AutismCRC

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FINAL REPORT

Dr Anne Masi
Professor Valsamma Eapen
Dr Ping-I Lin
Dr Mohammad Ali Moni
Associate Professor Helen Heussler

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Understanding the biological and behavioural attributes leading to sleep difficulties in children on the autism spectrum

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Dr Anne Masi
University of NSW | Autism CRC

Professor Valsamma Eapen
University of NSW | Autism CRC

Dr Ping-I Lin
University of NSW

Dr Mohammad Ali Moni
The University of Queensland

Associate Professor Helen Heussler
The University of Queensland | Autism CRC

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The Cooperative Research Centre for Living with Autism (Autism CRC)

The Cooperative Research Centre for Living with Autism (Autism CRC) is the world's first national, cooperative research effort focused on autism. Taking a whole-of-life approach to autism focusing on diagnosis, education and adult life, Autism CRC researchers are working with end-users to provide evidence-based outcomes which can be translated into practical solutions for governments, service providers, education and health professionals, families and people on the autism spectrum.

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A note on terminology

We recognise that when referring to individuals on the autism spectrum, there is no one term that suits all people. In our published material and other work, when speaking of children, we use the terms 'autistic child', 'child on the autism spectrum' or 'person on the spectrum'. The term 'autistic person' uses identity first language, which reflects the belief that being autistic is a core part of a person's identity. Autism Spectrum Disorder (ASD) is diagnostic terminology used by the healthcare sector, and is used in the context of a person being 'diagnosed with Autism Spectrum Disorder'.

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1. Background

Sleep is now recognised as a fundamental factor contributing to health and well-being outcomes. Inadequate sleep and disrupted patterns of sleep are associated with poor mental health across the life span. Children on the autism spectrum and their families and carers are already at high risk of poorer mental health. A comorbid sleep disorder is likely to compound these risks and significantly impact the quality of life and functioning of the whole family. The debilitating nature of sleep disturbances can also lead to a number of other biological and emotional based health and well-being difficulties for both child and family.

The prevalence of sleep disorders in children on the autism spectrum ranges from approximately 50% to 80% compared to less than 40% in non-autistic or children not on the autism spectrum, where, unlike in autism, the sleep difficulties usually lessen with age[1-4]. Sleep disorders in autism can manifest in various ways such as insomnia, sleep-onset delay, bedtime resistance, sleep anxiety, daytime sleepiness, and parasomnias[5-7]. The presence of sleep difficulties has been shown to have a substantial impact on children's quality of life but have also shown a specific impact on autism characteristics and behaviours, and quality of life of family members[8-10]. Children on the autism spectrum who are poor sleepers manifest greater levels of traits associated with autism compared to good sleepers[11] and are likely to present with increased prevalence of internalising behaviours such as anxiety, withdrawal, depression and externalising behaviours such as aggression, tantrums and inattention.[4, 12-14]

Given the prevalence of disordered sleep in this population it is imperative that a deeper, more comprehensive understanding of the contributing factors are elucidated. It has been hypothesised that behavioural attributes in the child including hyperactivity and difficulty settling, sensory difference, social cueing for sleep or night-time anxieties are exacerbated in this group. Furthermore, it is considered that there may be a biological basis for these sleep disturbances, with the identification of polymorphisms and mutations in a number of genes relating to the melatonin pathway.

Melatonin is considered to be one of the biomarkers strongly correlated with sleep quality and lower levels of the major metabolite of melatonin (urinary 6-sulfatoxymelatonin) measured by radioimmunoassay (RIA), have also been identified in autistic individuals.[31] The role of the genes in the melatonin metabolism pathways – such as Acetylserotonin O-Methyltransferase (ASMT) gene that encode the last enzyme of melatonin synthesis[32, 33] – may contribute to sleep difficulties in individuals on the autism spectrum. The identification of genetic variants associated with sleep

disturbance and melatonin levels in individuals on the autism spectrum may cast some insights into biological predispositions to sleep disturbances in autism – which in turn can pave the way for novel therapeutic strategies.

Whilst there are widely used treatments for sleep disorder, the problem continues to be exacerbated in this population. There is a unique opportunity to address this 'felt' need by the community using the Autism CRC's resource of the Australian Autism Biobank (AAB) as large numbers of children with genetic profiling and urine samples and detailed phenotypic (clinical) data is available through the AAB. Given that "autism is autisms", this project will aid in understanding the clinical and genetic heterogeneity of autism, as well as the factors associated with the varying clinical presentations and comorbidities, in this case being sleep. It is expected that understanding the pathogenesis of sleep as a comorbidity in autism can help predict the neurodevelopmental trajectories over time. This project will also provide important information on the most appropriate ways to potentially subgroup individuals on the autism spectrum based on shared characteristics such as sleep difficulties.

This project sought to identify the proportion of contribution of each of these data to the final sleep problem which will offer a more targeted approach to intervention. For example, if behaviour is the main contributor, then the targeted support would be a behavioural treatment program however if metabolite levels were significantly reduced or there are polymorphisms and mutations in a number of genes relating to the melatonin pathway, then melatonin could be the targeted intervention.

A well-defined genomics/metabolomics profile will enable clinicians to understand the pathophysiology behind sleep traits in children on the autism spectrum and the responses to treatments.

1.1 Project objectives

The main objectives of this project were:

1. To define the sleep difficulties in children on the autism spectrum aged 2-17 years, including sleep latency, waking after sleep onset, and sleep duration.
2. To comprehensively evaluate the relationship between sleep difficulties and clinical phenotype including levels of autism traits cognitive level, gender, adaptive behaviour, and sensory profile.
3. To measure melatonin and melatonin metabolites in urine samples and identify the relationship between melatonin level in urine and sleep disturbances in children on the autism spectrum.

4. To identify genetic variants associated with sleep disturbances in children on the autism spectrum.
5. To identify genetic variants associated with the variation in melatonin levels in children on the autism spectrum.

2. Research design and methods

2.1 Setting and participants

The Australian Autism Biobank (AAB) data was collected from 2013 to 2018 across four sites in Australia using a cross-sectional study comprising children and adolescents on the autism spectrum aged 2-17, and their parents and unaffected siblings as well as control children with no personal or family history of autism[15]. The ethics approval for this study utilising a subset of the data in the AAB repository was obtained through the UNSW Human Research Advisory Panel (HC190923).

Initial inclusion criteria to the AAB study comprised three groups of children and adolescents aged between 2 to 17: (i) children with an autism diagnosis (probands), (ii) siblings without an autism diagnosis and (iii) children without a diagnosis of autism and no first-degree relative on the autism spectrum (controls). The proband group had to meet the diagnostic criteria for autism according to DSM-IV or DSM-5. Participant data for this study was selected using the subjective sampling method with only children with a Children's Sleep Habits Questionnaire (CSHQ) included. Demographic characteristics were collected using a family history questionnaire (FHQ), a parental-report questionnaire specifically developed for the AAB study to collect demographic and family history information of the participants. A subset of variables, including age, gender, family income and the presence of unusual development within the first 12 months were extracted for analysis. To align developmental stages with educational attainment stages, age was categorised into trichotomous variables: preschool (2-5 years old), primary school (6-12 years old) and adolescents (13-18 years old). Family income was re-categorised from five levels to three levels, due to the low frequency of cases in some categories (Table 1). We combined "unusual development by first 6 months" and "6 to 12 months" to "unusual development by first 12 months".

2.2 Measures

2.2.1 Sleep difficulties

The Childhood Sleep Habits Questionnaire (CSHQ) was used to measure the primary outcome for this study[16]. The CSHQ is a retrospective caregiver-reported questionnaire that assesses child's sleep behaviours during a typical week. It contains 34 items that are divided into 8 subscales (bedtime resistance, sleep onset delay, sleep duration, sleep anxiety, night waking, parasomnia, sleep disordered breathing and day time sleepiness) and scored on a 3-point scale. Previous studies identified that CSHQ has moderate internal consistency with a Cronbach's alpha 0.671 and Guttman split-half coefficient of 0.563[17].

2.2.2 Autism-related clinical features

The Autism Diagnostic Observation Schedule-2nd Edition (ADOS-2) is a semi-structured assessment tool used to determine appropriate supports and strategies[18]. Children on the autism spectrum were administered one of four modules based on their chronological age and expressive language abilities. Each module assesses behaviours across two distinct domains: Social Affect (SA) and Restricted, Repetitive Behaviours (RRB). Subsequently, SA score and RRB score were converted to a calibrated severity score for each domain[19].

The Mullen Scales of Early Learning (MSEL) is a measure of language and cognitive function and assesses development across key domains including language, motor and perceptual abilities[20]. For each subscale, a raw score, and a corresponding age equivalence score is obtained. Developmental Quotients (DQ) were calculated for the MSEL subscales, visual reception, fine motor, receptive language and expressive language, using age equivalent scores to avoid floor and ceiling effects: DQ = DA/CA × 100, where DA = developmental age and CA = chronological age[21].

The Vineland Adaptive Behavioral Scale-2nd Edition (VABS-II) is a parental questionnaire used to measure a child's adaptive behaviours in everyday life[22]. Adaptive behaviours across four domains: communication, daily living skills (DLS), socialisation and motor skills (MS) were assessed and recorded as standard scores. The VABS-II standard score were utilised as an indication of the level of adaptive functioning in each of the four domains. The standard scores were kept as continuous variables with higher scores representing higher levels of adaptive functioning.

The Short Sensory Profile –2 (SSP-2) is a standardized questionnaire which assesses the sensory profile of children (3.0-14.11 years) based on their neurological threshold to sensory input and their method of self-regulation[23]. The parent-completed questionnaire contains 34 items distributed across the four quadrants of Dunn's Sensory Processing Framework: seeking (7 items), avoiding (9 items), sensitivity (10 items) and registration (8 items). The frequency with which the child displays each item is scored on a Likert scale 1 (*almost never* = 10% or less) to 5 (*almost always* = 90% or more).

2.3 Procedures and analysis

2.3.1 The phenotype and genotype data and urine were extracted from the AAB.

A participant's CSHQ questionnaire was excluded from the analysis if more than 20% of responses to items were missing[24]. After filtering data with the number of missing items >6 a forward imputation algorithm was used to impute missing values into the dataset[25]. This algorithm alternates nonlinear principal component analysis on a subset of the data with no missing data and sequential imputations of missing values by the nearest neighbour method. This sequential process starts from the units with the lowest number of missing values and ends with the units with the highest number of missing values.

