A Genome-Wide Association Study for Melatonin Secretion

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Abstract

Summary

Purpose: Melatonin exerts a wide range of effects among various tissues and organs. However, there is currently no study to investigate the genetic determinants of melatonin secretion. Here, we conducted a genome-wide association study (GWAS) for melatonin secretion using morning urine 6-hydroxymelatonin sulfate-to-creatinine ratio (UMCR).

Methods: We initially enrolled 5,000 participants from Taiwan Biobank in this study. After excluding individuals that did not have their urine collected in the morning and those who failed to pass quality control, association of single nucleotide polymorphisms with log-transformed UMCR adjusted for age, sex and principal components of ancestry were analyzed. A second model additionally adjusted for estimated glomerular filtration rate (eGFR).

Results: A total of 2,504 participants underwent the genome-wide analysis. Six candidate loci associated with log UMCR (P value ranging from 7.54×10^{-7} to 4.65×10^{-6}) encompassing *GALNT15*, *ZFHX3*, *NKAIN2*, *MME* and *NBPF22P* were identified. Similar results were yielded with further adjustment for eGFR. Interestingly, the identified genes are associated with central nervous system function and clinical condition such as Alzheimer's disease or sleep disorders.

Conclusions: We conducted the first GWAS for melatonin secretion and identified six candidate genetic loci associated with melatonin level. Replication and functional studies are needed in the future.

Introduction

Melatonin is a pleiotropic hormone primarily synthesized and secreted from the pineal gland. It can also be produced by many other tissues including leukocytes, bone marrow, gastrointestinal tract, neuronal cells and gonads [1–3]. Melatonin regulates a range of physiological process including circadian and seasonal rhythms, energy and glucose metabolism, antioxidant effects, anti-inflammatory actions, and immune function [1, 3–6]. There are many studies showing associations between melatonin and many disorders, including certain types of mental illness, cancer, cardiovascular disease, metabolic syndrome, type 2 diabetes, and obesity [6–11]. Melatonin is secreted into the circulation following a circadian rhythm with peak levels at night [12]. Aging was once thought to be directly associated with decreased melatonin secretion. However, there was no significant difference between circadian amplitude of the plasma melatonin between healthy elderlies and young adults [13]. Instead of aging, the degree of pineal calcification was associated with melatonin excretion amount [14].

Substantial evidence suggests genetic factor also play a major role in melatonin secretion [15, 16]. Genome-wide association study (GWAS) has been introduced as a powerful tool to identify common genetic variants of complex diseases or quantitative traits [17]. Currently there is no published GWAS regarding to melatonin level. Here, we conducted the first GWAS of urine melatonin metabolite, 6-hydroxymelatonin sulfate (aMT6s), which serves as an estimation of circulating melatonin level [18].

Materials And Methods

Study population

Five thousand individuals who are 30 to 70 years of age and without cancer history were enrolled from Taiwan Biobank. Biological specimens, personal and clinical information as delinked data were used in this study. This study was approved by the Institutional Review Board of Chang Gung Medical Foundation and the Institutional Review Board of National Taiwan University Hospital. All subjects have provided written informed consent and all methods were carried out in accordance with relevant guidelines and regulations.

Urine aMT6s and creatinine measurement

It is infeasible to draw blood samples from volunteers in the middle of the night for serum melatonin level. Urinary aMT6s is the major metabolite of melatonin excreted from the kidneys [19]. Thus, measuring morning urine aMT6s level is a practical

alternative for serum melatonin level at night [18]. For better correlation, spot urine aMT6s level should be creatinine-corrected to adjust the effect of variable urinary dilution [20]. Urine aMT6s-to-creatinine ratio (UMCR) was calculated from urinary aMT6s divided by urine creatinine level. The concentration of aMT6s was measured in the urine of Taiwan Biobank subjects by an enzyme-linked immunosorbent assay (ELISA) kit using the manufacturer's protocol (Human Melatonin Sulfate ELISA kit, Elab science). No significant cross-reactivity or interference between melatonin sulfate and analogues was observed. All standards via serial dilution were assayed in duplicates. The urine creatinine level was measured using a chemistry analyzer (AU5800, Beckman Coulter) with compensated Jaffe method.

