Original Article

A panel of rhythm gene polymorphisms is involved in susceptibility to type 2 diabetes mellitus and bipolar disorder

Wenjiao Min[1,2], Nie Tang[3], Zhili Zou[1], Yuexin Chen[2], Xu Zhang[1], Yulan Huang[1], Jinyu Wang[1], Yaoyin Zhang[1], Bo Zhou[1], Xueli Sun[2]

[1]Psychosomatic Department, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chinese Academy of Sciences Sichuan Translational Medicine Research Hospital, Chengdu, China; [2]Mental Health Center, West China University Hospital, Sichuan University, Chengdu, China; [3]Department of Endocrinology, Sichuan Provincial People's Hospital, Chengdu, China

Contributions: (I) Conception and design: W Min, X Sun, B Zhou; (II) Administrative support: B Zhou; (III) Provision of study materials or patients: N Tang, Y Zhang, Z Zou; (IV) Collection and assembly of data: X Zhang, Y Huang, J Wang; (V) Data analysis and interpretation: W Min, Y Chen; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Bo Zhou, MD. Psychosomatic Department, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chinese Academy of Sciences Sichuan Translational Medicine Research Hospital, Chengdu, China. Email: jiaojiao1766@163.com; Xueli Sun, MD. Mental Health Center, West China University Hospital, Sichuan University, Chengdu, China. Email: sunxueli58@163.com.

Background: Biological rhythm is closely related to health. We aimed to identify the potential correlations of rhythm gene polymorphisms to type 2 diabetes mellitus (DM) or bipolar disorder (BD), which both have many abnormal rhythmic activities, in a sample of Chinese Han origin.

Methods: A total of 136 patients with BD, 166 patients with DM, and 130 healthy controls were collected. We screened 28 single nucleotide polymorphisms (SNPs) located in rhythm genes CLOCK, ARNTL, PER2, PER3, CRY1, and CRY2 respectively. Snapshot typing technology was used for genotyping.

Results: Both the rs10832022-G and rs11022765-A allele frequencies of the ARNTL gene were significantly higher in patients with DM than in those with BD (corrected P=0.03, 0.004, respectively). The frequency of rs10832022-G, rs1022765-A, and rs11022762-T haplotypes, which was significantly lower in patients with BD than in controls (P=0.003, OR =0.579), was significantly higher in patients with DM than in those with BD (P=0.0002, OR =1.878). The rs2292910-CC genotypic frequency of the CRY2 gene was significantly higher in patients with BD than in controls (OR of regression =2.203, P=0.01), while the frequency of the AA genotype was significantly lower than in patients with DM (P=0.01). The frequency of rs1972874-G and rs36124720-G haplotype of the PER2 gene was significantly higher in patients with DM than in controls (P=0.01, OR =1.577).

Conclusions: Our study preliminarily suggested that both BD and type 2 DM could be considered as dysrhythmias with different rhythmic genetic backgrounds, which contribute to the early prediction of an individual's susceptibility to different rhythm disorders and early intervention.

Keywords: Type 2 diabetes mellitus (type 2DM); bipolar disorder; rhythm; gene

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Introduction

Biological rhythm regulates physiological activities of the body and is closely related to health (1). The suprachiasmatic nucleus (SCN) in the front of the hypothalamus is currently recognized as the rhythm center of mammals (2). The central oscillator of the SCN contains several rhythm genes, including CLOCK, ARNTL, PER1/2/3 and CRY1/2, constituting a "transcription-translation-feedback" loop, which plays an important role in the regulation of circadian activities of the body (3). When

the rhythm regulation loop turns abnormal, many disorders of rhythm activities may occur, including fluctuations of blood pressure and blood glucose (4).

Type 2 diabetes mellitus (DM) is considered as a typical psychosomatic disease characterized by abnormal blood glucose. Many researchers have found that patients with DM often have circadian rhythm disorder, or hormones of hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-thyroid (HPT) axes secretion rhythm disorder (5,6). Variations in the Rev-erb alpha and Rev-erb beta genes which are both associated with circadian rhythm could affect metabolic changes in DM (7). The rs4580704 single nucleotide polymorphism (SNP) in rhythm gene CLOCK was closely related to the occurrence of type 2 DM. The susceptibility of CC genotype carriers to DM was significantly higher than the other genotype carriers (8).

Bipolar disorder (BD) was a common severe mental disorder characterized by recurrent episodes of hyperthymia and hypothymia or mixed. Epidemiology showed that BD has been the second mental disorder in China with high rates of recurrence, disability and suicide (9). Recent years, BD has been considered to be a typical emotional rhythm disorder (10). The amplitude of body temperature rhythm decreased in BD patients (11). The rs2304672SNP in PER2 rhythm gene was associated with the susceptibility of BD (12). Besides, similarly to DM, disorder of neuroendocrine axes secretion rhythms (13), and circadian dysrhythmia (14) were also common in patients with BD. Mutations in the CLOCK rhythm gene in mice might lead to hyperglycemia, hyperlipidemia (15), or manic and excitatory behaviors (16). These suggest that the biological rhythm changes are closely related to both DM and BD. The variation of rhythm genes may affect the susceptibility of individuals to different diseases.

Through previous study on the identification and optimization of atypical symptoms of BD, our research team has proposed the theoretical hypothesis of "stress-dysrhythmia", supposing that stress and genetic factors are the pathogenesis of chronic non-infectious diseases, where dysfunctions of rhythms in the body may play an important role in the occurrence and development of disease (17). Different genetic backgrounds of rhythm may be the basis of different rhythm disorders. DM and BD are both common chronic non-infectious diseases, with heavy disease burdens and high rates of disability. They have a high rate of co-morbidity clinically (18), which may affect the treatment and prognosis of patients. Therefore, exploring the influence of rhythm genetic backgrounds on the susceptibilities of BD and DM would be helpful for the early prediction and intervention of disease. However, there are still few studies about the association of rhythm gene polymorphisms and BD or DM, with inconsistent conclusions and lack of repeated validation. Moreover, there is a lack of comparison of the differences of rhythm genetic background among different diseases.

Therefore, this study chose the four core circadian rhythm genes CLOCK, ARNTL, PER and CRY, taking healthy people as a control group, to explore the distribution characteristics of rhythm gene SNPs in patients with DM or BD, to preliminarily verify the influence of different rhythm genetic background on the susceptibilities of different rhythm disorders. We present the following article in accordance with the STREGA reporting checklist (available at https://dx.doi.org/10.21037/atm-21-4803).

