Melatonin in Children with Autism Spectrum Disorders: How Does the Evidence Fit Together?

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Autism spectrum disorders (ASD) are prevalent neurodevelopmental conditions, affecting 1 in 68 children in the United States alone. Sleep disturbance, particularly insomnia, is very common in children diagnosed with ASD, with evidence supporting overlapping neurobiological and genetic underpinnings. One of the most well studied mechanisms related to ASD and insomnia is dysregulation of the melatonin pathway, which has been observed in many individuals with ASD compared to typically developing controls. Furthermore, variation in genes whose products regulate endogenous melatonin modify sleep patterns in humans and have also been implicated in some cases of ASD. However, the relationship between comorbid insomnia, melatonin processing, and genes that regulate endogenous melatonin levels in ASD is complex and requires further study to fully elucidate. The aim of this review is to provide an overview of the current findings related to the effects of genetic variation in the melatonergic pathway on risk for expression of sleep disorders in children with ASD. In addition, functional findings related to endogenous levels of melatonin and pharmacokinetic profiles in this patient population are evaluated. Journal of Nature and Science, 1(7):e125, 2015.

Autism | insomnia | genetics | pharmacokinetics

Background

Autism spectrum disorder (ASD) is characterized by impairments in social communication and the presence of restricted and repetitive behavioral patterns.[1] Within this unified definition, the severity of clinical presentation is variable and many individuals present with a number of comorbidities, endophenotypes, and biomarkers.[2-6] Sleep disorders, particularly insomnia, are comorbidities commonly observed in patients with ASD and prevalence estimates range from 50-80%.[7-12] Disturbed sleep potential exacerbates ASD-related symptoms such as impaired social interactions, presence of repetitive behaviors, mood disorders, and inattention or hyperactivity.[9, 13-15]

The involvement of melatonin in regulating the sleep-wake cycle is well-known. There is also strong evidence supporting an involvement of the serotonin-N-acetylserotonin-melatonin pathway in the etiology of ASD.[16] Evidence for the involvement of the melatonergic system in ASD includes lower levels of blood melatonin and its primary metabolite 6-sulfoxymelatonin in many individuals.[17, 18] Additionally, polymorphisms, mutations and copy number variation located in genes that encode enzymes known to regulate endogenous melatonin levels have also been associated with ASD.

However, findings from most studies evaluating contributions of the melatonergic system to risk for ASD have been inconsistent and the relationship of this system to expression of comorbid sleep problems in individuals with ASD is yet to be fully understood.[19, 20] To understand these seemingly contradictory findings, it is helpful to review some background information on melatonin and the potential link to ASD. The aim of this review is to critically analyze some of the current genetic and functional findings in children with ASD and comorbid expression of insomnia. The ultimate goal is to help identify areas of research that will be most useful to future development of personalized approaches to treating sleep disorders in this patient population.

Dysregulation of Melatonergic Pathways in ASD

Melatonin is synthesized in the pineal gland and is involved in many functional processes including regulating the sleep-wake cycle in humans.[21-23] Synthesis of melatonin begins with the active uptake of the amino acid tryptophan into the gland. Tryptophan is then hydroxylated and decarboxylated to serotonin, another molecule with ample evidence for involvement in ASD.[24] Serotonin is then N-acetylated by arylalkylamine (AANAT), and subsequently converted to melatonin by the acetylserotonin O-methyltransferase (ASMT) enzyme, also known as hydroxyindole-O-methyltransferase.[21] Melatonin is then primarily metabolized in the liver by cytochrome P450 1A2 (CYP1A2).[25, 26]

