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ORIGINAL ARTICLE

CRY1, *CRY2* and *PRKCDBP* genetic variants in metabolic syndrome

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The circadian clock affects metabolic cycles, and there is a link between circadian clock genes and metabolic syndrome. Therefore, we wanted to investigate whether variants of the core circadian clock genes, cryptochrome circadian clocks 1 and 2 (*CRY1* and *CRY2*), or those of protein kinase C, delta binding protein (PRKCDBP), which regulate the interactions and abundance of dimers of the period and cryptochrome proteins, are associated with metabolic syndrome or its components. The association of 48 single-nucleotide polymorphisms (SNPs) from *CRY1*, *CRY2* and *PRKCDBP* genes with metabolic disorder or its components was analyzed in a sample of 5910 individuals. Genotyping was performed using the Sequenom MassARRAY system. SNPs and haplotypes were analyzed using linear or logistic regression with additive models controlling for age and sex. Continuous phenotypes were permuted 10 000 times. False discovery rate *q*-values were calculated to correct for multiple testing. Overall, *CRY1* and C*RY2* variants showed nominal association with the metabolic syndrome components, hypertension and triglyceride levels, and one *CRY2* variant had an association with metabolic syndrome, although none of these associations yielded significant *q*-values. However, the haplotype analysis of these variants supported the association of *CRY1* with arterial hypertension and elevated blood pressure. Further studies are warranted regarding the role of *CRY1* in cardiovascular diseases.
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INTRODUCTION

Metabolic cycles are influenced by the circadian clock to such an extent that the demarcation between metabolic and circadian oscillations can be regarded as somewhat arbitrary.[1] Although the links from genetic variants through transcription to physiologic functions are most likely less linear than predicted,[2] a landmark study identified obesity and features of metabolic syndrome as characteristics of clock circadian regulator (CLOCK)-deficient mice.[3] Since then, human studies have identified genetic variants and expression patterns of circadian clock genes, such as aryl hydrocarbon receptor nuclear translocator-like (*ARNTL or BMAL1)*, *CLOCK*, neuronal PAS domain protein 2 (*NPAS2*) or period circadian clock 2 (*PER2*), that are associated with metabolic syndrome, hypertension or type 2 diabetes.[4–11]

However, as cryptochrome circadian clock 1 (CRY1) and cryptochrome circadian clock 2 (CRY2) have a key role in the reciprocal regulation of the metabolic and circadian networks,[12,13] we wanted to analyze whether *CRY1* or *CRY2* genetic variants are associated with metabolic syndrome or its components. In addition, because protein kinase C, delta binding protein (PRKCDBP or CAVIN-3) regulates not only the circadian period but also interactions and abundance of the period-cryptochrome protein dimers,[14] we studied the associations of *PRKCDBP* genetic variants with metabolic syndrome and its components. Furthermore, because *CRY2* has been indicated in the control of fasting glucose levels,[15] to confirm and extend these findings, we analyzed whether *CRY2* genetic variants are associated with a range of glucose metabolism indicators in our sample.

METHODS

Subjects

Our sample included 5910 individuals (56% women) with available blood samples, the Munich-Composite International Diagnostic Interview (M-CIDI)[16] and the self-report on seasonal changes in mood and behavior. The sample is part of a national health survey, Health 2000, of the population aged ⩾30 years ($n=8028$; http://www.terveys2000.fi/indexe.html) that was approved by the ethics committees of the National Public Health Institute and the Helsinki and Uusimaa Hospital District. All participants provided written informed consent.

Phenotypes

Routine fasting laboratory tests included the concentrations of blood glucose, serum insulin, serum total cholesterol, triglycerides and high-density lipoprotein cholesterol. Blood glucose was measured one hour after the oral glucose intake. The Homeostasis Model Assessment insulin resistance and beta-cell function indexes were then computed. Blood pressure and waist circumference were also measured.

