Studies on circadian rhythm disturbances and melatonin in delirium

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Chapter 3
Polymorphisms in the melatonin receptor 1B gene and the risk of delirium
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Chapter 3

Abstract

Background/aims: A disturbed sleep-wake rhythm cycle can be seen in delirium and as melatonin regulates this cycle via melatonin receptors, genetic variations in these receptors may contribute to delirium susceptibility. The purpose of this study was to investigate whether genetic variants in the melatonin receptor 1B (MTNR1B) gene are associated with delirium.

Methods: Older medical and hip surgery patients were included. Five single nucleotide polymorphisms were determined in the MTNR1B gene (rs18030962, rs3781638, rs10830963, rs156244 and rs4753426).

Results: In total, 53% of 171 hip fracture patients and 33% of 699 medical patients were diagnosed with delirium. None of the polymorphisms were found to be associated with the occurrence of delirium.

Conclusion: Future research could focus on sequencing this gene to look for other functional SNPs in relation to delirium.
Polymorphisms in MTNR1B gene and delirium

Introduction
Delirium is the most common neuropsychiatric complication in hospitalized elderly patients. Although it has long been regarded as a transient disorder, it has become clear that these patients face substantially increased risks of mortality, institutionalization and dementia (1). Delirium is often precipitated by an acute infection, an operation, or an intensive care unit (ICU) stay, and it is associated with predisposing factors such as age, the presence of premorbid conditions like dementia and genetic predisposition (2). Because delirium can have far-reaching consequences for patients, further unraveling of the pathophysiology is necessary to develop effective and safe preventive and treatment options.

Delirium symptoms often include a disturbance of the circadian rhythm. The circadian rhythm is dictated by the suprachiasmatic nucleus (SCN), and the hormone melatonin regulates this cerebral biological clock via melatonin receptors (3). Melatonin is secreted by the pineal gland in response to darkness, and it is metabolized from serotonin, which is derived from tryptophan (4). Melatonin has various physiological functions, including neuromodulatory and vasoactive actions and also antioxidative and neuroprotective properties, but its main effect is that it positively affects sleep onset via a synchronizing effect on the biological clock (5). Endogenous melatonin production decreases with age (6), and lower nighttime melatonin levels were found in healthy elderly people with insomnia than in age-matched controls who had no sleep disorders (7).

A relationship between sleep disturbances and delirium was already established (8). The disruption of the circadian rhythm in delirium may be related to abnormal melatonin secretion patterns as low melatonin concentrations have been found in post-surgical conditions and in ICU patients (9, 10). A relationship between abnormal melatonin secretion and postoperative delirium was also found (11).

Dementia and particularly Alzheimer’s disease are associated with an increased risk of delirium (12). Low melatonin levels were associated with sleep and other circadian rhythm disturbances in dementia and were identified before clinical symptoms of dementia began to show (13). (14). Studies investigating the effect of melatonin supplementation in patients with dementia are however inconclusive (15-17). The loss of melatonin receptors in the SCN and the pineal and cortical regions as dementia progresses might explain these results (14, 18).

In addition to abnormal secretion patterns, and declining number of receptors, polymorphisms in the genes that encode for melatonin receptors could lead to abnormalities in the function of these receptors, and may also explain no straight forward response to melatonin.

There are two melatonin receptors that have known effects in humans: MTNR1A and MTNR1B. They are expressed in various regions of the brain, including the hippocampus and the thalamus (3). At the time this study was conducted, no associations had been found between single nucleotide polymorphisms (SNPs) in the MTNR1a gene and diseases.
Chapter 3

However, regarding the MTNR1B gene, the rs18030962, rs3781638, rs10830963, rs156244 and rs4753426 SNPs, have been shown to be associated with several diseases (19-21), among which the finding in three independent genome wide association studies of the association between variations in the MTNR1B gene, hyperglycemia, impaired early phase insulin secretion and beta cell function. These findings suggest that these SNPs have a functional role in melatonin metabolism and as the MTNR1B gene is an eligible candidate gene for the development of delirium these SNPs are a sensible target. The aim of this study was therefore to investigate whether these polymorphisms in the MTNR1B gene are associated with delirium in a population of acutely admitted older patients with or without preexisting cognitive impairment.

Methods

Patients and procedures
Between April 2003 and August 2008, medical patients aged 65 years or older who were acutely admitted to the Academic Medical Center, Amsterdam, The Netherlands, were consecutively included in the study. From June 2005 through August 2008, hip fracture patients aged 65 years and above scheduled for surgery were also included. Informed consent was obtained from all patients, or closest proxy in cases of cognitive impairment. Patients were excluded if they were unable to speak or understand Dutch or English. The institutional medical ethics committee approved the study (22).

