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Research Letter

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Novel *CLOCK* and *NR1D2* variants in 64 sighted Japanese individuals with non-24-hour sleep–wake rhythm disorder

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Circadian rhythm sleep–wake disorders (CRSWDs) are characterized by an inability to fall asleep and awaken at desired times. A subtype of CRSWDs, non-24-hour sleep–wake rhythm disorder (N24SWD), exhibits a free-running pattern of sleep–wake cycles that are not synchronized with the external 24-hour day. N24SWD occurs commonly in visually impaired individuals and rarely in sighted individuals. We have shown that the intrinsic circadian period (τ) determined under a forced desynchrony protocol is longer in sighted individuals with N24SWD than intermediate-type controls [1]. Therefore, it appears that prolonged τ and/or impaired entrainment mechanisms contribute to the pathogenesis of N24SWD. Some clock gene variants are associated with CRSWDs and transgenic animals carrying human clock variants exhibit altered τ [2]. These findings suggest that clock gene variants might disrupt the circadian clock system and lead to the onset of CRSWDs.

The study population consisted of 64 participants with N24SWD (45 males and 19 females; mean ± SD age: 27.7 ± 9.61 years). Most of the participants were examined in our previous studies [1, 3]. They were all unrelated and sighted Japanese. They were diagnosed by trained psychiatrists according to the International Classification of Sleep Disorders' second edition. This study was approved by the Ethics Committee of National Center of Neurology and Psychiatry and was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from each participant. DNA samples were extracted from the participants' blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN). Targeted sequencing of 76 genes was performed in 17 individuals with N24SWD (11 males and six females; mean ± SD age: 32.82 ± 10.05 years) using next-generation sequencing by RIKEN GENESIS (RIKEN GENESIS CO., LTD.). Briefly, DNA samples were captured using the SureSelect DNA Capture Custom Kit (Agilent Technologies) and sequenced on the MiSeq system (Illumina) with 151 bp paired-end reads. The reads were aligned to a human reference sequence (University of California Santa Cruz assembly GRCh37/hg19). The 76 genes examined in this study are listed in Supplementary Table 1. Sanger sequencing was performed using BigDye™ Terminator v1.1 Ready Reaction Mix (ThermoFisher Scientific) and an ABI Prism 3130 DNA Analyzer (Applied Biosystems). All primers were designed by Primer3Plus. The possible impact of amino acid substitutions on protein function was tested by Polyphen-2 and PROVEAN (Protein Variation Effect Analyzer).

In this study, we performed targeted sequencing of 76 genes by next-generation sequencing in 17 N24SWD individuals and found a total of 94 variants (Supplementary Table 2). A novel missense variant was initially found in each of the *APOE* (*apolipoprotein E*), *CLOCK* (*CLOCK*), *NR1D2* (*nuclear receptor subfamily 1 group D member 2*), and *PER1* genes The *NR1D2* (PERIOD1) and *PER1* missense variants were found in an individual with N24SWD. These four variants were further evaluated by public databases, the Genome Aggregation Database, 1000 Genomes and Human Genetic Variation Database, and the Japanese Whole Genome Reference Panel 8.3KJPN. The *APOE* and *CLOCK* variants (designated as rs1969838696 and rs1723064872, respectively) were recently observed as rare variants in 8.3KJPN (alternative allele frequency = 0.00012), while the *NR1D2* and *PER1* variants were not found in any database. Previous studies using animal models have suggested that *Clock* and *REV-ERBβ* (*NR1D2*) are involved in some mechanisms that regulate circadian rhythms and

sleep–wakefulness [2, 4, 5]. In contrast, *APOE*-deficient and *Per1*-null mice show a robust behavioral rhythm comparable to that of WT mice [6, 7]. Therefore, our initial focus was on the *CLOCK* and *NR1D2* genes, which have been implicated as core components of negative transcriptional feedback loops regulating multiple clock genes in the circadian clock system [2, 4, 5]. We subsequently performed Sanger sequencing on the coding regions of *CLOCK* and *NR1D2* in a total of 64 N24SWD individuals, including 17 N24SWD individuals.