Dysregulation of biological pathways maintaining proper levels of endogenous melatonin have been proposed to contribute to the expression of sleep disturbances in ASD.[27] Significantly lower levels of nocturnal [17, 28] and daytime [29] blood melatonin levels and lower levels of its primary metabolite 6-sulfoxymelatonin [16, 18] have been observed in many individuals with ASD when compared to typically developing controls. Interestingly, a study conducted by our group in a small subset of children (n = 9) with ASD and well-defined comorbid sleep onset insomnia observed normal overnight blood melatonin profiles.[7, 30] Furthermore, in endogenous samples maximal melatonin concentration and time to peak concentration were comparable to those previously published in the literature for typically developing children. Dim light melatonin onset sets were also captured in the majority of children and did not appear to be abnormal. In children treated with 1-3mgs of supplemental melatonin, melatonin pharmacokinetic parameters were also comparable to previously published parameters for typically developing children.[30] When evaluating efficacy of supplemental melatonin treatment in this sample of children having normal endogenous melatonin levels at night, it was observed that all of the patients responded to supplemental melatonin treatment and were able to fall asleep more quickly. This indicates that supplemental melatonin may be treating something other than simply a deficiency state. For example, melatonin may be acting as a hypnotic independent of a deficiency state (i.e., in children with normal endogenous melatonin levels).[31] Melatonin may also be acting as a chronobiotic to produce circadian phase-shifting effects in children with a delayed sleep onset.[32] Melatonin has also been shown to have antianxiolytic effects which may help settle a child with ASD and comorbid anxiety.[33] Finally, melatonin may mitigate hyperarousal-related insomnia through its effects on the hypothalamic pituitary adrenal axis as melatonin has been shown to suppress ACTH in animal models.[34]

In contrast to our findings of a beneficial response to supplemental melatonin, some patients with ASD have been identified as non-responders or exhibit disappearing effectiveness. [35-37] The phenomenon of disappearing effectiveness may be related to dysfunction of CYP1A2 and is discussed further below. As the currently evaluated patient populations exhibiting traits like...
the presence of normal overnight blood melatonin profiles in children with comorbid sleep onset insomnia are small, further investigation in this area is warranted.

Melatonin Pathway Genes and the Link to ASD
In reference to the relationship of melatonin synthesis to risk for ASD, a few groups have observed effects related to variation in genes involved in this pathway.[16, 29, 36, 38–40] It should be noted that the majority of studies investigating melatonin-related genes in ASD have focused on the ASMT gene and additional study needs to be conducted evaluating other genes in the melatonergic system related to synthesis, transport, and degradation. However, one group observed a relationship between the AANAT gene and risk for severe language deficits in ASD.[38] ASD individuals were subgrouped based on symptoms reported in the Autism Diagnostic Interview-Revised prior to genome-wide gene expression analyses. The authors observed that 15 circadian rhythm regulatory or responsive genes were among those genes identified as being differentially expressed in the most severe ASD subgroup but not in the mild or savant subgroups, suggesting a connection between dysregulation of circadian rhythms and the severity of language impairment. In particular, a significant decrease was observed for the gene encoding AANAT. [38, 41] A reduction in this enzyme would be consistent with the abnormally low levels of melatonin reported in a number of studies of ASD patients, and with the high incidence of sleep disorders reported in ASD.[42] These findings have yet to be replicated.

With regard to findings related to risk for ASD and variation in the ASMT gene, a previous study observed a relationship between two single nucleotide polymorphisms (SNPs), rs4446909 and rs59868681, located in the promoter of ASMT and increased risk for ASD (n=250).[29] Additionally, the authors observed that homozygous presence of the ASD-associated alleles at both SNPs (i.e., rs4446909GG, rs59868681GG) related to significant decreases in ASMT expression. Decreased expression of the ASMT transcript was correlated with decreased blood melatonin levels in individuals with ASD and their family members.[29] The ASMT promoter B polymorphisms are located in transcription factor binding sites for nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and specificity protein 1 (Sp1).[39] As such, the reported SNPs are thought to alter gene expression by disrupting transcription factor binding. An ASMT promoter B polymorphism has also been reported for the ASMT gene that includes the promoter B SNPs and a third SNP, rs6644635, located in the 5′-untranslated region (UTR) of the only known functional isoform of ASMT.[29, 43, 44] Further studies in independent ASD datasets have failed to replicate the statistical associations of these common SNPs in ASMT and ASD risk.[44, 45] However, most of these previous studies do not report evidence of sleep disruption in any of the individuals screened and presence of sleep disorders was not a primary focus for case criteria.

