



HHS Public Access

Author manuscript

J Autism Dev Disord. Author manuscript; available in PMC 2021 May 01.

Published in final edited form as:

J Autism Dev Disord. 2020 May ; 50(5): 1834–1840. doi:10.1007/s10803-019-03921-5.

Brief Report: A Gene Enrichment Approach Applied to Sleep and Autism

Emily A. Abel,

Department of Human Development and Family Studies, Purdue University, Yale Child Study Center, Yale University

A.J. Schwichtenberg,

Department of Human Development and Family Studies, Purdue University

Olivia R. Mannin,

Department of Human Development and Family Studies, Purdue University

Kristine Marceau

Departments of Human Development and Family Studies, and Statistics, Purdue University

Abstract

Sleep disorders (SD) are common in autism spectrum disorder (ASD), yet relatively little is known about the potential genetic mechanisms involved in SD and ASD comorbidity. The current study begins to fill this gap with a gene enrichment study that 1) identifies risk genes that contribute to both SD and ASD which implicate circadian entrainment, melatonin synthesis, and several genetic syndromes. An over-representation analysis identified several enriched pathways that suggest dopamine and serotonin synapses as potential shared SD and ASD mechanisms. This overlapping gene set and the highlighted biological pathways may serve as a preliminary stepping-stone for new genetic investigations of SD and ASD comorbidity.

Keywords

sleep; gene; syndrome; autism spectrum disorder; circadian; melatonin

Sleep problems/disorders (SD) are common in autism spectrum disorder (ASD), and up to 80% of individuals diagnosed with ASD will experience a SD within their lifetime (Baker & Richdale, 2017; Richdale & Schreck, 2009). These SD's regularly include increased sleep onset latency, frequent and prolonged night awakenings (Allik, Larsson, & Smedje, 2008; Krakowiak, Goodlin-Jones, Hertz-Picciotto, Croen, & Hansen, 2008), insomnia (Cortesi, Giannotti, Ivanenko, & Johnson, 2010; Richdale & Baglin, 2015), and/or early morning rise

Correspondence concerning this article should be addressed to Emily A. Abel, Department of Human Development and Family Studies, Purdue University, 1202 West State Street, West Lafayette, Indiana. 765-494-6610. EmilyAbel@purdue.edu or Emily.abel@yale.edu.

Compliance with Ethical Standards:

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest: Emily A. Abel declares that she has no conflict of interest. A.J. Schwichtenberg declares that she has no conflict of interest. Olivia Mannin declares that she has no conflict of interest. Kristine Marceau declares that she has no conflict of interest.

times (Richdale & Baglin, 2015). Although SD and ASD are highly comorbid, relatively little is known about the mechanistic intersections of these two disorder classes. The present study aims to inform this gap using a gene enrichment analysis to determine whether genes associated with both SD and ASD risk overlap to a higher degree than expected by chance. This analysis will identify a set of specific risk genes that contribute to both phenotypes, which may help to identify potential mechanistic pathways linking SD and ASD.

In studies of sleep and ASD, genetic mechanisms are often discussed as potentially important (Souders et al., 2017); however, studies directly assessing genes and sleep in ASD are still relatively uncommon. Most often, researchers have assessed genes in the circadian system including CLOCK-related genes (Nicholas et al., 2007), and genes in the melatonin pathway (Jonsson et al., 2010; Veatch et al., 2015). Notably, the steady stream of genetic studies informing our understanding of CLOCK-related or circadian sleep genes in ASD use very small sample sizes (e.g., N s between 28 and 110) and are thus vastly underpowered to detect the small effect that a specific gene is expected to exert on a complex phenotype. In order to generate new biologically plausible mechanisms to guide future studies, we identified genes that have been empirically linked to both SD and ASD and subjected these genes to an enrichment analysis that uses summarized empirical evidence and accumulated bioinformatics knowledge to understand the downstream impacts of these risk genes.

Methods

Data

This study utilized data from a previously published autism gene database (http://db.cbi.pku.edu.cn/autismkb_v2; Xu et al., 2011) and a hand-curated sleep gene set.

