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LETTER TO THE EDITOR

Case Report: Whole Exome Sequencing Unveils an Inherited Truncating Variant in *CNTN6* (p.Ser189Ter) in a Mexican Child with Autism Spectrum Disorder

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Autism spectrum disorders (ASDs) are a group of heterogeneous neurodevelopmental disorders with hundreds of related genes. Among these, *CNTN6* (Contactin-6) has recently been associated. Herein, we describe a paternally inherited *CNTN6* variant predicted *in silico* to be deleterious in a patient presenting with language delay, poor social interaction, stereotypic behavior, and sensory-motor and hearing problems. Additional genomic data by whole-exome sequencing (WES) suggest, however, that a concomitant pathogenic genetic background would be needed to explain the phenotype along with this *CNTN6* variant.

Case Report

A 10-year-old male patient was referred due to autistic spectrum. His parents were young (25 and 24 years of age) and healthy at periconception; no consanguinity was referred and there was no other family member affected with neuropsychological anomalies. He was the product of a second gestation with adequate prenatal care; vaginal delivery occurred without incident at the 39th week of gestation. Birth weight was 3400 g and height was 51 cm. No congenital anomalies were recorded. His psychomotor development was normal until age 16 months when he exhibited seizure episodes and regression of language. Soon after, he showed aggressive behavior and social retraction, overreaction to noise, stereotyped and ritualized activities, and at age 3 years, he suffered from deglutition dysfunction and was diagnosed as ASD by DSM-IV-TR and ADOS criteria. He was hospitalized at age 6 due to intestinal pseudo-obstruction. Later, he was evaluated by the Pediatric Neurology Service and was treated with risperidone, magnesium valproate, levetiracetam and methylphenidate. At physical examination (9.11 years), he presented with a weight, height, and occipitofrontal circumference of 26 kg (Z score − 0.2), 139 cm (Z score − 2.07), and 50.5 cm (Z score − 2.16), respectively. In addition to microcephaly, he only showed some minor facial dysmorphia. Muscle strength was 5/5 in all extremities; fine motor skills and coordination were slightly affected whereas ataxic gait was noticed. The Miller–Fisher test was positive. A brain MRI showed right frontal pachygyria and Sylvian dysplasia. The EEG exhibited irritative subcortical activity with slow acute bilateral waves, evoked auditory potentials with left hypoacusia for acute tones and delayed right central conduction in the inferior portion of the brainstem. Newborn metabolic and thyroid function screening were normal. A DSM-IV diagnosis of ASD was made based

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on deficits in social-emotional reciprocity with reduced sharing of interests, emotions or affect and failure to initiate or respond to social interactions at age 12 months, a lack of facial expressions and nonverbal communication at age 18 months followed by difficulties to adjust behavior to suit various social contexts; difficulties in sharing imaginative play or in making friends; stereotyped motor movements and use of objects, extreme distress at small changes, difficulty with transitions, rigid thinking patterns, integrative sensorial disorder (adverse response to acute sounds and rough textures) and visual fascination with lights or movement since age 2 years.

