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Retinoic Acid-Induced 1 gene variants associated with Smith–Magenis syndrome circadian phenotypes enriched in autism spectrum disorder: whole-genome sequencing study

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Abstract

Background This study aimed to characterize the frequency of *RAI1* genetic aberrations associated with Smith–Magenis syndrome (SMS), in a large cohort of autism spectrum disorder (ASD) whole-genome sequencing samples. We aimed to determine the frequencies of *RAI1* single-nucleotide variants (SNVs) and copy number variants (CNVs).

Results We report a 2.5 × enrichment of the major deletion and a > 5 × enrichment of the frameshift variants as compared to the known prevalence of SMS 1/15,000. Additionally, we report a significant enrichment of *RAI1* rare missense variants in ASD subjects with respect to controls (54 variants/6080 ASD subjects and 6 variants/2541 controls, *p*-value < 0.002, OR 3.78, CI 1.62–8–81).

Conclusions The SMS phenotype including circadian dysregulation and associated sleep disturbances is mainly caused by *RAI1* haploinsufficiency. Sleep disturbances as seen in SMS may overlap in ASD, especially in patients with consequential variants in *RAI1* gene.

Keywords *RAI1* gene, SMS, ASD, Circadian clock, Melatonin

Introduction

Smith–Magenis syndrome (SMS; OMIM 182290) is a rare genetic disorder that results from an interstitial deletion of 17p11.2 and, in rare cases, from a Retinoic Acid-Induced 1 (*RAI1*) gene variants [15]. The prevalence is estimated to be 1/15,000–25,000 [8, 16]. Haploinsufficiency of *RAI1* is the primary cause of the neurobehavioral and metabolic phenotype in SMS [8, 16]. Patients with SMS are characterized by a distinct pattern of mild to moderate intellectual disability as well as delayed speech and language skills, distinctive craniofacial and skeletal abnormalities, behavioral disturbances, and with significant sleep disturbances [16]. Alterations in *RAI1* copy number have been also linked to a number of neurodevelopmental disorders including ASD [9]. In fact, 90% of SMS patients meet diagnostic criteria for ASD at one point in their lives [9].

ASD comprises a complex of neurodevelopmental disorders primarily characterized by deficits in verbal communication, impaired social interactions, and repetitive behaviors [5, 6]. The profound clinical heterogeneity of ASD poses challenges in diagnosis and treatment. Heritable factors account for at up to 80% of ASD risk with the remainder attributable to environmental factors acting alone or through interaction with genetics [23].

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Tremendous progress has been made in understanding the genetic underpinnings of ASD with potential variants usually covering the entire spectrum of mutations from single-nucleotide variants to loss/gain of copy number effects. In addition to inherited variants, genomes of probands are enriched in de novo genetic variants [1, 23]. Genetic studies have pointed to hundreds of presumptive causative or susceptibility variants in ASD, making it difficult to find common underlying pathogenic mechanisms and suggesting that multiple different genetic etiologies for ASDs influence a continuum of traits. Sleep problems are almost twice as common in children with ASD compared to ancestry matched controls with no diagnosis of ASD. Different aspects of sleep are aberrant including inability to initiate sleep, delayed sleep, and fragmented sleep. About 50% of ASD children meet the diagnostic criteria for insomnia as defined by sleep latency greater than 30 min.

RAI1 is a dosage-sensitive gene expressed in many tissues. It is highly conserved among species. Multiple studies have demonstrated that *RAI1* and its homologs act as a transcriptional factor implicated in embryonic neurodevelopment and neuronal differentiation, as well as behavioral functions and importantly in circadian activity. Patients with *RAI1* pathogenic point mutations, show some phenotypic differences when compared to those carrying the larger typical deletion; however, haploinsufficiency of *RAI1* is the main cause of the neurobehavioral and metabolic phenotype in SMS [7]. Based on the current data, over 90% of cases present with a large deletion [17]. As exon 3 constitutes 95% of the coding sequence, that is where majority of the *RAI1* variants have been reported to date. The purpose of this study was to examine the frequency of *RAI1* consequential SNVs as well as CNVs in a large whole-genome sequencing set of ASD patients.

Results

We conducted a large-scale association analysis of the ASD MSSNG whole-genome sequencing data to determine the frequency of *RAI1* SNVs and CNVs. We accessed the MSSNG database hosting over 11,000 genomes (6080 probands) and queried both SNVs and CNVs.