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Table 1 Criteria for metabolic disorder and its risk factors used in this study and number of subjects in each of them

	Cases (N)	*Controls* (N)
The US Adult Treatment Panel III of the NCEP-ATPIII criteria	1835	4037
Three or more of the following risk factors:		
Fasting blood glucose $\geqslant 6.1$ mmol l^{-1}	870	5036
Systolic blood pressure $\geqslant 130$ mm Hg or diastolic blood pressure $\geqslant 85$ mm Hg	3616	2276
Serum triglycerides $\geqslant 1.7$ mmol l^{-1}	1993	3913
Serum HDL cholesterol of <1.0 mmol l^{-1} for men or <1.3 mmol l^{-1} for women	2048	3858
Waistline of >102 cm for men or >88 cm for women	2348	3494
IDF criteria	2521	3339
Waistline $\geqslant 94$ cm for men or $\geqslant 80$ cm for women and at least two of the following risk factors:	3988	1854
Serum triglycerides $\geqslant 1.7$ mmol l^{-1}	1993	3913
Serum HDL cholesterol $\leqslant 1.02$ mmol l^{-1} for men or $\leqslant 1.29$ mmol l^{-1} for women	2126	3780
Systolic blood pressure $\geqslant 130$ mm Hg or diastolic blood pressure $\geqslant 85$ mm Hg or treatment for previously diagnosed hypertension	3753	2143
Fasting plasma glucose level $\geqslant 5.6$ mmol l^{-1} or previously diagnosed type 2 diabetes	2149	3758

Abbreviations: HDL, high-density lipoprotein; IDF, The International Diabetes Federations; NCEP-ATPIII, National Cholesterol Education Program.

Metabolic disorder was assessed using two sets of criteria (Table 1): those of the US Adult Treatment Panel III of the National Cholesterol Education Program (NCEP-ATPIII)[17] and those of the International Diabetes Federation (IDF).[18] As a *post-hoc* test, elevated blood pressure was also analyzed, to dissect the parameter from the metabolic syndrome components, using the current WHO (World Health Organization)-based definition of 140/90 mm Hg or over.[19]

Gene and SNP selection

CRY1, *CRY2* and *PRKCDBP* single-nucleotide polymorphism (SNP) selection was based on HapMap phase 3 data (http://www.hapmap.org/) and performed using the Tagger program in the Haploview 4.1 software.[20] The linkage disequilibrium within the genes and within 10 kb of their 5′ and 3′ flanking regions, that is, 122 kb for *CRY1* (chr12:105 899–106 021 kb, NCBI36/hg18 assembly), 56 kb for *CRY2* (chr11:45 815–45 871 kb) and 22 kb for *PRKCDBP* (chr11: 6286–6308 kb), was used to select SNPs capturing most of the genetic variation.

The aim was to capture all the SNPs with a minor allele frequency $>5\%$ in the European population (CEU and TSI) in the HapMap database by setting the pair-wise r^2 to $\geqslant 0.9$. Ten out of 21 *CRY1* and 10 out of 34 *CRY2* 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 SNP[24] databases and were included in the study. Table 2 presents the 48 successfully genotyped SNPs.

Genotyping

Genomic DNA was isolated from whole blood according to standard procedures. The SNPs were genotyped at the Institute for Molecular Medicine Finland, Technology Centre, University of Helsinki, using the MassARRAY iPLEX method (Sequenom, San Diego, CA, USA),[25] with excellent success ($>95\%$) and accuracy (100%) rates.[26] For quality control purposes, positive (CEPH) and negative water controls were included in each 384-plate. Genotyping was performed blind to phenotypic information.

For *CRY1* and *CRY2* and for *PRKCDBP* and *CRY1* rs11113153 and rs10861695, 173 and 238 individuals were removed, respectively, due to a high missing genotype rate (that is, >0.1). The total genotyping rate in the remaining individuals was 0.999. Three SNPs were removed because the minor allele frequency was <0.01: *CRY2* rs3747548, *CRY2* rs35488012, *CRY1* rs7294758. Finally, there were 5737 (*CRY1* and *CRY2*) or 5672 (*PRKCDBP* and *CRY1* rs11113153 and rs10861695) individuals and 45 SNPs used in the statistical analysis.

Statistical analysis

Statistical analysis was performed using logistic or linear regression and additive genetic models controlling for age and sex with PLINK software v1.07.[27] Haplotype blocks were defined using Haploview software[20] and the confidence interval algorithm. For continuous phenotypes, 10 000 permutations were used to produce empirical *P*-values to relax the assumption of normality. The results of each set of analyses were corrected for multiple testing by calculating false discovery rate *q*-values[28] using R software (http://www.r-project.org/). The *q*-values of <0.05 were considered to be significant, and the *P*-values of <0.05 were considered to be nominally significant.

