Published in final edited form as:

Am J Med Genet A. 2021 March ; 185(3): 889–893. doi:10.1002/ajmg.a.62023.

Further delineation of a recognizable type of syndromic short stature caused by biallelic *SEMA3A* loss-of-function variants

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Abstract

The semaphorin protein family is a diverse set of extracellular signaling proteins that perform fundamental roles in the development and operation of numerous biological systems, notably the nervous, musculoskeletal, cardiovascular, endocrine, and reproductive systems. Recently, recessive loss-of-function (LoF) variants in *SEMA3A* (semaphorin 3A) have been shown to result in a recognizable syndrome characterized by short stature, skeletal abnormalities, congenital heart defects, and variable additional anomalies. Here, we describe the clinical and molecular characterization of a female patient presenting with skeletal dysplasia, hypogonadotropic hypogonadism (HH), and anosmia who harbors a nonsense variant c.1633C>T (p.Arg555*) and a deletion of exons 15, 16, and 17 in *SEMA3A* in the compound heterozygous state. These variants were identified through next-generation sequencing analysis of a panel of 26 genes known to be associated with HH/Kallmann syndrome. Our findings further substantiate the notion that biallelic LoF *SEMA3A* variants cause a syndromic form of short stature and expand the phenotypic spectrum associated with this condition to include features of Kallmann syndrome.

Keywords

anosmia; hypogonadotropic hypogonadism; Kallmann syndrome; SEMA3A; short stature

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AUTHOR CONTRIBUTIONS

Alexander F. Gileta, Maria L. Helgeson, Jacqueline M. M. Leonard, and Louise C. Pyle: Responsible for drafting the manuscript and figures. Kelly Arndt: Oversaw and performed data collection from the patient's specimen. Hari P. Subramanian: Responsible for NGS data analysis. Daniela del Gaudio and Colin P. Hawkes: Critically reviewed the manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

1 | INTRODUCTION

Skeletal dysplasias are a clinically and genetically diverse group of heritable disorders affecting the development of the bones and cartilage (Mortier et al., 2019). There is a wide phenotypic continuum, ranging from mild, proportionate, or disproportionate short stature to disorders that are lethal in the perinatal period (Argente, Tatton-Brown, Lehwalder, & Pfäffle, 2019). Although many skeletal dysplasias are characterized by isolated skeletal findings, some may involve additional organ systems including the cardiovascular, genitourinary, and central nervous systems. One such example is CHARGE (Coloboma, Heart defect, choanal Atresia, Retardation of growth and development, Genital hypoplasia, Ear anomalies) syndrome, caused by pathogenic variants in the *CHD7* gene. This gene encodes a transcriptional regulator with a wealth of enhancer binding sites, suggesting that multisystemic skeletal disorders may in part occur due to dysregulation of one or more downstream target genes (Feng et al., 2017).

Recently, two publications reported prepubescent male children who presented with syndromic short stature with cardiovascular anomalies, dysmorphic features, and other minor abnormalities, both of whom had biallelic loss-of-function (LoF) variants in semaphorin 3A (SEMA3A) (Baumann, Steichen-Gersdorf, Krabichler, Müller, & Janecke, 2017; Hofmann et al., 2013). SEMA3A is a member of the semaphorin protein family, all of which contain a characteristic, wellconserved 500 amino acid extracellular semaphorin (sema) domain and act as axonal guidance factors through attractant and repellant cues (Raper, 2000). SEMA3A, specifically, controls autocrine signaling networks responsible for essential biological functions such as innervation and blood vessel invasion in bone, neuronal control of osteoblast differentiation and inhibition of osteoclast resorption, cardiovascular sympathetic innervation, and patterning of olfactory, vomeronasal, and terminal nerve fibers to direct cellular migration during development, among others (Alto & Terman, 2017; Fukuda et al., 2013; Ieda et al., 2007; Negishi-Koga & Takayanagi, 2012). Of note, expression of *SEMA3A* is also directly regulated by CHD7 and both genes are known to harbor pathogenic variants contributing to hypogonadotropic hypogonadism (HH) with anosmia (Kallmann syndrome) (Ufartes et al., 2018).

Here, we report on a patient that is a compound heterozygote for a nonsense variant and a multiexonic deletion of *SEMA3A*. The patient is female and presents with short stature, cardiac anomalies, and dysmorphic features. Unlike the two previously described cases, our patient also presented with HH and anosmia. Interestingly, while *SEMA3A* has previously been implicated in HH and Kallmann syndrome, its contribution was believed to be oligogenic in nature (Cassatella et al., 2018; Valdes-Socin et al., 2014; Young et al., 2012). However, our findings corroborate those from the two previously described cases, reinforcing that biallelic *SEMA3A* variants represent a newly recognizable form of syndromic short stature and widens the clinical spectrum associated with this condition to include features of Kallmann syndrome.