Methods

Subjects

We enrolled 136 patients with BD and 166 patients with DM from southwest China between December 2018 and December 2019. Patients with BD fulfilled the Diagnostic and Statistical Manual of Mental Disorder, Fourth Edition (DSM-IV) criteria, and the diagnosis was independently assigned by two senior psychiatrists using the Structured Clinical Interview for DSM-IV Disorders-Clinician Version (the Kappa value of the consistency check was greater than 0.8). Patients with DM fulfilled the World Health Organization (WHO) diagnostic criteria for type 2 DM [1999]. The exclusion criteria were as follows: BD group: (I) with other Axis I psychiatric disorders including depression, schizophrenia, anxiety disorder, and substance abuse; (II) with Axis II disorders including personality disorders; (III) with any history of major medical/neurological disorders; (IV) using any drugs that may affect metabolism, such as glucocorticoids in the past 3 months; (V) pregnancy or lactation. For the DM group, the exclusion criteria were: (I) type 1 DM or some other special type of DM; (II) with acute complications of type 2 DM or severe chronic complications; (III) using β blockers or glucocorticoids in the past 3 months; (IV) with any history of other endocrine or autoimmune diseases, such as adrenal, thyroid, or gonad disorders; (V) with any history of major medical/neurological disorders; (VI) with any history of schizophrenia and its spectrum disorders, organic mental disorders, or substance abuse; (VII) pregnancy or lactation.

We also screened 130 race-matched healthy people as controls and ruled out current or past serious physical illnesses or DSM-IV Axis I Disorders through the Structural Clinical Interview for DSM-IV Axis I Disorders, non-patient edition (SCID-NP) (19).

All subjects were unrelated (no blood relationship) and of Chinese Han origin, sharing similar geographic and sociodemographic data. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Institutional Ethics Committee of the Sichuan Provincial People's Hospital [Ethic review (Research) No. 18.2018]. All methods were performed in accordance with the relevant guidelines and regulations, and written informed consent was obtained from each participant.

DNA extraction and genotyping

Five milliliters of peripheral venous blood were collected from each participant with EDTA anticoagulation on enrollment. Genomic DNAs were extracted from the blood samples using the standard phenol-chloroform method.

We screened 28 single nucleotide polymorphisms (SNPs), which located in rhythm genes CLOCK, ARNTL, PER2, PER3, CRY1, and CRY2, respectively, with the minor allele frequency (MAF) greater than 0.2 for genotyping (Reference website: https://www.stat.ubc.ca/rollin/stats/ssize/caco.html) (Table S1).

Snapshot typing technology was applied to type the 28 SNP sites, and primer sequences were made using online Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/) (Table S2). The typing process included polymerase chain reaction (PCR), restriction digestion, and multiple single base extension reaction with Snapshot. The extension products were sequenced using ABI3730XL sequencer, and the original data collected were analyzed by Gene Mapper 4.1 (Applied Bio-systems Co., Ltd., USA).

Statistical analysis

The study used χ^2 test to calculate Hardy-Weinberg equilibrium (HWE) and SHEsis software for Linkage disequilibrium (LD) analysis (20). PLINK software was used to conduct logistic regression statistics of SNP alleles, genotypes, and interactions among each group, and χ^2 test or ANOVA was used for comparison of the basic demographic data of the three groups in SPSS 18.0 software package. All the tests were two tailed, with alpha set at 0.05.

Results

Although there was no significant difference in sex distribution between the DM group and control group (P>0.05), women greatly outnumbered men in the BD group compared to the DM and control groups (P<0.05). The mean age of patients in the BD group was the youngest (29.97±13.460 years), followed by the control group (38.96 ±8.204 years), and that in the DM group was the oldest (58.81±10.369 years).

HWE analysis showed that except rs55794336 (P=0.007713), the P value of the other 27 SNP sites were all greater than 0.05, suggesting that all were consistent with HWE (Table S3). The MAF values of the 27 SNPs in both control and case groups were all similar to those in the gene bank, as were the LD distributions, indicating the samples tested had good reliability and representability.

Comparisons of allele frequencies of SNP sites among three groups

Both the allele frequencies of rs10832022 and rs11022765 were significantly different between the BD group and DM group. The G allele frequency of rs10832022 in the BD group (36.76%) was significantly lower than in the DM group (50.00%), while the A allele frequency (63.24%) was significantly higher than in the DM group (50.00%) (adjusted P=0.03). The A allele frequency of rs11022765 in the BD group (30.15%) was significantly lower than in the DM group (45.18%), while the C allele frequency was significantly higher than in the DM group (69.85% in the BD group and 54.82% in the DM group, adjusted P=0.004).

Comparisons of SNP genotypic distributions between BD group and control group

Genotypic distributions of the 27 SNPs in patients with BD and the controls are summarized in *Table 1*. Both the genotypic distributions of rs10832022 and rs11022765 were statistically different between the BD group and control group (χ^2=8.793, P=0.01 for the former, χ^2=9.009, P=0.01 for the latter). Moreover, after the regression analysis including each SNP genotypic distributions, the differences were still statistically significant (OR =0.614, P=0.01 for rs10832022, OR =0.589, P=0.00 for rs11022765). However, after the clinical phenotypic regression analysis including

Table 1 Genotypic distributions of 27 single nucleotide polymorphisms (SNPs) in the bipolar disorder (BD) group and the control group