RESULTS

Genotypes, allele frequencies and Hardy–Weinberg equilibrium estimates are shown in Table 2. The associations presented in the text refer to the NCEP-ATPIII criteria of metabolic syndrome, and the results for IDF criteria are presented in the Supplementary Material. Both criteria produced similar results. Five intronic *CRY1* SNPs (rs4964513, rs11613557, rs59790130, rs4964518 and rs12821586) and two *CRY2* SNPs (rs7121611 and rs7945565) had nominally significant associations with hypertension (Table 3 and Supplementary File 1). Three *CRY1* SNPs (rs2888896, rs10746077 and rs2078074) and one *CRY2* SNP (rs75065406) had nominally significant associations with elevated triglycerides, and the *CRY2* SNP (rs75065406) had a nominally significant association with metabolic syndrome as well. However, after correcting for multiple testing, none of the associations remained significant.

In the haplotype analysis, three haplotype blocks were formed for *CRY1* (Figure 1), and one each for *CRY2* (Figure 2) and *PRKCDBP* (Supplementary File 2). Table 4 (see also Supplementary File 3) presents the nominally significant haplotype associations ($P<0.05$). The haplotype analysis supported the association of *CRY1* 5′-*CGG*-3′ and 5′-*TGA*-3′ (Block 1) and 5′-*CTTCGTCCTTAG*-3′ (Block 3) haplotypes with hypertension. *CRY1* 5′-*CCCCACTCTCAG*-3′ and 5′-*GTCTGCCCCCAT*-3′ haplotypes associated with elevated triglycerides. *CRY2* 5′-*ATTTGCGGTGGCACG*-3′ haplotype associated with elevated triglycerides and metabolic syndrome.

A priori, we planned to extend the metabolic findings of *CRY2* and analyze *CRY2* SNPs in relation to indicators of glucose metabolism. *CRY2* SNPs had nominally significant associations with the indicators, but these associations did not survive after correction for multiple testing (Supplementary Files 4 and 5).

Table 2 Successfully genotyped SNPs, their selection criteria, allele and genotype frequencies and Hardy–Weinberg equilibrium *P*-values