Autism gene set.—Based on a comprehensive literature review, Xu and colleagues (2011) created the Autism KB database (newest version, Autism KB 2.0), which includes candidate gene lists that were curated from several studies (e.g., clinical features, experimental design). Meta-data from each study regarding the methodological approach were also recorded and scored in order to generate evidence scores for each gene found by the study. Documentation is available online at http://db.cbi.pku.edu.cn/autismkb_v2/document.php, containing a detailed outline of their search and scoring methodology. Autism KB 2.0 encompasses multiple evidence-based datasets. For the purposes of the current study, we used the ASD ‘Core Dataset’ (hereafter referred to as the *ASD core gene set*). The ASD core gene set includes 228 genes (see Supplemental Table 2), each with a total evidence score of 16 or higher (the higher the score, the more confident the gene is related to ASD). Total evidence scores above 16 are generally considered pathology-linked or significantly associated (Xu et al., 2011). Information regarding database construction and analyses for the ASD core gene set can be found in the online manual for Autism KB 2.0 (http://db.cbi.pku.edu.cn/autismkb_v2/document.php) and in the supplemental online materials of Xu et al. (2011).

Sleep gene set.—We hand-curated a set of genes likely related to sleep problem/disorder phenotypes (henceforth referred to as the *sleep phenotype data set*) from existing literature. The first step in this process involved identifying genes potentially related to sleep by searching 1) the Genome Wide-Association Study (GWAS) catalog (<https://www.ebi.ac.uk/>

gwas/) using the keyword ‘sleep’, 2) National Center for Biotechnology Information (NCBI) Gene (<https://www.ncbi.nlm.nih.gov/gene>) using the keyword ‘sleep’, and 3) PubMed and Google Scholar using the search terms ‘sleep’ and ‘genetics’. Searches were conducted between June and September 2017. Inclusion in the final gene set required a minimum of two peer-reviewed empirical findings (per gene) indicating the association of structural variations in that gene with sleep phenotypes (rather than the sleep phenotype influencing gene expression), or at least one peer-reviewed GWAS finding. The final sleep gene set contained 154 genes (for a complete list see Supplemental Table 1).

Analytic Strategy

Following Marceau & Abel (2018), our analytic strategy comprised two steps. First, we determined the number of genes appearing in both the sleep and ASD core gene datasets and calculated whether the ASD gene set was enriched for sleep-related genes (e.g., whether more genes were in both gene sets than expected by chance). Specifically, we compared our final sleep gene set against the published ASD core gene set. We used the *Nematode bioinformatics analysis tools and data* (Lund, n.d.) to calculate the statistical significance of genetic overlap between the two gene sets. Consistent with current estimates of protein-coding genes in the human genome (Ezkurdia et al., 2014), we estimated 19,000 genes to calculate the number of overlapping genes expected by chance.

Then, to further elucidate the biological function of the genes implicated in both sleep and ASD, we subjected the list of overlapping genes to an over-representation pathway analysis (see Marceau & Abel, 2018). Over-representation pathway analysis is a systems biology tool that identifies whether sets of genes are differentially expressed in any particular biological pathways (Kamburov et al., 2011; Kamburov, Wierling, Lehrach, & Herwig, 2009). A pathway can be defined as a set of “functionally-related” genes (Jin et al., 2014, p. 211), which may be more informative regarding genetically influenced biological mechanisms related to both sleep and ASD than single genes. The over-representation analysis was conducted by uploading the overlapping genes into the online over-representation tool maintained by the *Consensus Pathway Database* (CPDB; <http://cpdb.molgen.mpg.de/>). We selected pathway-based sets wherein at least 25% of the genes on the overlap list were included in the pathway to ensure high relevance of the pathway to identify potential new mechanisms of association of ASD and SD.

Results

Enrichment Analyses

There were 15 overlapping genes in our *sleep phenotype gene set* and the *ASD core gene set*, listed in Table 1. The expected number of overlapping genes was $(154[\text{sleep gene set size}] * 228[\text{ASD core gene set size}] / 19,000[\text{total genes in genome}]) = 1.848$. The representation factor was therefore $15[\text{identified genes}] / 1.848[\text{expected genes}] = 8.11$, $p < 5.466e-10$, indicating significantly more overlap than expected by chance.

Over-representation Pathway Analysis with Core ASD Gene Set

Using a threshold of 25% (a minimum of 4 genes from the overlap list), the over-representation analysis revealed four enriched pathways: *Dopaminergic Synapse*, *Serotonergic Synapse*, *MECP2 and Associated Rett Syndrome*, and *Sudden Infant Death Syndrome Susceptibility Pathways*. Table 2 describes each pathway. Table 3 summarizes the genes on the overlap list appearing in each pathway and the affiliated sleep problems/disorders or neurodevelopmental syndrome. Because the WikiPathways database (included in the CPDB search) is community-based without verifiable scientific oversight, we exclude *MECP2 and Associated Rett Syndrome* and *Sudden Infant Death Syndrome Susceptibility Pathways* (which were identified only via WikiPathways) from our discussion, as their veracity is less certain.