Since both the GTG-banded (450–550) karyotype and an *FMR1* CGG repeats screening were normal, we performed a genomic scan by WES (Illumina, San Diego, CA, USA) to identify single nucleotide variants (SNVs), copy number variants (CNVs) and/or structural variants (SVs) potentially related to the patient's phenotype. The BWA Enrichment app analysis from this WES demonstrated a high-quality Q30 score > 30 for 96.3% of reads, with a mean coverage depth of 57.2x; the variants summary revealed 28,843 SNVs. From these results, we focused on heterozygous and homozygous variants with a population frequency < 0.01% (global minor allele frequency < 0.01) and predicted to be pathogenic, likely pathogenic or a variant of uncertain significance (VUS) by the SIFT and PolyPhen-2 (as coupled in Variant Interpreter app), Mutation Taster 2, and CADD platforms. Thereby, we identified deleterious SNVs in nine known and/or potential ASD-risk genes (Table 1) (Mercati et al. 2017; Smith 2016; Stessman et al. 2017; Yuen et al. 2015). Among these, we observed a heterozygous SNV—with the highest CADD score (48)—in exon 6 of *CNTN6* whose consequence was a gained stop codon (c.566C > A, p.Ser189Ter; with genotype quality (GQX) = 99, alternative variant depth = 41, and total read depth = 94 scores) (Fig. 1). This variant was predicted to disrupt CNTN6 in the second Ig-like domain and produce a shorter protein with a length < 200 amino acids (189/1028). Although this SNV was previously reported by 1000 genomes (from the dbSNP database, rs773080572), its frequency and validation is not yet provided, and in other databases, such as ExAC, gnomaD, ClinVar, and Exome Variant Server, this SNV had not yet been reported; thus we annotated it at the ClinVar database (entry SCV000786639.1). Sanger sequencing confirmed the variant in *CNTN6* in the patient but, surprisingly, also evidenced it in his father (Fig. 1). Then, we also analyzed the patient's father DNA by WES. Among ASD-risk variants found in the patient, his father only presented—in addition to the *CNTN6* variant—the same variant in *COL11A1* (Table 1 footnote). No other truncating ASD-related SNV was observed in the patient's exome. Strikingly, an Enrichment app analysis disclosed an 824-bp inversion partially involving the exon 4 of *CNTN5* (breakpoints at chr11:99,690,484–99,691,307; hg19) in the patient when comparing his exome against four reference exome samples (including his father). No relevant CNV was found.

Discussion

CNTN6, which encodes the Contactin-6 protein, is highly expressed in different brain tissues including the cortex, hippocampus, and cerebellum (granule cells and inferior colliculus) and has been involved in processes of dendrite growth and synapse formation (Mercati et al. 2017). Rarely reported (Fig. 1), de novo or inherited loss-of-function SNVs in this gene appear to have a central pathogenic role in a few ASD patients with sensory-motor and auditory alterations (Mercati et al. 2017); yet, as occur with several other ASD-related hits, its actual clinical impact is often inconsistent. Notwithstanding that sensory-motor behaviors and hearing problems in the present ASD patient are comparable with such findings, the absence of any symptom in his father harboring the same truncating variant in *CNTN6* is unexpected and even contradicts the suggested impact of such mutations in ASD (Cheng et al. 2018; van Daalen et al. 2011).

It has been suggested that several mutations in *CNTN6* are not fully penetrant (even some related to deletions), and require the presence of concomitant variants—that in some cases can be in other contactin-related genes—to cause ASD (Mercati et al. 2017; Oguro-Ando et al. 2017; van Daalen et al. 2011). In this regard, our search for variants in other already reported ASD-risk genes demonstrated, among others, likely pathogenic variants in *CNTN5, DNAH10, NEO1*, and *NRXN2* in the patient, which were absent in his father (Table 1) and in other exome-sequenced healthy and non-ASD subjects in our lab (n = 15, data not shown). Alike *CNTN6, CNTN5* has relevant expression patterns and functional roles in the development of sensory-motor neuronal pathways; therefore, alterations of this gene may also lead to ASD involving sensory-motor and auditory affectations (Mercati et al. 2017). Although the pathogenicity of the *CNTN5* SV found in this patient is not clear (it was previously described by DGV (dgv705n106/nsv1078300) in at least two patients without available neurological data), the envisaged damage in exon 4/intron 4 (Suppl. Fig. 1) could produce an aberrant protein and add to the truncated CNTN6 effect. Moreover, *DNAH10* encodes for a dynein expressed in brain (UniProtKB, ID Q8IVF4). Neuron dyneins participate in establishing and maintaining the complex morphology of axons and dendrites (Kapitein et al. 2010). Significantly, two brothers with ASD inherited from their affected mother both a truncating *CNTN6* and a VUS in *DNAH10* as well (Mercati et al. 2017). *NEO1* and *NRXN2* encode for neuronal cell surface proteins; NEO1 has been involved in cell adhesion, neural migration, and axon guidance, whereas NRXN2 in

Table 1 Additional SNVs found in the present patient (but not in his father) related and/or potentially related to ASD phenotypes