Gene	*SNP*	*BP NCBI36/hg18*	*A1*	*A2*	*MAF*	*A1A1*	*A1A2*	*A2A2*	*P*	*Selection criteria*
PRKCDBP	RS1488864	6298905	*A*	*C*	0.08	38 (0.01)	810 (0.14)	4813 (0.85)	0.52	LD
PRKCDBP	RS2634183	6292850	*G*	*A*	0.24	304 (0.05)	2063 (0.36)	3302 (0.58)	0.46	LD
PRKCDBP	RS4758095	6294711	*A*	*G*	0.5	1409 (0.25)	2839 (0.5)	1414 (0.25)	0.85	LD
PRKCDBP	RS10839553	6307367	*C*	*A*	0.08	41 (0.01)	865 (0.15)	4764 (0.84)	0.79	LD
PRKCDBP	RS1051992	6297282	*T*	*C*	0.36	742 (0.13)	2558 (0.46)	2320 (0.41)	0.38	Missense
PRKCDBP	RS2634184	6292138	*G*	*A*	0.31	499 (0.09)	2455 (0.43)	2706 (0.48)	0.08	LD
PRKCDBP	RS16911940	6304374	*A*	*T*	0.14	106 (0.02)	1334 (0.24)	4229 (0.75)	0.96	LD
PRKCDBP	RS2947030	6300440	*T*	*G*	0.26	397 (0.07)	2203 (0.39)	3071 (0.54)	0.95	LD
CRY2	RS7121611	45820718	*A*	*T*	0.46	1218 (0.21)	2883 (0.5)	1711 (0.29)	0.96	LD
CRY2	RS7121775	45820899	*C*	*T*	0.27	384 (0.07)	2326 (0.4)	3100 (0.53)	0.06	LD
CRY2	RS61884508	45821508	*G*	*T*	0.02	1 (0)	241 (0.04)	5600 (0.96)	0.52	Pupasuite OregannoFilter TFBS
CRY2	RS75065406	45821518	*T*	*C*	0.04	13 (0)	421 (0.07)	5414 (0.93)	0.11	TFBS, MAF
CRY2	RS3747548	45825589	*A*	*C*	0	0 (0)	1 (0)	5847 (1)	1	Pupasuite non-synonymous and VarioWatch
CRY2	RS10838524	45826753	*G*	*A*	0.48	1337 (0.23)	2897 (0.5)	1579 (0.27)	0.92	LD and Lavebratt *et al.*[51]
CRY2	RS2292913	45834105	*T*	*C*	0.05	18 (0)	590 (0.1)	5233 (0.9)	0.7	LD and splice site
CRY2	RS7945565	45835568	*G*	*A*	0.46	1213 (0.21)	2890 (0.5)	1695 (0.29)	0.79	Pupasuite triplex
CRY2	RS1401419	45836315	*G*	*A*	0.46	1211 (0.21)	2909 (0.5)	1681 (0.29)	0.48	Pupasuite triplex
CRY2	RS72902437	45838834	*C*	*T*	0.03	2 (0)	313 (0.05)	5499 (0.95)	0.45	Pupasuite triplex
CRY2	RS35488012	45845804	*G*		0	0 (0)	0 (0)	5854 (1)	1	Variowatch synonymous
CRY2	RS7123390	45847994	*A*	*G*	0.29	431 (0.07)	2445 (0.42)	2915 (0.5)	0.01	LD and Lavebratt *et al.*[51]
CRY2	RS4755345	45848084	*A*	*G*	0.05	18 (0)	598 (0.1)	5229 (0.89)	0.8	LD
CRY2	RS17787136	45851212	*G*	*C*	0.28	409 (0.07)	2385 (0.41)	3014 (0.52)	0.03	Pupasuite TFBS
CRY2	RS10838527	45859770	*G*	*A*	0.12	89 (0.02)	1236 (0.21)	4509 (0.77)	0.67	LD and Lavebratt *et al.*[51]
CRY2	RS2292910	45860189	*A*	*C*	0.34	650 (0.11)	2707 (0.47)	2455 (0.42)	0.02	LD and dbSMR miRNA target site
CRY2	RS3824872	45862181	*T*	*G*	0.25	372 (0.06)	2173 (0.37)	3276 (0.56)	0.65	LD and Lavebratt *et al.*[51]
CRY2	RS1554338	45863406	*G*	*A*	0.05	14 (0)	528 (0.09)	5289 (0.91)	0.77	LD
CRY1	RS4964513	105899888	*C*	*T*	0.12	84 (0.01)	1224 (0.21)	4492 (0.77)	0.95	LD
CRY1	RS714359	105902975	*A*	*G*	0.22	276 (0.05)	2006 (0.35)	3516 (0.61)	0.67	LD
CRY1	RS12821586	105904582	*A*	*G*	0.11	72 (0.01)	1138 (0.19)	4629 (0.79)	0.84	LD
CRY1	RS2287161	105905270	*C*	*G*	0.5	1408 (0.24)	2930 (0.51)	1461 (0.25)	0.43	Soria *et al.*[52] and Utge *et al.*[53] and Pupasuite triplex
CRY1	RS11113153	105905900	*T*	*C*	0.17	178 (0.03)	1600 (0.28)	3888 (0.69)	0.4	LD
CRY1	RS8192441	105909584	*C*	*A*	0.01	1 (0)	136 (0.02)	5712 (0.98)	0.56	miRNASNP miRNA target site
CRY1	RS3741892	105911293	*C*	*G*	0.49	1395 (0.24)	2937 (0.51)	1477 (0.25)	0.4	Pupasuite triplex
CRY1	RS10861688	105918178	*T*	*C*	0.17	164 (0.03)	1642 (0.28)	4011 (0.69)	0.82	LD
CRY1	RS10861695	105939203	*A*	*G*	0.49	1372 (0.24)	2842 (0.5)	1452 (0.26)	0.81	Pupasuite triplex
CRY1	RS10861697	105943792	*C*	*G*	0.49	1352 (0.23)	2923 (0.5)	1521 (0.26)	0.48	Pupasuite triplex
CRY1	RS2078074	105960936	*C*	*T*	0.42	1017 (0.18)	2815 (0.49)	1930 (0.33)	0.87	Pupasuite transfac
CRY1	RS59790130	105964433	*T*	*C*	0.06	26 (0)	692 (0.12)	5131 (0.88)	0.58	Pupasuite transfac
CRY1	RS10437895	105964954	*C*	*T*	0.49	1398 (0.24)	2936 (0.5)	1482 (0.25)	0.46	Pupasuite transfac
CRY1	RS10746077	105965682	*A*	*G*	0.42	1027 (0.18)	2832 (0.49)	1960 (0.34)	0.96	Pupasuite transfac
CRY1	RS11613557	105966445	*T*	*C*	0.06	26 (0)	692 (0.12)	5130 (0.88)	0.58	Pupasuite triplex
CRY1	RS2888896	105970712	*T*	*C*	0.42	1019 (0.18)	2830 (0.49)	1947 (0.34)	0.87	LD
CRY1	RS11113179	105976915	*T*	*C*	0.08	39 (0.01)	833 (0.14)	4942 (0.85)	0.53	LD and Utge *et al.*[53]
CRY1	RS10746083	105978532	*T*	*C*	0.49	1391 (0.24)	2941 (0.51)	1481 (0.25)	0.37	Pupasuite triplex
CRY1	RS4964518	105990347	*T*	*C*	0.07	30 (0.01)	778 (0.13)	5027 (0.86)	1	LD
CRY1	RS7294758	105991959	*A*	*T*	0.01	0 (0)	97 (0.02)	5758 (0.98)	1	Pupasuite triplex
CRY1	RS17289712	105993098	*G*	*A*	0.05	6 (0)	524 (0.09)	5308 (0.91)	0.07	LD
CRY1	RS10778528	105998092	*G*	*T*	0.48	1358 (0.23)	2926 (0.5)	1533 (0.26)	0.62	LD

