030|rs1691940|rs10839553) associated with the increased odds for major depressive disorder (OR=1.6, p=0.0003, q=0.02) and depressive disorders (OR=1.5, p=0.001, q=0.02). _CRY2_ haplotype _ATGGGGCCGCAGG_ (rs7121611|rs7121775|rs7945565|rs10838524|rs2292919|rs9778136|rs10838527|rs2292910|rs3824872) associated with dysthymia (OR=1.5, p=0.0004, q=0.02).

### 4. Discussion

_CRY2_ rs10838524 _G_ allele, rs7121611 _A_ allele, rs7945565 _G_ allele, rs1401419 _G_ allele and rs3824872 _G_ allele were associated with dysthymia, which was supported by haplotype analysis. _CRY2_ rs7123390 _G_ allele and rs2292910 _C_ allele were associated with major depressive disorder. _CRY2_ rs7121611 _A_ allele, rs7945565 _G_ allele, rs1401419 _G_ allele and rs2292910 _C_ allele were associated with depressive disorders. _PRKDCBP_ rs1488864 _A_ allele was associated both with major depressive disorder and with depressive disorders (depressive disorders, dysthymia or both), which were supported by haplotype analysis.

We have earlier reported four of the SNPs (_CRY2_ SNPs rs10838524, rs7121611, rs7945565 and rs1401419) to associate with dysthymia (Kovanen et al., 2013). During the 11-year follow-up period, the number of individuals with dysthymia increased from 136 to 186. The fifth _CRY2_ tag-SNP rs3824872 now associated with dysthymia is
located downstream of CRY2 gene and upstream of MAPK8IP1 gene. Earlier, we have also reported the associations of rs10838524 allele G and rs7123390 allele G in a Finnish sample, and those of rs10838524 allele A, rs10838527 allele G and rs8284872 allele A in a Swedish sample with winter depression (Lavebratt et al., 2010). In addition, CRY2 rs10838524 A allele has been associated with bipolar disorder with rapid cycling, and CRY2 haplotype GGAC (of SNPs rs10838524, rs7123390, rs10838527 and rs8284872) was under-represented among the rapid-cycling cases (Sjoholm et al., 2010). Furthermore, rs7123390, rs10838527 and rs3824872) was under-represented also reports of no association of CRY2 awakening have been reported (Utge et al., 2010). However, there are Concerning CRY2, there is currently no information whether the SNPs we analyzed have any functional relevance to mood or behavior. Of the 12 SNPs forming the dysthymia-associated haplotype, however, five (two in specific, i.e., rs10838524 and rs8284872) were significantly associated with dysthymia, two (one in specific, i.e., rs7123390) with major depressive disorder, and four (i.e., rs7121611, rs7945565, rs1401419 and rs2292910) with depressive disorders (herein, dysthymia, major depressive disorder, or both). To have an idea for a mechanistic hypothesis of the impact of these genetic variants on the clinical phenotypes, a search from a bioinformatics database (HoploReg, v4.1, www.broadinstitute.org) yielded some information about these SNPs as follows. CRY2 rs10838524 as an intronic SNP influences the binding of the glucocorticoid receptor (NR3C1) which is implicated in depressive disorders (Goodwin et al., 1992) and known to interact reciprocally with CRY2 (Lamia et al., 2011; Torres-Farfan et al., 2009), thus having an impact on physiology and possibly on mood regulation as well. CRY2 rs10838524 also influences the binding of the CCCTC-binding factor (CTCF) and may thereby have an effect on transcriptional regulation of CRY2.

With regard to PRKCDBP, this is the first time that polymorphisms within this gene are linked to depressive disorders. The protein has earlier been shown to affect circadian clock functions (Schneider et al., 2012): PER2 complex interacts with PRKCDBP, and PER-to-CRY protein abundance and interactions are affected by PRKCDBP. In addition, the circadian period length is influenced by PRKCDBP such that the loss-of-function shortened the period (Schneider et al., 2012), similar to knockout animal models of each key clock gene except that of Cry2 (Ko and Takahashi, 2006). Cry2 knockout mice have a prolonged circadian period which as such resembles the gain-of-function for PRKCDBP that is the lengthened circadian period. A search from the aforementioned bioinformatics database yielded that PRKCDBP rs1488864 influences the binding of 10 proteins, among them NFKB1 which is known to interact with cryptochromes (Lee and Suncar, 2011; Narasimamurthy et al., 2012) and implicated in depressive disorders (Miklowitz et al., 2016) but with conflicting data (Mellon et al., 2016), and BCL11A which is relevant to a characterized at-risk phenotype for anxiety and depressive disorders (Alisch et al., 2014).

Our study has several strengths. We based our study on a large random sample representative of the Finnish adult population and its 11-year follow-up data. The diagnoses of mental health were assessed with a structured and validated diagnostic interview method which yielded reliable diagnoses of depressive disorders based on the DSM-IV classification. We had well-covered genetic information for variants in both cryptochrome genes and SDPR gene. We applied strict multiple statistical testing correction by the false-discovery rate method. Despite the increased number of cases from the follow-up, Finland is still a relatively small country by population and therefore the number of cases remains moderate. Further, some SNPs in PRKCDBP were not successfully included in the genotyping multiplexes.

To conclude, our study supports the role of CRY2 in depressive disorders and reports a novel association of PRKCDBP with major depressive disorder.

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Author contributions

T.P. and L.K. designed the study. M.K. and K.D. designed and conducted the genotyping. L.K. performed statistical analysis. All authors interpreted the results and participated to manuscript preparation and approved its final version.

Competing financial interests statement

None to report.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jad.2016.09.034.

References


Table 4

Significant (q-value < 0.05) haplotype associations of the adjusted model.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phenotype</th>
<th>SNPs</th>
<th>SNP1</th>
<th>SNP2</th>
<th>Haplotype</th>
<th>F</th>
<th>OR</th>
<th>P-value</th>
<th>q-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRY2</td>
<td>Dysthymia</td>
<td>12</td>
<td>rs7121611</td>
<td>rs8284872</td>
<td>ATTCGGGCGAGC</td>
<td>0.45</td>
<td>1.48</td>
<td>0.0004</td>
<td>0.02</td>
</tr>
<tr>
<td>PRKCDBP</td>
<td>Major depressive disorder</td>
<td>4</td>
<td>rs1488864</td>
<td>rs10839553</td>
<td>ATTGA</td>
<td>0.08</td>
<td>1.57</td>
<td>0.0003</td>
<td>0.02</td>
</tr>
<tr>
<td>PRKCDBP</td>
<td>Depressive disorders</td>
<td>4</td>
<td>rs1488864</td>
<td>rs10839553</td>
<td>ATTGA</td>
<td>0.08</td>
<td>1.46</td>
<td>0.001</td>
<td>0.02</td>
</tr>
</tbody>
</table>

SNPs, number of SNPs in the haplotype.

SNP1, 5’ SNP.

SNP2, 3’ SNP.

F, haplotype frequency.

OR, estimated odds ratio.
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