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Tiivistelmä – Referat – Abstract

Melatoniini on vuorokausirytmiä säätelevä hormoni, jolla on myös hermostoa suojaavia ominaisuuksia. Olemme aiemmissa tutkimuksissamme osoittaneet assosiaation geneettisen variantin rs12506228 (sijaitsee lähellä melatoniinireseptori 1A geeniä, *MTNR1A*) ja työuupumuksen sekä Alzheimerin taudin välillä. Tutkimuksessamme selvitimme yhteyttä rs12506228:n ja lapsen varhaisen kehityksen välillä, sillä melatoniinisignalointi on aiemmin liitetty sekä sikiön neurokognitiiviseen kehitykseen että unen kehitykseen. Tutkimukseen osallistui 8-kuukautisia lapsia, jotka ovat mukana suomalaisessa CHILD-SLEEP kohortissa (n=1301). Tutkimus suoritettiin kyselytutkimuksena; vanhemmille lähetettiin kyselylomakkeita, jotka mittaavat sosioemotionaalista, vuorovaikutuksellista ja motorista kehitystä sekä unen pituutta ja yöllisten heräämisten määrää. Tuloksissa havaitsimme assosiaation rs12506228:n A-alleelin ja sosioemotionaalisen (P=0.025) ja vuorovaikutuksellisen (P=0.0098) kehityksen välillä, mutta yhteyttä uneen ei voitu osoittaa. Havaitsimme kuitenkin interaktion geenivariantin kanssa tutkiessamme yhteyttä vuodenajan ja lapsen unen keston välillä. Geneettinen variantti rs12506228, jonka on aikaisemmissa tutkimuksissa osoitettu vaikuttavan aikuis- ja vanhuusiän ominaisuuksiin, osoittautui tutkimuksessamme olevan yhteydessä myös hidastuneeseen varhaiseen sosioemotionaaliseen kehitykseen. Lisäksi tuloksemme viittaavat siihen, että pimeät vuodenajat ovat yhteydessä pidentyneeseen kokonaisuniaikaan yksilöillä, joilla ei ole rs12506228-variantin AA-genotyyppiä. Rs12506228 on aiemmissa tutkimuksissa liitetty vähentyneeseen MT1-melatoniinireseptorien ilmentymiseen. Tämän pohjalta löydöksemme voivat olla seurausta heikentyneestä melatoniinisignaloinnista varhaisen kehityksen aikana.

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Variation near *MTNR1A* associates with early development and interacts with seasons

Short title: *MTNR1A* variation and early development

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Abstract

Melatonin is a circadian regulatory hormone with neuroprotective properties. We have previously demonstrated the association of genetic variant rs12506228 near the melatonin receptor 1A gene (*MTNR1A*) with intolerance to shift work. Furthermore, this variant has been connected to Alzheimer's disease. Because of the previously suggested role of melatonin signalling in foetal neurocognitive and sleep development, we studied here the association of rs12506228 with early development. The study sample comprised 8-month-old infants from the Finnish CHILD-SLEEP birth cohort (n=1301). Parental questionnaires assessed socioemotional, communication and motor development, as well as sleep length and night awakenings. The A allele of rs12506228 showed an association with slower socioemotional (P=0.025) and communication (P=0.0098) development but no direct association with sleep. However, the association of the Finnish seasons with infant sleep length interacted with rs12506228. Taken together, rs12506228 near *MTNR1A*, which has been previously linked to adult and elderly traits, is shown here to associate with slower early cognitive development. In addition, these results suggest that the darker seasons associate with longer infant sleep time, but only in the absence of the rs12506228 AA genotype. Since the risk allele has been connected to fewer brain MT1 melatonin receptors, these associations may reflect the influence of decreased melatonin signalling in early development.

Key words: *MTNR1A*, infants, sleep, genetics, cognitive development, seasonal variation

1 Introduction

A newborn sleeps about 15 hours a day and acquires a diurnal sleep–wake rhythm usually within the first 6 months of age (Mindell et al., 2016). The rhythmic secretion of melatonin from the pineal gland also develops during this period. (Kennaway, Goble, & Stamp, 1996; Kennaway, Stamp, & Goble, 1992). Prenatally, melatonin is acquired through the placenta from the mother, who produces it in high levels at the end of the pregnancy (Nakamura et al., 2001).