Abbreviations: dbSMR, database of SNPs affecting miR Regulation; LD, linkage disequilibrium; MAF, minor allele frequency; miRNA, microRNA; P, Hardy–Weinberg *P*-value; SNP, single-nucleotide polymorphism; TFBS, transcription factor binding site.

Post-hoc, we wanted to dissect the parameter of elevated blood pressure from the metabolic syndrome components. Four *CRY1* SNPs (rs17289712, rs4964513, rs59790130 and rs11613557), one *CRY2* SNP (rs75065406) and two *PRKCDBP* SNPs (rs2947030 and rs4758095) had nominally significant associations with elevated blood pressure (Supplementary File 6). After correcting for multiple testing, none of the associations remained significant. The haplotype analysis, however, supported the association of *CRY1* and *CRY2* with elevated blood pressure, as the 5′-*CGG*-3′ (*CRY1* Block 1), 5′-*CCCCACTCTCGG*-3′ and 5′-*CTTCGTCCTTAG*-3′ (*CRY1* Block 3) and 5′-*ATTT GCGGTGGCACG*-3′ (*CRY2*) haplotypes associated with elevated blood pressure (Supplementary File 7).

Table 3 Nominally significant single SNP associations using the NCEP-ATPIII criteria for metabolic syndrome

Phenotype	*Gene*	*SNP*	*A1*	N	*Odds ratio*	*L95*	*U95*	P-*value*	q-*value*
Elevated blood pressure	*CRY1*	rs4964513	*C*	5688	1.23	1.08	1.4	0.0013	0.21
Elevated blood pressure	*CRY1*	rs11613557	*T*	5717	1.3	1.1	1.55	0.0023	0.21
Elevated blood pressure	*CRY1*	rs59790130	*T*	5718	1.3	1.1	1.55	0.0024	0.21
Elevated blood pressure	*CRY1*	rs4964518	*T*	5719	1.23	1.045	1.44	0.012	0.8
High triglycerides	*CRY1*	rs2888896	*T*	5726	0.91	0.84	0.99	0.021	0.91
High triglycerides	*CRY1*	rs10746077	*A*	5728	0.91	0.84	0.99	0.022	0.91
High triglycerides	*CRY1*	rs2078074	*C*	5713	0.92	0.85	0.99	0.033	0.91
Metabolic syndrome	*CRY2*	rs75065406	*T*	5698	1.25	1.02	1.53	0.034	0.91
Elevated blood pressure	*CRY2*	rs7121611	*A*	5712	1.09	1.01	1.19	0.036	0.91
High triglycerides	*CRY2*	rs75065406	*T*	5732	1.23	1.01	1.5	0.041	0.91
Elevated blood pressure	*CRY1*	rs12821586	*A*	5717	0.87	0.77	1	0.043	0.91
Elevated blood pressure	*CRY2*	rs7945565	*G*	5699	1.09	1	1.18	0.048	0.91

Abbreviations: A1, tested allele (minor allele); L95, lower bound of 95% confidence interval for odds ratio; N, number of genotypes for the phenotype; NCEP-ATPIII, National Cholesterol Education Program; SNP, single-nucleotide polymorphism; U95, upper bound of 95% confidence interval for odds ratio.