Discussion

This study demonstrated significant overlap in genes related to both SD and ASD risk, highlighting potential genetic and downstream biological mechanisms that may be involved in increased rates of comorbid SD in ASD samples. Our findings serve as a first, *preliminary* step in understanding the genetic overlap in ASD and SD, which we hope will facilitate interest in new empirical studies on gene mutations and expression in individuals with ASD and comorbid SD. The field is well poised to conduct such studies given our expanding access to the largest autism genome databanks in history (e.g., *MSSNG*; *SPARK*, *Simons Simplex Collection*).

Unexpectedly, the overlapping genes identified in this study did not include those most commonly studied in relation to sleep in ASD samples (e.g., *CLOCK*). This finding does not imply that *CLOCK*-related genes do not influence sleep in ASD, but rather, they may not be part of a shared SD-ASD mechanistic pathway. We did identify one overlapping gene that supports circadian entrainment: *CACNA1C*. *CACNA1C* aids in the regulation of calcium channels and has a circadian expression pattern. In mouse models, *CACNA1C* is associated with deficits in phase shifting (Schmutz et al., 2014) and reduced lower spectral power and impaired REM during recovery sleep (Kumar et al., 2015). In individuals with ASD, some studies have noted circadian-based sleep difficulties and atypical EEG patterns during REM and non-REM sleep phases (Godbout, Bergeron, Limoges, Stip, & Mottron, 2000; Godbout, Bergeron, Stip, & Mottron, 1998; Limoges, Mottron, Bolduc, Berthiaume, & Godbout, 2005). Notably, these differences are not consistent across the ASD literature as some studies reported no remarkable EEG differences from comparison groups (e.g., Tani et al., 2003, 2004). Further explorations of *CACNA1C* and ASD could inform these inconsistencies.

One overlapping gene, *ASMT*, is involved in melatonin synthesis. Dysregulation of melatonin synthesis is well documented in individuals with ASD (Tordjman et al., 2013; Veatch et al., 2015) and the findings from the present study implicate one (of several) genes involved in melatonin synthesis. It is unclear what mechanistic role melatonin synthesis may have in ASD phenotypes, but it is likely a contributing factor in the high rates of comorbidity in SD and ASD (Veatch et al., 2015).

Neurotransmitter dysregulation is commonly documented in ASD—with noted differences in the dopamine, serotonin, glutamate, and GABA systems (Horder et al., 2018; Muller, Anacker, & Veenstra-VanderWeele, 2016; Paval, 2017). Within this exploratory study, dopamine and serotonin systems were implicated in our pathway analysis—including genes that aid in regulating calcium channels (CACNA1C, SCN1A), mitochondrial enzyme encoding (MAOA), dopamine transmission (SLC6A3), and serotonin receptors and transmission (HTR2A, SLC6A4). Future SD and ASD research can build on this study by giving attention to the dopamine and serotonin systems and how they may impact sleep and ASD phenotypes. From a treatment approach, pharmacological therapies that promote dopamine or serotonin regulation may have the potential to improve both sleep and ASD-related behaviors. Indeed, several commonly used medications target these systems (e.g., selective serotonin reuptake inhibitors) but additional studies are needed to inform if upregulation, downregulation, or adjusting interacting mechanisms will best support balance in these systems (e.g., Volkow et al., 2012).

It is important to note that the employed pathway analysis can be prone to identifying large-scale pathways involved in fundamental biological processes that therefore may not be specific to ASD and SD. Thus, our analysis may simply reflect that brain-based pathways are necessary for all behavior and higher-order cognitive function. The high percentage of syndromic or neurological conditions associated with ASD in our gene list (10 out of 16 genes) also alludes to a more general brain-based dysregulation, which may manifest as behavioral difficulties during the day and at night. Although the aim of this study was to narrow potential future targets for SD and ASD research, the identified genes may only influence both daytime (ASD behaviors) and nighttime (sleep) behaviors when large-scale brain-based dysregulation is present.