Adequate sleep is essential for normal brain development and synaptic plasticity (Tononi & Cirelli, 2014). Previous studies have demonstrated that sleep is associated with enhanced development of memory, learning and language (Tham, Schneider, & Broekman, 2017). Sleep traits have also been used to predict the cognitive, psychomotor and temperament development of children (Ednick et al., 2009). According to a twin-study of 18-month-old infants, genetic factors have a moderate effect on sleep early in life (Brescianini et al., 2011). However, only a few studies have addressed the effects of genes on sleep in infancy.

The foundations of developmental domains are built during the first year of life: children begin to engage in social interactions, cooing changes to babbling, they become able to adapt posturally to different situations, and emotions start to take shape (Zeanah, Boris, & Larrieu, 1997). Synaptogenesis is at its highest in the first year of life, peaking in the Angular gyrus and Broca's area at the age of 8 months, coinciding with the substantial development of higher cognition (Thompson & Nelson, 2001). Similarly to socioemotional development, a child's vocalisation as part of their communication abilities develops dramatically during the first year (D. Levine, Strother-Garcia, Golinkoff, & Hirsh-Pasek, 2016). Both the environment and genetics affect a child's socioemotional (DiLalla, Mullineaux, & Biebl, 2012) and communication development (Hayiou-Thomas, 2008).

In the present study, we focussed on the associations of rs12506228 with child development and sleep in the first 8 months of life. Rs12506228 is located in the q arm of chromosome 4, 72 kb downstream of *MTNR1A*, which is the closest confirmed

protein coding gene. This variant was previously identified as a top finding in a genome-wide study of job-related exhaustion in shift workers (as an estimate of sensitivity to circadian disruption) (Sulkava et al., 2017). A strong association with old age Alzheimer's disease has also been reported for the A allele of the variant (Sulkava et al., 2018) (Fig. 1). In previous studies, risk genes associated with Alzheimer's disease and multiple mental illnesses have been suggested to affect the brain as early as infancy (Knickmeyer et al., 2014). It has also been suggested that the A allele of rs12506228 decreases the expression of *MTNR1A* by methylation of the gene (Sulkava et al., 2017). Lower melatonin signalling has been linked to problems in foetal rhythmicity and normal neurocognitive development during the foetal period (Reiter, Tan, Korkmaz, & Rosales-Corral, 2014).

We hypothesised that the *MTNR1A* variant would have a negative effect on sleep quality because of the association with lower brain expression of MT1 melatonin receptors. If expression of these receptors is reduced, the circadian cycle would consequently be weaker (Sulkava et al., 2017), which could lead to disrupted sleep. Because of the suggested importance of melatonin signalling in brain development during the foetal period, we further hypothesised that the *MTNR1A* risk variant would also be associated with early development in infants. In this study, we investigated the association of rs12506228 with sleep traits (total sleep time, diurnal / total sleep time ratio and night awakenings) and with socioemotional, communication and motor development in 8-month-old infants using questionnaire-based data as well as actigraphy-based data for sleep length. As seasonal light conditions regulate the amount of melatonin secreted (Luboshitzky et al., 1998), we also studied the effects of seasons on infant sleep and interactions with the *MTNR1A* genetic variant.

2 Materials and Methods

2.1 Participants

This study is part of the CHILD-SLEEP cohort study of infants born between April 2011 and February 2013 in the Pirkanmaa area in Finland. The Ethical Committee of Pirkanmaa Hospital District approved the study protocol (R11032/9.3.2011), and the principles of the Declaration of Helsinki were followed. The parents gave their written informed consent. In our study, we examined the development and sleep of these infants at the age of 8 months. The initial number of participating families was 1673, and at the age of 8 months the response rate was 77.8% (N=1301). Prenatal characteristics for the study sample and those who dropped out are shown in Supplementary Table 1. No differences in the allele frequency of rs12506228 were detected between the groups. Families which dropped out were less likely to have parental university education, more likely to have maternal depression and at least one older sibling. However, these characteristics did not associate with our explanatory variables (rs12506228 and season of examination) thus they are not confounding factors in this study. Of the families participating in the 8-month data point, 87.5% (N=1139) had provided umbilical cord blood samples for DNA extraction and genotyping. Six participants, whose ages were over 10 months, were removed from the study. Sample sizes varied slightly depending on the variable, ranging from 1034 (night awakenings) to 1124 (socioemotional development). We also used actigraphy to objectively analyze the nocturnal sleep time of 354 infants, 314 of which were aged between 7-10 months and had genetic data available for rs12506228 (Martin & Hakim, 2011). Most of the infants (n=1290, 99.5%) were at home with their parent, and only 6 infants (0.2%) were in day care fulltime or part-time.