SNP	Genotype	BD group	Control group	Genotypic distributions		Regressions for SNPs variations		Regressions for clinical phenotype	
				χ^2	P_1	OR_2	P_2	OR_3	P_3
rs10832020	CC/CT/TT	2/66/68	8/60/62	4.029	0.13	0.805	0.32	0.848	0.58
rs10832022	GG/GA/AA	18/64/54	35/58/37	8.793	0.01	0.614	0.01	0.646	0.06
	G-/AA	82/54	93/37	3.734	0.05				
	GG/A-	18/118	35/95	7.805	0.01				
rs10832027	AA/AG/GG	16/74/46	14/65/51	0.839	0.65	1.168	0.42	1.153	0.58
rs1026071	GG/GA/AA	26/68/42	24/69/37	0.269	0.87	0.963	0.83	1.105	0.69
rs10832030	GG/GA/AA	18/86/32	39/58/33	13.070	0.00	0.720	0.07	0.891	0.63
rs11022762	CC/CT/TT	20/70/46	16/56/58	3.251	0.19	1.334	0.12	1.310	0.27
rs11022765	AA/AC/CC	12/58/66	26/59/45	9.009	0.01	0.589	0.00	0.625	0.05
	A-/CC	70/66	85/45	5.292	0.02				
	AA/C-	12/124	26/104	6.780	0.01				
rs11894491	AA/AG/GG	26/60/50	19/56/55	1.330	0.51	1.217	0.25	1.190	0.46
rs1868049	CC/CT/TT	18/84/34	32/64/34	6.491	0.04	0.791	0.21	1.003	0.99
rs1972874	CC/CG/GG	14/52/70	17/58/55	2.283	0.32	0.771	0.15	0.834	0.46
rs2253820	CC/CT/TT	18/62/56	12/54/64	2.151	0.34	1.310	0.14	1.209	0.45
rs2292910	CC/CA/AA	20/66/50	8/64/58	5.634	0.06	1.484	0.04	2.203	0.01
	C-/AA	86/50	72/58	1.699	0.19				
	CC/A-	20/116	8/122	4.230	0.04				
rs2640908	TT/TC/CC	30/70/36	37/59/34	1.592	0.45	0.878	0.45	0.842	0.46
rs34862781	AA/AG/GG	16/56/64	10/50/70	1.859	0.39	1.284	0.18	1.326	0.28
rs36124720	GG/GC/CC	14/54/68	14/46/70	0.534	0.77	1.077	0.68	1.046	0.86
rs2585405	CC/CG/GG	24/64/48	35/59/36	3.835	0.15	0.725	0.06	0.756	0.23
rs4757142	AA/AG/GG	24/66/46	25/59/46	0.277	0.87	1.000	1.0	1.041	0.86
rs3736544	AA/AG/GG	16/56/64	18/64/48	2.803	0.25	0.767	0.14	0.711	0.18
rs3789327	GG/GA/AA	8/76/52	15/57/58	5.039	0.08	1.019	0.93	1.010	0.97
rs4757139	CC/CT/TT	24/64/48	26/73/31	4.196	0.12	0.746	0.10	0.683	0.13
rs4757145	GG/GA/AA	32/64/40	17/66/47	5.053	0.08	1.425	0.05	1.343	0.22
rs7950226	GG/GA/AA	20/72/44	14/64/52	2.062	0.36	1.308	0.15	1.313	0.29
rs4757151	GG/GA/AA	14/82/40	30/56/44	10.78	0.00	0.833	0.31	0.866	0.55
rs5863	AA/AG/GG	20/62/54	23/68/39	2.772	0.25	0.765	0.14	0.807	0.39
rs707463	TT/TC/CC	22/76/38	28/54/48	5.473	0.06	1.076	0.67	1.029	0.90
rs6486116	AA/AC/CC	20/76/40	35/64/31	6.128	0.04	0.682	0.04	0.700	0.14
	A-/CC	96/40	99/31	1.052	0.31				
	AA/C-	20/116	35/95	6.049	0.01				
rs6798	TT/TC/CC	30/72/34	28/77/25	1.475	0.48	0.887	0.52	0.779	0.33

gender and age, the differences were both not statistically significant. The frequencies of the rs10832022 A- genotype and rs11022765 C- genotypes were both much higher in the BD group than in the control group (86.8% in the BD group and 73.1% in the control group, χ^2=7.805, P=0.01 for the former; 91.2% in the BD group and 80.0% in the control group, χ^2=6.78, P=0.01 for the latter).

Distributions of the rs2292910 A allele carriers (A-) and CC genotype carriers were both statistically significant between the BD group and the control group (χ^2=4.230, P=0.04), and the frequency of the CC genotype in the BD group (14.7%) was significantly higher than in the control group (6.7%). Moreover, after both the regression including each SNP genotypic distributions and clinical phenotypic regression including gender and age, these differences were still statistically significant (OR =1.484, P=0.04 for the former, OR =2.203, P=0.01 for the latter).

Rs10832022, rs11022765, and rs11022762 had LD through LD analysis (*Figure 1A*). Haplotype analysis showed that the distribution frequency of rs10832022-G, rs11022765-A, and rs11022762-T haplotype in the BD group (30.1%) was significantly lower than that in the control group (42.7%) (χ^2=9.050, P=0.003, OR =0.579, 95% CI: 0.405–0.828).

Comparisons of SNP genotypic distributions between DM group and control group

Genotypic distributions of the 27 SNPs in patients with DM and the controls are summarized in *Table 2*. The genotypic distribution of rs1972874 was statistically different between the DM group and control group (χ^2=6.212, P=0.04). Moreover, after both the regression including each SNP genotypic distributions and clinical phenotypic regression including gender and age, the difference was still statistically significant (OR =0.655, P=0.02 for the former, OR =0.407, P=0.02 for the latter). The frequency of the CC genotype was much higher in the DM group (56.5%) than in the control group (42.3%) (χ^2=5.979, P=0.01), and the genotypic distribution of rs36124720 was also statistically different between the DM group and the control group (χ^2=6.227, P=0.04). While after the regression including each SNP genotypic distributions, the difference was still statistically significant (OR =1.512, P=0.02), after the clinical phenotypic regression including gender and age, the difference was not statistically significant. The frequency of the G- genotype was much higher in the DM group (60.2%) than in the control group (46.2%) (χ^2=5.825, P=0.02).

The distributions of rs7950226 G allele carriers (G-) and AA genotype carriers were statistically significant between the DM group and control group (χ^2=4.004, P=0.04), and the frequency of the G- genotype in the DM group (71.1%) was significantly higher than in the control group (60.0%). Moreover, after both regression including each SNP genotypic distributions and clinical phenotypic regression including gender and age, the difference was still statistically significant (OR =1.456, P=0.04 for the former, OR =2.909, P=0.01 for the latter).

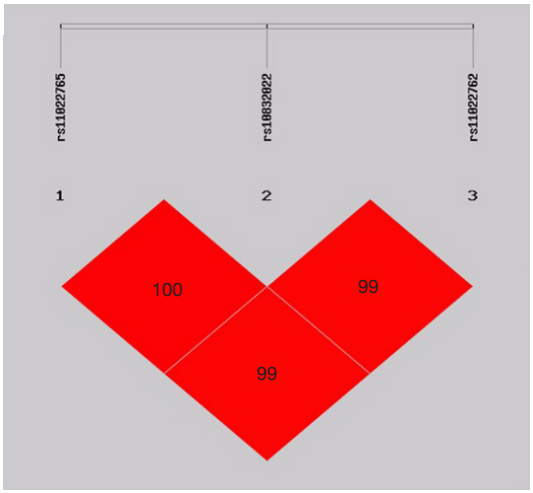
Rs1972874 and rs36124720 had LD through LD analysis (*Figure 1B*). Haplotype analysis showed that the distribution frequency of rs1972874-C and rs36124720-C haplotype in the DM group (25.9%) was significantly lower than in the control group (35.4%) (χ^2=6.233, P=0.01, OR =0.638, 95% CI: 0.448–0.909), while the distribution frequency of rs1972874-G and rs36124720-G haplotype in the DM group (38.6%) was significantly higher than in the control group (28.5%) (χ^2=6.608, P=0.01, OR =1.577, 95% CI: 2.223–2.235).

