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Whole-Exome Sequencing Identifies a de novo *AHDC1* Mutation in a Colombian Patient with Xia-Gibbs Syndrome

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Keywords

AHDC1 mutation · Whole-exome sequencing · Xia-Gibbs syndrome

Abstract

Xia-Gibbs syndrome is an autosomal dominant multisystem developmental disorder characterized by global developmental delay, hypotonia, obstructive sleep apnea, seizures, retrocerebellar cysts, delayed myelination, micrognathia, and mild dysmorphic features. Using whole-exome sequencing, we identified a de novo *AHDC1* frameshift mutation c.2030_2030delG (p.G677Afs*52) in a Colombian patient, which was absent in both parents. Furthermore, we summarized the phenotypes of patients reported in the literature.

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Many patients living with rare genetic diseases have gone through several rounds of genetic testing (e.g., karyotyping, candidate gene sequencing, aCGH, next-generation sequencing), frequently not receiving an accurate diagnosis or proper information regarding the prognosis of the disease. This may lead to treatment delay and a risk of morbidity/mortality [Gahl et al., 2012].

aCGH offers a higher diagnostic rate (15–20%) for genetic testing of individuals with unexplained developmental delay (DD) and/or intellectual disability (ID) compared to karyotyping, primarily because of its higher sensitivity for submicroscopic deletions and duplications [Miller et al., 2010]. However, whole-exome sequencing (WES) in DD/ID revealed de novo mutations in protein coding genes in 60% of individuals, having a higher performance [Hochstenbach et al., 2011].

Next-generation sequencing is a powerful tool for the diagnosis of mendelian disorders with variable phenotypes without a family history, leading to the discovery of new genes associated with diseases or a new disease associated with specific loci [Yang et al., 2013; Xue et al., 2015; Retterer et al., 2016]. The clinical yield of this test will continue to increase over time, allowing providers to efficiently arrive at a diagnosis [Lee et al., 2014; Xue et al., 2015]. According to Yang et al. [2014], this method identifies underlying genetic defects in 25% of patients referred for a possible genetic condition.

According to the American College of Medical Genetics (ACMG), indications for WES [ACMG, 2012] are when (1) clinical diagnostic assessment of a phenotypically affected individual with history data strongly implicate a genetic etiology, but the phenotype does not correspond with a specific disorder for a specific gene, (2) the phenotype demonstrates a high degree of genetic heterogeneity without a specific test available, and (3) specific genetics tests available for the phenotype have failed.

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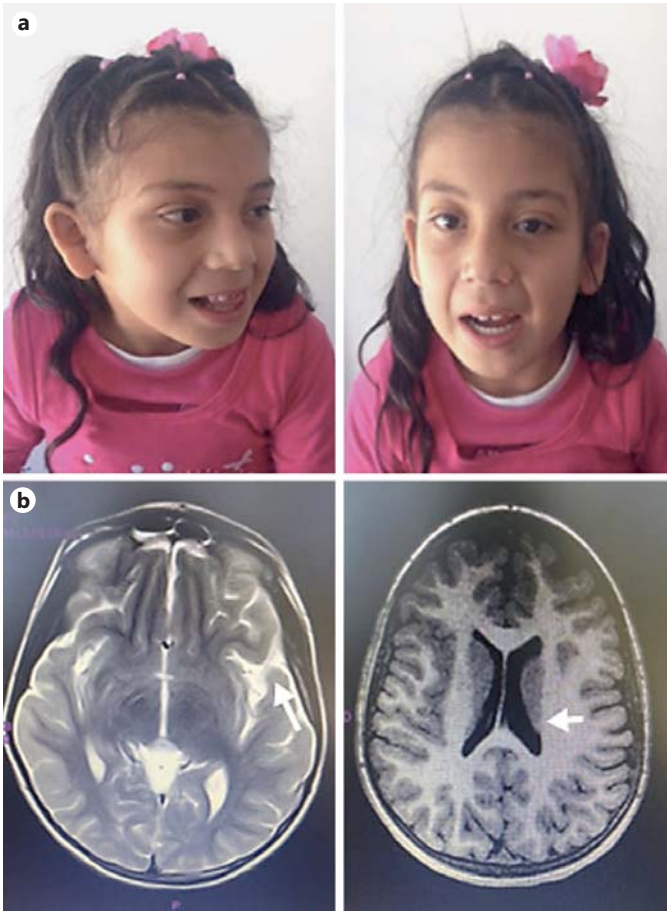

Fig. 1. a Patient at the age of 5 years. Facial features showing midfacial hypoplasia, hypertelorism, micrognathia, epicanthic fold, prominent teeth, and upslanting palpebral fissures. **b** Brain MRI showing frontal and temporal cortical atrophy, with loss of posterior ventricular white matter (arrows).

