Evidence for a Pathogenic Role of *CSMD1* in Childhood Apraxia of Speech

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Abstract

Childhood apraxia of speech (CAS) is a pediatric motor speech disorder. The genetic etiology of this complex neurological condition is not yet well understood, although some genes have been linked to it.

We describe the case of a boy with a severe and persistent motor speech disorder, consistent with CAS, and a coexisting language impairment.

Whole exome sequencing in our case revealed a *de novo* and splicing mutation in the *CSMD1* gene.

Keywords

- Childhood apraxia of speech
- language impairment
- *CSMD1*
- array-CGH
- WES

Introduction

Childhood apraxia of speech (CAS) is a subtype of motor speech disorder defined by the American Speech-Language-Hearing Association[1] as "a neurological childhood (pediatric) speech sound disorder, in which the precision and consistency of movements underlying speech are impaired in the absence of neuromuscular deficits (e.g., abnormal reflexes, abnormal tone)". According to the ASHA consensus, three speech features are characteristic of CAS: "Inconsistent errors on consonants and vowels in repeated productions of syllables or words", (b) "Lengthened and disrupted coarticulatory transitions between sounds and syllables", and (c) "Inappropriate prosody, especially in the realization of lexical or phrasal stress". Other speech features variably found in CAS are: increased difficulty with longer words and phrases, slow speech and diadochokinetic rate, intrusive sounds, voicing errors, difficulty achieving initial articulatory configurations, scanned speech, and lexical stress assignment errors.[2] CAS may co-occur with persistent language and learning disorders,[3] resulting in effortful, poorly intelligible speech which negatively impacts the child's social communication in daily activities, peer interactions, and literacy. With regard to the etiology of the condition, increasing evidence suggests that CAS has a genetic basis.

We report the case of a boy with a severe and persistent motor speech disorder, consistent with CAS, and a co-occurrent language impairment, in whom a novel *de novo*, probably pathogenic variant in the *CSMD1* gene was detected by WES.

Methods

Having received written parental informed consent, we performed genome-wide array-CGH in the proband and Whole Exome Sequencing analysis on the family trio. The study received local ethics committee approval.

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Array-CGH was performed as previously described to identify potential genomic rearrangements. The hg19 human genome build GRCh37 was used as the consensus reference sequence. Data analyses were performed using Agilent Cytogenomics V.2.5.8.1. (Agilent, Santa Clara, CA).

Copy number variants (CNVs) were classified as "causative" or "non-causative" according to the American College of Medical Genetics and Genomics (ACMG) guidelines. Segregation analyses in parental DNA were performed by quantitative real-time polymerase chain reaction (qPCR).

We performed WES using NimbleGen SeqCap EZ Exome v3 capture kits (Roche, Italy) of family trio by assuming de novo, autosomal recessive, and X-linked mode of inheritance and by standard filtering methodologies outlined elsewhere.[4]

Variants were prioritized according to the following criteria:

(1) not present in gnomAD or present with a mean allelic frequency $<0.05\%$;
(2) predicted as damaging with Combined Annotation-Dependent Depletion score ≥ 20;
(3) (for splicing variants) predicted to affect splicing by AdaBoost and Random Forest software (we selected variants with a score ≥ 0.6);
(4) classification as pathogenic, likely pathogenic, or of uncertain significance according to ACMG guidelines.

Results

This patient has been followed by our institute (IRCSS Fondazione Stella Maris) for his language impairment from the age of 4 years, when he was administered a thorough speech and language examination[3] according to the standard of care in our hospital.

The boy was born at term by C-section due to breech presentation after an uneventful pregnancy in which his mother was treated with levothyroxine because of hypothyroidism. His weight at birth was 3690 g.; his neonatal and perinatal history was unremarkable. His family history was unremarkable for speech, language and cognitive disorders. Motor development was normal, with independent walking achieved at 12 months, whilst oral language acquisition was delayed from the early vocal stages: canonical babbling was scarce and showed little variation. Up to 30 months of age, the child produced only single vocalic sounds and some isolated syllables. His social and communicative skills were normal: he used several nonverbal strategies to communicate (signs, mimicking, eye contact). When first examined at the age of 4 years and 5 months, neurological examination detected only mild limb hypotonia. Routine blood tests were also unremarkable. We observed normal nonverbal cognitive skills (WPPSI-III PIQ = 107). The child had no orofacial abnormalities and his hearing was within the normal range. Brain MRI revealed a small area of signal hyperintensity on T1-weighted images, involving the deep white matter adjacent to the superior external angle of the left ventricle. This punctiform non-specific alteration can be interpreted either as a prominent vascular space or as a small gliotic area. No other structural brain abnormalities were detected.