2.2 Collection of data

2.2.1 Measures

The domains of development and sleep were measured by delivering specific questionnaires to the parents. The questions regarding sleep were multifaceted and designed to map out disturbances in different aspects of infant sleep behaviour (Sadeh, 2004). We were primarily interested in sleeping time including total sleep time as well

as differences in nocturnal and diurnal sleep. Nocturnal sleep time was measured between 19.00 and 07.00, and diurnal sleep between 07.00 and 19.00. Total sleep time was calculated as the sum of diurnal and nocturnal sleep time. Children with total sleep time > 17 h were removed as outliers (n=3). In addition to time spent sleeping, we assessed the number of night awakenings according to the Infant Sleep Questionnaire (Morrell, 1999) by asking, "How many times does your baby wake each night and need resettling on average" between 24.00 and 06.00. Response choices ranged from 0 to 5 or more times. In addition to these subjective questionnaires, we were able to objectively measure sleep with actigraphy; 353 participants aged from 7 to 10 months wore an actigraphy bracelet on their thigh for three days and nights. This was used for secondary analysis to confirm the questionnaire's results. Finnish 8-month-old infants sleep their daytime naps not only in bed but often also in a moving pram, which makes the actigraphy measurement of day sleep less reliable compared to nocturnal sleep. Therefore, only the actigraphy measurement of the nocturnal actual sleep time was considered in this study. Figures for individual actograms were made with MotionWare Software (version 1.1.26,
CamNTech Ltd., Cambridge, U**K:** https://www.camntech.com/products/motionwatch/motionware-software).

Socioemotional, communication and gross motor development were assessed according to a specific Finnish parent-rated questionnaire modified for the study (Lyytinen, 2000; Nieminen & Korpela, 2004). Our main interest was cognitive development, but gross motor development was included as a non-cognitive domain of development to study the specificity of the associations with cognitive development. The questionnaire included 13, 10 and 12 statements of socioemotional, communication and gross motor development, respectively. The statements were designed to be easy to observe by the parents, for example "Shows fear of strangers by getting serious or crying when a new adult approaches" in the socioemotional scale or "Can get to sitting position without help" in the gross motor scale. The parents were asked to rate the statements about their infant's development according to three choices: never detected (0 points), detected once (1 point), and detected multiple times (2 points). The answers were rated from 0 to 2 and summed together within each scale.

Table 1. Key values of the main variables at 8 months-of-age.

	Total sample			CC genotype of rs12506228			AC genotype of rs12506228			AC genotype of rs12506228		
	N[a]	Skewness	Kurtosis	N[a]	Mean	SD	N[a]	Mean	SD	N[a]	Mean	SD
Total sleep time (h)	1102	-0.26	0.65	554	13.3	1.14	463	13.3	1.15	85	13.3	1.2
Diurnal/total sleep time	1102	0.51	0.59	554	0.26	0.07	463	0.26	0.06	85	0.26	0.08
Night awakenings	1033	0.65	-0.23	519	1.9	1.4	432	2.0	1.4	82	1.9	1.4
Socioemotional development	1123	-0.33	-0.06	568	21.0	2.4	456	20.7	2.3	90	20.5	2.4
Communication development	1116	-0.11	-0.41	563	13.7	3.6	463	13.3	3.4	90	12.6	3.3
Motor development	1116	0.08	-0.92	563	15.2	4.1	465	15.0	4.0	88	15.1	4.2
Actigraphy nocturnal sleep time (h)	315	-0.32	0.77	142	8.6	0.94	147	8.7	0.96	26	8.4	0.92

[a]Individuals with genetic data and age between 7-10 months.

2.2.2 Measures for seasonal analyses

The study subjects were living in the Pirkanmaa region of Finland, situated in latitude 61°N. We divided the participants into three groups according to the season when they answered the questionnaire: winter (November to January), summer (May to July) and autumn/spring (August to October and February to April). Summer was coded as 1, spring and fall 2 and winter 3 according to increasing amount of darkness. We studied the association of seasons with sleep variables using linear regression analysis and, in addition, studied the interaction of the genetic variant and seasons by adding an interaction term to the linear regression. Stratified analyses for the association of seasons with sleep were performed in the rs12506228 genotype groups.