One novel and four known *CLOCK* variants were identified in our study population of 64 N24SWD individuals (Supplementary Table 3). A novel variant in exon 18 was identified in one of the additional 47 N24SWD individuals (NM_004898.3: c.1488C>G: p.Q[Gln]496H[His]) (Figure 1A). The amino acids Q and H differ in isoelectric point and the Q496H substitution is predicted to be possibly damaging (0.887) by Polyphen-2 and deleterious (–2.854) by PROVEAN. The Q496H substitution occurs in the domain that potentially associates with SIRT1, a NAD+-dependent histone deacetylase. SIRT1 is recruited to the CLOCK:BMAL1 chromatin complex and regulates target gene transcription by modulating the histone acetyltransferase function of CLOCK [8]. The Q496H substitution could alter the binding interaction between CLOCK and SIRT1 thereby disrupting chromatin remodeling. The known variant in exon 22 (rs1723064872) causes the amino acid substitution of glutamine to glutamate (NM_004898.3: c.2278C>G: p.Q[Gln]760E[Glu]). The amino acids Q and E differ in isoelectric point, although the Q760E substitution is predicted to be benign (0.033) by Polyphen-2 and neutral (–1.289) by PROVEAN. The Q760E substitution is located in the poly-Q region of the C-terminal domain. The Q-rich motif is known to characterize the activation domain of transcription factors. Furthermore, *Clock* mutant mice show a longer period of behavioral rhythms than wild-type mice. The genetic variant carried by *Clock* mutants results in exon skipping and a deletion of 51 amino acids within the CLOCK transactivation domain [4]. Notably, the missense variants in the potentially functional domains of CLOCK were identified in two N24SWD individuals. These *CLOCK* missense variants might contribute to the N24SWD phenotype.

One novel and eight known *NR1D* variants were identified in our study population of 64 N24SWD individuals (Supplementary Table 3). The novel variant in exon 2 causes an amino acid substitution of glycine to serine (NM_005126.4: c.274G>A: p.G[Gly]92S[Ser]) (Figure 1B). The amino acids G and S differ in polarity, although the G92S substitution is predicted to be benign (0.011) by Polyphen-2 and neutral (-1.094) by PROVEAN. A known variant in exon 5 (rs139583758) causes the amino acid substitution of glutamine to histidine (NM_005126.4: c.696A>C: p.Q[Gln]232H[His]). The amino acids Q and H differ in isoelectric point and the Q232H substitution is predicted to be possibly damaging (0.925) by Polyphen-2 and be deleterious (–2.518) by PROVEAN. Another known variant in exon 7 (rs78292562) causes the amino acid substitution of alanine to threonine (NM_005126.4: c.1351G>A: p.A[Ala]451T[Thr]). Amino acids A and T differ in polarity. Furthermore, the A451T substitution is predicted to be probably damaging (0.995) by Polyphen-2 and deleterious (–3.157) by PROVEAN. The A451T substitution occurs in the potential ligand-binding domain of NR1D2. Heme binds to the ligand-binding domain and modulates the ability of NR1D2 to recruit the corepressor and repress target gene transcription [9]. NR1D1 (REV-ERBα) and NR1D2 (REV-ERBβ) regulate sleep architecture and emotional behavior in mice [5]. REV-ERB agonists induce wakefulness and reduce rapid eye movement and slow-wave sleep. Intriguingly, a pharmacological study in REV-ERBβ-deficient mice suggests that REV-ERBβ modulates the maintenance of wakefulness during the activity period [10]. These *NR1D2* missense variants could alter the function of NR1D2, resulting in impaired sleep regulation. Also, the novel *PER1* variant was found in the N24SWD individual carrying the novel *NR1D2* variant in exon 2. The *PER1* variant in exon 10 (NM_002616.2: c.1198G>A: p.E[Glu]400K[Lys]) was confirmed by Sanger sequencing in the N24SWD individual (Figure 1C).

Further analysis is required to demonstrate that these variants contribute to the N24SWD phenotype. However, our findings will provide potential genetic factors associated with the N24SWD phenotype and expand the current understanding of circadian and sleep regulation in humans.

Supplementary Material

Supplementary material is available at *SLEEP* online.