At 5 years of age his speech was markedly impaired and showed all the three ASHA features and several of the ten speech characteristics listed in Strand's 10-point checklist[2] (►**Table 1**). His phonetic inventory included 12/21 consonant

Table 1 CAS speech features shown by the patient

		Patient 1 S.Q.
ASHA: 3 speech features	1) Inconsistent errors on consonants and vowels in repeated productions of syllables or words.	+
	2) Lengthened and disrupted co-articulatory transitions between sounds and syllables.	+
	3) Inappropriate prosody, especially in the realization of lexical or phrasal stress.	+
Strand's 10-point checklist		
	1. Vowel or consonant errors including distorted substitutions.	+
	2. Intrusive schwa.	−
	3. Voicing errors.	−
	4. Lexical stress errors or equal stress.	−
	5. Syllable segregation.	+
	6. Slow rate.	−
	7. Difficulty achieving initial articulatory configurations and transitions into vowels.	+
	8. Slow DDK rate.	+
	9. Groping (articulatory searching prior to phonating).	+
	10. Increased difficulty with longer or more phonetically complex words.	+

ASHA: American Speech-Language-Hearing Association; CAS: childhood apraxia of speech. + indicates the presence of a speech feature.

sounds. His speech production was characterized by a 57% rate of inaccurate repetitions of two-syllable nonwords over successive attempts at the same target, and 21% of the errors were inconsistent. The diadochokinetic rate of three-syllable nonword sequences was markedly inaccurate, inconsistent, and slow for the child's age (11 repetitions over 20 seconds). In repeated production of real two- and three-syllable words, 55% of productions were inaccurate and 19% of the errors were inconsistent. Inaccuracy and inconsistency increased in longer utterances and the child's verbal production became poorly intelligible, scanned, and slow. On the Verbal Motor Production Assessment for Children[5] his verbal motor skills were globally impaired.

The child's receptive lexicon was within the normal limits, as were his receptive grammar skills.[6] His expressive lexicon was normal, whilst his expressive grammar was impaired. Analysis of semi-spontaneous language samples showed that his expressive language was below the expected level for his age and corresponded to Grid of Analysis of Spontaneous Speech (GASS) level 4,[7–8] characterized by poor control of grammar rules in complex phrases, with frequent omission of free morphemes and of subordinate clause functions. GASS level 4 is usually achieved by typically developing children by 32 months of age (a sample of his speech is presented online as ►**Supplementary Material 1**).

Array-CGH analysis did not show any potentially relevant CNVs. Conversely, WES analysis identified the rare *de novo* variant c.9280 + 1G > A in *CSMD1* (►**Figure 1**).

This variant was not present in the gnomAD database, nor was it found in in-house WES data of >3500 individuals with unrelated genetic diseases. The variant c.9280 + 1G > A is located in canonical donor splicing site and different algorithms predict the effect of sequence changes on RNA splicing (SpliceAI Donor Loss = 1.0, Ada_score = 0.999915711 and rf_score = 0.916); it is expected that exon 59 skipping does not disrupt reading frame. The role of exon 59 on CSMD1 protein function is unknown. Currently, pathogenic missense variants are not known in exon 59 and a single missense variant in Clinvar database was reported in Sushi 24 domain (amino acids 3035–3094 on protein Q96PZ7). However, exon 59 is present in biologically-relevant transcript and exon 59 skipping removes about <10% of CSMD1 protein. This mutation, meeting the PVS1-PM2 -PS2 criteria of the ACMG, was considered "likely pathogenic".

Discussion

Thiese findings add a potentially new gene to the list of etiological factors in CAS and language impairment.

We found a likely pathogenic *de novo* splice-site variant in *CSMD1*. *CSMD1* is classified as an autism spectrum disorder candidate gene in the SFARI (Simons Foundation Autism Research Initiative) database. The gene is expressed at intermediate levels in the brain, including the cerebellum, substantia nigra, and hippocampus, as well as in the fetal brain. It lies at 8p23.2 and *CSMD1* genetic variants have been shown to be associated with autism.[9,10]

CSMD1 (OMIM ID 608397; 8p23.2) and its paralog *CSMD2* (OMIM ID 608398; 1p35.1) are members of the CUB and Sushi multiple domain protein family, and are described as regulators of complement activation and inflammation in the developing central nervous system.[11] Complement activation is gaining much attention because it is essential for synaptic plasticity and has recently been implicated in several brain-related conditions such as schizophrenia,[11] a condition characterized by poor and disorganized speech, and impairment of cognitive domains, including attention, memory, learning, and executive functions.