Comparisons of SNP genotypic distributions between DM and BD groups

Genotypic distributions of the 27 SNPs in DM and BD groups are summarized in *Table 3*. Besides the significant differences of allele frequencies of rs10832022 and rs11022765, the genotypic distributions of the above two SNPs were also both statistically different between the DM group and BD group (χ^2=11.450, P=0.00 for the former, χ^2=15.380, P=0.00 for the latter). Moreover, after the regression including each SNP genotypic distributions, the differences were still statistically significant (OR =1.774, P=0.00 for rs10832022, OR =1.991, P=0.00 for rs11022765). However, after the clinical phenotypic regression including gender and age, the differences were not statistically significant.

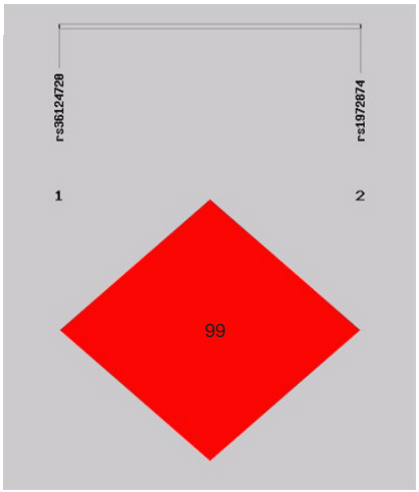
The frequency of the rs10832022 G- genotype in the DM group (77.1%) was significantly higher than that in the BD group (60.3%) (χ^2=9.977, P=0.00), and the frequency of the GG genotype in the DM group (22.9%) was also significantly higher than that in the BD group (13.2%) (χ^2=4.615, P=0.03). The frequency of the rs11022765 A-genotype in the DM group (72.3%) was significantly higher than in the BD group (51.5%) (χ^2=13.890, P=0.0002), and the frequency of the AA genotype in the DM group (18.1%) was also significantly higher than that in the BD group (8.8%) (χ^2=5.341, P=0.02).

A



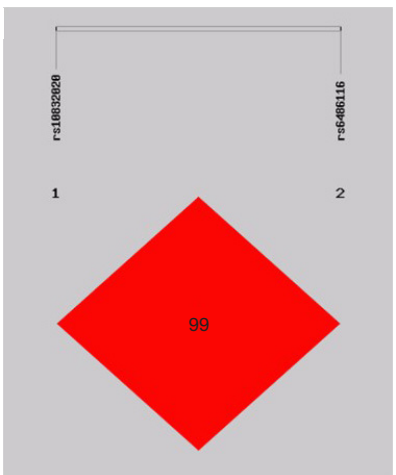
D'	rs10832022	rs11022762
rs11022765	1.000	1.000
rs10832022		1.000
r^2	rs10832022	rs11022762
rs11022765	0.768	0.381
rs10832022		0.496

B

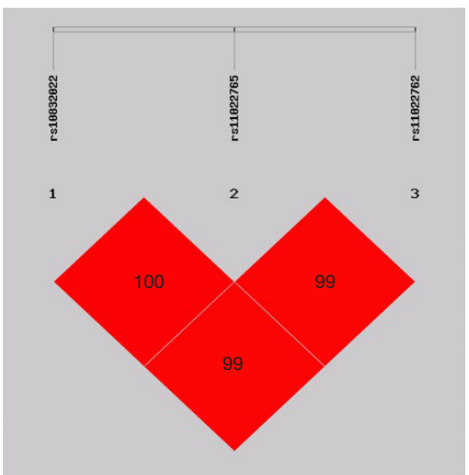


rs1972874–rs36124720	
D'=1.000	r^2=0.223

C



rs10832020–rs6486116	
D'=1.000	r^2=0.388



D'	rs10832022	rs11022762
rs11022765	1.000	1.000
rs10832022	–	1.000
r^2	rs10832022	rs11022762
rs11022765	0.768	0.381
rs10832022		0.496

Figure 1 Linkage disequilibrium (LD) analysis of single nucleotide polymorphisms (SNPs) among groups. (A) LD analysis of SNPs in the bipolar disorder (BD) group and the control group; (B) LD analysis of SNPs in the diabetes mellitus (DM) group and the control group; (C) LD analysis of SNPs in the DM group and the BD group.

Table 2 Genotypic distributions of 27 single nucleotide polymorphisms (SNPs) in the diabetes mellitus (DM) group and the control group