Descriptive statistics were then prepared for the demographic factors and the total CSHQ score. Corresponding frequencies were calculated to illustrate sample characteristics. One-way analysis of variance (ANOVA) was used to compare the differences in continuous variables between autistic children, their siblings, and the control group. Independent samples T-test and a one-way ANOVA were also performed to compare the mean CSHQ total score and subscale scores. Pearson correlation tests were performed to select potential sleep-associated variables from domains of the ADOS-2 and VABS-II, subscales of the MSEL based on development quotients, and quadrants of the SSP-2, with the CSHQ total score and CSHQ subscale values. Finally, multiple regression models were used to determine clinical predictors of the severity of sleep difficulties adjusting for age and gender. The significance cut-off was set at a two-sided alpha value of 0.05 and all statistical analyses were performed using IBM SPSS Statistics Version 26.0 (IBM Corp., Armonk, NY, USA) or R packages[26].

2.3.2 Melatonin analysis

Concentrations of the primary metabolite of melatonin - 6-sulfatoxymelatonin - were determined using an enzyme-linked immunosorbent assay (ELISA). Since melatonin levels were measured using the urine samples from the participants were collected in two different times of the day (5.00 AM to 11.55 AM and 12.00 PM to 9.02 PM) the batch effect of metabolite levels was corrected using the proportion of maximum scoring (POMS) or min-max scaling approach in rescaling all features to 0 – 1 range by applying the following transformation:

$$z_i = \frac{x_i - x_{min}}{x_{max} - x_{min}}$$

However, normalised melatonin levels were still highly skewed, so the data on melatonin levels were processed using the rank-based inverse normal transformation technique.

To examine the relationship between melatonin levels and total CSHQ scores, we used general linear model to regress transformed melatonin levels against normalized melatonin levels adjusting for the age, gender, and the level of autism traits (indicated by the ADOS comparison score). Additionally, since melatonin rhythms are altered in a variety of circadian rhythm, we also adjusted for the time of sample collection (i.e., morning versus afternoon) in the general linear model. The two-sided alpha value of 0.05 was used to determine the level of statistical significance.

2.3.3 Genome-wide association study (GWAS)

Genotype data were also extracted from the AAB. Preparation for genotyping analysis including pre-imputation QC, ethnicity derivations, imputation (using ENIGMA genetics protocols) and post-imputation QC were undertaken.

Two quantitative trait genome wide association studied (GWAS) were carried out to identify genetic variants associated with sleep quality (measured with the CSHQ scores) and the variation in melatonin levels, respectively. Participants were genotyped using either the Global Screening Array v1 or Global Screening Array v2 (Illumina, San Diego, CA). SNPs with a genotyping frequency < 95%, Hardy-Weinberg Equilibrium (HWE) P-value < $1x10^{-5}$, or minor allele frequency (MAF) < 0.1%, were eliminated from the GWAS. The analyses were carried out using the PLINK (v1.90b5 64-bit (14 Nov 2017)) software package. Manhattan, Q-Q and p-value distribution plots were generated using R version 3.6.3 [36, 37] with the qqman package[37]. We consider that the traditional GWAS significance threshold, P-value < 5×10^{-8}, might be too conservative because it

controls type 1 errors (false-positives) at the price of inflating type 2 errors (false negatives)[38]. As an exploratory study, we also considered using 1 x 10^{-5} as a significance threshold.

2.3.4 Gene set analysis

We used an exploratory and less stringent significance threshold to select the top 0.01% of the SNPs as loci associated with the individual phenotype to identify genetic networks. Therefore, a total of 25 SNPs were selected from each phenotype. No trait-associated SNPs were shared by the two sets of loci associated with sleep quality (i.e., CSHQ score) and melatonin level, respectively. We found the p-value cut-off for sleep data was <2.5 x 10^{-5}) and the p-value cut-off for melatonin data was <3.5 x 10^{-5}. We have used the Ensemble Variant Effect Predictor (http://asia.ensembl.org/info/docs/tools/vep/index.html) to predict and identify the mapped genes. We then performed the gene set enrichment analysis of Gene Ontology and cell signalling pathways to evaluate the biological relevance and functional pathways of these mapped genes from both the mapped gene lists. The GO terms and pathways enriched by the list of genes were identified using the hypergeometric analyses with an adjusted P ≤ 0.05 was considered as statistically significant.

3. Findings

3.1 Clinical and behavioural attributes leading to sleep disorders in children on the autism spectrum

The sample consists of 969 children on the autism spectrum, 188 siblings and 111 unrelated controls. The proband group was younger than the sibling group (proband: 7.63 ± 3.86, sibling: 8.07 ± 4.26, p = 0.031), but older than the control group (5.99 ± 3.12, p = 0.001), respectively. Sample characteristics and demographic variables and their associations with the total CSHQ score are shown in Table 1. Using a clinical cutoff score of 41, 73.4% of probands had total CSHQ scores above the clinical cutoff whilst 54.3% and 43.2% of siblings and controls respectively had scores above the clinical cutoff.

Autistic children had statistically significantly higher scores than unaffected siblings and controls for most of the CSHQ subclasses except that children on the spectrum and controls did not have statistically significant difference in the problem of sleep disordered breathing (Table 2, Figure 1). Siblings and controls had similar levels for most CSHQ subclasses except for the problem of sleep duration. Compared to siblings, children on the spectrum had statistically significantly poorer sleep

quality in terms of bedtime resistance ($p < 0.001$), sleep onset delay ($p < 0.001$), sleep duration ($p < 0.001$), sleep anxiety ($p = 0.001$), night wakings ($p = 0.002$), parasomnias ($p < 0.001$), sleep disordered breathing ($p < 0.001$) and daytime sleepiness ($p = 0.007$). Compared to the control group, the group of children on the autism spectrum was also positively associated with bedtime resistance ($p < 0.001$), sleep onset delay ($p = 0.004$), sleep duration ($p < 0.001$), sleep anxiety ($p < 0.001$), night wakings ($p < 0.001$), parasomnias ($p < 0.001$) and daytime sleepiness ($p < 0.001$). The only significant correlation between the sibling and control groups was for the sleep duration subscale, in that reduced sleep duration was reported for siblings relative to controls ($p = 0.034$).

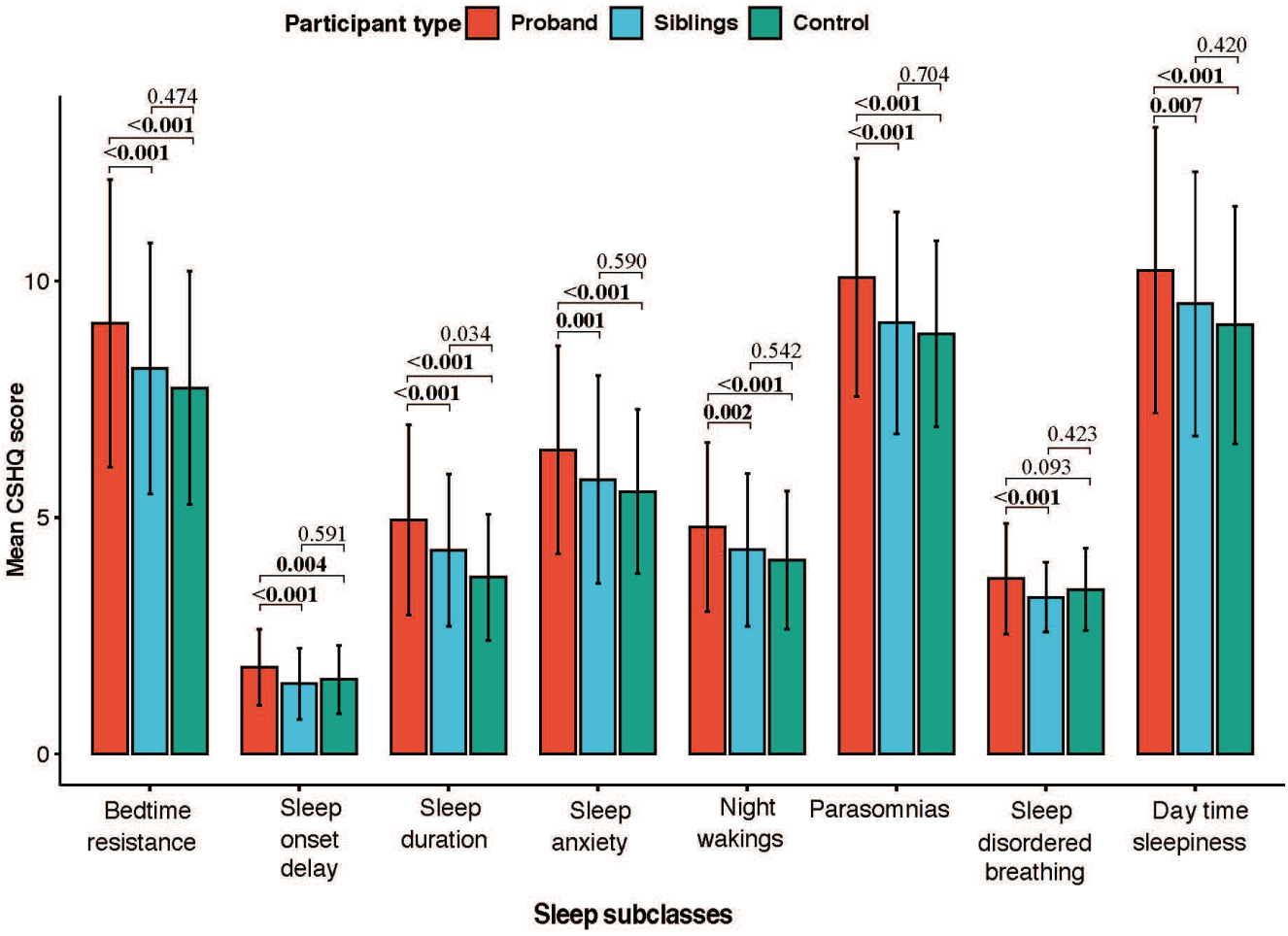
Table 1. Relationships between participant characteristics, sociodemographics and Total CSHQ score.