Gene/SNV	S, P-2, MT, and CADD scores	Zygosity in patient	ASD-risk genes (known)	Potential ASD-risk genes	Present in subjects without ASD	References
DNAH10 Missense c.6117G > C/p.Met2039Ile	–, 0.815, 10, 26.2	Heterozygous	Yes	–	Not in our samples	Yuen et al. (2015)
KMT2C Missense (all) c.2573G > T/p.Trp858Leu c.2578C > T/p.Pro860Ser c.2645T > C/p.Ile882Thr	–, 0.935, 25.1, 89 –, 0.945, 19.05, 74 –, 1, 61, 26.4	Heterozygous	Yes	–	Yes	Stessman et al. (2017)
ANKRD13C Splice region c.1216-6delT	–	Heterozygous	–	Yes	Yes	This report
DNAH7 Missense c.11720A > G/p.Tyr3907Cys	0, 0.975, 10, 28.9	Heterozygous	-	Yes	Not in our samples	This report
NEO1 Missense c.1112C > T/p.Thr371Ile	0.03, 0.97, 89, 26.9	Heterozygous	Yes	–	Not in our samples	Siu et al. (2016)
NRXN2 Missense c.1975C > T/p.Arg659Trp	0, 31, 101, 20.9	Heterozygous	Yes	–	Not in our samples	SFARI database[a]
SLC45A1 Missense c.2080G > C/p.Val694Leu	0.01, 0.095, 32, 23.6	Heterozygous	Yes	–	Not in our samples	SFARI database[a]
MTNR1A Missense c.287G > A/p.Gly96Asp	0, 0.86, 94, 24.4	Heterozygous	Yes	–	Not in our samples	AutismKB database[a]

The variant found in *COL11A1* (c.652-8_652-6dupTTT) was omitted from this table because of it was present in the patient's father

S SIFT, *P-2* Polyphen-2, *MT* mutation taster, *CADD (PHRED)* combined annotation dependent depletion

[a]https://gene.sfari.org/database/human-gene/NRXN2; https://gene.sfari.org/database/human-gene/SLC45A1; http://autismkb.cbi.pku.edu.cn/gene_evidence_detail.php?entrez_id=4543

cell recognition and adhesion (UniProtKB, ID Q9P2S2; Siu et al. 2016; Stessman et al. 2017). Another gene harboring a likely pathogenic variant—but not yet reported as ASD-risk factor—was *DNAH7* (Table 1).

Even though most of the ASD-related genes mentioned in this report appear to have incomplete penetrance or a less defined association (e.g., Mercati et al. 2017; Yuen et al. 2015), it is tempting to speculate on the participation of more than one of these and perhaps other genes (probably not yet related to ASD) (Table 1 and Suppl. Table 1) in the pathogenesis of this patient, mainly through subtle additive effects in axon and/or dendrite behavior. This is consistent with the functional overlapping of autism-risk genes (e.g., those encoding contactins and neurexins) implicated in regulating the structural stability of neurons (Lin et al. 2016).

In summary, we identified a loss-of-function variant in *CNTN6* related to an ASD phenotype. Although this finding may reinforce the link between *CNTN6* mutations and ASD with sensory perception alterations, the presence of concomitant likely pathogenic variants—including a SV affecting another closely related contactin—seems to be indispensable to complete the pathogenesis in this, and perhaps, other patients. Thus, our findings further support the contention that *CNTN6*-related ASD requires a specific genetic (and/or even epigenetic) background to manifest and would lead us to the question: Does *CNTN6* represent an additive or a causative gene? In this regard, further studies of