Specifically, we focused on the frequency of the classic SMS large deletions, microdeletions of (exon 3) and of the rare (as defined by gnomAD [10] Max < 0.005) missense, frameshift, and splicing variants. We report a single case of classic SMS deletion spanning (17p11.2 critical region (chr17:16845401-20516200)). We also report 2 frameshifts and one known splicing variant. Given that the SMS deletion frequency is ~ 1:15,000, we observe a 2.5 × enrichment of the major deletion and 2:6080 > 5 × enrichment of the frameshift variants (Table 1). The two reported frameshifts are loss of function variants predicted to result in a premature stop codon. Table 1 supplement includes CADD scores as well as SIFT scores which predict whether an amino acid substitution is likely to affect protein function based on sequence homology and the physico-chemical similarity between the alternate amino acids. Figure 1 depicts how the identified *RAI1* variants are localized across domains.

In a set of 6080 probands we also observed 54 unique missense variants, which constitute 84 alleles out of 6080 individuals located within exon 3 of *RAI1* gene. We observe a significant enrichment of rare *RAI1* missense variants in comparison with the control dataset (confirmed lack of diagnosis SMS and ASD) (54 variants/6080 ASD subjects and 6 variants/2541 controls, p-value < 0.002, OR 3.78 CI 1.62–8–81). This effect persists when dosage effect is tested. Altogether 11% of these variants are de novo. The variants detected in the ASD set are not present in the control dataset of 2541 whole-genome sequencing mixed ancestry samples (see Methods section). The identified variants of interest are depicted in Table 1. We therefore observe enrichment of *RAI1* genetic aberrations (CNVs and SNVs) as compared to the known prevalence of SMS (1:15,000) as well as enrichment of *RAI1* variants as compared to a set of non-ASD non-SMS ancestry matched controls.

Discussion

Both ASD patients and SMS patients suffer from sleep disturbances. Currently, the prevailing theory is that there is an underlying circadian pathophysiology causing sleep disturbances in SMS associated with *RAI1* haploinsufficiency, as these patients exhibit low overall melatonin concentrations and abnormal timing of peak plasma melatonin concentrations. This abnormal inverted circadian rhythm is estimated to occur in 95% of individuals with SMS [3, 18]. Variation of sleep disturbances as seen in SMS may overlap in ASD, especially in patients with consequential variants in *RAI1* gene—this could be tested as part of the future follow-up studies. ChIP-Chip and reporter studies showed that *RAI1* binds, directly or in a complex, to the first intron of *CLOCK* gene, enhancing transcriptional activity [22]. Reduced expression of *RAI1* results in reduced *CLOCK* expression both in the animal models and in the SMS patient-derived cell lines. This is supportive of the fact that treatment with a circadian regulator such as melatonin agonist can, in part, correct the deficiencies caused by *RAI1* abnormalities, providing further evidence of *RAI1* interaction with the molecular clock and the impact on circadian rhythm.

Interestingly two recent studies showed the association between methylation status of *RAI1* and sleepiness scores

Table 1 All rare (MAF < 0.005) missense, splicing, and frameshift variants detected in the ASD WGS set