SNP	Genotype	DM group	Control group	Genotypic distributions		Regressions for SNPs variations		Regressions for clinical phenotype	
				χ^2	P_1	OR_2	P_2	OR_3	P_3
rs10832020	CC/CT/TT	14/84/68	8/60/62	1.558	0.46	1.270	0.21	1.425	0.36
rs10832022	GG/GA/AA	38/90/38	35/58/37	2.717	0.26	1.031	0.85	1.069	0.85
rs1026071	GG/GA/AA	40/90/36	24/69/37	2.445	0.29	1.310	0.12	1.121	0.73
rs10832027	AA/AG/GG	16/84/66	14/65/51	0.102	0.95	0.960	0.83	0.930	0.84
rs10832030	AA/AG/GG	28/94/44	33/58/39	4.932	0.08	0.901	0.54	1.392	0.34
rs11022765	AA/AC/CC	30/90/46	26/59/45	2.404	0.30	1.109	0.54	1.168	0.67
rs1868049	TT/TC/CC	40/82/44	34/64/32	0.225	0.89	0.925	0.64	0.977	0.94
rs1972874	CC/CG/GG	14/58/94	17/58/55	6.212	0.04	0.655	0.02	0.407	0.02
	C-/GG	72/94	75/55	5.979	0.01				
	CC/G-	14/152	17/113	1.676	0.20				
rs11894491	AA/AG/GG	24/72/70	19/56/55	0.003	1.00	1.000	1.00	1.248	0.51
rs2253820	CC/CT/TT	10/70/86	12/54/64	1.111	0.57	0.864	0.43	0.900	0.77
rs2292910	CC/CA/AA	24/54/88	8/64/58	10.790	0.01	0.998	0.99	0.757	0.44
rs2585405	GG/GC/CC	36/86/44	36/59/35	1.700	0.43	0.896	0.50	0.880	0.69
rs2640908	TT/TC/CC	32/88/46	37/59/34	3.558	0.17	0.807	0.20	0.893	0.73
rs34862781	AA/AG/GG	16/78/72	10/50/70	3.207	0.20	1.356	0.10	2.752	0.01
rs36124720	GG/GC/CC	28/72/66	14/46/70	6.227	0.04	1.512	0.02	1.924	0.07
	G-/CC	100/66	60/70	5.825	0.02				
	GG/C-	28/138	14/116	2.227	0.14				
rs11022762	CC/CT/TT	20/66/80	16/56/58	0.399	0.82	0.922	0.63	1.240	0.55
rs3789327	GG/GA/AA	26/60/80	15/57/58	2.189	0.33	1.011	0.95	1.490	0.23
rs4757139	CC/CT/TT	32/82/52	26/73/31	2.109	0.35	0.839	0.31	0.615	0.18
rs4757142	AA/AG/GG	22/94/50	25/59/46	4.046	0.13	0.985	0.93	1.428	0.31
rs4757151	GG/GA/AA	26/80/60	30/56/44	2.643	0.27	0.828	0.25	0.692	0.26
rs5863	AA/AG/GG	26/68/72	23/68/39	5.700	0.06	0.733	0.06	0.541	0.08
rs6486116	CC/CA/AA	34/96/36	31/64/35	2.207	0.33	1.042	0.81	1.174	0.67
rs6798	CC/CT/TT	36/80/50	25/77/28	3.926	0.14	0.877	0.44	0.833	0.62
rs707463	TT/TC/CC	20/92/54	28/54/48	7.306	0.03	0.898	0.53	0.968	0.92
rs7950226	GG/GA/AA	26/92/48	14/64/52	4.473	0.11	1.456	0.04	2.909	0.01
	G-/AA	118/48	78/52	4.004	0.04				
	GG/A-	26/140	14/116	1.494	0.22				
rs3736544	AA/AG/GG	22/62/82	18/64/48	5.02	0.08	0.762	0.11	0.503	0.06
rs4757145	GG/GA/AA	20/76/70	17/66/47	1.107	0.58	0.854	0.37	1.078	0.84

Table 3 Genotypic distributions of 27 single nucleotide polymorphisms (SNPs) in the diabetes mellitus (DM) group and the bipolar disorder (BD) group

SNP	Genotype	BD group	DM group	Genotypic distributions		Regressions for SNPs variations		Regressions for clinical phenotype	
				χ^2	P_1	OR_2*	P_2	OR_3*	P_3
rs10832020	CC/CT/TT	2/66/68	14/84/68	8.261	0.02	1.603	0.02	1.736	0.27
	C-/TT	68/68	98/68	2.466	0.12				
	CC/T-	2/134	14/152	7.224	0.01				
rs10832022	GG/GA/AA	18/64/54	38/90/38	11.450	0.00	1.774	0.00	1.333	0.48
	G-/AA	82/54	128/38	9.977	0.00				
	GG/A-	18/118	38/128	4.615	0.03				
rs1026071	GG/GA/AA	26/68/42	40/90/36	3.549	0.17	1.351	0.08	0.630	0.24
rs10832027	AA/AG/GG	16/74/46	16/84/66	1.236	0.54	0.820	0.28	1.405	0.42
rs10832030	AA/AG/GG	32/86/18	28/94/44	8.630	0.01	0.604	0.01	1.091	0.84
	A-/GG	118/18	122/44	8.069	0.01				
	AA/G-	32/104	28/138	2.084	0.15				
rs36124720	GG/GC/CC	14/54/68	28/72/66	4.331	0.11	1.417	0.04	2.112	0.08
rs11022762	CC/CT/TT	20/70/46	20/66/80	6.375	0.04	0.694	0.03	1.162	0.73
	C-/TT	90/46	86/80	6.348	0.01				
	CC/T-	20/116	20/146	0.460	0.50				
rs11022765	AA/AC/CC	12/58/66	30/90/46	15.380	0.00	1.991	0.00	1.538	0.31
	A-/CC	70/66	120/46	13.890	0.00				
	AA/C-	12/124	30/136	5.341	0.02				
rs11894491	AA/AG/GG	26/60/50	24/72/70	1.539	0.46	0.821	0.22	0.736	0.43
rs1868049	CC/CT/TT	18/84/34	44/82/40	8.518	0.01	1.375	0.07	0.928	0.86
rs1972874	CC/CG/GG	14/52/70	14/58/94	0.868	0.65	0.851	0.36	0.573	0.18
rs2253820	CC/CT/TT	18/62/56	10/70/86	6.190	0.04	0.652	0.02	0.721	0.44
	C-/TT	80/56	80/86	3.391	0.07				
	CC/T-	18/118	10/156	4.621	0.03				
rs2292910	CC/CA/AA	20/66/50	24/54/88	9.137	0.01	0.721	0.04	0.246	0.00
	C-/AA	86/50	78/88	7.952	0.01				
	CC/A-	20/116	24/142	0.004	0.95				
rs4757139	CC/CT/TT	24/64/48	32/82/52	0.547	0.76	1.120	0.49	1.439	0.40
rs2585405	CC/CG/GG	24/64/48	44/86/36	7.921	0.02	1.580	0.01	1.635	0.22
	C-/GG	88/48	130/36	6.894	0.01				
	CC/G-	24/112	44/122	3.363	0.07				
rs2640908	TT/TC/CC	30/70/36	32/88/46	0.358	0.84	0.918	0.61	1.053	0.90
rs34862781	AA/AG/GG	16/56/64	16/78/72	1.113	0.57	1.036	0.84	1.424	0.45