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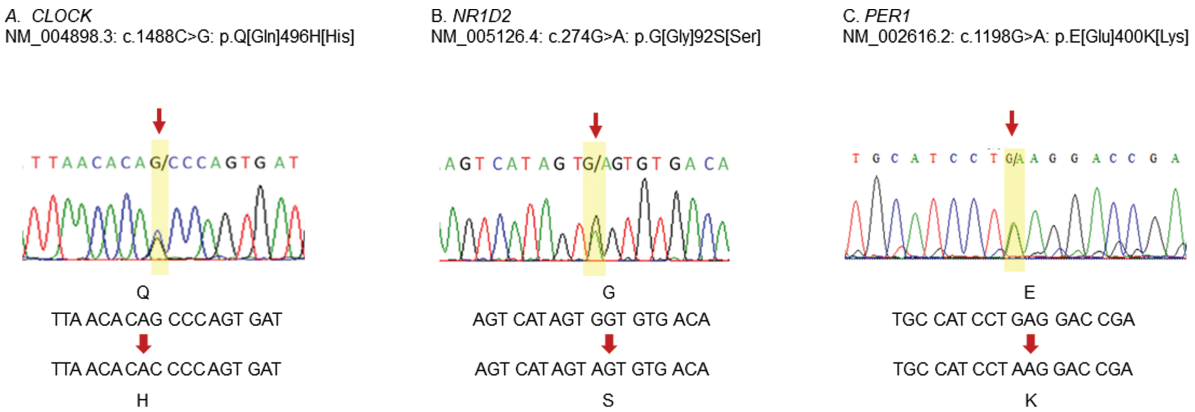

Figure 1. Novel missense variants of the CLOCK, *NR1D2*, and *PER1* genes in N24SWD individuals. Sanger sequencing confirms a novel missense variant in each of *CLOCK* (A), *NR1D2* (B) and *PER1* (C) as indicated by an arrow.

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Disclosure Statement

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Data Availability

The data underlying this article are available in the article and in its online supplementary material.

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Supplementary Table 1: 76 circadian- and sleep-related genes examined in 17 subjects with N24SWD

Chromosome	Gene
chr17	*AANAT*
chr19	*APOE*
chr11	*ARNTL*
chr12	*ARNTL2*
chrX	*ASMT*
chr20	*AVP*
chr12	*AVPR1A*
chr1	*AVPR1B*
chrX	*AVPR2*
chr11	*BDNF*
chr3	*BHLHE40*
chr12	*BHLHE41*
chr4	*CLOCK*
chr12	*CRY1*
chr11	*CRY2*
chr17	*CSNK1D*
chr22	*CSNK1E*
chr3	*GSK3B*
chr17	*HCRT*
chr6	*HCRTR2*
chr6	*HLA-A*
chr6	*HLA-B*
chr6	*HLA-C*
chr6	*HLA-DMA*
chr6	*HLA-DMB*
chr6	*HLA-DOA*
chr6	*HLA-DOB*
chr6	*HLA-DPA1*
chr6	*HLA-DPB1*
chr6	*HLA-DPB2*
chr6	*HLA-DQA1*
chr6	*HLA-DQA2*
chr6	*HLA-DQB1*
chr6	*HLA-DQB2*
chr6	*HLA-DRA*
chr6	*HLA-DRB1*
chr6	*HLA-DRB5*
chr6	*HLA-DRB6*
chr6	*HLA-E*
chr6	*HLA-F*
chr6	*HLA-F-AS1*
chr6	*HLA-G*
chr6	*HLA-H*
chr6	*HLA-L*
chr5	*HTR1A*
chr13	*HTR2A*
chrX	*HTR2C*
chr11	*HTR3A*
chr11	*HTR3B*
chr3	*HTR3C*
chr3	*HTR3D*
chr3	*HTR3E*
chr5	*HTR4*
chr7	*HTR5A*
chr2	*HTR5B*
chr1	*HTR6*
chr10	*HTR7*
chr7	*IL6*
chr4	*MTNR1A*
chr11	*MTNR1B*
chr2	*NPAS2*
chr17	*NR1D1*
chr3	*NR1D2*
chr10	*OPN4*
chr17	*PER1*
chr2	*PER2*
chr1	*PER3*
chr22	*PPARA*
chr15	*RORA*
chr9	*RORB*
chr12	*TIMELESS*
chr6	*TNF*
chr6	*VIP*
chr3	*VIPR1*
chr7	*VIPR2*

Supplementary Table 2: 94 variants of circadian- and sleep-related genes in 17 individuals with N24SWD