	N (%)	M (SD)		
Participant type				
Proband	969 (76.4%)	47.92 (9.98)	36.46	<0.001[a]
Siblings	188 (14.8%)	43.12 (9.08)		
Controls	111 (8.8%)	41.50 (7.97)		
CSHQ				
Age group (*N* = 969)				
Preschool (2-5 Years Old)	428 (44.1%)	47.93 (10.09)	0.01	0.995[a]
Primary School (6-12 Years Old)	427 (44.1%)	47.93 (10.17)		
Adolescents (13-18 Years Old)	114 (11.8%)	47.83 (8.87)		
Gender (*N* = 969)				
Male	763 (78.7%)	47.26 (9.74)	3.4	0.065[b]
Female	206 (21.3%)	50.38 (10.46)		
Family Income (*N* = 785)				
$70,000 per year or lower	234 (29.8%)	51.51 (10.08)	27.52	<0.001[a]
$70,000-$104,000 per year	203 (25.6%)	48.25 (10.02)		
$104001 per year or more	348 (44.3%)	45.48 (9.07)		
Unusual development by first 12 months (*N* = 958)				
No	283 (29.5%)	46.05 (9.59)	1.77	0.184[b]
Yes	675 (70.5%)	48.73 (10.05)		
Autistic Children Good sleepers vs Bad sleepers (*N* = 969)				
No	256 (26.4%)			
Yes	713 (73.6%)			

Table 2. Relationships between participant type and CSHQ subclass

CSHQ Subclass	Mean±SD	Mean±SD	Mean±SD	Mean Square	F	P Value
Bedtime resistance	9.11±3.04	8.15±2.66	7.74±2.47	147.33	17.07	**<0.001**
Sleep onset delay	1.83±0.81	1.48±0.75	1.58±0.72	11.45	18.32	**<0.001**
Sleep duration	4.94±2.01	4.31±1.61	3.74±1.33	93.35	25.72	**<0.001**
Sleep anxiety	6.43±2.20	5.80±2.20	5.55±1.74	61.52	13.15	**<0.001**
Night wakings	4.80±1.79	4.32±1.62	4.10±1.46	37.69	12.43	**<0.001**
Parasomnias	10.08±2.52	9.12±2.35	8.88±1.96	126.73	21.16	**<0.001**
Sleep disordered breathing	3.71±1.17	3.31±0.74	3.48±0.87	13.47	11.31	**<0.001**
Day time sleepiness	10.23±3.02	9.52±2.80	9.07±2.51	94.73	10.9	**<0.001**

Figure 1. Distributions of CSHQ subclass scores classified by participant type



There were statistically significant associations between the age group of children on the spectrum(preschool/primary/adolescents) and the scores on six of the eight CHSQ subscales (Figure 2, Table 3). Preschool aged autistic children were found to have higher level of bedtime resistance relative to primary school aged children ($p < 0.001$) and adolescents ($p < 0.001$), increased sleep duration relative to primary school aged children ($p = 0.029$) and adolescents ($p = 0.018$), higher levels of sleep anxiety relative to adolescents ($p < 0.001$), and more night wakings relative to both primary school aged ($p < 0.001$) and adolescents ($p = 0.01$). Relative to preschool aged children, adolescents were found to have greater sleep onset delays ($p = 0.005$), and increased daytime sleepiness ($p < 0.001$). Relative to primary school aged children, adolescents also had increased daytime sleepiness ($p < 0.001$) but less bedtime resistance ($p = 0.002$) and suffered reduced levels of sleep anxiety ($p < 0.001$).

Figure 2. Distributions of CSHQ subclass scores classified by age groups for children on the autism spectrum group

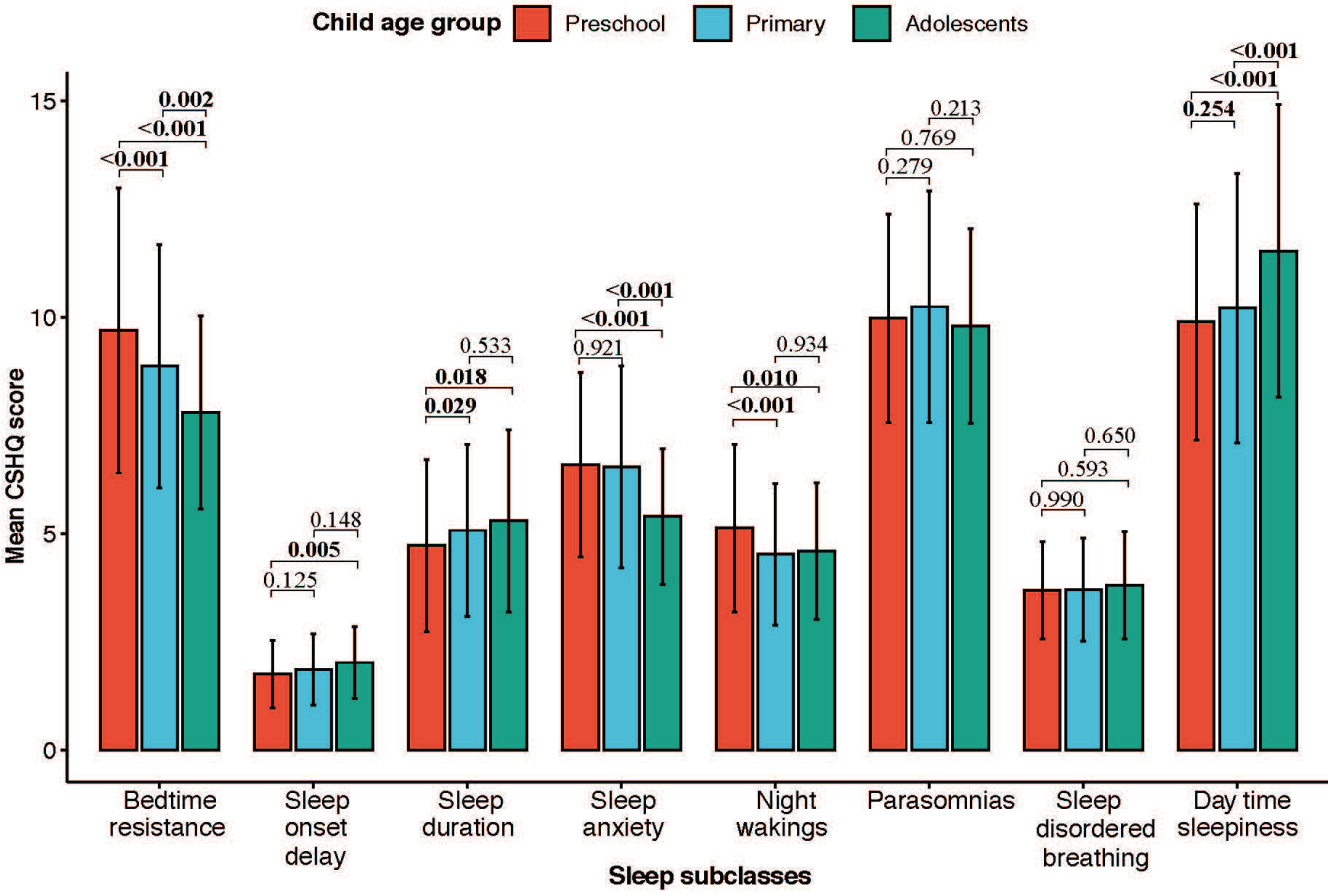


Table 3. Relationships between proband participant age group and CSHQ subclass

	Preschool (N=428)	Primary (N=427)	Adolescents (N=114)			
CSHQ Subclass	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean Square**	**F**	**P Value**
Bedtime resistance	9.69±3.30	8.87±2.81	7.81±2.23	181.92	20.5	**<0.001**
Sleep onset delay	1.75±0.78	1.86±0.82	2.02±0.83	3.48	5.41	0.005
Sleep duration	4.72±1.99	5.07±1.99	5.30±2.11	21.23	5.3	0.005
Sleep anxiety	6.60±2.14	6.54±2.33	5.39±1.57	69.49	14.79	**<0.001**
Night wakings	5.13±1.94	4.52±1.63	4.59±1.58	42.46	13.56	**<0.001**
Parasomnias	9.98±2.41	10.24±2.68	9.80±2.24	12.31	1.95	0.143
Sleep disordered breathing	3.69±1.13	3.70±1.19	3.81±1.25	0.67	0.49	0.611
Day time sleepiness	9.89±2.73	10.22±3.11	11.54±3.39	121.5	13.65	**<0.001**

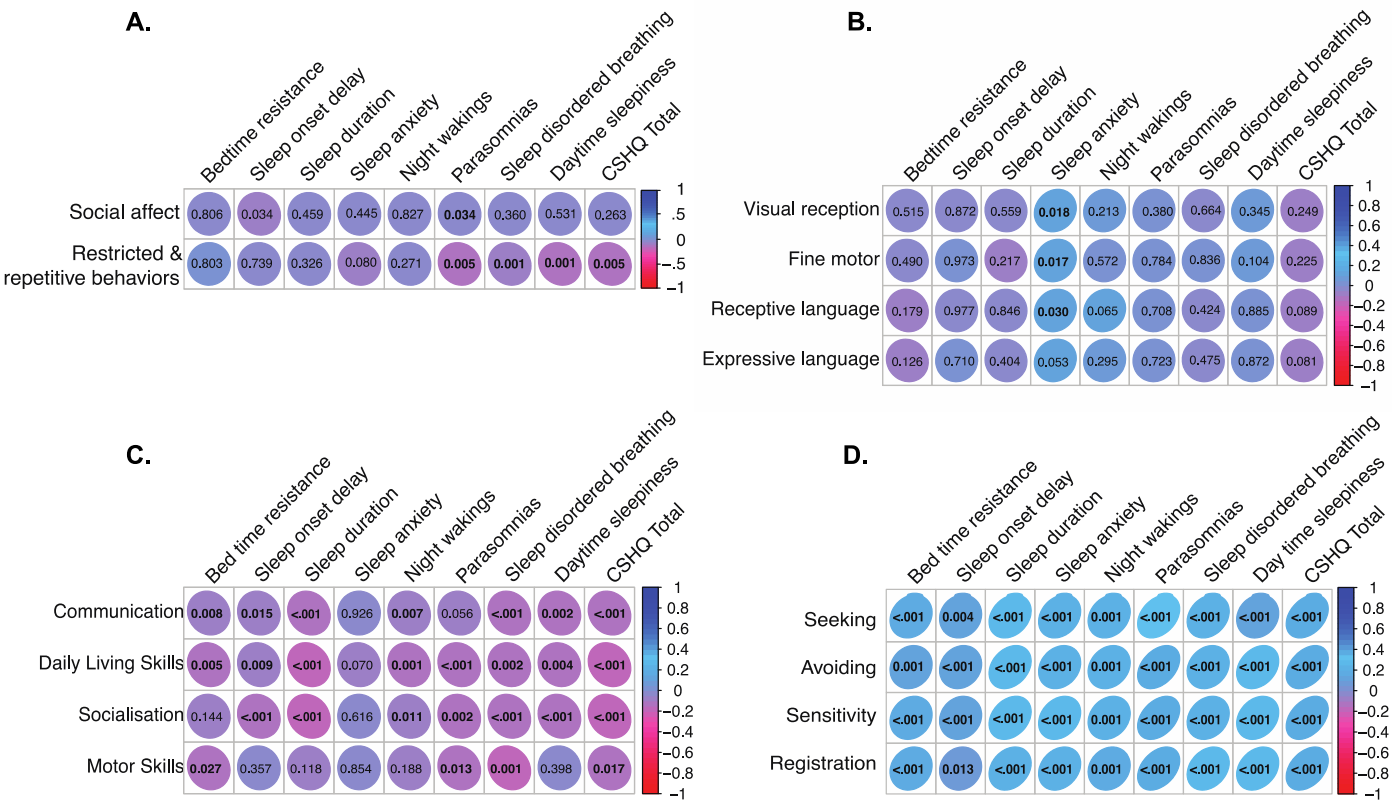
Females were identified as having more sleep difficulties than males based on CHSQ total scores (females: 50.38 ± 10.46, males: 47.26 ± 9.74, $p = 0.065$). Gender was significantly associated with the severity of sleep difficulties in four of the CSHQ subscales with females exhibiting higher levels of bedtime resistance ($p = 0.048$), reduced sleep duration ($p = 0.003$), and increased levels of sleep anxiety ($p = 0.033$), and daytime sleepiness ($p < 0.001$) (Table 4). Family income level was significantly associated with greater levels of bedtime resistance ($p < 0.001$), increased sleep onset delay ($p = 0.010$), reduced sleep duration ($p < 0.001$), higher levels of sleep anxiety ($p = 0.003$), night wakings ($p = 0.002$), parasomnias ($p < 0.001$), more sleep disordered breathing ($p < 0.001$), and higher levels of daytime sleepiness ($p < 0.001$). Unusual development in the first 12 months was significantly associated with higher levels of bedtime resistance ($p = 0.002$), reduced sleep duration ($p < 0.001$), increased levels of sleep anxiety ($p = 0.002$), more night wakings ($p = 0.003$) and parasomnias ($p < 0.001$) and worse disordered breathing during sleep ($p < 0.001$).