Table 3 *(continued)*

Table 3 *(continued)*

SNP	Genotype	BD group	DM group	Genotypic distributions		Regressions for SNPs variations		Regressions for clinical phenotype	
				χ^2	P_1	OR_2*	P_2	OR_3*	P_3
rs3736544	AA/AG/GG	16/56/64	22/62/82	0.496	0.78	0.983	0.92	0.986	0.98
rs3789327	GG/GA/AA	8/76/52	26/60/80	14.510	0.00	0.996	0.98	1.141	0.75
rs4757142	AA/AG/GG	24/66/46	22/94/50	2.195	0.33	0.985	0.93	1.163	0.74
rs4757145	GG/GA/AA	32/64/40	20/76/70	9.089	0.01	0.611	0.00	1.043	0.92
	G-/AA	96/40	96/70	5.254	0.02				
	GG/A-	32/104	20/146	6.913	0.01				
rs4757151	GG/GA/AA	14/82/40	26/80/60	4.691	0.01	0.968	0.86	0.792	0.58
rs7950226	GG/GA/AA	20/72/44	26/92/48	0.420	0.81	1.107	0.56	2.237	0.08
rs5863	AA/AG/GG	20/62/54	26/68/72	0.657	0.72	0.947	0.74	0.673	0.35
rs6486116	AA/AC/CC	20/76/40	36/96/34	4.447	0.11	1.458	0.04	1.074	0.87
rs6798	CC/CT/TT	34/72/30	36/80/50	2.523	0.28	0.794	0.16	0.319	0.01
rs707463	TT/TC/CC	22/76/38	20/92/54	1.436	0.49	0.811	0.24	1.232	0.67

* OR (odds ratio): risk value of DM group compared with BD group.

The genotypic distribution of rs2292910 was statistically different between the DM group and BD group (χ^2=9.137, P=0.01). Moreover, after both the regression including each SNP genotypic distributions, and clinical phenotypic regression including gender and age, the difference was still statistically significant (OR =0.712, P=0.04 for the former, OR =0.246, P=0.00 for the latter). The frequency of the AA genotype was significantly higher in the DM group (53.0%) than that in the BD group (36.8%) (χ^2=7.952, P=0.01).

The genotype distribution of rs11022762 was also statistically different between the DM group and BD group (χ^2=6.375, P=0.04), and after regression including each SNP genotypic distributions, the difference was still statistically significant (OR =0.694, P=0.03). However, after the clinical phenotypic regression including gender and age, the difference was not statistically significant. The frequency of the TT genotype in the DM group (48.2%) was significantly higher than that in the BD group (33.8%) (χ^2=6.348, P=0.01).

The genotypic distribution of rs10832020 was statistically different between the DM group and BD group (χ^2=8.261, P=0.02), and after regression including each SNP genotypic distributions, the difference was still statistically significant (OR =1.03, P=0.02). The frequency of the CC genotype in the DM group was significantly higher than in the BD group (8.4% for the former and 1.5% for the latter; χ^2=7.224, P=0.01).

Rs10832022, rs11022765, and rs11022762 had LD through LD analysis (*Figure 1C*), and haplotype analysis showed that the distribution frequency of rs10832022-A, rs11022765-C, and rs11022762-C haplotype in the DM group (31.2%) was significantly lower than in the BD group (40.4%) (χ^2=5.309, P=0.02, OR =0.674, 95% CI: 0.482–0.944). The distribution frequency of rs10832022-G, rs11022765-A, and rs11022762-T haplotype in the DM group (44.4%) was significantly higher than in the BD group (30.1%) (χ^2=13.489, P=0.0002, OR =1.878, 95% CI: 1.339–2.633).

In addition, rs10832020 and rs6486116 also had LD through LD analysis (*Figure 1C*), and haplotype analysis showed that the distribution frequency of rs10832020-C and rs6486116-A haplotype in the DM group (33.7%) was significantly higher than in the BD group (25.7%) (χ^2=4.545, P=0.03, OR =1.469, 95% CI: 1.031–2.094).

Discussion

Both the frequencies of the rs10832022 G allele/G- genotype and rs11022765 A allele/A- genotype were significantly higher in patients with DM than in those

with BD. This result suggests that the two alleles might be closely related to the occurrence of type 2 DM. The frequency of the rs11022762 TT genotype, as well as that of the rs10832022-G, rs11022765-A, and rs11022762-T haplotype was both significantly higher in patients with DM than in those with BD. Rs10832022, rs11022765, and rs11022762 were all located in the ARNTL gene intron 2 region. The ARNTL gene, also known as the BMAL1 gene, is one of the core components of the biological rhythm oscillator of the body, and the transcription factor protein encoded by ARNTL can generate circadian rhythm through its positive and negative feedback pathways (21). The ARNTL gene was also thought to be associated with blood pressure fluctuations and metabolic syndrome. Previous animal experiments have found that some genetic variants of this gene might induce spontaneous hypertension and significantly affect the transcriptional activation of Gata-4 in rats (22), while mice with ARNTL gene knockout developed endothelial dysfunction, leading to increased vascular damage (23). In addition, mice with ARNTL gene knockout also had abnormal glucose tolerance and significantly decreased insulin secretion *in vivo*, and some mutations in the ARNTL gene could increase the risk of hypertension and type 2 DM in individuals (22). Moreover, diabetic mice showed a disturbance of heart rate, blood pressure, and circadian rhythm when expression of the ARNTL gene in arteries was inhibited (24). Our results add support to the observation that the ARNTL gene, especially the variations in its intron region, may be related to the occurrence and development of type 2 DM.

It is worth noting that the genotypic distributions of rs10832022 and rs11022765 in ANRTL gene also showed significant differences between patients with BD and controls. Frequencies of the rs10832022 GG genotype and rs11022765 AA genotype were much lower in the BD group, and the frequency of rs10832022-G, rs11022765-A, and rs11022762-T haplotype was also significantly lower in the BD group. Some SNP variations in the ARNTL gene have been linked to insomnia in women (25). A recent study further found that sleep could drive the rhythmic migration of the ARNTL gene in splenic monocytes and improve the function of immune cells (26), suggesting a role in the regulation of sleep rhythm of this gene. As sleep disorders are common in patients with DM or BD, our results demonstrated to some extent the hypothesis that DM and BD are rhythm disorders. Variations in the intron region of the ARNTL gene may be one of the genetic backgrounds of emotional rhythm disorder or glucometabolic rhythm disorder in individuals. Although the intron region of the gene was not involved in the transcription and translation of the corresponding RNA, it might still affect the linear expression of the gene and the selective splicing of the transcription and translation, affecting the occurrence of rhythm disorders (27). In the future, with the combination of downstream functional changes, we will further explore the role of the ARNTL gene in the occurrence and development of rhythm abnormalities.