Chromosome	Start Position	End Position	Reference Allele (0)	Alternative Allele (1)	Type	Known /Novel	Gene	Function	dbSNP	Reference Sequence	Genotype Count			Average Read Depth
											0|0	0|1	1|1	
chr17	74465813	74465813	G	A	SNV	Known	*AANAT*	Missense	rs28936679	NM_001166579.1	16	1	0	190.00
chr19	45409167	45409167	C	G	SNV	Known	*APOE*	Missense	rs440446	NM_001302688.1	4	11	2	88.59
chr19	45411139	45411139	C	T	SNV	Novel*	*APOE*	Missense	rs1969838696	NM_001302688.1	16	1	0	232.59
chr19	45411941	45411941	T	C	SNV	Known	*APOE*	Missense	rs429358	NM_001302688.1	16	1	0	199.24
chr19	45412079	45412079	C	T	SNV	Known	*APOE*	Missense	rs7412	NM_001302688.1	16	1	0	315.24
chr11	13398129	13398129	T	A	SNV	Known	*ARNTL*	Missense	rs10741617	NM_001297719.1	0	0	17	309.94
chr12	63541279	63541279	A	T	SNV	Known	*AVPR1A*	Missense	rs183401493	NM_000706.4	15	2	0	429.76
chr11	27679916	27679916	C	T	SNV	Known	*BDNF*	Missense	rs6265	NM_001143810.1	10	5	2	406.76
chr12	26277654	26277654	A	G	SNV	Known	*BHLHE41*	Missense	rs201545489	NM_030762.2	16	1	0	360.82
chr4	56304532	56304532	G	C	SNV	Novel*	*CLOCK*	Missense	rs1723064872	NM_004898.3	16	1	0	233.59
chr11	45869013	45869013	C	A	SNV	Known	*CRY2*	Missense	rs3747548	NM_021117.3	15	2	0	308.06
chr6	55142337	55142337	A	G	SNV	Known	*HCRTR2*	Missense	rs2653349	NM_001526.3	0	3	14	359.53
chr6	29912395	29912396	-	GG	INS	Known	*HLA-A*	Frameshift	rs545184037	NM_002116.7	11	6	0	409.59
chr6	29912414	29912414	G	-	DEL	Known	*HLA-A*	Splice donor site	rs9278466	NM_002116.7	5	6	6	350.76
chr6	31237124	31237124	T	C	SNV	Known	*HLA-C*	Missense	rs1130838	NM_002117.5	0	5	12	321.18
chr6	31239449	31239449	C	G	SNV	Known	*HLA-C*	Missense	rs28626310	NM_002117.5	16	1	0	512.59
chr6	32917544	32917544	C	T	SNV	Known	*HLA-DMA*	Missense	rs1063478	NM_006120.3	13	4	0	392.29
chr6	32904981	32904981	G	A	SNV	Known	*HLA-DMB*	Missense	rs1042337	NM_002118.4	1	7	9	326.00
chr6	32905086	32905086	G	T	SNV	Known	*HLA-DMB*	Missense	rs2071555	NM_002118.4	9	8	0	333.82
chr6	32974551	32974551	G	T	SNV	Known	*HLA-DOA*	Missense	rs2582	NM_002119.3	7	9	1	259.18
chr6	32781554	32781554	G	A	SNV	Known	*HLA-DOB*	Missense	rs2070121	NM_002120.3	15	2	0	326.94
chr6	32784676	32784676	C	T	SNV	Known	*HLA-DOB*	Missense	rs2071554	NM_002120.3	12	5	0	338.53
chr6	33036435	33036435	T	G	SNV	Known	*HLA-DPA1*	Missense	rs1126769	NM_001242524.1	5	7	5	290.18
chr6	33036853	33036853	A	C	SNV	Known	*HLA-DPA1*	Missense	rs1042308	NM_001242524.1	5	7	5	324.18
chr6	33036999	33036999	T	C	SNV	Known	*HLA-DPA1*	Missense	rs1042190	NM_001242524.1	5	7	5	360.12
chr6	33037080	33037080	G	A	SNV	Known	*HLA-DPA1*	Splice acceptor site	rs73739671	NM_001242524.1	5	7	5	257.76
chr6	33037412	33037412	G	C	SNV	Known	*HLA-DPA1*	Splice donor site	rs114707319	NM_001242524.1	5	7	5	165.59
chr6	33037413	33037413	C	A	SNV	Known	*HLA-DPA1*	Splice donor site	rs116509985	NM_001242524.1	5	7	5	167.53
chr6	33037419	33037419	G	A	SNV	Known	*HLA-DPA1*	Splice donor site	rs1126543	NM_001242524.1	5	7	5	182.18
chr6	33037424	33037424	T	C	SNV	Known	*HLA-DPA1*	Missense	rs1126542	NM_001242524.1	5	7	5	191.82