2 | CLINICAL REPORT

The patient is the product of a naturally conceived single gestation to a then 30-year-old G2P1-P2. Ultrasounds and prenatal testing were unremarkable, and she was born at 40 weeks via emergent C-section delivery for decreased fetal heart rate. She was born weighing 2.98 kg (25th–50th percentile) and measuring 50.8 cm in length (75th percentile). Following delivery, she had desaturations with noted airway hypotonia requiring intubation. She had a prolonged NICU stay of 4 months following birth. Evaluation for her respiratory distress included cardiac magnetic resonance imaging which identified a common origin of the right and left carotid arteries with a hypoplastic right carotid artery, a left aortic arch with no evidence of a vascular ring, and a patent foramen ovale. She required tracheostomy placement due to continued respiratory distress and hypopharyngeal collapse, which was decannulated by 1 year of age. During her NICU stay, she was noted to have a barrel-like chest, bluish conjunctiva, unilateral talipes equinovarus, and unilateral clinodactyly of one finger. A sacral dimple was also noted, but no tethered cord was identified on further imaging.

Through childhood, she reached developmental milestones as expected with the exception of mild fine motor delays secondary to hypotonia. She walked at 11–12 months and had normal speech development with first words at 12 months of age after her tracheostomy was removed, and sentences emerging at 18 months. She had no behavioral or early cognitive concerns. At 16 years of age, she performs at grade level and is in college-level courses with a 504 plan in place for anxiety and stress support. Full psychoeducational testing has revealed cognitive deficits and an individualized education plan has been implemented.

The patient was followed regularly by cardiology for her congenital cardiac defects and was subsequently discharged from follow up. Her medical history is notable for severe 90° kyphoscoliosis that was diagnosed at age 14, for which she underwent posterior spinal fusion. She additionally has a history of pectus carinatum requiring surgical repair. She has tight ligaments and weak knee muscles that required bracing followed by physical therapy. She developed hyperopia during childhood and has been noted to have bilateral small ear canals. She has a history of autoimmune thyroiditis.

The patient was noted to have delayed puberty with no breast development or menses by age 16. Pelvic ultrasound was performed and noted a prepubertal uterus that was small for age and small, but normal appearing, ovaries, consistent with delayed puberty. Bone age was also delayed, with an estimated bone age of 12 years on dual-energy X-ray absorptiometry (DEXA) scan performed at 16 years 2 months. She was additionally noted to have anosmia through standardized history-taking. An MRI of the pituitary gland noted heterogeneous enhancement of the pituitary gland with more hypoenhancing tissue on the left aspect of the sella and absence of the olfactory nerves. She was referred for evaluation by endocrinology at the age of 16 years due to her history of anosmia, delayed puberty, and short stature.

At the age of 16 years, height was 140.5 cm (0.04th percentile, −3.38 *SD*), weight was 32.3 kg (<0.01st percentile, −5.13 *SD*), and head circumference was 51.7 cm (0.99th percentile, −2.33 *SD*). Her height also reflected effects of her scoliosis, which caused further

height reduction. Physical exam revealed short stature with microcephaly, mild telecanthus, “moon”-like eyelid shape, protuberant ears, a small unilateral preauricular pit, high narrow palate, dental crowding, long and slim fingers with mild contractures of the fingers and toes, and tight high arches in feet. Family history is significant for delayed puberty and delayed growth spurt in her father, and thyroid disease in her mother. No anosmia was reported in either parent, her maternal half-sister, or in further extended family. Skeletal survey recommended post evaluation was notable for spinal fusion hardware, hypotonic appearance of the chest, mild levocurvature of the thoracolumbar spine, and slightly dysmorphic pelvis (appearing tall and narrow on frontal radiograph) with transitional lumbosacral anatomy. Based on the patient’s history of anosmia and delayed puberty, a hypogonadotropic hypogonadism gene sequencing panel was ordered.

3 | MATERIALS AND METHODS

All molecular methods and sample data processing steps are outlined in the Supporting Information. Exonic numbering and variant annotations for *SEMA3A* were defined based on transcript NM_006080.2 and genomic reference NG_011489.1.

3.1. | Editorial policies and ethical considerations

Written informed consent was obtained from the patient’s parents to allow for the use of the medical history and genetic testing results as approved by the local Institutional Review Board.

3.2 | Data availability

Data available on request due to privacy/ethical restrictions.