Cryptochrome 2 gene (CRY2), a transcriptional inhibitor, is one component in the negative regulation loop of biological rhythm, playing an important role in maintaining circadian stability and regulating cell cycle and DNA repair. It is widely expressed in the liver, pancreases, heart, and other tissues (28). The expression of this gene was down-regulated in some tumor tissues (29) and might influence the occurrence of breast cancer through hormone-mediated signal transduction (30). A previous study has found that rs2292910 SNP of this gene may affect bone formation and resorption, and its C allele might be a protective factor in the pathogenesis of osteoporosis (31). We found that the frequency of the rs2292910 CC genotype was significantly higher in patients with BD than in controls, and frequency of the AA genotype was significantly higher in patients with type 2 DM than with BD. Furthermore, the above differences were not affected by the clinical phenotype of diseases and other detected SNPs variations. Therefore, rs2292910 in CRY2 gene might be used as a genetic marker to predict the risk of somatic or emotional rhythm disorder in individuals. The CC genotype may be the risk genotype for BD (an emotional rhythm disorder), while the AA genotype may be a risk predictor for individuals to differentiate into type 2 DM (a glucose metabolism rhythm disorder) rather than the emotional rhythm disorder. As rs2292910 was located in the exon 13 region of the CRY2 gene 3'-UTR, its variation might regulate the expression of CRY2, thereby affecting the individual's rhythmic function. The rs11605924 SNP of the CRY2 gene was thought to be associated with increased fasting glucose level in the general population and was a risk factor for metabolic syndrome. It was further speculated that the CRY2 gene might be related to glucose metabolism and energy balance and affect the susceptibility of individuals to obesity (32). Our results further support the view that the CRY2 gene may play an important role in the development of type 2 DM, as its different genetic variants had a certain predictive value for the risk of somatic glucose metabolism rhythm disturbance.

This study showed that the rs7950226 G allele carriers

were much more prevalent in patients with type 2 DM than in controls, and the OR value reached 2.909, suggesting the G- genotype was a risk genotype for type 2 DM. Rs7950226 is also located in the intron 1 region of the ARNTL gene, and its A allele was found to associated with the occurrence of type 2 DM in a Greek population (33), contrary to our result. While this contradiction may be attributed to ethnic differences, both results suggest the genetic variability in the intron region of the ARNTL gene may be the rhythm-related genetic background for the occurrence of type 2 DM. In addition, rs10832020 and rs6486116, two SNPs also located in the intron 1 region of the ARNTL gene and having significantly different distributions between patients with type2 DM and with BD, had no LD with rs7950226. Therefore, we believe that the different genotypic variation of rs7950226 may be an independent risk factor associated with rhythm heredity in the occurrence of type 2 DM.

Rs1972874 and rs36124720 were both located in the intron region of PER2 gene and had a strong LD in this study. The distribution frequency of the G-G haplotype in patients with type 2 DM was significantly higher than in controls, with an OR value of 1.577, suggesting a high-risk genotype for the occurrence of type 2 DM. The intron region of PER2 gene may be correlated with the susceptibility to type 2 DM. PER2 belongs to the mammalian Period family, which can regulate its own transcription in both direct and indirect ways, and form an endogenous biological clock with CRY/BMAL1 and CLOCK to regulate body rhythm (28). Sevoflurane could cause PER2 to lose its rhythm, which could be restored by light (34). The serum cortisol peak was delayed in the diabetic depression model rats, and the expression of PER2 in SCN was up-regulated compared with a control model, which suggested that overexpression of the PER2 gene might inhibit the positive activation process and change the HPA axis rhythm of the body, leading to fluctuations of blood sugar and mood (35). Although the intron region did not participate in the final expression of PER2, its different gene mutations might still influence the abnormal regulation of downstream rhythm through the intervention of splicing (36). Further studies will focus on the effect of PER2 gene variations to the dysrhythmia of body glucose metabolism by combining epigenetics.

Unfortunately, this study did not find any risk allele for type 2 DM or BD. On this basis, we consider that only a single SNP mutation might not be enough to cause these diseases. As abnormal expressions of some rhythm genes are found in many chronic non-infectious diseases, including type 2 DM (37), it is necessary to combine epigenetic indicators and other clinical rhythm changes to further explore the genetic mechanism of different rhythm disorders. It is worth noting that the genotypic distributions of rs10832022, rs11022765, and rs2292910 SNPs were not only significantly different between patients with type 2 DM and those with BD, but also between patients with BD and controls. This suggests that the three SNPs may be relatively independent genetic mutation sites for BD, being worthy of further study.

In conclusion, the present study preliminarily demonstrates that some variations of rhythm genes, especially the SNPs located in rhythm gene ARNTL intron region, CRY2 3'-UTR and PER2 intron region are associated with the susceptibility to type 2 DM or BD. Meanwhile, these variations may predict the risk of developing glucometabolic or emotional dysrhythmia in individuals, and be the specific rhythm genetic background of the two diseases. These results preliminarily verified the theoretical hypothesis of "stress-dysrhythmia" about the mechanisms of chronic non-infectious diseases (17). Based on the different rhythm genetic variations, individuals may have different biological rhythm dysfunction and lead to different diseases with various stress factors.

Our study not only suggests both type 2 DM and BD could be considered as rhythmic disorders with different genetic backgrounds of rhythm, but also is helpful for the early prediction of individual susceptibility to physical or emotional rhythm disorder and for early intervention.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/atm-21-4803). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Institutional Ethics Committee of the Sichuan Provincial People's Hospital [Ethic review (Research) No. 18.2018]. All methods were performed in accordance with the relevant guidelines and regulations, and written informed consent was obtained from each participant.

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Supplementary

Table S1 Basic information of the 29 selected SNP sites

SNP	CHR*	Gene Name	Position	MAF# (Gene pool)	
rs4757139	11	ARNTL	13300456	C	0.423
rs4757142	11	ARNTL	13325695	A	0.416
rs4757145	11	ARNTL	13331324	G	0.435
rs1026071	11	ARNTL	13364712	G	0.488
rs10832020	11	ARNTL	13321343	C	0.313
rs10832022	11	ARNTL	13339272	G	0.445
rs10832027	11	ARNTL	13357183	A	0.399
rs10832030	11	ARNTL	13399791	G	0.490
rs11022762	11	ARNTL	13335926	C	0.392
rs11022765	11	ARNTL	13345115	A	0.373
rs11894491	2	PER2	239198325	A	0.341
rs1868049	11	ARNTL	13383682	C	0.454
rs1972874	2	PER2	239178402	C	0.315
rs2253820	17	PER1	8048169	C	0.310
rs2292910	11	CRY2	45903613	C	0.325
rs2585405	17	PER1	8046772	C	0.440
rs2640908	1	PER3	7889941	T	0.486
rs34862781	11	ARNTL	13305263	A	0.339
rs36124720	2	PER2	239155971	G	0.361
rs3736544	4	CLOCK	56309992	A	0.334
rs3789327	11	ARNTL	13385316	G	0.332
rs4757151	11	ARNTL	13392213	G	0.399
rs55794336	12	CRY1	107445977	G	0.423
rs5863	4	CLOCK	56296907	A	0.404
rs6486116	11	ARNTL	13319838	A	0.490
rs6798	11	CRY2	45904477	C	0.452
rs707463	1	PER3	7850062	T	0.450
rs7950226	11	ARNTL	13318139	G	0.392

CHR, chromosome; MAF, minor allele frequency.