2.2.3 Genotyping

An umbilical cord blood sample was drawn from each newborn at birth. DNA was extracted according to standard procedures at the National Institute for Health and Welfare. Genotyping of the rs12506228 variant was based on genome-wide genetic mapping with the Illumina Infinium PsychArray BeadChip, which comprises 603132 single nucleotide polymorphisms (SNPs). Genotyping was processed at the Estonian Genome Centre, University of Tartu. Quality control (QC) was performed using PLINK 1.9 (http://www.cog-genomics.org/plink/1.9/) (Purcell et al., 2007). Markers were removed for missingness (>5%) and Hardy-Weinberg equilibrium (P-value < 1×10^{-6}). Individuals were checked for missing genotypes (>5%), relatedness (identical by descent calculation, PI_HAT > 0.2) and population stratification (multidimensional scaling).

2.3 Statistical analysis

All the data was analysed with SPSS (IBM SPSS Statistics for Windows, version 25, IBM Corp. Armonk, NY, USA). We used linear regression models in all of the analyses, with age and gender as covariates. An additive model for genetic association analyses was used, meaning that the genotypes were coded based on the number of the minor allele A in rs12506228 (CC=0, AC=1, AA=2).

To enable the use of a linear regression model in our statistical analysis, we tested the normality of the variables by observing skewness and kurtosis. Kurtosis and skewness values between -0.92 and 0.77 were detected (Table 1). Because a linear regression model is not sensitive for small deviations from normality (Jupiter, 2017), all the variables could be used in the model without transformation. A nominal P-value of 0.05 was used as the threshold of significance in all the analyses. In addition, we used FDR correction for our 6 main variables (socioemotional, communication, gross motor development, total sleep time, diurnal sleep time/total sleep time, and night awakenings) using the FDR online calculator (FDR online calculator www.sdmproject.com/utilities/?show=FDR, n.d.). The FDR correction is, however, likely to be a rather conservative correction because of the correlation of the traits (e.g.

Pearson correlation coefficient for socioemotional and communication development = 0.44, and for total sleep time and ratio of diurnal sleep/total sleep time = -0.35). Therefore, a significance level of 0.1 was selected.

3 Results

3.1 Association of rs12506228 with sleep traits

The additive effects of the rs12506228 genetic variant on sleep were analysed with linear regression models adjusted for age and gender. No significant association was detected with sleep length or night awakenings in the questionnaire data or nocturnal sleep length measured with actigraphy (Table 2).

3.2 Association of rs12506228 with child development

We detected an association of rs12506228 with cognitive development: in the additive model, the minor allele A of rs12506228 was associated with slower socioemotional ($P = 0.025$, $P_{FDR} = 0.075$, beta = -0.24) and communication development ($P = 0.0098$, $P_{FDR} = 0.059$, beta = -0.42). No significant association with gross motor development was detected. The results can be examined in Table 2. Adjusting the model with potential environmental factors affecting development and sleep (poor family environment, maternal depression, maternal age, breastfeeding, number of siblings, birth season) did not change the associations of rs12506228 (Supplementary Table 2).

Table 2. Association of rs12506228 with development and sleep traits.

	N	Beta	SE	P-value
Total sleep time (h)	1102	0.030	0.055	0.58
Diurnal/total sleep time	1102	0.001	0.003	0.84
Night awakenings	1033	0.019	0.069	0.78
Socioemotional development	1123	-0.24	0.11	**0.025**[a]
Communication development	1116	-0.42	0.16	**0.0098**[a]
Motor development	1116	0.009	0.19	0.96
Actigraphy nocturnal sleep time (h)	312	0.007	0.085	0.94

Age and gender used as covariates in the analyses. [a] Significant with FDR correction P<0.1

3.3 Association of seasons with sleep traits and interaction with rs12506228

We also examined the seasonal differences in infant sleep by analysing the association between the season during which the assessment occurred and sleep traits. The results of the linear regression analyses showed that the questionnaire-based total sleep time as well as the actigraphy-based nocturnal actual sleep time were significantly longer during the darker months of the year (i.e., in winter). Moreover, night awakenings were more abundant in the darker months and the proportion of diurnal sleep from the total sleep time was higher. These results are presented in Table 3.

Table 3. Association of seasons with sleep traits.

	N	Beta	SE	P-value
Total sleep time (h)	1102	0.18	0.051	**0.00033**
Diurnal/total sleep time	1102	0.008	0.003	**0.0070**
Night awakenings	1033	0.16	0.064	**0.014**
Actigraphy nocturnal sleep time (h)	349	0.15	0.075	**0.048**

Covariates of age and gender used in the analyses. Winter coded 3, spring and fall coded 2, summer coded 1.