chr6	33037522	33037522	T	C	SNV	Known	*HLA-DPA1*	Missense	rs1042178	NM_001242524.1	5	7	5	331.71
chr6	33037579	33037579	A	T	SNV	Known	*HLA-DPA1*	Missense	rs2308912	NM_001242524.1	5	7	5	314.76
chr6	33037580	33037580	T	G	SNV	Known	*HLA-DPA1*	Missense	rs2308911	NM_001242524.1	5	7	5	313.65
chr6	33053609	33053609	G	A	SNV	Known	*HLA-DPB1*	Missense	rs11551421	NM_002121.5	8	6	3	273.59
chr6	33048661	33048661	A	G	SNV	Known	*HLA-DPB1*	Missense	rs1042151	NM_002121.5	13	4	0	435.12
chr6	33048663	33048663	G	A	SNV	Known	*HLA-DPB1*	Missense	rs1042153	NM_002121.5	13	4	0	433.53
chr6	32611068	32611068	A	G	SNV	Known	*HLA-DQA1*	Splice acceptor site	rs707947	NM_002122.3	7	7	3	355.24
chr6	32634447	32634447	C	G	SNV	Known	*HLA-DQB1*	Splice acceptor site	rs3210146	NM_001243961.1	12	5	0	252.29
chr6	32628633	32628633	T	C	SNV	Known	*HLA-DQB1*	Splice donor site	rs9273528	NM_001243961.1	6	9	2	313.18
chr6	32628660	32628660	T	C	SNV	Known	*HLA-DQB1*	Splice acceptor site	rs28688207	NM_001243961.1	11	6	0	317.76
chr6	32407773	32407773	G	C	SNV	Known	*HLA-DRA*	Missense	rs16822586	NM_019111.4	16	1	0	338.00
chr6	32411035	32411035	A	C	SNV	Known	*HLA-DRA*	Splice acceptor site	rs8084	NM_019111.4	4	6	7	337.53
chr6	32411646	32411646	T	G	SNV	Known	*HLA-DRA*	Missense	rs7192	NM_019111.4	4	6	7	348.41
chr6	32552094	32552099	CCGCAC	-	DEL	Known	*HLA-DRB1*	Deletion	rs201929829	NM_002124.3	7	10	0	319.24
chr6	30458064	30458064	G	A	SNV	Known	*HLA-E*	Missense	rs1264457	NM_005516.5	9	7	1	259.88
chr6	30459317	30459317	C	A	SNV	Known	*HLA-E*	Missense	rs148162840	NM_005516.5	16	1	0	355.82
chr6	29693011	29693011	C	T	SNV	Known	*HLA-F*	Missense	rs1736924	NM_001098479.1	0	0	17	461.76
chr6	29693233	29693233	C	T	SNV	Known	*HLA-F*	Missense	rs57237143	NM_001098479.1	16	1	0	431.82
chr6	29694680	29694680	A	C	SNV	Known	*HLA-F-AS1*	Missense	rs3734814	NM_001098479.1	9	5	3	326.53
chr6	29694681	29694681	A	T	SNV	Known	*HLA-F-AS1*	Missense	rs3734815	NM_001098479.1	9	5	3	327.18
chr6	29796376	29796376	C	A	SNV	Known	*HLA-G*	Missense	rs12722477	NM_002127.5	5	7	5	319.88
chr5	63256729	63256729	C	T	SNV	Known	*HTR1A*	Missense	rs1800042	NM_000524.3	13	4	0	343.00
chr13	47470824	47470824	C	T	SNV	Known	*HTR2A*	Missense	rs6312	NM_001165947.2	0	0	17	349.35
chr11	113846041	113846041	G	T	SNV	Known	*HTR3A*	Missense	rs190646973	NM_213621.3	16	1	0	235.41
chr11	113803008	113803008	T	C	SNV	Known	*HTR3B*	Splice acceptor site	rs11214773	NM_006028.4	16	1	0	305.71
chr11	113803028	113803028	A	C	SNV	Known	*HTR3B*	Missense	rs1176744	NM_006028.4	10	6	1	342.29
chr3	183774762	183774762	C	A	SNV	Known	*HTR3C*	Missense	rs6766410	NM_130770.2	3	7	7	358.88
chr3	183778010	183778010	G	C	SNV	Known	*HTR3C*	Missense	rs6807362	NM_130770.2	0	8	9	264.12
chr3	183753777	183753777	G	A	SNV	Known	*HTR3D*	Missense	rs36092077	NM_001163646.1	11	4	2	169.47
chr3	183754278	183754278	C	G	SNV	Known	*HTR3D*	Missense	rs73183412	NM_001163646.1	12	5	0	327.88
chr3	183755822	183755822	G	A	SNV	Known	*HTR3D*	Missense	rs1000952	NM_001163646.1	0	1	16	173.00
chr3	183818416	183818416	G	A	SNV	Known	*HTR3E*	Missense	rs7627615	NM_001256614.1	0	6	11	311.71