Chr	Start	Reference allele	Alternate allele	Inheritance	Variant	Gene symbol	Effect-impact	Max frequency GnomAD genome
chr17	N/A	N/A	N/A	De novo	g.16845401-20516200del	RAI1/others	Haploinsufficiency	N/A
chr17	17683704	T	C	Paternal	RAI1:splicing	RAI1	Predicted Splicing-High	0
chr17	17793391	GA	AGC	Denovo	RAI1:NM_030665:exon3:c.444_445delinsAGC	RAI1	Frameshift-High	0
chr17	17798167	C	CA	Unknown	RAI1:NM_030665:exon3:c.5221dupA:p.L1740fs	RAI1	Frameshift-High	0
chr17	17793068	G	A	Maternal	RAI1:NM_030665:exon3:c.G121A:p.D41N	RAI1	Missense-High	2.52E−05
chr17	17793078	G	A	Paternal	RAI1:NM_030665:exon3:c.G131A:p.R44Q	RAI1	Missense-High	2.05E−05
chr17	17793105	C	T	Unknown	RAI1:NM_030665:exon3:c.C158T:p.P53L	RAI1	Missense-High	1.23E−05
chr17	17793193	C	G	Paternal	RAI1:NM_030665:exon3:c.C246G:p.S82R	RAI1	Missense-High	0
chr17	17793258	A	G	Paternal	RAI1:NM_030665:exon3:c.A311G:p.D104G	RAI1	Missense-High	0
chr17	17793297	G	C	Maternal	RAI1:NM_030665:exon3:c.G350C:p.S117T	RAI1	Missense-High	0
chr17	17793048	G	A	Paternal	RAI1:NM_030665:exon3:c.G101A:p.S34N	RAI1	Missense-High	2.05E−05
chr17	17793435	A	G	Paternal	RAI1:NM_030665:exon3:c.A488G:p.Q163R	RAI1	Missense-High	1.23E−05
chr17	17793666	C	T	Unknown	RAI1:NM_030665:exon3:c.C719T:p.T240I	RAI1	Missense-High	2.52E−05
chr17	17793729	A	G	Paternal	RAI1:NM_030665:exon3:c.A782G:p.Q261R	RAI1	Missense-High	0
chr17	17793773	T	C	Denovo	RAI1:NM_030665:exon3:c.T826C:p.Y276H	RAI1	Missense-High	0
chr17	17793890	G	A	Paternal	RAI1:NM_030665:exon3:c.G943A:p.G315R	RAI1	Missense-High	0
chr17	17793918	C	T	Unknown	RAI1:NM_030665:exon3:c.C971T:p.P324L	RAI1	Missense-High	5.14E−06
chr17	17793929	G	A	Maternal	RAI1:NM_030665:exon3:c.G982A:p.V328I	RAI1	Missense-High	0
chr17	17793986	G	A	Unknown	RAI1:NM_030665:exon3:c.G1039A:p.A347T	RAI1	Missense-High	0
chr17	17793990	G	A	Denovo	RAI1:NM_030665:exon3:c.G1043A:p.R348H	RAI1	Missense-High	0
chr17	17794257	C	G	Paternal	RAI1:NM_030665:exon3:c.C1310G:p.A437G	RAI1	Missense-High	1.23E−05
chr17	17794280	A	G	Maternal	RAI1:NM_030665:exon3:c.A1333G:p.I445V	RAI1	Missense-High	0
chr17	17794415	G	A	Paternal	RAI1:NM_030665:exon3:c.G1468A:p.A490T	RAI1	Missense-High	0
chr17	17794434	C	T	Unknown	RAI1:NM_030665:exon3:c.C1487T:p.P496L	RAI1	Missense-High	0
chr17	17794505	G	A	Paternal	RAI1:NM_030665:exon3:c.G1558A:p.G520S	RAI1	Missense-High	3.22E−05
chr17	17794526	C	T	Paternal	RAI1:NM_030665:exon3:c.C1579T:p.R527C	RAI1	Missense-High	0
chr17	17794563	C	T	Unknown	RAI1:NM_030665:exon3:c.C1616T:p.P539L	RAI1	Missense-High	0
chr17	17794722	G	C	Paternal	RAI1:NM_030665:exon3:c.G1775C:p.R592P	RAI1	Missense-High	0.00017955
chr17	17794734	G	A	Unknown	RAI1:NM_030665:exon3:c.G1787A:p.R596Q	RAI1	Missense-High	1.23E−05
chr17	17794784	G	A	Paternal	RAI1:NM_030665:exon3:c.G1837A:p.G613R	RAI1	Missense-High	4.03E−05

Table 1 (continued)