We then examined an interaction term for season and rs12506228 in predicting the sleep traits. The interaction term was significant for the actigraphy-measured sleep length (P = 0.041, beta = -0.25), and a trend was visible for the questionnaire-based sleep length (P = 0.063, beta = -0.15). Diurnal/total sleep time ratio and night awakenings did not show significant interaction terms (P = 0.77 and P = 0.97). In addition to examining the interaction term, we performed stratified analyses to show the effects of the seasons on sleep separately within the genotype groups of rs12506228. Regarding sleep length (questionnaire-based and actigraphy), a significant association of seasons with sleep was detected among carriers of the CC genotype (P = 0.01, beta = 0.24 and P = 0.016, beta = 0.29) and for the questionnaire-based trait, a weaker association among the carriers of the AC genotype was visible (P = 0.018, beta = 0.19). Among carriers of the AA genotype, no significant association of seasons with any sleep trait was detected and the effect was to the opposite direction. Thus, we showed that the association of seasons on sleep length was not visible among carriers of the AA genotype of rs12506228 and, when compared to the CC group, it was weaker among the carriers of the AC genotype. The results of the association of seasons with the sleep traits by genotype groups are presented in Table 4 and examples of actograms for different seasons and different genotype groups are shown in Figure 1.

Table 4. Association of seasons with sleep traits in the genotype groups of rs12506228.

		rs12506228 CC				rs12506228 AC				rs12506228 AA		
Trait	N	Beta	SE	P-value	N	Beta	SE	P-value	N	Beta	SE	P-value
Total sleep time	554	0.24	0.07	**0.001**	463	0.19	0.08	**0.018**	85	-0.25	0.20	0.20
Diurnal/total sleep time	554	0.005	0.004	0.203	463	0.013	0.004	**0.0036**	85	-0.002	0.013	0.89
Night awakenings	519	0.122	0.09	0.17	432	0.27	0.10	**0.010**	82	-0.07	0.21	0.74
Actigraphy nocturnal sleep time	142	0.29	0.12	**0.016**	146	0.15	0.12	0.20	26	-0.25	0.28	0.38

Age and gender used as covariates in the analyses

Winter coded 3, spring and fall coded 2, summer coded 1.

4 Discussion

Variant rs12506228 near the *MTNR1A* gene has previously been connected to sensitivity to circadian disruption as well as Alzheimer's disease (Sulkava et al., 2017, 2018). In this study, we examined the association of the variant with infants' sleep and development at the age of 8 months. We found that the previous risk allele (minor allele A) of rs12506228 was associated with slower socioemotional and communication development. No direct association with sleep traits was detected; however, rs12506228 was found to regulate the association of seasons on infants' sleep length (Fig. 1).

The A allele of rs12506228 is potentially linked to lower expression of *MTNR1A* in the brain (Sulkava et al., 2017). Based on this, we anticipated that carrying the minor allele A of rs12506228 would have negative effects on the development of sleep due to weaker melatonin signalling. However, we could not find support for this hypothesis as no connection between the genetic variant and sleep traits was detected.

Our results show that seasons affect sleep traits: darker months of the year were associated with longer total sleep time (both questionnaire-based and actigraphy-measured), a higher number of night awakenings, and a higher proportion of diurnal sleep from the total sleep time This may reflect weaker consolidation of nocturnal sleep in infants. The study subjects were living in the Pirkanmaa region of Finland, in latitude 61°N where considerable seasonal changes in day length occur.

Lengthening of the total sleep time during winter has previously been observed in the adult population (Cepeda et al., 2018), however, a large questionnaire-based population study from Norway did not find this effect (Sivertsen, Øverland, Krokstad, & Mykletun, 2011). In school-aged children, a small increase in the sleep length has been suggested to occur during the winter months (Hjorth et al., 2013). In line with this finding, a study of 5-12 year-old Finnish children showed less actigraphy-measured activity during the darker months (Aronen, Fjallberg, Paavonen, & Soininen, 2002). A study of 34 7-month-old infants did not show seasonal differences in sleep length or the number of awakenings (Cohen, Atun-einy, & Scher, 2012). However, this could be due to lack of power or less variation in day length as the study was carried out in Israel. In a large study of 1-month-old infants in the southern part of Japan, longer sleep time was observed in those born in spring compared to those born in autumn (Iwata et al., 2017).