chr3	183822699	183822699	G	A	SNV	Known	*HTR3E*	Missense	rs146851567	NM_001256614.1	16	1	0	295.59
chr3	183822730	183822731	-	ATTCCTCT	INS	Known	*HTR3E*	Frameshift	rs560673114	NM_001256614.1	16	1	0	263.65
chr5	147928225	147928225	C	T	SNV	Known	*HTR4*	Splice donor site	rs2278392	NM_001040173.2	11	4	2	247.53
chr10	92503408	92503408	C	G	SNV	Known	*HTR7*	Missense	rs138700544	NM_019859.3	16	1	0	336.00
chr10	92503619	92503619	T	C	SNV	Known	*HTR7*	Missense	rs3740048	NM_019859.3	14	2	1	340.88
chr7	22771039	22771039	T	A	SNV	Known	*IL6*	Missense	rs13306435	NM_000600.3	16	1	0	328.06
chr4	187455426	187455426	G	A	SNV	Known	*MTNR1A*	Missense	rs1800884	NM_005958.3	15	2	0	386.71
chr4	187476360	187476360	G	A	SNV	Known	*MTNR1A*	Missense	rs1800885	NM_005958.3	15	2	0	266.12
chr11	92714996	92714996	G	A	SNV	Known	*MTNR1B*	Missense	rs772328752	NM_005959.3	16	1	0	323.29
chr2	101584895	101584895	C	T	SNV	Known	*NPAS2*	Splice donor site	rs2289950	NM_002518.3	4	9	4	240.76
chr2	101437601	101437601	C	G	SNV	Known	*NPAS2*	Missense	rs6542990	NM_002518.3	1	9	7	280.00
chr2	101591304	101591304	A	G	SNV	Known	*NPAS2*	Missense	rs2305160	NM_002518.3	1	6	10	277.82
chr3	23996285	23996285	G	A	SNV	Novel	*NR1D2*	Missense		NM_005126.4	16	1	0	235.82
chr3	24006477	24006477	T	A	5' UTR	Known	*NR1D2*	Missense	rs4858097	NM_005126.4	0	0	17	269.76
chr10	88416963	88416963	G	A	SNV	Known	*OPN4*	Missense	rs7901458	NM_001030015.2	16	1	0	309.94
chr10	88419062	88419062	G	A	SNV	Known	*OPN4*	Missense	rs202171086	NM_001030015.2	16	1	0	275.00
chr10	88422116	88422116	C	T	SNV	Known	*OPN4*	Missense	rs1079610	NM_001030015.2	0	5	12	206.82
chr17	8046772	8046772	C	G	SNV	Known	*PER1*	Missense	rs2585405	NM_002616.2	2	7	8	250.76
chr17	8051351	8051351	C	T	SNV	Novel	*PER1*	Missense		NM_002616.2	16	1	0	286.53
chr2	239168651	239168651	T	C	SNV	Known	*PER2*	Missense	rs758749027	NM_022817.2	16	1	0	355.12
chr2	239161869	239161869	G	A	SNV	Known	*PER2*	Missense	rs199746230	NM_022817.2	15	2	0	330.00
chr2	239162312	239162312	T	C	SNV	Known	*PER2*	Missense	rs763347228	NM_022817.2	16	1	0	288.12
chr1	7889973	7890026	AGAATCCATCCC ATCCTACTGCCA GCGCTCTGTCC ACAGGATCGCC TCCCATGA	-	DEL	Known	*PER3*	Deletion	rs57875989	NM_001289862.1	0	4	13	307.06
chr1	7887493	7887493	T	C	SNV	Known	*PER3*	Missense	rs228696	NM_001289862.1	0	0	17	431.76
chr1	7887579	7887579	C	G	SNV	Known	*PER3*	Missense	rs228697	NM_001289862.1	11	6	0	457.59
chr22	46615880	46615880	T	C	SNV	Known	*PPARA*	Missense	rs1800234	NM_005036.4	16	1	0	316.35
chr12	56814653	56814653	G	A	SNV	Known	*TIMELESS*	Missense	rs2291739	NM_003920.3	3	11	3	340.24
chr12	56815922	56815922	C	T	SNV	Known	*TIMELESS*	Missense	rs774047	NM_003920.3	1	12	4	297.65
chr12	56818610	56818610	G	A	SNV	Known	*TIMELESS*	Missense	rs151238555	NM_003920.3	16	1	0	288.47
chr12	56822378	56822378	T	A	SNV	Known	*TIMELESS*	Missense	rs774027	NM_003920.3	1	12	4	278.00
chr3	42567463	42567463	A	G	SNV	Known	*VIPR1*	Missense	rs144670843	NM_004624.3	15	2	0	305.12
chr7	158835860	158835860	G	A	SNV	Known	*VIPR2*	Missense	rs534492909	NM_003382.4	16	1	0	154.82