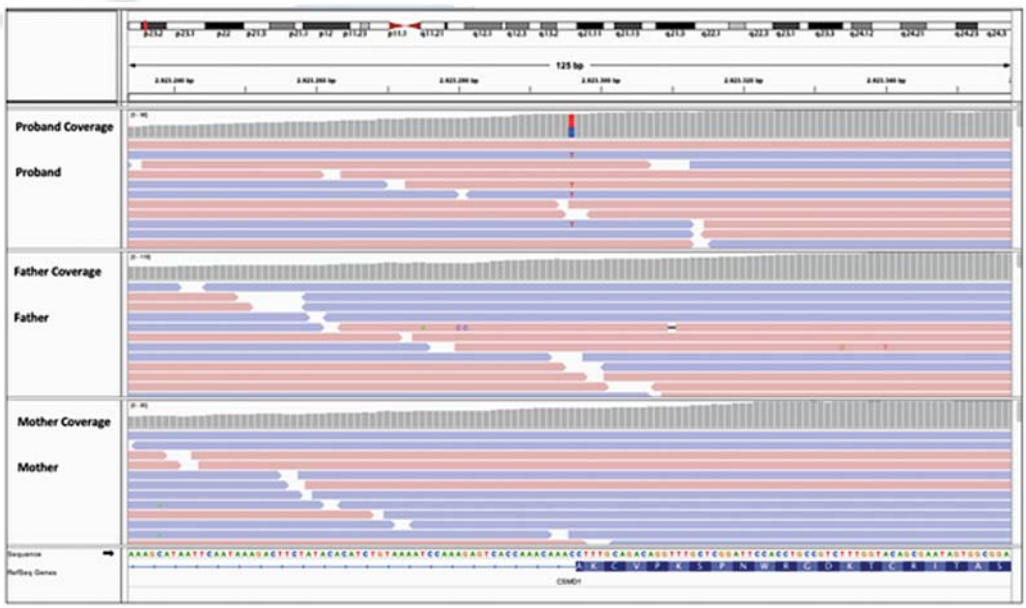

Fig. 1 IGV screenshot of WES alignments for the proband (top track), father, (middle track), and mother (bottom track). Each track comprises two parts: a histogram of the read depth and the reads as aligned to the reference sequence. Reads are colored according to the aligned strand (red = forward strand; blue = reverse strand).

A study on *CSMD1* knockout mice suggested that depletion of *CSMD1* expression is linked to abnormal emotional/affective responses, hyperactivity, and increased anxiety-related response.[12] Koiliari and colleagues highlighted the involvement of the *CSMD1* gene in cognitive disorders, reporting associations of *CSMD1* variants with poorer neurocognitive functioning, such as impaired general cognitive abilities, strategy formation, planning, set shifting, and episodic verbal memory.[13,14]

Additionally, in a recent review by Akyuz and Bell[15] the role of *CSMD1* is described in neurodevelopmental disorders and in some pathological conditions, such as Parkinson disease and schizophrenia. *CSMD1* variants are associated with immediate episodic memory and cognitive functions[14] and *CSMD1* expression is high in the amygdala, thus affecting social behavior.[16] Moreover, the fact that during early development, neuronal connections between the amygdala and the language processing centers are strengthened in the brain, highlights the role of the amygdala in language learning processes and also the probably potential involvement of *CSMD1* in speech impairment.

Our child meets the clinical criteria for a diagnosis of CAS. We performed in-depth language phenotyping, which helped us to prioritize variants in WES. A similar approach might allow future larger studies to define additional monogenic forms of CAS and/or language impairment. Interestingly, scrutiny of the pertinent literature revealed a description of a genomic duplication at the 8p23.locus encompassing the *CSMD1* gene in a patient who showed speech delay and learning difficulties.[17] Even more interestingly, upon revision of our own database of 2715 children with neurodevelopmental disorders and speech impairment, we found an additional case with normal WES and a similar genomic rearrangement encompassing *CSMD1* and with some features resembling CAS (data not shown). Moreover, the DECIPHER database, too, includes patients (ID_394741, ID_287658, ID_254189, ID_379314) with speech delay, language impairment, and intellectual disability, who have 8p23.2 duplications involving *CSMD1*. Thus, forms related to this gene may be more common than originally believed.

In summary, our findings show that *CSMD1* may play a role in language development, and suggest it may be involved in the mechanism underlying CAS phenotype. These findings add to existing knowledge on the diagnostic genetic markers of CAS, and contribute to efforts to ensure that pediatric patients with speech disorders receive the most appropriate treatment options.

Author Contributions

AC, FMS, DF and IP drafted the manuscript. DF performed wet-lab (sample processing steps) and WES data analysis. MP performed array-CGH. AC and IP collected and analyzed clinical data. DL performed genetic counseling. AC, FMS, DF, SG and EA critically reviewed the manuscript. AC and DF designed the study. FMS reviewed the study design. All authors have read and approved the final version of the manuscript.

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Conflict of Interest[Q4]

None declared.

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