Chr	Start	Reference allele	Alternate allele	Inheritance	Variant	Gene symbol	Effect-impact	Max frequency GnomAD genome
chr17	17794797	C	A	Maternal	RAI1:NM_030665:exon3:c.C1850A:p.A617D	RAI1	Missense-High	0
chr17	17794829	G	A	Paternal	RAI1:NM_030665:exon3:c.G1882A:p.E628K	RAI1	Missense-High	6.11E−05
chr17	17794851	A	T	Unknown	RAI1:NM_030665:exon3:c.A1904T:p.D635V	RAI1	Missense-High	0.00173152
chr17	17794872	C	T	Paternal	RAI1:NM_030665:exon3:c.C1925T:p.S642L	RAI1	Missense-High	0.00024018
chr17	17794995	G	C	Unknown	RAI1:NM_030665:exon3:c.G2048C:p.S683T	RAI1	Missense-High	0.00011326
chr17	17795172	A	G	Unknown	RAI1:NM_030665:exon3:c.A2225G:p.N742S	RAI1	Missense-High	5.00E−05
chr17	17795495	T	C	Denovo	RAI1:NM_030665:exon3:c.T2548C:p.F850L	RAI1	Missense-High	0
chr17	17795541	T	C	Unknown	RAI1:NM_030665:exon3:c.T2594C:p.L865P	RAI1	Missense-High	6.11E−05
chr17	17795682	C	T	Paternal	RAI1:NM_030665:exon3:c.C2735T:p.A912V	RAI1	Missense-High	0
chr17	17795742	T	C	Denovo	RAI1:NM_030665:exon3:c.T2795C:p.V932A	RAI1	Missense-High	0
chr17	17795886	C	A	Unknown	RAI1:NM_030665:exon3:c.C2939A:p.A980D	RAI1	Missense-High	0
chr17	17795906	G	A	Unknown	RAI1:NM_030665:exon3:c.G2959A:p.A987T	RAI1	Missense-High	0.00083379
chr17	17796143	C	A	Unknown	RAI1:NM_030665:exon3:c.C3196A:p.P1066T	RAI1	Missense-High	0
chr17	17796155	G	A	Maternal	RAI1:NM_030665:exon3:c.G3208A:p.G1070R	RAI1	Missense-High	0.00026899
chr17	17796428	C	T	Unknown	RAI1:NM_030665:exon3:c.C3481T:p.R1161W	RAI1	Missense-High	0
chr17	17796491	C	T	Paternal	RAI1:NM_030665:exon3:c.C3544T:p.H1182Y	RAI1	Missense-High	0
chr17	17796552	G	A	Unknown	RAI1:NM_030665:exon3:c.G3605A:p.R1202Q	RAI1	Missense-High	0
chr17	17796596	C	T	Unknown	RAI1:NM_030665:exon3:c.C3649T:p.R1217W	RAI1	Missense-High	7.88E−06
chr17	17796609	C	A	Maternal	RAI1:NM_030665:exon3:c.C3662A:p.A1221E	RAI1	Missense-High	0
chr17	17797196	A	G	Maternal	RAI1:NM_030665:exon3:c.A4249G:p.K1417E	RAI1	Missense-High	0
chr17	17797293	G	A	Unknown	RAI1:NM_030665:exon3:c.G4346A:p.R1449Q	RAI1	Missense-High	5.90E−05
chr17	17797557	G	A	Maternal	RAI1:NM_030665:exon3:c.G4610A:p.R1537Q	RAI1	Missense-High	2.52E−05
chr17	17798082	T	A	Denovo	RAI1:NM_030665:exon3:c.T5135A:p.L1712H	RAI1	Missense-High	0
chr17	17798117	C	T	Unknown	RAI1:NM_030665:exon3:c.C5170T:p.R1724W	RAI1	Missense-High	7.88E−06
chr17	17803779	G	A	Maternal	RAI1:NM_030665:exon4:c.G5590A:p.G1864R	RAI1	Missense-High	7.88E−06
chr17	17798435	G	A	Maternal	RAI1:NM_030665:exon3:c.G5488A:p.V1830M	RAI1	Missense-High	0
chr17	17803842	G	A	Maternal	RAI1:NM_030665:exon4:c.G5653A:p.D1885N	RAI1	Missense-High	0.00016678

[2, 11]. In another recent GWAS study of self-reported daytime sleepiness, authors reported a significant association of variant rs11078398 (MAF 0.04) with daytime sleepiness in the general population [20]. These reports further support the role of *RAI1* gene variants in the regulation of sleep and circadian rhythms in the general population.

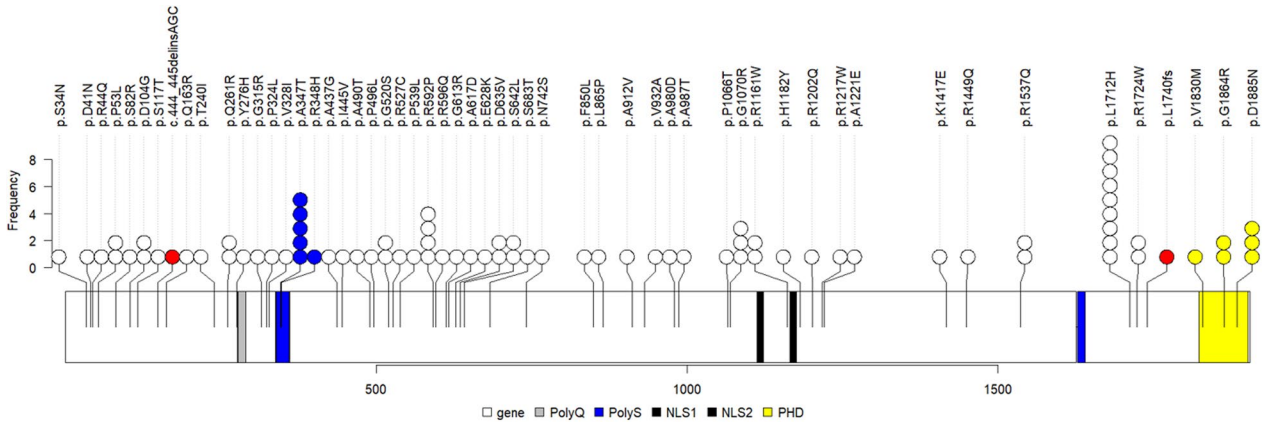

Fig. 1 All rare (MAF < 0.005) missense, splicing, and frameshift variants detected in the ASD cases are depicted as localized across domains