Human genome sequence was referred to UCSC assembly GRCh37/hg19.; *These variants were recently observed in the Japanese Whole Genome Reference Panel 8.3KJPN.

Supplementary Table 3: Allele frequency of the *CLOCK* and *NR1D2* variants in 64 individuals with N24SWD

Gene	Reference Sequence	Chromosome Position	Exon	Reference Allele	Alternative Allele	Known /Novel	dbSNP	Function	Alternative Allele Frequency (AAF)	AAF in public databases (population)			
										gnomAD-Exomes (Global)	1000Genomes (Global)	HGVD (Japanese)	8.3KJPN (Japanese)
CLOCK	NM_004898.3	56314997	18	C	G	Novel		missense	0.0078125				
CLOCK	NM_004898.3	56309992	21	A	G	Known	rs3736544	synonymous	0.8359375	0.695777	0.723	0.7728	0.79075
CLOCK	NM_004898.3	56304532	22	G	C	Known	rs1723064872	missense	0.0078125				0.00012
CLOCK	NM_004898.3	56301663	23	T	C	Known	rs192938463	synonymous	0.0078125	0.00002	0.0002	0.0045	0.004
CLOCK	NM_004898.3	56301369	23	A	G	Known	rs1801260	3' UTR	0.1640625	0.242754*	0.2296	0.16	0.17208
NR1D2	NM_005126.4	23986895	1	C	T	Known	rs536068754	5' UTR	0.0078125	0.000424*	0.0002		0.02011
NR1D2	NM_005126.4	23986930	1	A	C	Known	rs4858565	5' UTR	0.953125	0.794666*	0.7704		0.58014
NR1D2	NM_005126.4	23986947	1	C	T	Known	rs575779558	5' UTR	0.015625	0.011979*	0.0254		0.03103
NR1D2	NM_005126.4	23986983	1	C	G	Known	rs7644275	5' UTR	0.0078125		0.244		0.05372
NR1D2	NM_005126.4	23996056	2	C	T	Known	rs72627100	synonymous	0.046875	0.009695	0.0184	0.0276	0.03126
NR1D2	NM_005126.4	23996285	2	G	A	Novel		missense	0.0078125				
NR1D2	NM_005126.4	24003646	5	A	C	Known	rs139583758	missense	0.015625		0.0026	0.0033	
NR1D2	NM_005126.4	24006497	6	G	A	Known	rs72628104	synonymous	0.046875	0.009537	0.018	0.0306	0.03138
NR1D2	NM_005126.4	24009322	7	G	A	Known	rs78292562	missense	0.0078125	0.000853	0.0024	0.0142	0.01426

Human genome sequence was referred to UCSC assembly GRCh37/hg19.; gnomAD: Genome Aggregation Database; HGDV: Human Genetic Variation Database; *AAF in gnomAD-Genomes