Additional limitations include that the statistical method for assessing gene overlap does not account for gene size, which could lead to increased type I error. Second, GWAS studies are difficult to replicate, and as such the identified genes may include some that are not robustly linked to sleep. Further, many of the large GWAS studies in the *sleep phenotype gene set* measured sleep problems using self-report methods. Although polysomnography and actigraphy may provide less biased sleep estimates than sleep diaries or self-report questionnaires, these gold-standard approaches are also costly and difficult to implement on a large scale. Our *sleep phenotype data set* also includes genes implicated in a broad range of SD's, which are each biologically distinct. Some of these SD's are more common in individuals with ASD than others. Thus, future research may benefit from a more concise gene set—including genes related to SD's with the highest rates of comorbidity in ASD, genes that are more heavily replicated in the peer-reviewed literature (e.g., two or more GWAS Studies and excluding genes with evidence only from candidate gene studies with small samples).

Summary and Future Directions

Our overlapping gene set may serve as a *preliminary* stepping-stone for new studies investigating genetic components of SD in ASD risk (including new gene sets that may be more specific to ASD), or may be useful in exploring existing autism genome databases to

answer critical questions about why children with ASD experience increased rates of SD. Future research exploring: 1) potential mutations in genes empirically found to influence SD and ASD, and 2) related neural underpinnings of SD in ASD may prove valuable for understanding mechanistic links between SD and ASD. This study should be replicated using other reputable ASD gene databases, such as SFARI, and should be repeated as more reliable gene discovery is completed in both disorders (particularly sleep).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant sponsor: The National Institute on Drug Abuse; Grant number: K01 DA039288 (Marceau).

Funding: This study was funded in part by The National Institute on Drug Abuse; Grant number: K01 DA039288 (PI: [removed for review]).

References

- Allik H, Larsson J-O, & Smedje H. (2008). Sleep patterns in school-age children with asperger syndrome or high-functioning autism: A follow-up study. *Journal of Autism and Developmental Disorders*, 38(9), 1625–1633. [PubMed: 18293072]
- Baker EK, & Richdale AL (2017). Examining the behavioural sleep-wake rhythm in adults with autism spectrum disorder and no comorbid intellectual disability. *Journal of Autism and Developmental Disorders*, 47(4), 1207–1222. [PubMed: 28160224]
- Cortesi F, Giannotti F, Ivanenko A, & Johnson K. (2010). Sleep in children with autistic spectrum disorder. *Sleep Medicine*, 11(7), 659–664. [PubMed: 20605110]
- Ezkurdia I, Juan D, Rodriguez JM, Frankish A, Diekhans M, Harrow J, ... Tress ML (2014). Multiple evidence strands suggest that there may be as few as 19,000 human protein-coding genes. *Human Molecular Genetics*, 23(22), 5866–5878. doi:10.1093/hmg/ddu309 [PubMed: 24939910]
- Godbout R, Bergeron C, Limoges E, Stip E, & Mottron L. (2000). A laboratory study of sleep in Asperger's syndrome. *NeuroReport*, 11(1), 127–130. [PubMed: 10683843]
- Godbout R, Bergeron C, Stip E, & Mottron L. (1998). A laboratory study of sleep and dreaming in a case of Asperger's syndrome. *Dreaming*, 8(2), 75–88. doi:10.1023/B:Drem.0000005898.95212.58
- Horner J, Petrinovic MM, Mendez MA, Bruns A, Takumi T, Spooren W, ... Murphy DG (2018). Glutamate and GABA in autism spectrum disorder—a translational magnetic resonance spectroscopy study in man and rodent models. *Translational Psychiatry*, 8(1), 106. doi:10.1038/s41398-018-0155-1 [PubMed: 29802263]
- Jin L, Zuo XY, Su WY, Zhao XL, Yuan MQ, Han LZ, ... Rao SQ (2014). Pathway-based analysis tools for complex diseases: A review. *Genomics Proteomics Bioinformatics*, 12(5), 210–220. doi:10.1016/j.gpb.2014.10.002 [PubMed: 25462153]
- Jonsson L, Ljunggren E, Bremer A, Pedersen C, Landén M, Thuresson K, ... Melke J. (2010). Mutation screening of melatonin-related genes in patients with autism spectrum disorders. *BMC Medical Genomics*, 3(1), 10. doi:10.1186/1755-8794-3-10 [PubMed: 20377855]
- Kamburov A, Pentchev K, Galicka H, Wierling C, Lehrach H, & Herwig R. (2011). ConsensusPathDB: Toward a more complete picture of cell biology. *Nucleic Acids Research*, 39, D712–717. doi:10.1093/nar/gkq1156 [PubMed: 21071422]
- Kamburov A, Wierling C, Lehrach H, & Herwig R. (2009). ConsensusPathDB—a database for integrating human functional interaction networks. *Nucleic Acids Research*, 37(Database issue), D623–628. doi:10.1093/nar/gkn698 [PubMed: 18940869]