Table 4. Relationships between proband participant gender and CSHQ subclass

	Male (N = 763)	Female (N=206)			
CSHQ Subclass	**Mean±SD**	**Mean±SD**	**F**	**P Value**	
Bedtime resistance	9.01±3.00	9.48±3.16	36.05	3.92	**0.048**
Sleep onset delay	1.81±0.81	1.91±0.77	1.75	2.71	0.1
Sleep duration	4.84±1.99	5.32±2.06	36.06	8.99	**0.003**
Sleep anxiety	6.35±2.15	6.72±2.35	21.87	4.54	**0.033**
Night wakings	4.74±1.78	5.00±1.84	11.11	3.47	0.063
Parasomnias	9.99±2.52	10.38±2.49	24.07	3.82	0.051
Sleep disordered breathing	3.67±1.14	3.83±1.27	4.36	3.2	0.074
Day time sleepiness	10.01±2.95	11.03±3.15	169.92	18.95	**<0.001**

Correlation and multiple regression analyses were conducted to examine the relationship between sleep disturbance in children on the autism spectrum as measured by the subscales of the CSHQ, and potential predictors of sleep difficulties. The ADOS-2 social affect domain (N = 699) was significantly negatively associated with sleep onset delay (R = -0.080, p = 0.034). Restricted and repetitive behaviour (N = 699) was also significantly inversely associated with parasomnias (R = -0.106, p = 0.005), sleep disordered breathing (R = -0.096, p = 0.011), daytime sleepiness (R = -0.127, p = 0.001) and the CSHQ total score (R = -0.106, p = 0.005) (Figure 3A). Table 5 summarises the results of a multiple linear regression. Sleep onset delay was the only CSHQ subscale found to be significantly predicted by social affect (B = -0.041, p = 0.017). Restricted and repetitive behaviours predicted sleep anxiety (B = -0.073, p = 0.047), parasomnias (B = -0.109, p = 0.008), sleep disordered breathing (B = -0.045, p = 0.021) and daytime sleepiness (B = -0.13, p = 0.009).

Figure 3. Correlations between the CSHQ subclasses and subclasses of ADOS-2, Mullen, VABS-II and SSP-2 for children on the autism spectrum group.



A) Correlations between the CSHQ subclasses and ADOS-2 domains.

B) Correlations between the CSHQ subclasses and MSEL subscale DQs.

C) Correlations between the CSHQ subclasses and VABS-II subdomains.

D) Correlations between the CSHQ subclasses and SSP-2 domains.

The *p*-values are used in this figure and colour is used for the correlations (-ve or +ve). More circular means less correlated whereas more oval means strong correlated.

Table 5. The association between the ADOS-2 domains and CSHQ subclasses based on the results of multiple regression models

	Bedtime resistance		Sleep onset delay		Sleep duration		Sleep anxiety		Night wakings		Parasomnias		Sleep disordered breathing		Daytime sleepiness		CSHQ Total	
	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*
Constant	10.63	<0.001	1.86	<0.001	4.62	<0.001	7.53	<0.001	5.44	<0.001	11.03	<0.001	4.06	<0.001	10.48	<0.001	51.61	<0.001
Age	-0.18	<0.001	0.02	0.02	0.07	<0.001	-0.07	0.01	-0.07	0.00	-0.03	0.34	0.00	0.74	0.08	0.02	-0.07	0.54
Gender	0.61	0.03	0.13	0.07	0.42	0.02	0.45	0.02	0.38	0.02	0.59	0.01	0.26	0.01	0.97	<0.001	3.63	<0.001
Social affect	0.01	0.89	-0.04	0.02	-0.04	0.32	-0.02	0.73	0.01	0.87	-0.03	0.55	-0.02	0.54	-0.03	0.61	-0.17	0.41
RRB	-0.03	0.59	0.01	0.60	-0.01	0.87	-0.07	0.05	-0.05	0.13	-0.11	0.01	-0.05	0.02	-0.13	0.01	-0.42	0.01

Note. RRB: Restricted and Repetitive Behaviours

Table 6. The association between the Mullen subscales and CSHQ subclasses based on the results of multiple regression models

	Bedtime resistance		Sleep onset delay		Sleep duration		Sleep anxiety		Night wakings		Parasomnias		Sleep disordered breathing		Daytime sleepiness		CSHQ_Total	
	Estimate	p	Estimate	p	Estimate	p	Estimate	p	Estimate	p	Estimate	p	Estimate	p	Estimate	p	Estimate	p
Constant	11.47	<0.001	1.99	<0.001	5.08	<0.001	5.82	<0.001	5.28	<0.001	9.4	<0.001	3.58	<0.001	9.16	<0.001	47.66	<0.001
Age	-0.21	0.15	-0.04	0.27	0.04	0.63	0.06	0.54	-0.04	0.64	0.09	0.40	0.02	0.70	0.05	0.66	0.05	0.91
Gender	0.02	0.95	0.14	0.08	0.05	0.80	-0.03	0.88	0.01	0.97	0.14	0.57	0.13	0.25	0.35	0.22	0.94	0.37
Visual Reception	0.01	0.58	0.00	0.55	0.00	0.77	0.00	0.94	0.00	0.80	0.01	0.17	0.00	0.85	0.01	0.51	0.02	0.57
Fine Motor	0.00	0.85	0.00	0.61	-0.01	0.21	0.01	0.28	-0.01	0.34	-0.01	0.42	0.00	0.44	0.02	0.12	0.01	0.87
Receptive Language	-0.02	0.21	0.00	0.51	0.01	0.37	-0.01	0.52	0.01	0.14	-0.01	0.47	-0.01	0.27	-0.02	0.11	-0.03	0.49
Expressive Language	-0.01	0.40	0.00	0.94	-0.01	0.33	0.00	0.86	0.00	0.55	0.00	0.97	0.00	0.89	0.00	0.67	-0.02	0.51

For the MSEL (*N* = 330), sleep anxiety was significantly positively correlated with visual reception skills (R = 0.130, *p* = 0.018), fine motor skills (R = 0.132, *p* = 0.017) and receptive language subscales (R = 0.120, *p* = 0.030), and almost reached significance with the expressive language subscale (R = 0.106, *p* = 0.053; N = 330) (Figure 3B). However, the multiple regression model with all four MSEL subscales, age, and gender as predictors of sleep difficulties was not significant (Table 6).

Significant associations between the VABS-II domains – communication (*N* = 763), daily living skills (*N* = 758), socialisation (*N* = 741), and motor skills domains (*N* = 465) - with the subclasses of CSHQ were identified for most of the relationships. Only the sleep anxiety subscale of the CSHQ was not significantly correlated with the VABS-II domains (Figure 3C). All relationships were indicative of improved adaptive behaviours reducing sleep difficulties. Multiple linear regression using the four VABS-II domains as predictors identified daily living skills as the domain predicting the most sleep problem behaviours (Table 7). Improved daily living skills predicted increased sleep duration (B = -0.026, *p* = 0.011), reduced sleep anxiety (B = -0.030, *p* = 0.007), reduced night waking (B = -0.021, *p* = 0.032), and reduced parasomnias (B = -0.053, *p* < 0.0001). The only other association in the regression model was motor skills, where better motor skills predicted increased daytime sleepiness (B = 0.026, *p* = 0.022).