Interestingly, the seasonal differences in sleep length we observed in our study seem to be dependent on the genotype of rs12506228. We found that the seasonal differences are not visible among carriers of the AA genotype of rs12506228. Longer sleep time during the Finnish winter could be caused by higher melatonin levels (Adamsson, Laike, & Morita, 2016; Kivelä, Kauppila, Ylöstalo, Vakkuri, & Leppäluoto, 1988), by the weaker direct activating effect of light, or by other seasonally dependent factors, such as temperature or the amount of outdoor activity. As the A allele of rs12506228 is linked to fewer MT1 melatonin receptors, our interaction results support the assumption that the seasonal effects on sleep length are caused by changes in melatonin levels.

Our findings confirm the hypothesis regarding developmental aspects: the A allele of rs12506228 was associated with slower socioemotional and communication development, but not with motor development in the first 8 months of life. The

association may be caused by a lower amount of melatonin receptor type 1A in the brain, which was previously linked to the A allele of rs12506228 (Sulkava et al., 2017). During the foetal period, melatonin and the melatonin receptor may play an important role in the development of the brain: in this period MT1 melatonin receptors are, unlike in adults, abundant in a number of discrete brain areas (Thomas, Purvis, Drew, Abramovich, & Williams, 2002). Infants' own melatonin production from the pineal gland begins postpartum, but maternal melatonin, which is present in high levels and has a clear circadian rhythm at the end of pregnancy (Kivela, 1991), crosses the placenta freely (Reppert, Chez, Anderson, & Klein, 1979) and is capable of synchronising foetal biological rhythms (Mendez et al., 2012) through these receptors. Animal models have suggested that melatonin has a pre- and perinatal neuroprotective effect against hypoxic-ischemic brain injury (Robertson et al., 2013), which could therefore protect consecutive cognitive development. In ovine models and in a pilot study in humans, melatonin has also been found to protect against intra-uterine growth restriction (Miller et al., 2014), a multifactorial condition linked to problems in neurocognitive development in infancy and childhood (T. A. Levine et al., 2015).

The association with early development is interesting when considering the previously demonstrated strong association of rs12506228 with clinical Alzheimer's disease and the amyloid and neurofibrillary pathology of Alzheimer's disease (Sulkava et al., 2018). Previously, studies on the strongest risk gene for sporadic late-onset Alzheimer's disease, APOE, have suggested expression of the genetic risk of Alzheimer's disease in childhood. In a large imaging study of 3- to 20-year-old participants, carriers of APOE genotypes ε4ε4, ε2ε4 and ε2ε2 showed an association with hippocampal volume and age-dependent brain maturation in ways that resemble the changes in Alzheimer's disease. The brain volume changes also correlated with poorer performance in attention tasks and working memory (Chang et al., 2016). In another study, MRI changes in APOE ε4 carriers were detected in 2-25 month-old children (Dean et al., 2014). Differences in grey matter volume in APOE ε4 carriers have been reported already in neonates (Knickmeyer et al., 2014). In contrast, association with faster mental development has been suggested in 24-month-old carriers of the ε4 risk genotype (Wright et al., 2003). Some studies have also examined another Alzheimer's risk gene, APP, and reported association of the risk SNPs with verbal and total level of intelligence in 8-year-old children (Myrum, Nikolaienko, Bramham, Haavik, & Zayats,

2017). Our findings suggest that not only genes related to APP metabolism, but also other Alzheimer risk genes, could manifest as early as childhood.

Figure 2. Associations of rs12506228 across the lifespan.

4.1 Limitations

Our study has some limitations to consider when interpreting the findings. Firstly, estimating an infant's nocturnal sleep is affected by many parent-related factors, such as their own ability to sleep and bed-sharing with an infant. Secondly, parental evaluations of their infant's development are vulnerable to subjective biases, even though the aspects of development used in the questionnaires were planned to be easily detectable by parents. Thirdly, the actigraphy data was considerably smaller (n = 314) than the questionnaire sleep data (n = 1103), although the actigraphy data managed to support our findings based on the questionnaire. Finally, the seasonal assessments were conducted on different infants studied in different seasons in contrast to following up the same infant. However, it must be stated that this prevents the aging of the infants from affecting the results.

4.2 Conclusions

In summary, we did not detect a direct association between the *MTNR1A* variant and sleep variables, but we showed that the darker months associate with longer sleep length only in the absence of the AA genotype of rs12506228, which is possibly linked to fewer MT1 melatonin receptors. We also showed an association between rs12506228 and slower socioemotional and communication development during the first 8 months of infancy, which may be linked to the importance of melatonin signalling during foetal development. These results may lead to a better understanding of different phenotypic expressions of genetic risks of adult conditions in the developing brain. However, to elucidate this more, longitudinal research across the lifespan is needed.