Genotyping, quality control and imputation

Genotyping with the Axiom-Taiwan Biobank Array Plate (TWB chip; Affymetrix Inc, Santa Clara, California) was performed at the National Center for Genome Medicine of Academia Sinica [21]. We use PLINK (version 1.07), an open-source whole-genome data analysis toolset, for quality control procedures [22]. For SNPs with batch effect, their genotypes were set as missing. SNPs were excluded if missing genotype rate was high ($> 5\%$), minor allele frequency was low ($< 1\%$) or deviated from Hardy-Weinberg equilibrium (P value $< 10^{-5}$). Individuals with high missing genotyping rate ($> 5\%$), extreme heterozygosity rate (more than 5 standard deviations away from the mean) or high identity-by-descent score (≥ 0.1875) implying close relatedness were excluded from subsequent analyses. We computed the principal components on a linkage disequilibrium (LD)-pruned ($r^2 < 0.2$) set of autosomal variants obtained by removing high-LD regions via PLINK. Genotype imputation was carried out with SHAPEIT [23] and IMPUTE2 [24]. We applied 1000 Genomes Project Phase 3 East Asian Ancestry as the reference population. For gene annotation, Genome Reference Consortium Human Build 37 was used. Imputed SNPs with low quality score (info score [25] lower than 0.3) were excluded. Indels were removed by using VCFtools [26].

Statistical analyses

Age and estimated glomerular filtration rate (eGFR) were expressed in terms of mean and standard deviation. Urine aMT6s and UMCR was expressed as median and interquartile range. Logarithmic transformation of UMCR was done to normalize the data. GWAS analysis was carried out via PLINK v1.07 with an additive genetic model. We applied linear regression for analyzing associations between SNPs and log UMCR. Covariate adjustment in Model 1 included age, sex and the first ten principal components of ancestry. eGFR was additionally adjusted in Model 2. We used a genome-wide significance threshold of $P < 5.0 \times 10^{-8}$ [27]. Since this threshold is very conservative for a small sample size, we set the level for suggestive significance at $P < 5 \times 10^{-6}$ [28, 29]. The Manhattan plot and quantile-quantile plot were generated by the qqman R package [30, 31]. Regional association plots were made via LocusZoom [32]. Using the following items, the proportion of phenotypic variance explained by SNP was calculated: effect size estimate of each minor allele on log UMCR, standard error of the effect size, sample size, and minor allele frequency for the SNP [33]. The array-based heritability was estimated by LD score regression [34].

Results

Five thousand subjects were enrolled from Taiwan Biobank initially. One withdrew from the study. 2,361 did not have their urine collected in the morning and were excluded. 134 did not pass quality control procedures. After imputation and quality control, 7,897,704 autosomal SNPs remained. We performed a GWAS analysis for log UMCR in the remaining 2,504 subjects. The characteristics of our study population are listed in Table 1. Age is borderline significantly associated with log UMCR (Pearson's $r = 0.038$; $P = 0.057$). There is no significant gender difference of log UMCR shown by T test (males 1.211, females 1.207; $P = 0.819$). eGFR is also not significantly associated with log UMCR (Pearson's $r = -0.001$; $P = 0.943$). Variants with the strongest association in each region regarding to log UMCR are shown in Table 2. Melatonin production is known to be decreased with advanced chronic kidney disease [35]; thus, we adjusted eGFR additionally in Model 2. Figures 1 and 2 are the Manhattan plot and quantile-quantile plot. Regional association plots of the top SNPs are shown in Fig. 3. Six loci showed suggestive significance, with one near the *GALNT15* gene on chromosome 3 (rs142037747; $P = 7.54 \times 10^{-7}$, Fig. 3a), another within the *ZFXH3* gene on chromosome 16 (rs17681554; $P = 3.29 \times 10^{-6}$, Fig. 3b), a third within the *NKAIN2* gene on chromosome 6 (rs74760291; $P = 3.33 \times 10^{-6}$, Fig. 3c), a fourth near the *MME* gene on chromosome 3 (rs7433686; $P = 4.09 \times 10^{-6}$, Fig. 3d), a

fifth being intergenic on chromosome 14 (rs11625484; $P = 4.37 \times 10^{-6}$, Fig. 3e) and the other near the *NBPF22P* gene on chromosome 5 (rs76204167; $P = 4.65 \times 10^{-6}$, Fig. 3f).