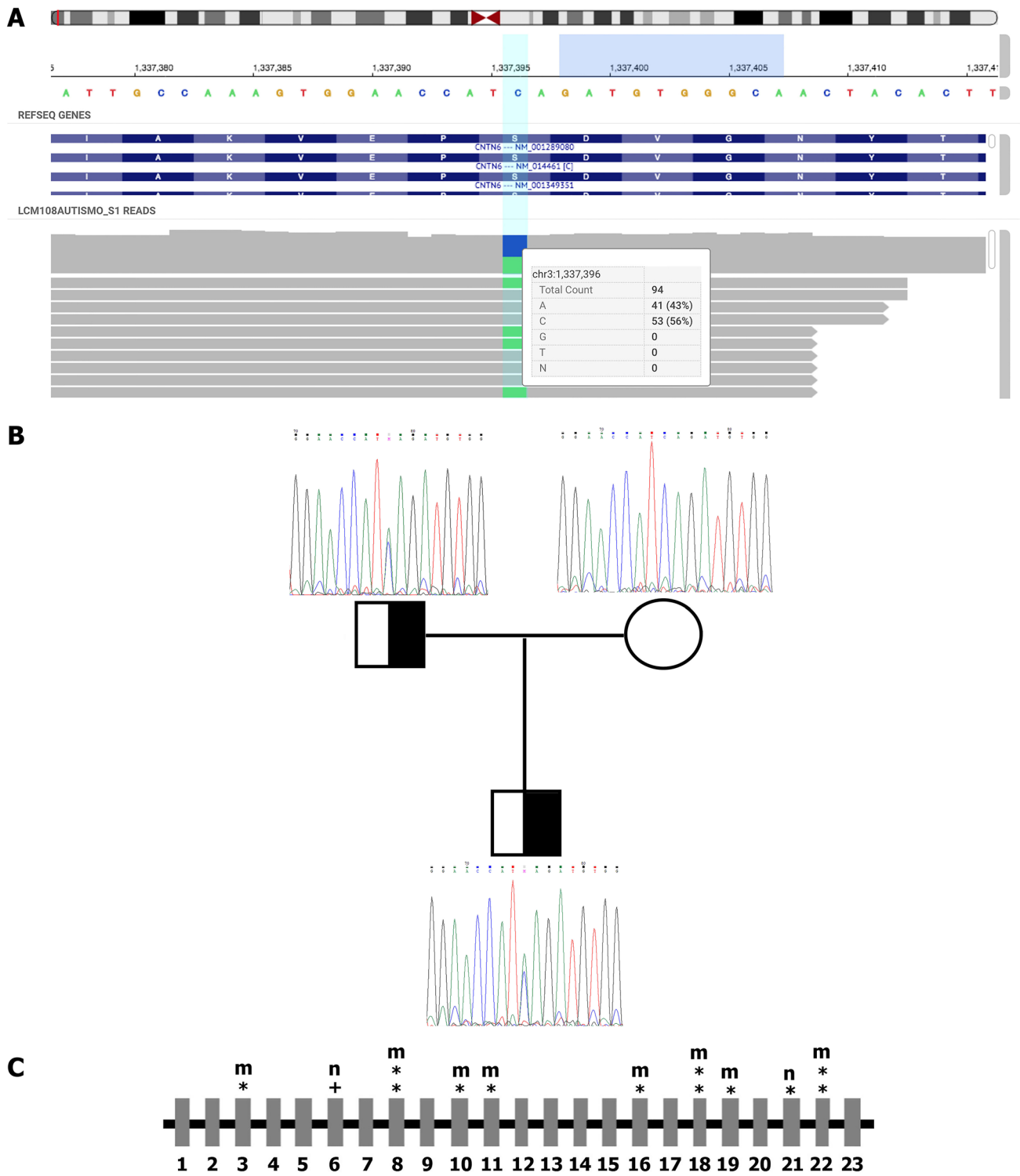

Fig. 1 **A** Integrative genome viewer showing the patient's variant, **B** family pedigree including chromatogram for the *CNTN6* variant, and **C** deleterious variants in *CNTN6* reported so far. +, patient's variant; * variants reported by Mercati et al. (2017); m, missense; n, nonsense

those ASD-related variants accompanying likely pathogenic *CNTN6* variants will be needed.

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Author Contributions JEG-O provided samples and clinical reports, participated in the coordination of the study, and helped to draft the manuscript. AIZ-N and EAR-O participated in the design, processing, and interpretation of the genomic data. AAH-O, CEP-Á, and LEB-S participated in the design, coordination, and interpretation of the clinical data. KAG-H and AMR-E participated in the validation of the genetic data. CC-F conceived of the study, participated in its design and coordination, and drafted the manuscript. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

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. This article does not contain any studies with animals performed by any of the authors.

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

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