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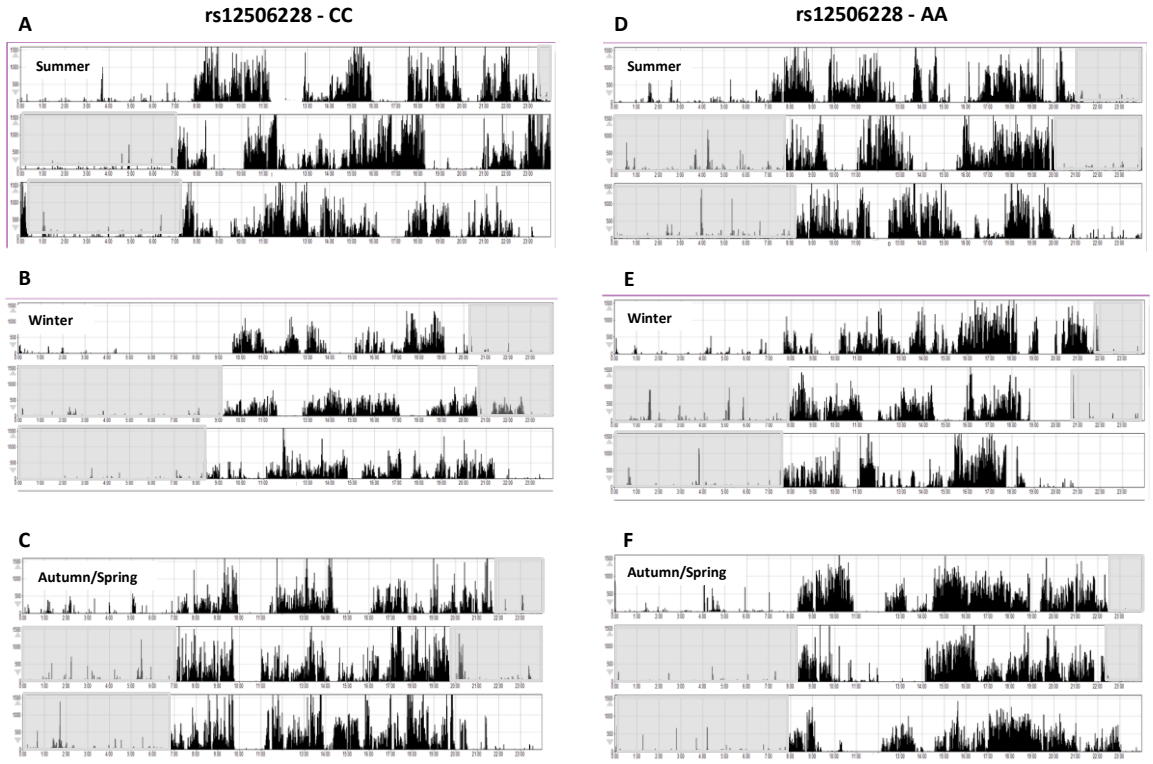

Figure 1. Six examples of actograms for different infants in summer, winter, and autumn/spring seasons, among carriers of the CC genotype and AA genotype of rs12506228. The period highlighted in grey indicates the nocturnal sleep period which was used for the analysis. The carrier of CC genotype of rs12506228 studied in summer (A) shows a shorter night time sleep duration (mean nocturnal sleep length = 5h 52mins) than the infant studied in winter (B, mean nocturnal sleep length = 10h 32mins), or in autumn/spring (C; mean nocturnal sleep length = 8h 23mins). Carriers of the AA genotype at 8 months of age have a more similar night time sleep duration, independent of the season of actigraphy assessment (D, mean nocturnal sleep length = 8h 48mins; E, mean nocturnal sleep length =8h 32mins; F, mean nocturnal sleep length 8h 56mins).