Table 1
Descriptive characteristics of study subjects

Characteristics	
Total participants, N	2,504
Age, year	50.81 ± 10.83
Males, N (%)	925 (36.94)
eGFR, ml/min/1.73 m ²	108.30 ± 28.00
Urine aMT6s, ng/ml	20.45 (11.99–30.19)
UMCR, ng/mg	17.00 (10.37–27.40)
Data are mean ± SD, median (IQR) or number (%), as appropriate. Age is at specimen collection. eGFR, estimated glomerular filtration rate (by Modification of Diet in Renal Disease equation); UMCR, urine aMT6s/creatinine ratio.	

Table 2
Association of genetic loci with log UMCR in a Taiwan Han Chinese population

SNP	Chr	Position	Nearest gene	UMCR increasing allele	Other allele	UMCR increasing allele frequency	Model 1		Model 2	
							P value	Beta (SEM)	P value	Beta (SEM)
rs142037747	3	16121712	<i>GALNT15</i>	G	A	0.989	7.54 x 10 ⁻⁷	0.249 (0.050)	7.46 x 10 ⁻⁷	0.249 (0.050)
rs17681554	16	73016768	<i>ZFHX3</i>	A	C	0.804	3.29 x 10 ⁻⁶	0.062 (0.013)	3.29 x 10 ⁻⁶	0.062 (0.013)
rs74760291	6	124526587	<i>NKAIN2</i>	A	C	0.941	3.33 x 10 ⁻⁶	0.108 (0.023)	3.34 x 10 ⁻⁶	0.108 (0.023)
rs7433686	3	155032307	<i>MME</i>	A	C	0.610	4.09 x 10 ⁻⁶	0.051 (0.011)	4.04 x 10 ⁻⁶	0.052 (0.011)
rs11625484	14	43065019	<i>intergenic</i>	A	C	0.050	4.37 x 10 ⁻⁶	0.050 (0.011)	4.33 x 10 ⁻⁶	0.050 (0.011)
rs76204167	5	85643507	<i>NBPF22P</i>	T	C	0.024	4.65 x 10 ⁻⁶	0.159 (0.035)	4.63 x 10 ⁻⁶	0.159 (0.035)
Model 1 was adjusted for age, sex and the first ten principal components of ancestry. Model 2 was additionally adjusted for eGFR. SNP, single nucleotide polymorphism; Chr, chromosome. SNPs are imputed with high info score (0.831, 0.992 and 0.978 for rs142037747, rs7433686 and rs76204167, respectively).										

The array-based heritability estimate for log UMCR is 0.1502. Proportion of variance explained by the individual SNPs are 1.00%, 0.87%, 0.87%, 0.87%, 0.85% and 0.84% for rs142037747, rs17681554, rs74760291, rs7433686, rs11625484 and

rs76204167 respectively.

Discussion

In this first GWAS on melatonin secretion, we identified six suggestive loci that were associated with variation in log UMCR. rs142037747 is located near *GALNT15* (polypeptide N-acetylgalactosaminyltransferase 15). *GALNT15* catalyzes initiation of mucin-type O-linked glycosylation by adding N-acetylgalactosamine to serine or threonine residues of the polypeptide chain [36]. Glycosylation is associated with cell adhesion, signal transduction, molecular trafficking and differentiation in central nervous system development [37]. It remains to be determined whether and how *GALNT15* significantly affects melatonin level.

rs17681554 is located within *ZFHX3* (Zinc Finger Homeobox 3). *ZFHX3* is a transcriptional regulator which contains four homeodomains and seventeen zinc fingers [38]. During neuronal differentiation, there is certain preferential expression pattern of *ZFHX3* isoforms [39]. In addition, circadian behavior alteration is shown in inducible conditional *Zfhx3* knockout adult mice [40]. Further studies are needed to elucidate if there is a direct linkage between *ZFHX3* and melatonin.

rs74760291 locates in the intronic region of *NKAIN2* (sodium/potassium-transporting ATPase subunit beta-1-interacting protein). *NKAIN2* is previously named T-cell lymphoma breakpoint-associated target protein 1 due to its association with lymphoma [41]. Genome-wide analyses demonstrated *NKAIN2* gene in association with sleep quality and insomnia [42, 43]. Since darkness is the most important regulator of melatonin secretion and light drastically reduced melatonin across the day, melatonin also reciprocally affected sleep quality and insomnia in large-scale meta-analysis [44]. From our study, it is highly likely that genetic variation of *NKAIN2* affects sleep via affecting melatonin secretion. However, further study is warranted as we cannot exclude the reverse causation that *NKAIN2* variation-associated sleep order results in disturbed melatonin secretion.