- Krakowiak P, Goodlin-Jones B, Hertz-Picciotto I, Croen LA, & Hansen RL (2008). Sleep problems in children with autism spectrum disorders, developmental delays, and typical development: A population-based study. *Journal of Sleep Research*, 17(2), 197–206. [PubMed: 18482108]
- Kumar D, Dedic N, Flachskamm C, Voule S, Deussing JM, & Kimura M. (2015). *Cacna1c* (Cav1.2) Modulates Electroencephalographic Rhythm and Rapid Eye Movement Sleep Recovery. *Sleep*, 38(9), 1371–1380. doi:10.5665/sleep.4972 [PubMed: 25845695]
- Limoges É, Mottron L, Bolduc C, Berthiaume C, & Godbout R. (2005). Atypical sleep architecture and the autism phenotype. *Brain*, 128(5), 1049–1061. [PubMed: 15705609]
- Marceau K, & Abel EA (2018). Mechanisms of cortisol - Substance use development associations: Hypothesis generation through gene enrichment analysis. *Neuroscience and Biobehavioral Reviews*, 92, 128–139. doi:10.1016/j.neubiorev.2018.05.020 [PubMed: 29802855]
- Muller CL, Anacker AMJ, & Veenstra-VanderWeele J. (2016). The serotonin system in autism spectrum disorder: From biomarker to animal models. *Neuroscience*, 321, 24–41. doi:10.1016/j.neuroscience.2015.11.010 [PubMed: 26577932]
- Nicholas B, Rudrasingham V, Nash S, Kirov G, Owen MJ, & Wimpory DC (2007). Association of *Per1* and *Npas2* with autistic disorder: support for the clock genes/social timing hypothesis. *Molecular Psychiatry*, 12(6), 581–592.
- Paval D. (2017). A Dopamine Hypothesis of Autism Spectrum Disorder. *Developmental Neuroscience*, 39(5), 355–360. doi:10.1159/000478725 [PubMed: 28750400]
- Richdale AL, & Baglin CL (2015). Self-report and caregiver-report of sleep and psychopathology in children with high-functioning autism spectrum disorder: A pilot study. *Developmental Neurorehabilitation*, 18(4), 272–279. [PubMed: 24087935]
- Richdale AL, & Schreck KA (2009). Sleep problems in autism spectrum disorders: prevalence, nature, & possible biopsychosocial aetiologies. *Sleep Medicine Reviews*, 13(6), 403–411. [PubMed: 19398354]
- Schmutz I, Chavan R, Ripperger JA, Maywood ES, Langwieser N, Jurik A, ... Albrecht U. (2014). A specific role for the REV-ERB α -controlled L-Type Voltage-Gated Calcium Channel CaV1.2 in resetting the circadian clock in the late night. *Journal of Biological Rhythms*, 29(4), 288–298. doi:10.1177/0748730414540453 [PubMed: 25238857]
- Souders MC, Zavodny S, Eriksen W, Sinko R, Connell J, Kerns C, ... Pinto-Martin J. (2017). Sleep in children with autism spectrum disorder. *Current Psychiatry Report*, 19(6), 34. doi:10.1007/s11920-017-0782-x
- Tani P, Lindberg N, Nieminen-von Wendt T, von Wendt L, Alanko L, Appelberg B, & Porkka-Heiskanen T. (2003). Insomnia is a frequent finding in adults with Asperger syndrome. *BMC Psychiatry*, 3, 12. doi:10.1186/1471-244X-3-12 [PubMed: 14563215]
- Tani P, Lindberg N, Nieminen-von Wendt T, von Wendt L, Virkkala J, Appelberg B, & Porkka-Heiskanen T. (2004). Sleep in young adults with Asperger syndrome. *Neuropsychobiology*, 50(2), 147–152. [PubMed: 15292669]
- Tordjman S, Najjar I, Bellissant E, Anderson GM, Barburoth M, Cohen D, ... Vernay-Leconte J. (2013). Advances in the research of melatonin in autism spectrum disorders: Literature review and new perspectives. *International Journal of Molecular Sciences*, 14(10), 20508–20542. doi:10.3390/ijms141020508 [PubMed: 24129182]
- Veatch OJ, Goldman SE, Adkins KW, & Malow BA (2015). Melatonin in children with autism spectrum disorders: How does the evidence fit together? *Journal of Nature and Science*, 1(7), e125.
- Veatch OJ, Pendergast JS, Allen MJ, Leu RM, Johnson CH, Elsea SH, & Malow BA (2015). Genetic variation in melatonin pathway enzymes in children with autism spectrum disorder and comorbid sleep onset delay. *Journal of Autism and Developmental Disorders*, 45(1), 100–110. [PubMed: 25059483]
- Volkow N, Tomasi D, Wang G, Telang F, Fowler J, Logan J, ...Ferre S. (2012). Evidence that sleep deprivation downregulates dopamine D2R in ventral striatum in the human brain. *Journal of Neuroscience*, 32(19), 6711–6717. doi: 10.1523/JNEUROSCI.0045–12.2012 [PubMed: 22573693]
- Xu L-M, Li J-R, Huang Y, Zhao M, Tang X, & Wei L. (2011). AutismKB: an evidence-based knowledgebase of autism genetics. *Nucleic Acids Research*, 40(D1), D1016–D1022. [PubMed: 22139918]