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Table S2 Primer sequences of SNPs detected

SNP	F (forward)	R (reverse)
rs4757139	ATCCCAGAGAGCTTCCCGTTTG	GGCCGGCGAGAACTTGACA
rs4757142	GCCTTGGGAATGACAAGCAGAG	TCTAACGGCTTCTCCCCACCTC
rs4757145	GCCACAACGTGCCATGTGTTAC	CCCTGTAAGCCTCCCCAGTGTT
rs1026071	CCAACATCACACGGCTGGTAAA	TTGGAGTCCCAGGAACCATCAA
rs10832020	GGTGGAGTTGTCTTGGGGACCT	ACCTGAAGGCCCAGCTCTCCTC
rs10832022	CCCTCTCCAAGTTTTGTGCCTTGTA	GCAGCCAGGGGGTAGACAGTTT
rs10832027	TGTGGGCAAAACCCTGCTTAAA	GGCACCGCTACACCAGAAAACA
rs10832030	TTTCCCTGCTGGAATGCCTTTT	GAACAGTGGGGTGGGTCCTCTT
rs11022762	TCTCTGTTCCAAGTGCCAGCATA	GGAGACACAACAGGCGTCACAA
rs11022765	AGAGGTGGAGGACCTAAGTGGCTA	GATGATACCATTGCACTCCAGC
rs11894491	CCGGCAGCTACCAAGTGACCTT	CCGTTGTAAGGCGTCCCTTTCT
rs1868049	CTGGGAATGGTGTTGGGAATTG	GCAGCTGCCCCAAATGATACAG
rs1972874	AGTGCACTGCTGCAAACACACC	TGGCCTCCACAGCTTTCTTTGT
rs2253820	AGGTCTCGGAAGCGGCTGA	CTACCGTCCAGTGGGGCTGAC
rs2292910	CCTGGTTTCTCTGGCCACACTC	CTCTGGGTCAAACCTCCCACCT
rs2585405	GCAGCAGATTGAGCTGGAGTGG	TGGCCTTGGTGCTCCCTAACTA
rs2640908	GCAACAATGGCAGTGAGAGCAGT	GTGACCCCGTGGACAGAACAGT
rs34862781	GCTGACAGCAATTGGGAACCAG	ATCCAGGTCCGCATCCCTTATG
rs36124720	TTTCAACTGGACATTCACAGCAG	CACTTGCAGGCCTCTCACACAC
rs3736544	TGTGCTGAGTTGTGCCAATGTGT	GGGTTGAATTTTGGTTCCGTTCA
rs3789327	CCTCAGCAATGCAAAATGGACA	TACCAGCACCACAGAGCCACAG
rs4757151	TGAAGTCCTGCCACAATGAGTCC	GGGCTGCATGGTCACGTTAAAT
rs55794336	CCCAAGTTTGCACGAGGTTTTTCTA	TGCCCTTGTGGTAAAGAGGTACTGTC
rs5863	GCCAACATTTCAGGGCACATTT	TGTGGACACCAAAAGAGACCAATG
rs6486116	TGGGTTCTGCACAGCTCATTG	GTCCAGGCCAAGGGAGCA
rs6798	CCTCCGCCTACTTCTCCACCAT	TCTACCCTGCCCTTCCCTCTTG
rs707463	TGTCATCCCTGCTTGCTTCTAGC	CCTGGGGAAAAAATGGGAAAGA
rs7950226	CTGAAGGGGTCTGGGGAATCAC	GGGCCACATTCACAAGGGAAAT

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Table S3 Hardy-Weinberg equilibrium (HWE) analysis

CHR	SNP	A1	A2	GENO[4]	O[1](HET)	E[2](HET)[3]	P
1	rs707463	T	C	28/54/48	0.4154	0.4882	0.1052
1	rs2640908	T	C	37/59/34	0.4538	0.4997	0.2959
2	rs36124720	G	C	14/46/70	0.3538	0.4072	0.1349
2	rs1972874	C	G	17/58/55	0.4462	0.4573	0.8478
2	rs11894491	A	G	19/56/55	0.4308	0.4617	0.4502
4	rs5863	A	G	23/68/39	0.5231	0.4924	0.593
4	rs3736544	A	G	18/64/48	0.4923	0.4734	0.7138
11	rs4757139	C	T	26/73/31	0.5615	0.4993	0.2179
11	rs34862781	A	G	10/50/70	0.3846	0.3935	0.824
11	rs7950226	G	A	14/64/52	0.4923	0.4573	0.4466
11	rs6486116	C	A	31/64/35	0.4923	0.4995	0.8618
11	rs10832020	C	T	8/60/62	0.4615	0.4137	0.2873
11	rs4757142	A	G	25/59/46	0.4538	0.487	0.4718
11	rs4757145	G	A	17/66/47	0.5077	0.4734	0.4629
11	rs11022762	C	T	16/56/58	0.4308	0.4478	0.6959
11	rs10832022	G	A	35/58/37	0.4462	0.4999	0.2225
11	rs11022765	A	C	26/59/45	0.4538	0.4893	0.4728
11	rs10832027	A	G	14/65/51	0.5	0.4595	0.4442
11	rs1026071	G	A	24/69/37	0.5308	0.495	0.4803
11	rs1868049	T	C	34/64/32	0.4923	0.4999	0.8618
11	rs3789327	G	A	15/57/58	0.4385	0.4453	0.8454
11	rs4757151	G	A	30/56/44	0.4308	0.4942	0.1562
11	rs10832030	A	G	33/58/39	0.4462	0.4989	0.2231
11	rs2292910	C	A	8/64/58	0.4923	0.426	0.1002
11	rs6798	C	T	25/77/28	0.5923	0.4997	0.05283
12	rs55794336	G	A	35/49/46	0.3769	0.4964	0.007713
17	rs2585405	G	C	36/59/35	0.4538	0.5	0.2958
17	rs2253820	C	T	12/54/64	0.4154	0.42	1

[1]O: observed frequency; [2]E: expected frequency; [3]HET: heterozygosity; [4]GENO: genotype.

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