rs7433686 is located near *MME* (membrane metalloendopeptidase; more commonly known as neprilysin). Neprilysin degrades amyloid beta-protein, which is the main constituent of amyloid plaques seen in patients with Alzheimer's disease (AD) [45]. A candidate gene-based association study suggested neprilysin was a susceptibility gene for AD [46]. Melatonin levels are altered in AD patients, possibly due to a decrease in suprachiasmatic nucleus cell number and functional pineal gland volume [47]. Our present unbiased genetic study, revealing the *MME* variant associated with melatonin secretion from pineal gland, provides additional evidence for potential mechanistic explanation in AD patients with altered melatonin levels.

rs11625484 is an intergenic SNP. Whether long non-coding RNAs or microRNAs encoded in this intergenic region might affect melatonin levels remains to be elucidated.

rs76204167 is located near *NBPF22P* (neuroblastoma breakpoint family, member 22, pseudogene). Members of *NBPF* are likely to be involved in neuronal development besides cancer occurrence [48]. A previous GWAS regarding to actigraphy-based sleep parameters has shown *NBPF22P* related to sleep quality [49]. The exact mechanism of relation between *NBPF22P* and melatonin is to be explored.

This study also showed borderline significance regarding to the positive correlation between age and log UMCR. Since aging causes sarcopenia, subsequent decreased creatinine excretion from urine tends to increase the substance-to-creatinine ratio. Our result support the current concept that aging itself will not cause a decrease in melatonin secretion or excretion.

There was a concern that aMT6s excretion may be altered when renal function declines. A previous study enrolling 20 elderlies demonstrated that 24-hour urine aMT6s was a reliable surrogate for plasma melatonin level, at least among individuals with GFR 24.6 ml/min or above [50]. Our study confirmed that morning UMCR is not significantly correlated with eGFR, and adjusting eGFR in GWAS analysis essentially did not have influence on the results.

There are limitations of our study. First, it lacks replication of the result in another cohort. We searched in the UK Biobank, but melatonin as phenotype does not exist in the database. Moreover, the sample size is relatively small, thus for the time being these SNPs can be only seen as suggestive signals, and true loci remain to be identified and validated.

In summary, we have performed the first GWAS regarding to melatonin secretion to date. This GWAS identified 6 highly suggested genetic loci encompassing genes that had been demonstrated potential functional connectivity between the genes-associated melatonin level and clinical documented disorders such as AD and sleep disorders. Replication and functional studies of these genetic variations are warranted to better understand the regulation of melatonin secretion and related clinical disorders.

Declarations

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Author contributions

P.H.L., G.T.C. and Y.C.C. contributed to the experimental design. Y.S.W., H.C.K., Y.C.L., Y.S.C., Y.Y.H., C.H.L., J.W.L., Chih-Neng H., J.J.H, M.L.H., H.L.L. and Y.C.C. contributed to sample procurement and data generation. G.T.C. was responsible for data analysis; P.H.L., Chia-Ni H. and C.Y.S. provided technical help. Manuscript writing was done by G.T.C., P.H.L. and Y.C.C.

Conflict of Interest Statement

The authors declare no conflicts of interest.

Data availability

Individual researchers may request to use the data for specific projects on a collaborative basis.

Bioethics Statement

This study was approved by the Institutional Review Board of Chang Gung Medical Foundation and the Institutional Review Board of National Taiwan University Hospital. All subjects have provided written informed consent and all methods were carried out in accordance with relevant guidelines and regulations.

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Figures

Figure 1

Manhattan plot of the GWAS results for log UMCR. SNPs are plotted on the x axis according to their chromosome position against association with log UMCR on the y axis. The red horizontal line represents the suggestive association threshold of $P =$

5.0 x 10⁻⁶.

Figure 2

Quantile-quantile plots of log UMCR.

Figure 3

Regional association plots of log UMCR. (A) rs142037747 (B) rs17681554 (C) rs74760291 (D) rs7433686 (E) rs11625484 (F) rs76204167.