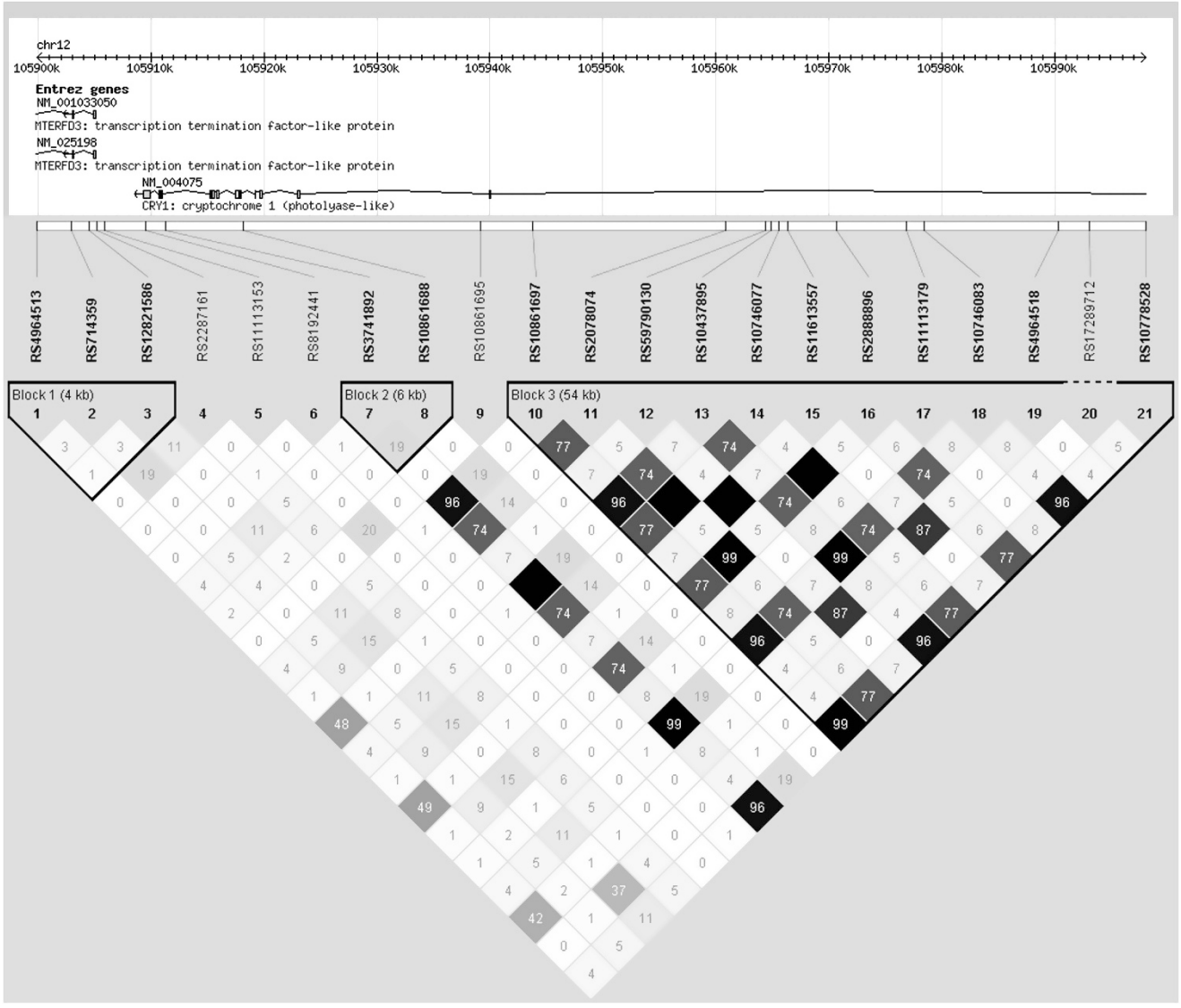

Figure 1 The analyzed circadian clock 1 single-nucleotide polymorphisms (*CRY1* SNPs) in this study, their location and the haplotype block structure of the area formed, based on our sample showing r^2-values.