Two geriatric physicians, a fellow in geriatric medicine, and four research nurses trained in geriatric medicine collected demographic and clinical data. The presence or absence of delirium was scored within 48 hr of admission using the Confusion Assessment Method (CAM) (23). We based our information for the diagnosis on a structured psychiatric examination of the patient according to the DSM-IV criteria for delirium (24), medical, and nursing records including the Delirium Observation Screening Scale (DOS) (25), and information given by the patient’s proxy.

Possible confounding factors, such as demography, reason for admission, premorbid cognitive and physical functioning, were recorded for all patients. The assessment of global cognitive functioning was based on the patient’s anamnesis and medical history and their results on the Informant Questionnaire on Cognitive Decline-Short Form (mean score of 3.9 or more points) (26) and the Mini-Mental State Examination (a cutoff score of less than 24 points) (27). To measure physical functionality, patients, or their closest relatives in cases of cognitive impairment, were asked to complete the 15-item Katz Activities of Daily Living (Katz-ADL) index score (28, 29) based on the patients performance two weeks before admission. Patients with a score of 7 or higher were considered to be functionally impaired. These procedures have been described in detail previously (30).
**Single Nucleotide Polymorphism (SNP) genotyping**

Genomic DNA was isolated from 10 ml whole blood on an AutopureLS apparatus according to the manufacturer’s protocol (Gentra Systems, Minneapolis, USA). Genomic DNA was isolated from 10 ml whole blood with Gentra Puregene chemistry on an Autopure 98 apparatus according to the manufacturer’s protocol (Qiagen, Venlo, The Netherlands). The genotypes of all SNPs were determined using Taqman Assay-By-Design (Applied Biosystems, Foster City, CA, USA) probes for allelic discrimination. The assay numbers for rs18030962, rs3781638, rs10830963, rs156244 and rs4753426 were C__11323152_10, C__27500317_10, C__3256858_10, C__8369474_10, C__289583_10, respectively. All samples were coded for research, and personnel were blinded to the statuses of the individuals.

**Statistics**

All statistical analyses were performed with SPSS version 16.0 for Windows. Differences in characteristics of patients with and without delirium were tested using T-tests, Mann-Whitney U Tests, and Chi-squared tests. Hardy-Weinberg equilibrium was tested using x² test. The association between each SNPs and delirium was investigated by multivariable logistic regression analysis and was adjusted for independent delirium risk factors. Independent risk factors were determined by a backward selection procedure of multivariable logistic regression analyses with the significant factors shown in Table 1. Haplotype effects and frequencies were estimated using the haplo.stats package (31, 32). A p-value at or below 0.05 was considered statistically significant.

**Power of the study**

With the present case and control sample sizes and minor allele frequencies ranging from 0.29 to 0.50 in the controls, the study had ≥ 80% power to detect an odds ratio of ≥ 1.4 (or ≤ 0.7) assuming a log-additive effect of the SNP and a two-sided significance threshold of 0.05.

**Results**

**Study population**

In total, 881 patients were recruited for genotyping, 11 of whom were missing CAM scores. Of these patients 53% of the 171 hip fracture patients and 33% of the 699 medical patients developed delirium. The characteristics of the patients with and without delirium are presented in Table 1. On average, delirious patients were older, 82.8 versus vs. 77.6 years, and they had more often pre-existing functional or cognitive impairments (P<0.001). The main reasons for admission to the hospital were significantly different overall (P<0.001); the patients with delirium were more often admitted with infectious diseases (39 vs. 36%), water- and electrolyte disturbances (11 vs. 7%), and digestive system diseases (16 vs. 6%) than patients without delirium.
### Table 1: Characteristics of patients with and without delirium

<table>
<thead>
<tr>
<th>Variable</th>
<th>Delirium (N= 311)</th>
<th>No Delirium (N= 559)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years (SD)</td>
<td>82.8 (7.4)</td>
<td>77.6 (8.6)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Male (%)</td>
<td>117 (38)</td>
<td>255 (46)</td>
<td>.02</td>
</tr>
<tr>
<td>Caucasian ethnicity (%)</td>
<td>267 (86)</td>
<td>494 (88)</td>
<td>.70</td>
</tr>
<tr>
<td>Living at home (%)</td>
<td>210 (68)</td>
<td>492 (88)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Functional impairment* (%)</td>
<td>192 (64)</td>
<td>108 (36)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cognitive impairment* (%)</td>
<td>256 (83)</td>
<td>141 (26)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