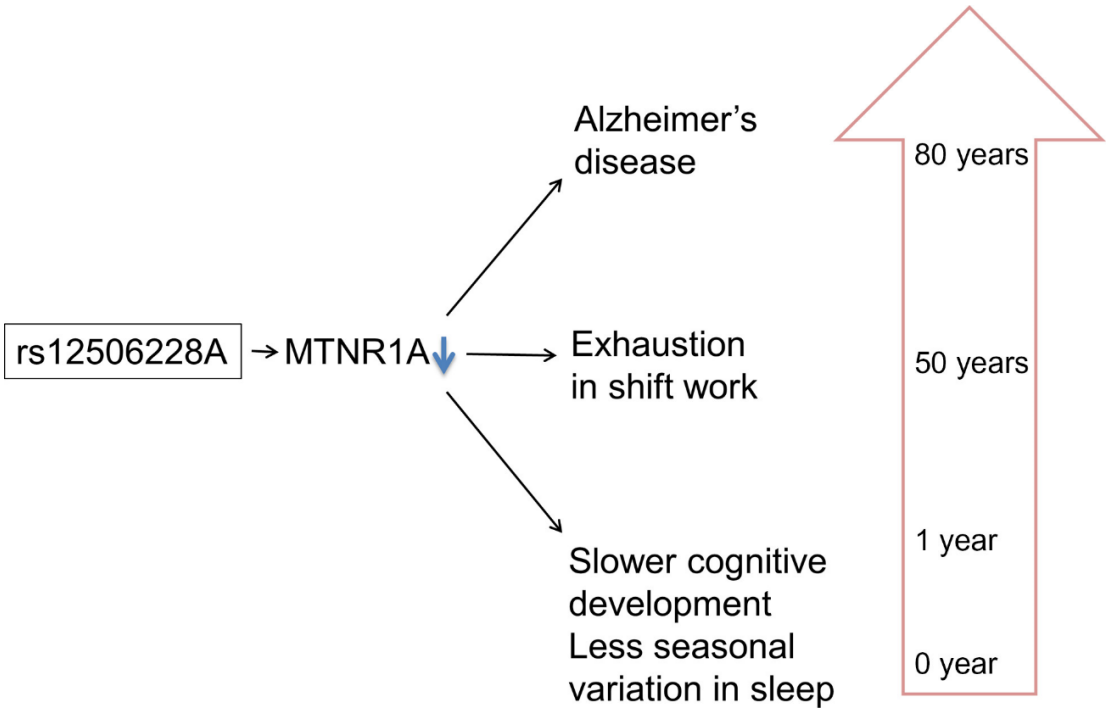

Figure 2. Associations of rs12506228 across the lifespan. The variant near melatonin receptor 1A gene (*MTNR1A*), rs12506228, was first discovered as a top finding of GWAS and replication studies for job-related exhaustion in shift workers (Sulkava et al., 2017). Subsequently, a strong association with clinical and neuropathological Alzheimer's disease was detected in old cohorts (Sulkava et al., 2018). Here, we demonstrated an association of the same risk variant with slower early cognitive development, as well as with decreased seasonal variation in infants' sleep. As the variant has an association with lower brain expression of *MTNR1A* (Sulkava et al., 2017), we suggest that the diverse associations of rs12506228 across the lifespan are mediated by weaker melatonin signalling due to a relative lack of receptors, which may cause different phenotypes at different ages. Molecular mechanisms mediating these phenotypes in the carriers of the risk allele may include the following: an increase in the rhythm-changing effect of light at night (Sulkava et al., 2017), increased processing of APP to amyloid (Sulkava et al., 2018), reduced neuroprotection during foetal brain development, and decreased transmission of the seasonal changes in the amount of melatonin.

Supplementary Table 1. Characteristics of the study sample (at 8 months) and those who dropped out (before 8 months)

	Study sample		Dropout		Test of difference
	Proportion of cases (N)	**mean**	**Proportion of cases (N)**	**mean**	**P**
Maternal age at delivery (y), mean		30.8		30.2	0.104[a]
Maternal university education	35 % (454)		27 % (101)		**0.005**[b]
Paternal university education	31 % (384)		23 % (80)		**0.002** [b]
Maternal depression, CES-D>10	10 % (124)		15 % (56)		**0.002** [b]
Paternal depression, CES-D>10	5 % (58)		8 % (11)		0.18 [b]
Older sibling(s)	53 % (582)		68 % (220)		**0.000002** [b]
rs12506228 CC	50 % (569)		52 % (72)		
AC	42 % (471)		38 % (53)		
AA	8 % (92)		10 % (14)		0.61 [b]
Birth season[c] summer	31 % (406)		29 % (107)		
spring/autumn	46 % (598)		47 % (172)		
Winter	23 % (292)		24 % (90)		0.62 [b]

[a] Mann-Whitney U test

[b] Chi^2 test

[c]Coded according to the darkness of months 1= summer (May-Jul), 2= spring/autumn (Feb-Apr/Aug-Oct), 3=winter (Nov-Jan)