4 | RESULTS AND DISCUSSION

Next-generation sequencing (NGS) analysis of 26 genes associated with hypogonadotropic hypogonadism identified a nonsense variant in *SEMA3A*, c.1633C>T (p.Arg555*), resulting in a premature stop codon in exon 15. The variant was confirmed by Sanger sequencing (Figure 1a).

This variant was present in one heterozygous individual of East Asian descent in the ExAC databases but had not been previously reported in ClinVar, HGMD, or other literature as associated with a clinical phenotype. Interestingly, the patient appeared to be homozygous for the variant, as well as for several other high-frequency variants in exons 15, 16, and 17.

Copy number variant (CNV) detection from targeted NGS data was performed using a custom CNV detection pipeline based on a synthesis of results from the R package ExomeDepth v1.1.12 and the commercially available software NxClinical (Biodiscovery, Inc.). CNV analysis demonstrated that the nonsense variant in exon 15 was in *trans* to a deletion of exons 15, 16, and 17 of *SEMA3A* (chr7: 83590677–83,606,680; Figure S1). This three-exon deletion was confirmed by a custom designed Multiplex Ligation-Dependent Probe Amplification assay (MLPA) (Figure 1b). The precise breakpoints of the deletion were not captured in the raw alignment NGS data; therefore, we were unable to confer

accurate HGVS nomenclature to this variant. Both the nonsense variant and deletion were interpreted as pathogenic based on the ACMG standards and guidelines for interpretation of sequence variants (Clinical Genome Resource Sequence Variant Interpretation Working Group et al., 2020).

This is the third literature-reported case of a syndrome caused by biallelic variants in *SEMA3A* (Baumann et al., 2017; Hofmann et al., 2013). The clinical summary presented in Table S1 has been adapted from Baumann et al. to allow for a direct comparison of the patient's clinical manifestations with both previously reported cases. There were numerous shared defining features of the syndrome, including short stature (below 2 SDs), thoracic bone abnormalities (kyphosis/scoliosis), arthrogryposis (camptodactyly/clinodactyly/talipes equinovarus), hypotonia, cardiac deformities, and various mild facial dysmorphisms. As previously mentioned by Baumann et al., Sema3a$^{-/-}$ mice showed an analogous phenotype consisting of increased bone resorption, reduced osteoblast differentiation, low bone mass, short stature, malformed thoracic bones, cardiac abnormalities, and as in this case, anosmia, and reproductive system dysfunction (Behar, Golden, Mashimo, Schoen, & Fishman, 1996; Cariboni et al., 2011; Fukuda et al., 2013; Hayashi et al., 2012).

There are a few notable differences in this patient compared with the past two reports. First, the patient is female, demonstrating that the syndromic presentation of biallelic pathogenic *SEMA3A* variants is consistent across sexes. Secondly, the patient has displayed delayed puberty due to hypogonadotropic hypogonadism (HH), having reached the age of 17 without endogenous menarche or breast development. Interestingly, both parents reported having experienced delayed pubertal progression. This phenomenon was also noted by Hofmann et al. with regard to the father of their proband who harbored a heterozygous 150-kb deletion encompassing exon 1 of *SEMA3A* (Hofmann et al., 2013). Together, these observations suggest that heterozygous carriers of loss-of-function (LoF) alleles in *SEMA3A* may have a mild haploinsufficiency of SEMA3A and be at risk for delayed-onset puberty.

Thirdly, the female patient presented here is anosmic, supported by the absence of olfactory nerves on her MRI. The combination of substantially delayed puberty and anosmia, along with the presence of two pathogenic variants in *SEMA3A*, draw strong parallels to Kallmann syndrome (KS). It is well-known that *SEMA3A* contributes to oligogenic inheritance of KS (Hanchate et al., 2012; Känsäkoski et al., 2014; Young et al., 2012). However, to our knowledge, this is the first reported case of both HH and anosmia being caused by biallelic pathogenic *SEMA3A* variants, as the two previous male cases were reported to have normal olfactometry. Etiologically, this aligns well with the known essential role of SEMA3A guidance of the vomeronasal axons and migration of gonadotropin-releasing hormone (GnRH) neurons from the nasal placode into the forebrain (Schwanzel-Fukuda & Pfaff, 1989; Teixeira et al., 2010). Knockout Sema3a mouse models exhibited abnormal innervation of the olfactory bulbs as well as a malformed GnRH neuronal system with mistargeted vomeronasal axons and GnRH neurons, leading to abnormal or absent olfaction and reproductive function in adulthood (Cariboni et al., 2011; Schwarting et al., 2000).