SD: standard deviation
*Impairment as determined two weeks prior to admission

The genotyping of the polymorphisms had success rates around 94%. The distributions of the SNPs were all in line with Hardy-Weinberg equilibrium. Table 2 shows the results of the analysis for possible association between the MTNR1B gene polymorphisms and delirium. None of the identified polymorphisms were associated with the presence of delirium (p>0.24). Delirium was independently associated with older age (Odds Ratio (OR) =1.03, 95% CI: 1.01-1.06, p=0.005), pre-admission cognitive impairment (OR=9.54, 95% CI: 6.49-14.01, p<0.001) and pre-admission functional impairment (OR=1.89 CI: 1.31-2.72, p<0.001) in logistic regression analyses. After adjusting for these three factors the polymorphisms were not associated with delirium (P>0.17).

### Table 2: Polymorphisms in the melatonin receptor 1B (MTNR1B) gene (11q21-q22) in patients with and without delirium

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Location</th>
<th>Alleles</th>
<th>Delirium (%)</th>
<th>No delirium (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1562444</td>
<td>3PRIME_UTR</td>
<td>AA, AG, GG</td>
<td>76 (26)</td>
<td>132 (26)</td>
<td>0.74</td>
</tr>
<tr>
<td>rs10830963</td>
<td>INTRONIC</td>
<td>CC, CG, GG</td>
<td>21 (7.0)</td>
<td>45 (8.7)</td>
<td>0.64</td>
</tr>
<tr>
<td>rs3781638</td>
<td>INTRONIC</td>
<td>AA, AC, CC</td>
<td>91 (30)</td>
<td>150 (29)</td>
<td>0.80</td>
</tr>
<tr>
<td>rs4753426</td>
<td>UPSTREAM</td>
<td>TT, CT, CC</td>
<td>97 (32)</td>
<td>151 (29)</td>
<td>0.53</td>
</tr>
<tr>
<td>rs10830962</td>
<td>UPSTREAM</td>
<td>CC, CG, GG</td>
<td>108 (36)</td>
<td>166 (32)</td>
<td>0.43</td>
</tr>
</tbody>
</table>

UTR= Untranslated Region
Nine different haplotypes were discriminated and analyzed for their possible association with delirium. None of these haplotypes was associated with delirium, even after adjusting for pre-existing cognitive impairment.

**Discussion**

In this study, we tested whether five SNPs in the MTNR1B gene were associated with delirium in a large cohort of elderly patients acutely admitted to the medical and surgical department. The results show that there is no association between these SNPs in the MTNR1B gene and delirium. The results were adjusted for age, cognitive impairment, and functional impairment. No other studies exist that directly investigate the association between the MTNR1B gene and delirium. Because delirium is a complex phenotype to define, heritability studies and linkage analyses in families are still lacking. However, there are a few studies that report on genetic associations with delirium. Carrying the Apolipoprotein E 4 (APOE 4) allele is probably associated with delirium, even after adjusting for pre-existing global cognitive impairment (33, 34). Furthermore, variations in the dopamine transporter (SLC6A3) gene and possibly the dopamine D2 receptor (DRD2) gene were found to be associated with delirium (35). Homozygous carriers of haplotype 4 of the glucocorticoid receptor were found to have a 92% lower risk of developing delirium compared to individuals carrying other haplotypes of this gene (36).

Unfortunately, information on melatonin levels in delirious patients is still scarce, probably because repetitive nightly blood or saliva sample tests are burdensome, especially for delirious patients. Also, melatonin metabolism is complex, and the melatonin hypothesis could still be correct for other reasons as melatonin can activate or inhibit signal transduction cascades independent of receptors or through receptors. The ability of melatonin to act independently from its receptors is attributed to its small and highly lipophilic nature and/or its active uptake mechanism (37). Thus, changes in melatonin metabolism could influence SCN and might provoke delirium independent of the function and number of melatonin receptors. Indirect evidence underlines this as recent findings suggest that melatonin supplementation is an effective strategy for preventing delirium (38, 39).

According to our power analysis the study had > 80% power of detecting an odds ratio of more than 1.4. Furthermore, we selected these five SNPs because they have been found before in studies in association with diseases, which makes functionality probable. However, we have not investigated the entire gene so it is possible that we could have missed certain (unknown) SNPs that were associated with delirium (40).
Chapter 3

Conclusion
Previous studies have demonstrated the role of genetics and melatonin in the occurrence of delirium. In the present study, we found no association between delirium and some specific polymorphisms in the MTNR1B receptor. However, as other SNPs in the MTNR1B gene have not been investigated, future research could also focus on sequencing the gene to look for other functional SNPs in relation to delirium.
Polymorphisms in MTNR1B gene and delirium

Reference List


Chapter 3

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