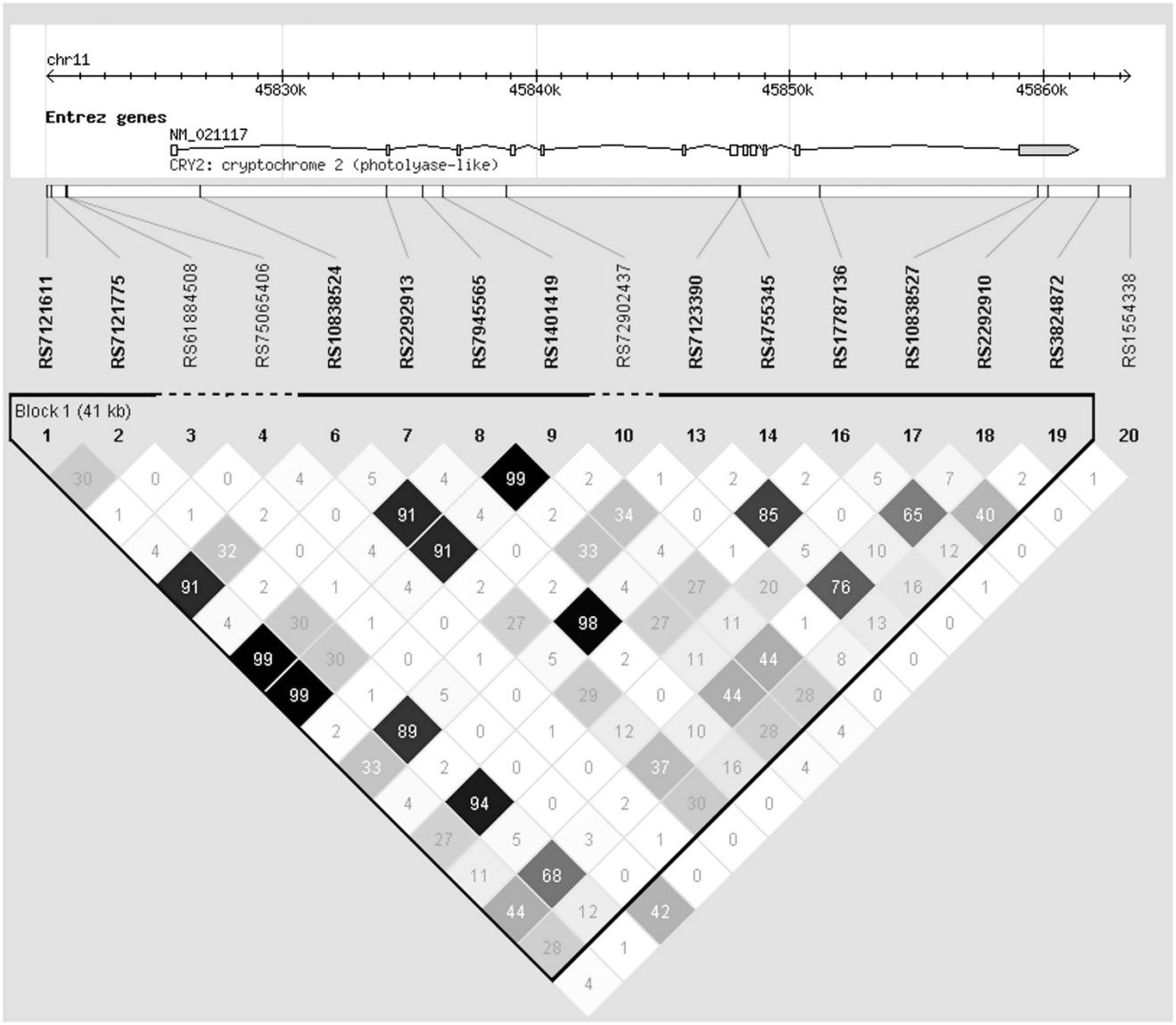

Figure 2 The analyzed circadian clock 2 single-nucleotide polymorphisms (*CRY2* SNPs), their location and the haplotype block structure constructed using the Haploview program showing r^2-values.

Table 4 Nominally significant haplotype associations using the NCEP-ATPIII criteria for metabolic syndrome

Phenotype	*NSNP*	*NHAP*	*Gene*	*SNP1*	*SNP2*	*HAPLOTYPE*	*Frequency*	*Odds ratio*	P-*value*	*q-value*
Elevated blood pressure	3	4	*CRY1*	RS4964513	RS12821586	5′-*CGG*-3′	0.12	1.24	0.00093	0.15
Elevated blood pressure	12	5	*CRY1*	RS10861697	RS10778528	5′-*CTTCGTCCTTAG*-3′	0.065	1.3	0.0026	0.21
High triglycerides	12	5	*CRY1*	RS10861697	RS10778528	5′-*CCCCACTCTCAG*-3′	0.38	0.9	0.012	0.66
Metabolic syndrome	15	10	*CRY2*	RS7121611	RS3824872	5′-*ATTTGCGGTGGCACG*-3′	0.039	1.24	0.039	0.77
High triglycerides	15	10	*CRY2*	RS7121611	RS3824872	5′-*ATTTGCGGTGGCACG*-3′	0.039	1.22	0.045	0.77
High triglycerides	12	5	*CRY1*	RS10861697	RS10778528	5′-*GTCTGCCCCCAT*-3′	0.43	1.08	0.047	0.77
Elevated blood pressure	3	4	*CRY1*	RS4964513	RS12821586	5′-*TGA*-3′	0.11	0.88	0.049	0.77