The four quadrants of the Short Sensory Profile-2 (seeking, avoiding, sensitivity and registration) were all statistically significantly correlated with all the subclasses of the CSHQ (*N* = 656; Figure 3D). All correlations were positive in that increases in all types of sensory behaviours were associated with increased scores on the CSHQ subscales. Multiple linear regression considered all SSP-2 quadrants as potential predictors of sleep difficulties (Table 8). Sensory seeking behaviours predicted more night waking (B = 0.033, *p* = 0.030) and reduced daytime sleepiness (B = -0.056, *p* = 0.026), whilst sensory avoiding behaviours predicted reduced sleep duration (B = 0.032, *p* = 0.031) and more parasomnias (B = 0.057, *p* = 0.001). Sleep anxiety was found to be significantly predicted by higher levels of sensory sensitivity (B = 0.038, *p* = 0.028), and higher levels of sensory registration was found to be associated with more parasomnia related behaviours (B = 0.054, *p* = 0.003), sleep disordered breathing (B = .033, *p* < 0.001) and daytime sleepiness (B = 0.080, *p* = 0.001)

Table 7. The association between the VABS-II domains and CSHQ subclasses based on the results of multiple regression models

	Bedtime resistance		Sleep onset delay		Sleep duration		Sleep anxiety		Night wakings		Parasomnias		Sleep disordered breathing		Daytime sleepiness		CSHQ Total	
	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*
Constant	12.66	<0.001	2.22	<0.001	6.00	<0.001	7.17	<0.001	6.43	<0.001	11.94	<0.001	4.90	<0.001	11.76	<0.001	58.76	<0.001
Age	-0.21	0.00	-0.01	0.62	0.06	0.14	-0.02	0.60	-0.09	0.02	0.00	0.97	-0.02	0.33	0.00	1.00	-0.20	0.30
Gender	0.24	0.51	0.12	0.20	0.00	1.00	0.14	0.59	0.04	0.85	0.33	0.24	0.31	0.02	0.79	0.02	2.00	0.08
Communication	-0.02	0.22	0.00	0.69	0.00	0.75	0.01	0.23	0.00	0.63	0.02	0.13	-0.01	0.18	-0.02	0.08	-0.02	0.62
DLS	-0.02	0.27	-0.01	0.06	-0.03	0.01	-0.03	0.01	-0.02	0.03	-0.05	<0.001	0.00	0.91	-0.02	0.15	-0.16	0.00
Socialisation	0.01	0.71	0.00	0.40	0.00	0.99	0.01	0.59	0.00	0.86	0.01	0.43	0.00	0.60	-0.01	0.35	-0.01	0.80
Motor Skills	0.00	0.88	0.00	0.26	0.01	0.33	0.01	0.33	0.01	0.13	0.00	0.76	-0.01	0.28	0.03	0.02	0.06	0.16

Note. DLS: Daily Living Skills

Table 8. The association between the SSP-2 quadrants and CSHQ subclasses based on the results of multiple regression models

	Bedtime resistance		Sleep onset delay		Sleep duration		Sleep anxiety		Night wakings		Parasomnias		Sleep disordered breathing		Daytime sleepiness		CSHQ Total	
	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*
Constant	8.38	<0.001	1.14	<0.001	2.01	<0.001	4.74	<0.001	3.44	<0.001	5.97	<0.001	2.63	<0.001	6.17	<0.001	31.19	<0.001
Age	-0.20	<0.001	0.02	0.01	0.04	0.03	-0.10	<0.001	-0.06	<0.001	-0.03	0.14	-0.01	0.37	0.10	0.00	-0.13	0.16
Gender	0.34	0.23	0.06	0.47	0.16	0.39	0.38	0.06	0.23	0.16	0.21	0.31	0.10	0.34	0.63	0.02	1.95	0.02
Seeking	0.04	0.15	0.01	0.36	0.03	0.05	0.03	0.14	0.03	0.03	0.02	0.23	0.02	0.09	-0.06	0.03	0.12	0.14
Avoiding	0.00	0.93	0.01	0.11	0.03	0.03	0.00	0.83	0.02	0.11	0.06	0.00	0.01	0.53	0.08	<0.001	0.22	0.00
Sensitivity	0.03	0.22	0.01	0.44	0.03	0.09	0.04	0.03	0.02	0.16	0.03	0.08	0.00	0.84	0.01	0.54	0.15	0.05
Registration	0.03	0.29	0.00	0.53	0.01	0.70	0.03	0.11	-0.01	0.60	0.05	0.00	0.03	<0.001	0.08	0.00	0.20	0.01

3.2 Genetic variants associated with sleep issues in children on the autism spectrum disorder

3.2.1 Characteristics of the sample

A total of 2,489 subjects had genotypic data at ABA. The analyses used to evaluate the association between sleep difficulties and genotypes were only conducted for the subjects with available CSHQ scores. The melatonin levels were available in 22.6% of probands (see Figure 4).

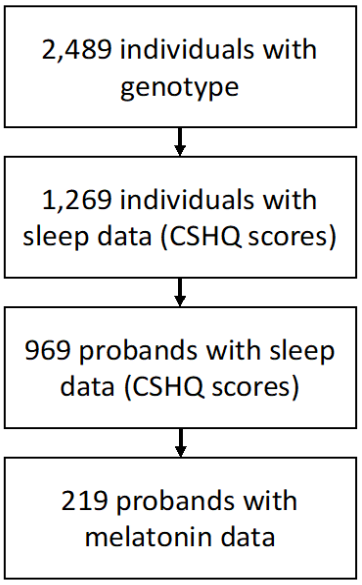


Figure 4 illustrates the sample sizes for each dataset given the available data on clinical features and biomarkers.

3.2.2 The relationship between the melatonin level and the CSHQ score

Normalised melatonin levels were statistically significantly associated with the CSHQ scores ($p = 0.0371$) after adjusting for data collection time, gender, age, and ADOS2 comparison score. The linear relationship indicates that a higher melatonin level was associated with a higher CSHQ total score (see Figure 5A).

Since melatonin levels vary by age, we further examined the relationship between CSHQ scores and melatonin levels by age group and found that the trend did vary by age (see Figure 5B). Therefore, the positive association between melatonin levels and CSHQ scores seemed to be driven by younger children.

Figure 5A **Figure 5B**

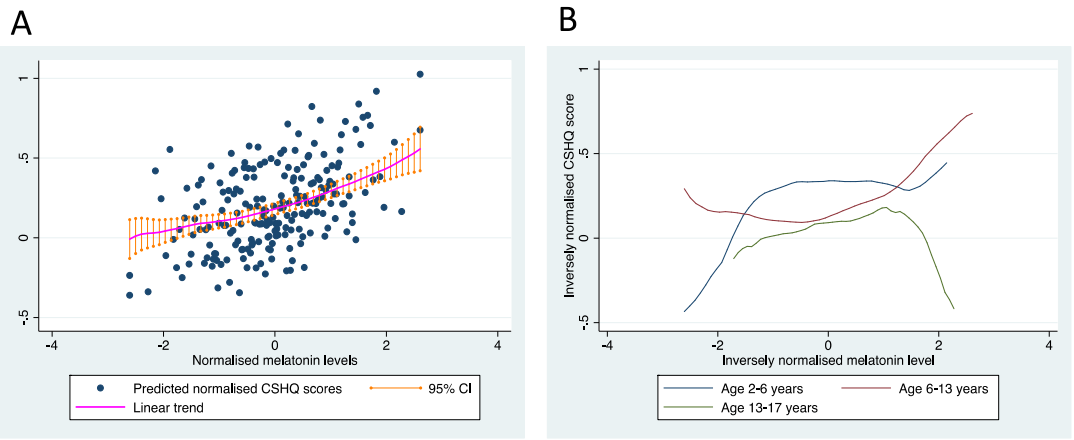


Figure 5. Panel A shows the overall trend for the relationship between melatonin levels and CSHQ scores. Panel B shows the trends stratified into three age groups.

3.3 Genetic variants associated with the target phenotypes.

When the CSHQ score was treated as a phenotype, the GWAS results indicate that none of the SNPs reached the stringent genome-wide significance level ($p < 5 \times 10^{-8}$). There was a total of nine SNPs with a p-value $< 1 \times 10^{-5}$. The SNP with the strongest evidence for association with the CSHQ score was rs13011288, which is located in the intronic region of the Stonin 1 (STON1) gene. When the melatonin level was treated as a phenotype, the GWAS results indicate that none of the SNPs reached the stringent genome-wide significance level ($p < 5 \times 10^{-8}$). The SNP with one of the strongest lines of evidence for its association with the melatonin level is rs1543334 located approximately 10,000 bp away from the Homo Sapiens Synaptosome Associated Protein 25 (SNAP25) gene ($p = 1.15 \times 10^{-5}$). The Manhattan plots of the two GWAS studies are shown in Figure 6, below.

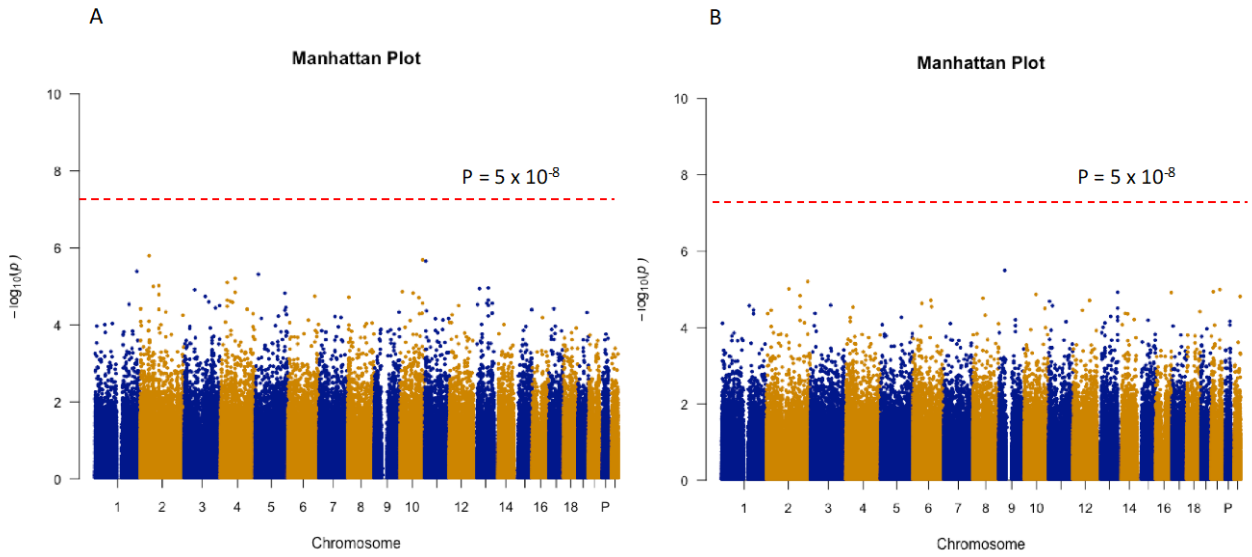


Figure 6. Panel A and panel B represent the GWAS findings with CSHQ scores and melatonin levels, respectively. Only autosomal chromosome results are shown here.