Table 1.

List of 15 overlapping genes including function and pathway(s).

Gene Symbol	Gene Function	Pathway(s)
ASMT	An encoded enzyme catalyst used in the final reaction in melatonin synthesis.	
BDNF	Encodes a member of the nerve growth factor family of proteins. Binding of this protein to its cognate receptor promotes neuronal survival in the adult brain.	3 and 4
CACNA1C ^a	This gene encodes an alpha-1 subunit of a voltage-dependent calcium channel. Calcium channels mediate the influx of calcium ions into the cell upon membrane polarization.	1 and 2
CDKL5 ^a	This gene is a member of the Ser/Thr protein kinase family and encodes a phosphorylated protein with protein kinase activity.	
DISC1	This gene encodes a protein located in the nucleus, cytoplasm, and mitochondria which is involved in neurite outgrowth and cortical development through its interaction with other proteins.	
DSCAM	The Down syndrome cell adhesion molecule (DSCAM) gene impacts cell adhesion and is expressed widely in the developing nervous system (implicated in both central and peripheral system development).	
FOXP2	This gene encodes a member of the forkhead/winged-helix (FOX) family of transcription factors. This gene is required for proper development of speech and language regions of the brain during embryogenesis and may be involved in a variety of biological pathways and cascades that may ultimately influence language development.	
HTR2A	This gene encodes one serotonin receptor. Mutations in this gene are associated with schizophrenia and obsessive-compulsive disorder.	2 and 4
MAOA ^a	This gene is one of two neighboring gene family members that encode mitochondrial enzymes which catalyze the oxidative deamination of amines, such as dopamine, norepinephrine, and serotonin.	1, 2, and 4
MECP2 ^a	MEP2 is essential for embryonic development and mutations in this gene cause most cases of Rett syndrome and some other neurodevelopmental disorders.	3 and 4
RFX1	This gene regulates tissue-specific alternative splicing in metazoa.	3
SCN1A ^a	Voltage-dependent sodium channels are heteromeric complexes that regulate sodium exchange between intracellular and extracellular spaces and are essential for the generation and propagation of action potentials in muscle cells and neurons.	1
SLC6A4	This gene encodes an integral membrane protein that transports the neurotransmitter serotonin from synaptic spaces into presynaptic neurons.	2 and 4
SLC6A3	The primary role of this gene is the generation of a dopamine transporter (DAT). Implicated in dopamine transporter deficiency syndrome and considered a risk factor for ADHD, ASD, and alcohol use disorder.	1
UBE3A ^a	This gene encodes E3 ubiquitin-protein ligase which is involved in the protein degradation process (to remove damaged proteins from the cell).	3

Note.

^a Genes linked with genetic syndromes that are highly comorbid with autism spectrum disorder. 1 = Dopaminergic Synapse, 2 = Serotonergic Synapse, 3 = MECP2 and Associated Rett Syndrome, 4 = Sudden Infant Death Syndrome (SIDS) Susceptibility Pathways.