Abbreviations: NCEP-ATPIII, National Cholesterol Education Program; NHAP, number of common haplotypes ($f>0.01$); NSNP, number of SNPs in this haplotype; SNP1, SNP ID of the first SNP (5′); SNP2, SNP ID of the last SNP (3′).

DISCUSSION

Our results suggest that *CRY1* genetic variants may have a role in elevated blood pressure and hypertension. Previously, other core circadian clock genes were implicated in the regulation of blood pressure whose systolic component follows a circadian rhythm.[29] Circadian clock disruption has been implicated in the pathogenesis of cardiovascular disease, for which hypertension is a major factor. We found no evidence in our study sample to support the findings that relate *CRY2* to fasting glucose levels or indices of glucose metabolism.

Our study has some limitations. Our systematic screening for the metabolic syndrome and its components in relation to the SNPs covering three genes yielded results that did not reach study-wide significance. Our results indicated that metabolic syndrome, as such, is not associated with the genetic variants of *CRY1*, *CRY2* or *PRKCDBP*. However, the false discovery rate procedure used does not take into account the linkage disequilibrium between the SNPs, and our haplotype analysis gave further support to the one-phenotype association of hypertension and elevated blood pressure with the *CRY1* SNPs.

Cryptochromes act as key repressors in the core of the circadian clocks.[30–33] Both CRY1 and CRY2 act as repressors, but the actions of CRY1 are opposed by CRY2.[33] Furthermore, PER1 antagonizes CRY2, through which PER1 target genes are activated.[34] Actions of PER1 have a potential contribution to visceral fat accumulation,[35] functions of beta-cells in the pancreas[36] and synchronization of the peripheral liver clock by insulin.[37] In addition to actions in the nucleus, the cryptochrome proteins act as inhibitors of adenylyl cyclase, thereby limiting cyclic adenosine monophosphate production,[38] and they act as inhibitors of G protein coupled receptor activity through a direct interaction with the G(s)alpha subunit.[39]

Genetic loss of cryptochromes does change physiology. Cryptochromes appear relevant to the pathogenesis of metabolic syndrome, as cryptochromes participate in glucocorticoid regulation of gluconeogenesis and steroidogenesis.[40] Cryptochrome-deficient mice have elevated sympathetic nerve activity and impaired glucose tolerance,[41] and increased susceptibility to glucocorticoid-induced hyperglycemia with glucose intolerance and constitutively high levels of circulating corticosterone.[40] When cryptochrome-deficient mice are challenged with a high-salt diet, they have hypertension due to abnormally high synthesis of the mineralocorticoid aldosterone by the adrenal gland,[42] and when challenged with a high-fat diet, obesity develops due to the increased insulin secretion and lipid storage in white adipose tissue.[43] Genetic loss of cryptochromes constitutively activates proinflammatory cytokine expression, and then the innate immune system becomes hypersensitive, the NF-κB signaling pathway is constantly activated and the PKA signaling activity is constitutive.[38]

In addition to these findings, in *CRY2* knock-down experiments, genes contributing to inflammation are upregulated, the proinflammatory cytokine activity through the actions of interleukin-6 and interleukin-18 is increased, and genes contributing to immune responses are upregulated.[44] All of these features are also part of metabolic syndrome, and here, we hypothesize that dysfunction of cryptochromes might be an overarching factor with a shared effect that contributes to the changes in physiology. Disruption of circadian clocks seems to affect not only the metabolic and cell-division cycles,[45,46] but also mood and behavior.[47,48] It is therefore likely that in humans, cryptochromes have a role in the pathogenesis of these medical conditions and mental disorders.[49,50] Based on our current results, the role of *CRY1* in the pathogenesis of cardiovascular diseases, and its contribution to elevated blood pressure deserve further study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Author contributions. TP conceived the study and coordinated the same. All authors designed the study, drafted the manuscript, read and approved the final manuscript. LK performed the statistical analysis. KD and MK carried out the molecular genetic studies.

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