Another unique facet of the case presented here is that both variants result in loss of the last three terminal exons (15/16/17) of *SEMA3A*, which code for the Ig and C-terminal basic domains of SEMA3A. These domains potentiate the effect of the sema domain in axonal growth cone retraction and collapse; without them, SEMA3A is essentially rendered nonfunctional (He & TessierLavigne, 1997; Kolodkin et al., 1997; Koppel, Feiner, Kobayashi, & Raper, 1997; Lee, Kreusch, McMullan, Ng, & Spraggon, 2003; Luo, Raible, & Raper, 1993). It is, therefore, possible that the observed clinical features in the patient result from the loss of the Ig and basic domains in a truncated protein and/or from nonsensemediated mRNA decay of the aberrant transcripts. Intriguingly, LoF variants in *CHD7*, another KS-associated gene, cause autosomal dominant CHARGE syndrome. The typical clinical presentation of CHARGE shares numerous features with our patient including skeletal abnormality, short stature, congenital heart defect, delayed puberty, genital abnormality, anosmia, and other variable malformations (Hsu et al., 2014). These shared features and affected tissue types might be expected in light of the fact that *CHD7* encodes a transcription factor that directly regulates the expression of *SEMA3A* (Schulz et al., 2014; Ufartes et al., 2018).

In summary, we present a new case of syndromic short stature caused by compound heterozygous LoF variants in *SEMA3A*. The patient's clinical features are similar to two previous reports of biallelic pathogenic SEMA3A variants, as well as homozygous *Sema3a* knock-out mouse models. However, our patient is believed to be the first recorded case in which biallelic pathogenic *SEMA3A* variants cause both HH and anosmia. This case helps to broaden the known phenotypic spectrum for this syndrome and provides insight into the etiology of this rare, multisystemic disorder.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions.

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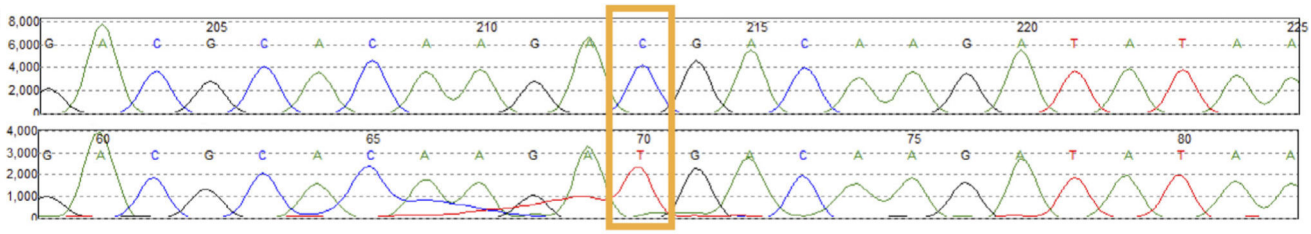
(a)



(b)

	Probe Name	Bin Size	19.1439_D05
1	**MYO5B**	138.1	0.978
2	**NCOA2**	129.5	0.849
3	**PCSK5**	105.2	1.007
4	**POMT2**	101.4	0.964
5	SEMA3A E15	108.5	0.477
6	SEMA3A E16	114.3	0.521
7	SEMA3A E17	124.2	0.514
8	SEMA3A I15	121.3	0.499
9	**SPAST**	134.2	1.052
10	X	96.7	0.983

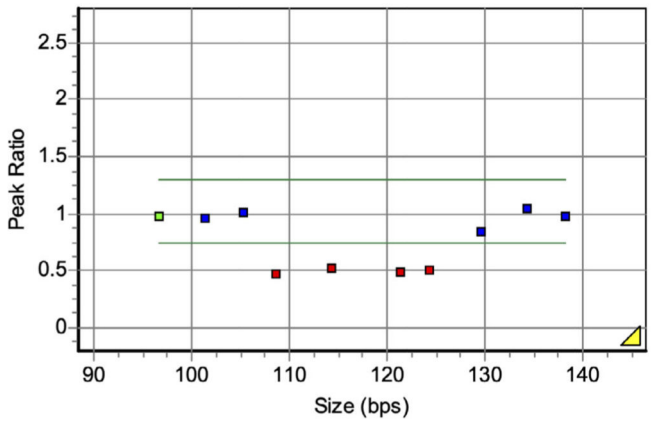


FIGURE 1.

Pathogenic *SEMA3A* variants identified in this patient. (a) Sanger sequencing results displaying confirmation of the c.1633C>T variant in the apparent homozygous state. The normal control sequence is shown in the upper panel. (b) MLPA results showing decreased peak ratio (0.5) corresponding to exons 15, 16, and 17 and intron 15 of *SEMA3A* consistent with a heterozygous deletion involving these exons. An X chromosome-specific probe also confirmed the accuracy of the patient's gender