* Gene descriptions modified from NCBI gene: <https://www.ncbi.nlm.nih.gov/gene>

Table 2.

Enriched pathways for the 15-gene overlap set.

Pathway Name	Set Size	Candidates Contained	p value	Pathway Source	Pathway Description
1) Dopaminergic Synapse	131	4 (3.1%)^a CACNA1C MAOA SCN1A SLC6A3	9.14e-06	KEGG	Dopamine is found in the brain and controls several functions such as locomotor activity, motivation and reward, learning and memory, and endocrine regulation.
2) Serotonergic Synapse	115	4 (3.5%)^a CACNA1C HTR2A MAOA SLC6A4	5.08e-6	KEGG	Serotonin is a neurotransmitter that has a key role in physiological functions including sleep, emotion, learning and memory, and pathology.
3) MECP2 and Associated Rett Syndrome	63	4 (6.3%)^a BDNF MECP2 RBFOX1 UBE3A	4.84e-07	Wikipathways	MECP2 regulates neuronal function and development. It effects all cell types, especially neurons but also astrocytes, oligodendrocytes, and glial cells. MECP2 is also involved in neuronal differentiation, maturation, morphology and function, and influences synaptic plasticity. Mutations in MECP2 are associated with Rett Syndrome, schizophrenia, PPM-X-syndrome, FASD, and ASD.
4) Sudden Infant Death Syndrome and (SIDS) Susceptibility Pathways	159	5 (3.1%)^a BDNF HTR2A MAOA MECP2 SLC6A4	4.72e-07	Wikipathways	SIDS is the sudden and unexpected death of an infant, almost always during sleep, where no cause of death can be found by autopsy. Factors that mediate SIDS are likely to be both biological and behavioral, such as sleeping position, environment and stress during a critical phase of infant development. This pathway describes candidate genes associated with neuronal signaling, cardiac contraction, and inflammatory response that are empirically related to SIDS.

Note. To be included in the manuscript, biological pathways had to contain at least 25% of the genes on the overlap list.

^aThese numbers refer to the percentage of each total pathway (set size) represented in our overlap list.

Table 3.

Candidate genes in each of the primary biological pathways.

Gene Symbol	Sleep Phenotype	Syndrome Associated with Autism
Dopaminergic Synapse		
MAOA	Poorer sleep quality; lower sleep efficiency (indexed by higher quantitative sleep disturbance scores); insomnia	Brunner Syndrome
SCN1A	Increased sleep disturbances; abnormal non-rapid eye movement sleep architecture; sleep dysregulation	Early Infantile Epileptic Encephalopathy; Dravet Syndrome
CACNA1C	Increased (longer) sleep latency; reduced REM Sleep	Timothy Syndrome
SLC6A3	Sleep-wake cycle; sleep architecture	N/A
Serotonergic Synapse		
MAOA	Sleep-wake cycle—including lower homeostatic drive; poorer sleep continuity.	N/A
CACNA1C	Shorter sleep duration; fatigue; early morning awakening	Yu Type of X-linked Syndromic Mental Retardation
HTR2A	Obstructive sleep apnea, chronic fatigue syndrome	N/A
SLC6A4	Increased risk of sleep bruxism; increased complaint of sleep onset problems; shorter sleep duration	N/A
MECP2 and Associated Rett Syndrome		
MECP2	Phase-specific sleep loss; sleep fragmentation; increased daytime napping with age;	Rett Syndrome
BDNF	Regulation of sleep slow wave oscillations; decreased spectral power in the alpha band in stage one and decreased theta power in stages two and three of nonrapid-eye-movement sleep	N/A
RBFOX1	Sleep timing	N/A
UBE3A	Short sleep duration; increased sleep onset latency; reduced capacity to accumulate sleep pressure	Angelman Syndrome
SIDS Susceptibility Pathways		
MECP2	Phase-specific sleep loss; sleep fragmentation; increased daytime napping with age;	Rett Syndrome
BDNF	Regulation of sleep slow wave oscillations; decreased spectral power in the alpha band in stage one and decreased theta power in stages two and three of nonrapid-eye-movement sleep	N/A
SLC6A4	Increased risk of sleep bruxism; increased complaint of sleep onset problems; shorter sleep duration	N/A
MAOA	Poorer sleep quality; lower sleep efficiency (indexed by higher quantitative sleep disturbance scores); insomnia	Brunner Syndrome