In our preliminary studies, which focused only on individuals with ASD and comorbid sleep onset insomnia, we observed higher frequencies than currently reported (p<0.04) for these previously reported common variants evidenced to decrease ASMT expression when compared to individuals with ASD without evidence of a sleep disturbance.[40] Our results indicate that sleep disorder data is useful for genetic studies of ASD. Also, it is possible within the genetic background of ASD that there is incomplete penetrance for variation in melatonin pathway genes and potentially undiscovered genetic modifiers.

In addition to the findings related to common variation observed in the ASMT gene, several rare variants having functional consequences have been observed.[29, 39, 44] These rare mutations include non-synonymous and splice-site mutations. In particular, a splice-site mutation reported by Melke et al., 2008 was exclusive to two ASD families and was shown in vitro to have functional consequence causing very low levels of ASMT transcript production.[29] This same year, a second group observed a rare microduplication in exon 8 of the ASMT gene in 16 children with ASD.[46] Nine of these children having the duplication in ASMT were reported to have sleep problems. We did not observe any novel or previously reported rare point mutations in our small set of individuals with well-defined sleep onset insomnia and we did not look for the microduplication in our sample.[40] Given that this type of variation is by definition rare it is not surprising that we did not observe any in our small dataset (n=15).

In reference to defects in melatonin degradation, there are numerous polymorphisms located either within the CYP1A2 gene, or in intronic regions, that are reported to influence subsequent enzymatic activity.[47–53] A potential relationship has also been implicated between presence of predicted slow-metabolizing alleles in CYP1A2 and susceptibility to ASD with comorbid sleep problem.[35, 36] Interestingly, all of the individuals included in the Braam et al., 2013 study (n=11) were diagnosed as slow melatonin metabolizers and it was observed for these children that the efficacy of supplemental melatonin exhibited disappearing effectiveness over the course of 4–8 weeks.

We also evaluated slow-metabolizing alleles in CYP1A2 and the relationship to expression of sleep onset insomnia in a small population of children with ASD and comorbid sleep onset insomnia (n=15).[40] While we were only able to evaluate a small sample of children, we observed increased frequencies for variants in the CYP1A2 gene related to decreased enzyme activity (p≤0.0007). Some patients evaluated in our genetic study were also included in the study of overnight endogenous and pharmacokinetetic melatonin profiles. There was no evidence indicating individuals with slow-metabolizing alleles in the CYP1A2 gene were actually slow melatonin metabolizers (T1/2 > 2 hours).[30] We also did not observe disappearing effectiveness of melatonin treatment in our patients over the course of 17 weeks. However, we observed that expression of insomnia in ASD, and response to supplemental melatonin treatment, was potentially related to dysfunctional variation in both the ASMT and CYP1A2 melatonin pathway genes. A relationship was observed between genotypes at SNP rs6644635 in the 5′-untranslated region of ASMT and genotypes at SNP rs2069514 in the promoter region of CYP1A2 (r2=0.63). This implicates a potential mechanism connecting lower levels of ASMT transcript production with reduced CYP1A2 metabolic activity in some children with ASD and comorbid sleep onset insomnia;[40] the net result may be normal nocturnal blood melatonin levels in these children. The Braam et al. study did not assess effects related to polymorphisms in the ASMT gene. Therefore, it is possible the patients with a slow melatonin metabolism only had dysfunctional alleles in CYP1A2 and not in ASMT. However, to fully understand this underlying relationship to the etiology of ASD with comorbid sleep onset insomnia, it will be necessary to evaluate larger ASD datasets focusing on children with comorbid sleep onset insomnia and incorporating assessment of melatonin pharmacokinetetic data.

Future Directions
To ultimately elucidate the relationship between genotypes in genes involved in melatonin synthesis and genotypes in the gene that metabolizes melatonin, larger sample sizes and additional genes in the melatonergic pathway need to be evaluated. In addition, functional experiments modeling specific risk genotypes will need to be conducted both in vitro and in vivo to evaluate the effects of the combination of ASD risk variants in both ASMT and CYP1A2. Additional study of individuals with ASD, concurrent reduced endogenous melatonin levels and the severity of sleep disturbance in ASD is also warranted. To ultimately parse out this relationship, endogenous melatonin levels should be evaluated in children with well-characterized phenotypes with respect to both the core deficits involved in ASD and the sleep problems observed in these patients.

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