3.4 Gene set analysis

We have found 24 and 16 mapped genes for the sleep and melatonin SNPs respectively. Although there was no common SNP shared by these two lists of SNPs, there was one common mapped gene: TBC1 Domain Family Member 1 gene (TBC1D1). For the sleep quality, we discovered 45 signalling pathways and 29 GO pathways over-represented by these 24 genes as shown in Figures 7 and 8, respectively. The signalling pathway with strongest evidence for enrichment with these genes is the pathway involved in platelet aggregation by Eph kinase and ephrins. The GO pathway with strongest evidence for enrichment with these genes is the pathway involved in regulation of T-helper 1 cell cytokine production. On the other hand, 60 signalling pathways and 51 GO pathways associated with the melatonin level were found to be over-represented by these 16 genes as shown in Figures 9 and 10, respectively. The signalling pathway with strongest evidence for enrichment with these genes is the differentiation pathway in PC12 cells. The GO pathway with strongest evidence for enrichment with these genes is the pathway involved in Schwann cell differentiation.

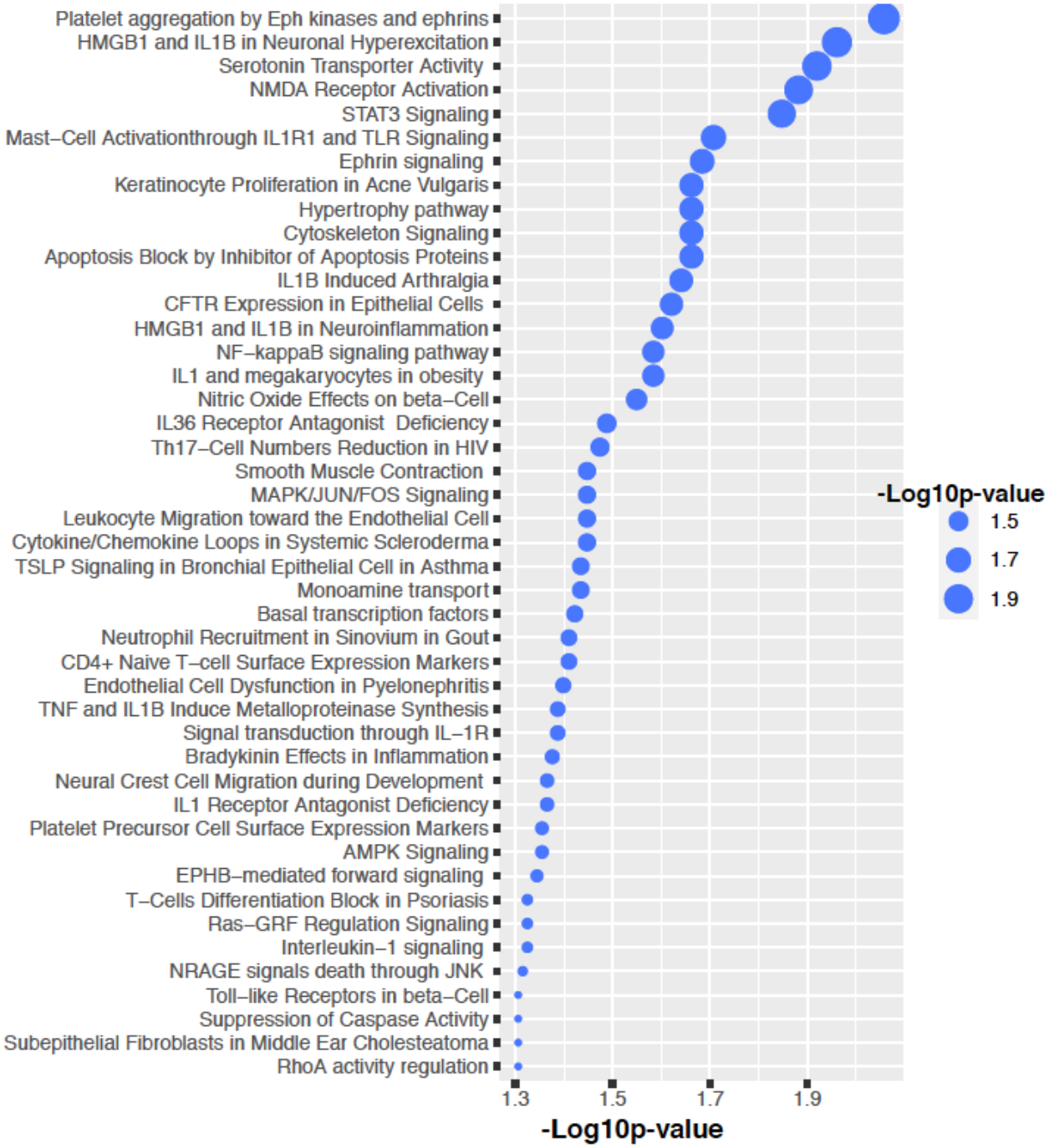


Figure 7: Signalling pathways over- represented in sleep quality problems

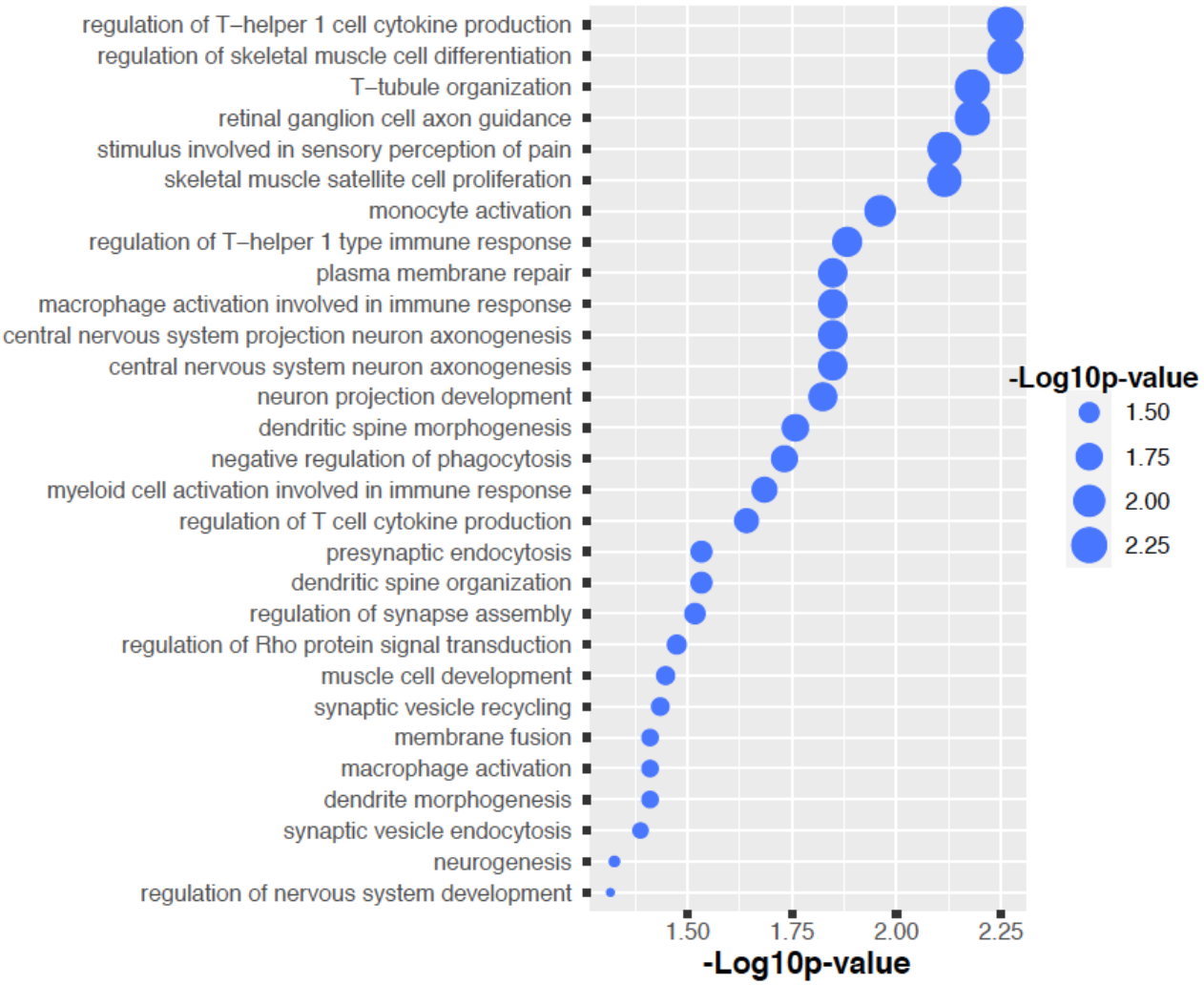


Figure 8: GO pathways over-represented in sleep quality problems

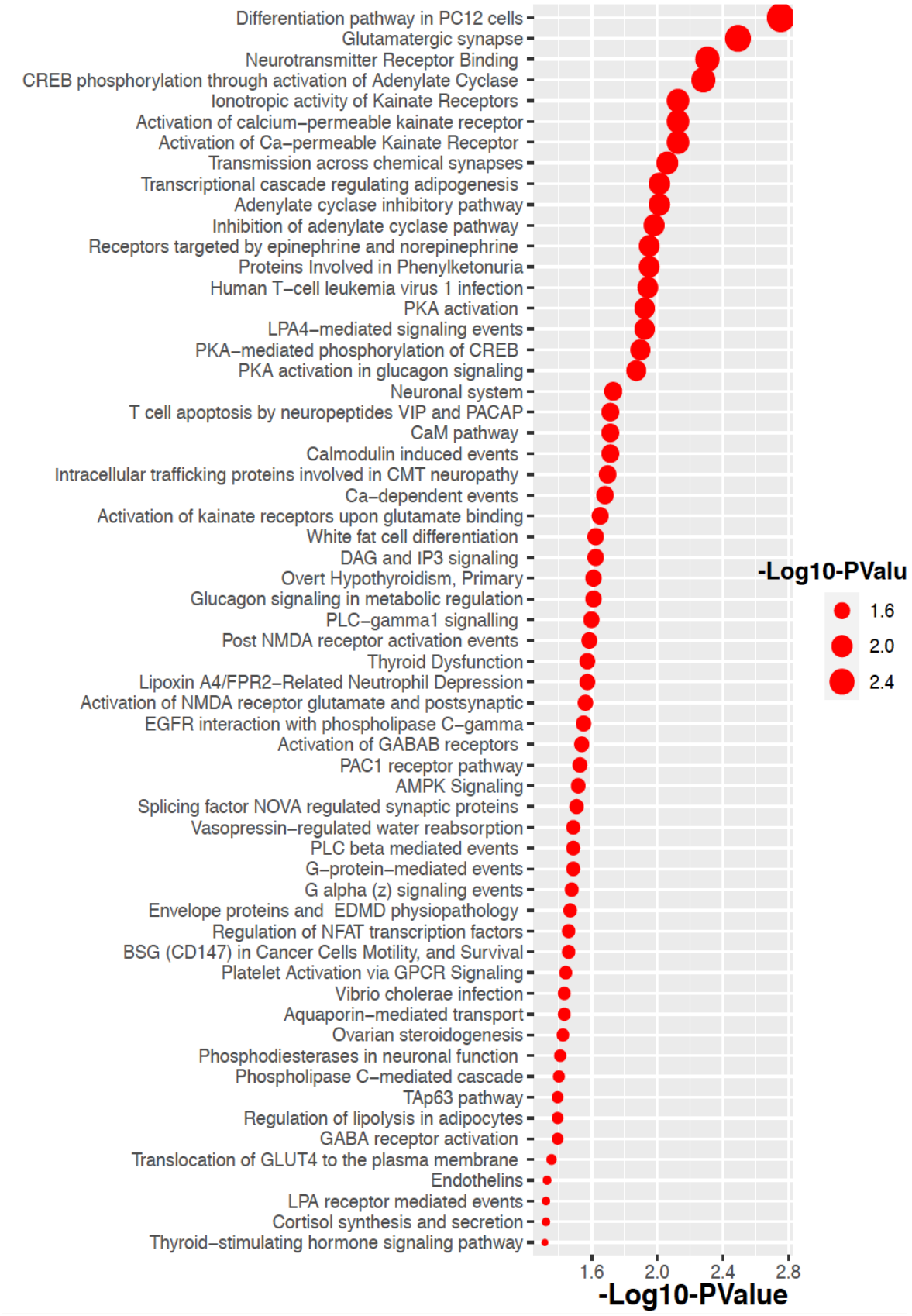


Figure 9: Signalling pathways associated with melatonin over-represented in sleep and melatonin SNPs

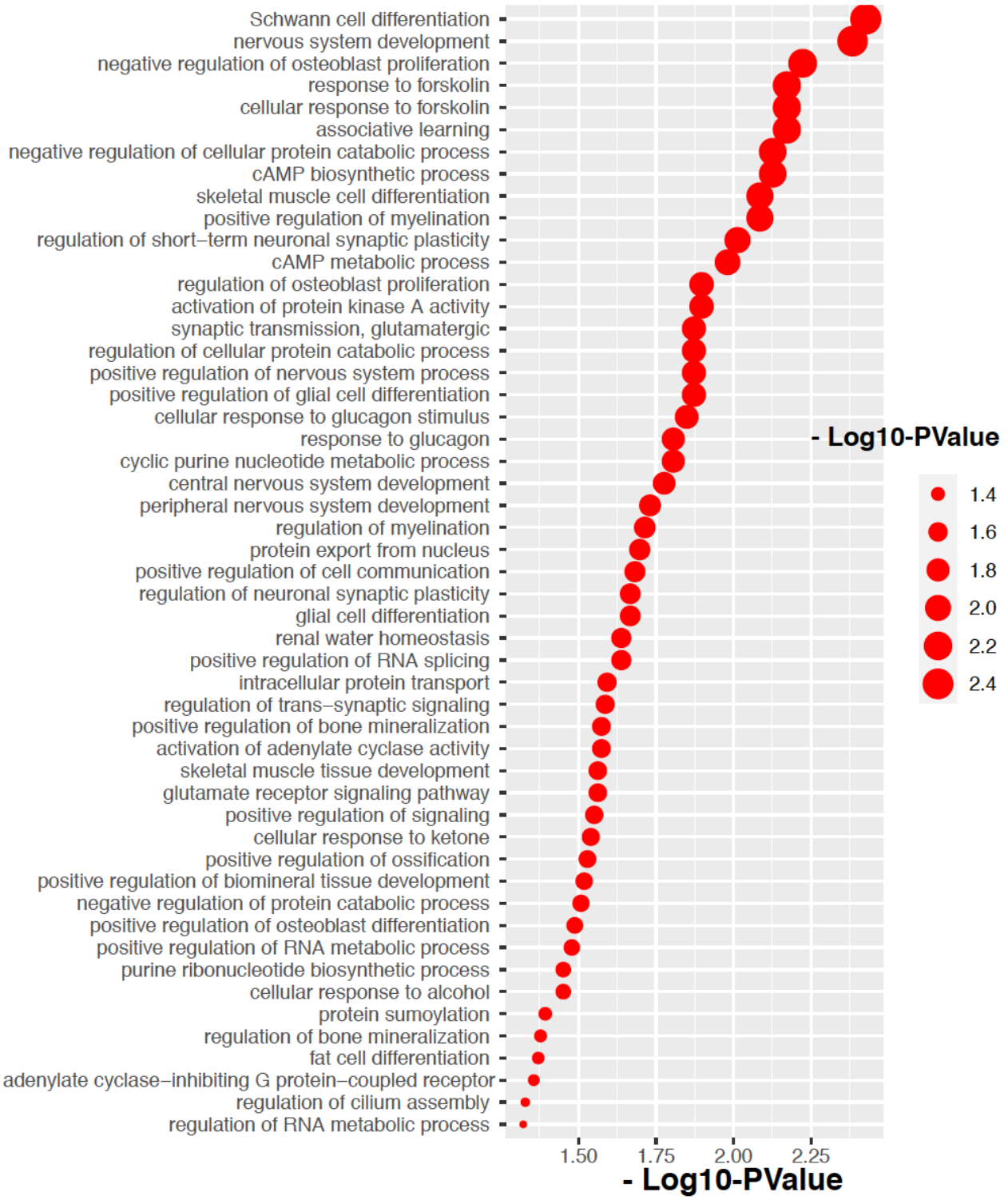


Figure 10: GO pathways associated with melatonin over-represented in sleep and melatonin SNPs

4. Discussion

Consistent with our hypothesis that children on the autism spectrum may experience more severe sleep difficulties, our results showed that children and adolescents with a diagnosis of autism were more likely to have greater severity of sleep difficulties compared to both siblings and unrelated children without an autism diagnosis. This was true based on the total score from the CSHQ and its subscales. Specifically, children on the autism spectrum had more bedtime resistance, greater sleep onset delay, reduced sleep duration, increased levels of sleep anxiety, more night awakenings and parasomnias and greater levels of daytime sleepiness than both siblings and unrelated children without an autism diagnosis. Children on the autism spectrum also had more disordered breathing difficulties while they slept compared to their siblings, but not compared to the control group.

In line with previous work, we found that sleep difficulties were common across all ages in children on the spectrum[39]. However, subscale-level analysis highlighted some differences across the age range with greater levels of bedtime resistance in preschool children while higher levels of daytime sleepiness, greater sleep onset delay and reduced sleep duration were observed in adolescents. Although our study found that children across the age groups presented with one or more sleep difficulties, given the differential age effects, in addition to screening for sleep difficulties in children on the spectrum, consideration should be given to providing developmentally appropriate resources and interventions[40].

Autistic females experienced greater severity of sleep difficulties than males, specifically with greater levels of bedtime resistance, reduced sleep duration, increased levels of sleep anxiety and higher levels of daytime sleepiness. Whilst the literature on sex differences of sleep difficulties in autism highlights mixed findings[41-43], there is some indication that there may be sex differences in the association with sleep difficulties and behaviour of children on the autism spectrum, with sex differences dependent on the behavioural domain being measured[44]. These mixed findings underscore the importance of working with datasets that include as many females as possible in order to further clarify whether there are specific gender dependent sleep phenotypes in autism.

Children from families with lower income levels were found to experience greater severity of sleep difficulties in our study. This is consistent with previous evidence suggesting that those from disadvantaged and low socioeconomic backgrounds have more sleep related issues and are more likely to be impacted[13]. However, other studies have failed to find such an association[39, 45]. Other demographic factors such as lower primary caregiver education level

and ethnicity, as well as lower family income have been found to be associated with increased severity of sleep difficulties in children on the autism spectrum[46, 47].