It is important to note there are other potential genetic causes of sleep disorders such as sleep-related breathing disorders, hypersomnolence, parasomnias, sleep-related movement disorders, and circadian rhythm sleep–wake disorders attributed to distinct variants and pathways [12]. Moreover, other genes have also been implicated as the underlying etiology behind sleep disturbances such as problems with sleep induction disturbances in healthy population as well as in individuals with neurodevelopmental disorders and comorbid sleep disturbances [14, 19]. In a study that examined 80 variants across 5 circadian clock genes in healthy Japanese individuals, the strongest association with sleep induction disorder was that of rs11113179 in *CRY1* and variants rs1026071 and rs1562438 in *BMAL1* [14]. Variants in melatonin receptors, specifically in *MTNR1B*, were observed in individuals with ASD and sleep disturbances [19]. This is suggestive of the fact the RAI1 variant carriers could be one of many underlying causes of comorbid sleep disorders and this is yet to be confirmed.

The results of this current study warrant further confirmation in ASD patients manifesting with sleep disturbances. Despite limitations and the need to confirm these findings in an independent cohort, our pilot findings offer promising insights. This studies laid the foundation for important follow-up studies such as one on ASD cases with and without sleep disturbance. Further studies will help discern the role of *RAI1* variants in ASD and particularly in patients with sleep disturbances.

Methods

Datasets and ethical considerations

MSSNG [4] constitutes a large whole-genome sequencing set of samples obtained from over 10,000 individuals from families from the Autism Genetic Research Exchange (AGRE) repository and from other well-phenotyped cohorts entering into this study. Vanda control dataset is a well-phenotyped cohort of whole-genome sequencing samples obtained from consented individuals participating in Vanda sleep studies. The controls have been selected to match the demographics of the cases. All individuals have consented to participate in genetic research. The controls were selected to match cases in terms of age, sex, and ancestry. Potential biases could have been caused by the ascertainment and recruitment of the cases limited geographically and sample selection criteria such a willingness to participate in research studies.

Genetic analysis of the Vanda dataset

Incoming nucleic acid samples are quantified using fluorescent-based assays (PicoGreen) to accurately determine whether sufficient material is available for library preparation and sequencing. DNA sample size distributions are profiled by a Fragment Analyzer (Advanced Analytics) or BioAnalyzer (Agilent Technologies), to assess sample quality and integrity. HumanCoreExome 24v1.3 array was performed on all human DNA samples sequenced. Whole-genome sequencing (WGS) libraries were prepared using the Truseq DNA PCR-free Library

Preparation Kit. Whole-Genome data were processed on NYGC automated pipeline. Paired-end 150-bp reads were aligned to the GRCh37 human reference (BWA-MEM v0.7.8) and processed with GATK best-practices workflow (GATK v3.4.0). The mean coverage was 35.8; it reflects the samples average. All high-quality variants obtained from GATK were annotated for functional effects (intronic, intergenic, splicing, non-synonymous, stop-gain, and frameshifts) based on RefSeq transcripts using Annovar [21]. Additionally, Annovar was used to match general population frequencies from public databases (Exac, gnomAD, ESP6500, 1000 g) and to prioritize

rare, loss-of-function variants. Linear models adjusted for PC, age, and sex were conducted in PLINK [13].

Enrichment analysis

The analysis in both datasets focused on rare (as defined by MAF < 0.005 in gnomAD) missense, frameshift, and splicing variants. Enrichment was defined and tested twofold: with regard to established SMS prevalence and with regard to ancestry matched non-SMS non-ASD set of control set of samples.

Abbreviations

ASD	Autism spectrum disorder
CNV	Copy number variant
MAF	Minor allele frequency
RAI1	Retinoic Acid-Induced 1 gene
SMS	Smith–Magenis syndrome
SNV	Single-nucleotide variant
WGS	Whole-genome sequencing

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Author contributions

SPS performed the analysis and wrote the manuscript.

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Availability of data and materials

Data are available upon request and pending application approval.

Declarations

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Competing interest

The author is an employee of Vanda Pharmaceuticals Inc.

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