Considered together, these relationships between demographic and developmental factors and the child's sleep difficulties, highlight that sleep difficulties cannot be solely explained by the presence of autism, and may instead have a multifactorial origin. For example, lower socioeconomic status may affect the risk of sleep disturbance through reduced access to healthcare services and family dysregulation, which may impact the severity of behavioural difficulties including arousal and dysregulation in autism. Further there may be other compounding factors in that sociodemographic characteristics including income level may have a direct or indirect impact on sleep due to the availability of quiet spaces and individual rooms to enable stable and suitable sleep routines which may interact with sociocultural practices of co-sleeping[48, 49].

It is noteworthy that children whose parents reported unusual development by 12 months had more severe sleep difficulties than children whose parents reported no unusual development by 12 months. In this regard there is some evidence, albeit limited to retrospective accounts[50, 51], suggesting that sleep difficulties can differentiate young children on the autism from those with other disabilities. This has significant implications in that sleep difficulties in the first and second year of life may warrant comprehensive developmental evaluation to identify early indicators of developmental differences that may signpost a differential diagnosis of autism spectrum disorder.

There was an association between increased sensitivities in all sensory domains (seeking, avoiding, sensitivity and registration) and higher levels of all types of sleep difficulties. However, when controlling for age and gender more specific relationships were identified. Children with patterns of sensory behaviour where they are quick to notice sensory stimuli i.e., low threshold, were found to have reduced sleep duration, more parasomnias and increased daytime sleepiness (all avoiding quadrant) and higher levels of sleep anxiety (sensitivity quadrant). Children with patterns of sensory behaviour where they are slow to notice sensory stimuli i.e., high threshold, were found to have more night waking (seeking quadrant) and more parasomnias, higher levels of disordered breathing and increased daytime sleepiness (all registration quadrant). However, in contrast, children with more sensory seeking patterns of behaviour, a type of high sensory threshold behaviour, were less likely to be identified as having daytime sleepiness. While the reason for such an association is not clear, it is possible that a child with high sensory threshold behaviour is overstimulated during the day as they engage in various sensory seeking activities and

hence are reported as having less daytime sleepiness. This also aligns with our finding that children with more restricted and repetitive behaviours, as measured by the ADOS–2, were also less likely to experience daytime sleepiness. Furthermore, increased levels of restricted and repetitive behaviours were predictors of reduced levels of sleep anxiety, fewer parasomnias, and less issues with disordered breathing. It is possible that restricted and repetitive behaviours serve a self-soothing or a calming effect, reducing levels of anxiety and thereby reducing associated sleep difficulties or offering a protective effect. Similarly, lower levels of social affect traits predicted reduced sleep onset delay. Therefore, in summary, higher levels of autism related traits in the two ADOS-2 domains of social affect and restricted and repetitive behaviours predicted reduced sleep difficulties. This seems to suggest that sleep is more impacted by the associated maladaptive behaviours and sensory issues in autism rather than the severity of social affect and restricted repetitive behaviours. On the other hand, children with improved levels of adaptive behaviour, specifically daily living skills, were likely to have less severe sleep difficulties relating to sleep duration, sleep anxiety, night waking and parasomnias. This is consistent with other studies that have found a positive association between better sleep and better cognitive skills and adaptive functioning but it is important to acknowledge that the relationship is bidirectional in that sleep difficulties are common in autism but poor sleep can worsen day time behaviours and functioning in autism[4]. In this regard, we observed that children who had higher levels of motor skills had increased levels of daytime sleepiness. This could simply be a reflection of the fact that children who are more active may fatigue more easily. Further research is indicated to understand the neurobiological underpinnings of such interaction as this may have a bearing on developing appropriate intervention and supports.

Given the predominant contribution of adaptive behaviours to reduced severity of sleep difficulties in autism, clinicians may be advised to routinely assess the level of maladaptive behaviours as part of a comprehensive diagnostic evaluation in this population[52]. Maladaptive behaviours may cause physiological hyperarousal[7, 53]. Behavioural interventions that reduce physiological hyperarousal such as sleep hygiene interventions (e.g., minimising light in bedroom, regular bedtime) and relaxation techniques (e.g., deep breathing) should be considered to reduce the severity of sleep difficulties in autism[54, 55]. In addition, given higher levels of adaptive functioning were associated with decreased severity of sleep difficulties in autism, specifically daily living, improving adaptive behavioural skills with the use of comprehensive educational program such as milieu teaching (e.g., teaching in natural communicative environments) would merit consideration in this population[56, 57].

The findings from the melatonin metabolite analysis indicate that greater melatonin levels might correlate with poorer sleep quality (i.e., higher CSHQ scores). These findings seem to contradict with a large proportion of prior evidence that shows an inverse relationship between melatonin levels and sleep disturbance. Notably, we found that the relationship between CSHQ scores and melatonin levels could vary by age in our sample, in which the positive association between melatonin levels and CSHQ scores primarily stemmed from the younger children (age: 2-6 years). The relationship between melatonin levels and sleep quality appeared to be a dome-shaped curve for the adolescents (aged 13-17 years), and hence the assumption of a linear relationship regardless of the age group might be problematic. An earlier study found that melatonin levels collected during the daytime might correlate with sleep quality in different ways from night time levels[58]. The authors inferred that higher anxiety level increases the oxidative stress in the body with consequent production of a larger volume of melatonin so that its antioxidant action serves to effectively protect the body. Thus, anxiety may lead to poorer sleep quality and vice versa, which can in turn trigger melatonin secretion. Taken together, these observations may account for the elusive or paradoxical relationship between melatonin levels and sleep quality.

The first GWAS reveals that the variants of the STON1 gene may be associated with the variation in sleep quality indicated by the CSHQ score amongst individuals on the autism spectrum. The STON1 gene is involved in endocytic machinery, which plays a key role in synaptic functions[59]. It is also highly expressed in the cerebellum, pons, and medulla regions. Although the variants of the STON1 gene have not been found to contribute to any behavioural or emotional traits, one recent study reports that DNA methylation patterns of this gene may correlate with the risk of schizophrenia[33, 60]. The signalling pathway analysis results reveal that T-helper 1 cell cytokine production may play a role in sleep disturbances of children on the autism spectrum. A few earlier studies found that production of pro-inflammatory cytokines could reach peaks during early nocturnal sleep[61]. Our data also suggest that the pathway involved in platelet aggregation by Eph kinase and ephrins. This finding may lend some support to the previous evidence from a mouse model that suggested the role of Eph kinase in rapid eye movement sleep[62].

The second GWAS indicates that one SNP near the SNAP25 gene may be associated with the melatonin level. Disruptions of the SNAP25 gene has been found to cause dysregulated circadian rhythm in the schizophrenia-related mouse model[63, 64]. Alternative splicing in the SNAP25 gene could also affect the circadian clock in mice[65]. The signalling pathway analysis results suggest that PC12 cells, a type of catecholamine cells that synthesise, store and release norepinephrine and dopamine, may be involved in melatonin levels of children

on the autism spectrum. Accumulating evidence reveals that melatonin can inhibit PC12 cell growth[66-68]. The GO pathway analysis results highlight the link between Schwann cells and melatonin, which may align with the findings that melatonin can promote Schwann cell proliferations[32, 69, 70].

The data seem to indicate that the relationship between melatonin and sleep quality in children on the autism spectrum may be complex due to the effect of other factors such as age. Although our GWAS findings do not provide strong or robust evidence for the association between common variants and either sleep quality or melatonin levels in children on the autism spectrum, our data do suggest potential roles of some genetic networks in either sleep quality or melatonin levels in children on the autism spectrum. GWAS studies using larger and independent samples to replicate these findings are warranted to clarify the relationship between genetic networks, melatonin levels and sleep disturbances, among individuals on the autism spectrum.

5. Limitations

Limitations to this study include the fact that CSHQ is a subjective parent report measure for assessing the impact of sleep difficulties[16]. While the CSHQ has well-established psychometric properties, parental recall bias is possible and no objective measures such as polysomnography were included to counter such bias. Hence, our findings may at best be interpreted as how autism-related clinical and behavioural features correlated with sleep issues observed by parents. Further, the current cross-sectional study design did not allow investigation of the temporal relationships between predictors and outcomes. As a result, we cannot assume any causal relationship due to the difficulty in clarifying the direction of the relationship with our findings and sleep difficulties are the consequence of poor sleep or vice versa. An additional limitation is the lack of information about mood and anxiety symptoms, which may also explain the link between autism and sleep difficulties. It is well established that depression/anxiety is correlated with autism traits and sleep disturbance, respectively.

The major limitation of our genetic association studies is the relatively small sample size compared with most of the recent large-scale GWAS. A small sample size could limit the robustness of findings. Additionally, a small sample size might be sensitive to clinical heterogeneity that predisposes to inconsistency across different studies because of difficulties in replicating results. Therefore, caution needs to be exercised particularly for our findings on melatonin based on 219 subjects.

Despite these limitations, our study has a number of strengths. Firstly, our study had a large sample size and the sample included siblings without an autism diagnosis and unrelated controls for comparison. There are a handful of studies exploring the behavioural attributes of sleep difficulties in autism but to the authors' knowledge, this is the largest study exploring the relationship between autism characteristics and sleep in children on the autism spectrum. In particular, the inclusion of motor skills and daily living skills adds to the limited knowledge base as motor and daily living skill domains have not been previously studied in the context of sleep in autism.

The inverse relationship between severity of sleep difficulties in autism and restricted and repetitive behaviours deserve further evaluation in an extended population, given the inconsistencies in the current literature and the current findings. Further, the association of motor skills and daily living skills with the severity of sleep difficulties also needs further exploration as there is a lack of comparable studies. Specific sleep information (e.g., sleep onset, sleep duration) collected via wearable devices would merit inclusion in future studies, and follow-ups scheduled to observe changes in the relationship between behaviour and severity of sleep difficulties over the developmental trajectory of children and their response to interventions would provide valuable insights.

6. Implications for research and practice

In summary, our finding that three quarters of children on the autism spectrum scored above the clinical cut off indicating poor sleep supports previous findings in the literature and has clinical implications. Given the significant functional impact of poor sleep on not only children but also on parents and care givers, assessment of sleep should form a critical part of the holistic assessment of children on the spectrum. We found that increased levels of adaptive behaviours were linked with reduced severity of sleep difficulties while increased levels of maladaptive behaviours and sensory issues predicted more severe sleep difficulties. This study also observed an inverse relationship between restricted and repetitive behaviours and severity of sleep difficulties which may indicate an adaptive role for such behaviours in autism especially in relation to sleep. It is possible that such behaviours may actually assist the child to settle at bedtime and this would merit further research. This coupled with our finding that there were differential impacts on different components of sleep behaviours based on age and sex highlights the need for better understanding of the unique sleep profile of the child as part of the holistic assessment in order to match appropriate sleep intervention and supports. In this regard, it is noteworthy that higher adaptive functioning

such as daily living skills was associated with decreased sleep difficulties and hence educational and skills building programs may have a positive impact on sleep in autism.

Our findings provide a tantalising preliminary evidence of higher anxiety increasing the oxidative stress in the body with consequent production of a larger volume of melatonin as a compensatory mechanism to protect the body. However, the anxiety in turn may lead to poorer sleep quality and vice versa. These effects are also influenced by age and biological sample collection time due to the diurnal variation in melatonin secretion. Such complex inter-relationships may explain the contradictory findings reported in the literature about the association between sleep and melatonin levels. Further, our genetic analysis seems to suggest potential roles of some genetic networks in either sleep quality or melatonin levels in children on the autism spectrum. In the context of high prevalence and significant impact of sleep difficulties, behavioural attributes as well as genetic and hormonal underpinnings of sleep disorders in autism warrant further exploration.

7. Key recommendations

Based on the outcomes and learnings from this study, the key recommendations are as follows.

- Increase awareness among parents and health professionals about the high occurrence of sleep difficulties as a co-occurring in children on the spectrum
- Given the significant impact of age on all aspects of sleep including behavioural attributes, co-occurring such as anxiety, genetic underpinnings and melatonin levels, future research on sleep disturbances in individuals on the autism spectrum should take age dependent impacts into consideration.
- The observations on the inverse and paradoxical relationship between melatonin levels and sleep quality highlight the complex and inter-connected link between sleep behaviours and co-morbidities such as anxiety, further compounded by the effects of age and sex, and these interrelationships deserve further exploration. Autistic females experienced greater severity of sleep difficulties than males, and preschool children had slightly different profile of sleep difficulties such as increased bedtime resistance, and hence it is critical to undertake a comprehensive assessment of not only the presence but also the nature of sleep difficulties taking into consideration also the age and sex, in order to provide matching intervention and developmentally appropriate resources and supports.

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AutismCRC

Autism CRC
The University of Queensland
Long Pocket Precinct
Level 3, Foxtail Building
80 Meiers Road
Indooroopilly Qld 4068
T +61 7 3377 0600
E info@autismcrc.com.au
W autismcrc.com.au

@autismcrc

Our values

Inclusion

Working together with those with the lived experience of autism in all we do

Innovation

New solutions for long term challenges

Evidence

Guided by evidence-based research and peer review

Independence

Maintaining autonomy and integrity

Cooperation

Bringing benefits to our partners; capturing opportunities they cannot capture alone

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