Xia-Gibbs syndrome (OMIM 615829) was mentioned for the first time by Xia et al. [2014] after analysis of parent-offspring trios of 4 probands with DD, hypotonia, mild dysmorphic features, sleep apnea, and other symptoms, finding 3 new allelic variants in the *ADHC1* (AT hook DNA-binding motif containing 1) gene likely to be pathogenic; it was the first report in genome databases. Yang et al. [2015] reported 6 frameshifts or nonsense deleterious de novo variants and 1 recurrent variant in the same gene, associated with expressive language delay, hypotonia, and sleep apnea. Recently, Bosch et al. [2016] identified a de novo variant in an older patient with a history of DD and speech delay as well as characteristic facies. Previously, a de novo missense variant in a case of schizophrenia [Guipponi et al., 2014] and a de novo balanced translocation with a breakpoint in the *AHDC1* intron 1 in a boy with bicuspid aortic valve, aortic coarctation, patent ductus arteriosus, and DD/ID have been reported in this gene [Quintero-Rivera et al., 2015]. Several large CNV deletions including the *AHDC1* gene have been described in pediatric patients with DD/ID [Itsara et al., 2009; Cooper et al., 2011; Coe et al., 2014]. Although due to the large size of the deletions, it is uncertain whether *AHDC1* is the only gene contributing to the neurodevelopmental phenotype. Xia et al. [2014] proposed that *AHDC1*-associated ID is due to a dominant-negative mechanism, given the autosomal-dominant inheritance and the single coding exon of this gene.

The *AHDC1* gene located in chromosome 1p36.11 encodes a protein of 1,603 amino acids, consisting of 7 exons with only 1 coding exon (exon 6), containing 2 AT-hooks, which likely function in DNA binding. AT-hook motifs are known as auxiliary protein motifs that cooperate with other DNA-binding activities and facilitate DNA structure changes [Aravind and Landsman, 1998]. The *AHDC1* gene is part of the CBX family of proteins associated with human chromodomain-containing Polycomb proteins [Vandamme et al., 2011]. In vivo assays have demonstrated that AHDC1 interacts with several nuclear proteins involved in epigenetic regulation during development [Vandamme et al., 2011], mainly in neural sites and neuron proteins for transport [Uhlén et al., 2015]. In mice, *Ahdc1* is expressed at embryonic day E11.5 and E16.5 in the developing brain [Quintero-Rivera et al., 2015], suggesting that AHDC1 may be involved in early brain development.

In this study, we described the first Colombian patient with Xia-Gibbs syndrome, with a new mutation in the *AHDC1* gene identified by WES and compared the 12 cases reported in the literature to better understand the clinical phenotype and the association with the *AHDC1* gene.

Patient and Methods

Case Report

The 8-year-old Colombian girl is the first child born at term to 27-year-old nonconsanguineous parents after prolonged labor and perinatal hypoxia, with an Apgar score of 6 at 10 min. The child was admitted to the neonatal intensive care unit requiring mechanical ventilation for 27 days. Clinical follow-up showed hypotonia and DD (head control at 7 months, sitting at 12 months, standing at 24 months, walking at 28 months, and currently speaking only 2 words) with dysmorphic features including midfacial hypoplasia, hypertelorism, micrognathia, epicanthic fold, prominent teeth, upslanting palpebral fissures (Fig. 1), and laryngoma-

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Table 1. Clinical featurs of patients with Xia-Gibbs syndrome

Reference	Case	Gender	Age	Variant in *AHDC1*	Developmental delay	Cognitive delay	Speech delay	Dysmorphic facial features	Sleep disturbance	Brain abnormality
Xia F et al. [2014]	1	F	18 mo	c.2373_2374 delTG (p.Cys791Trpfs*57)	no sitting at 18 mo	unknown	no words at 18 mo	low-set ears, exotropia, upslanting palpebral fissures, micrognathia, flat nasal bridge	obstructive sleep apnea	retrocerebellar cyst
	2	F	4 yrs	c.2898delC (p.Tyr967Thrfs*175)	sitting at 19 mo, walking at 2 yrs	moderate	2 words at 4 yrs	protuberant ears, upslanting palpebral fissures, flat nasal bridge	obstructive sleep apnea	thinning of the corpus callosum
	3	M	8 yrs	c.2373_2374 delTG (p.Cys791Trpfs*57)	sitting at 9 mo, walking at 18 mo	mild	first words after 1 y, persistent speech therapy	protuberant low-set ears, small earlobes, hypertelorism, downslanting palpebral fissures, mild ptosis, micrognathia	obstructive sleep apnea	thinning of the corpus callosum
	4	M	11 yrs	c.2547delC (p.Ser850 Profs*82)	sitting at 15 mo, no independent ambulation	moderate to severe	no words, noncommunicating autism	upturned earlobes, hypertelorism, exotropia, flat nasal bridge	snoring	thinning of the corpus callosum
Yang et al. [2015]	5	M	2 yrs	c.1945delG (p.A649Pfs*83)	no independent walking	unknown	no verbal speech	bitemporal hollowing, brachycephaly, plagiocephaly, short nose, narrow mouth, high-arched palate, tented upper lip, and high, prominent forehead with prominent glabella	sleep apnea	normal
	6	F	7 yrs	c.2529_ 2545del17 (p.D845Rfs*40)	walking at 3 yrs 10 mo	yes	current 2–3-word sentences	bitemporal hollowing, brachycephaly, plagiocephaly, short nose, narrow mouth, high-arched palate, tented upper lip, and high, prominent forehead with prominent glabella	restless sleeper	periventricular leukomalacia, thin CC, subependymal cysts
	7	M	5 yrs	c.1881delG (p.Q627Hfs*105)	unknown	moderate	unknown	bitemporal hollowing, brachycephaly, plagiocephaly, short nose, narrow mouth, high-arched palate, tented upper lip, and high, prominent forehead with prominent glabella	unknown	unknown
	8	F	4 yrs	c.1122dupC (p.G375Rfs* 3)	walking at 20 mo	yes	current single words	normal	unknown	small CC, loss of posterior ventricular white matter
	9	M	9 yrs	c.3809delA (p.Q1270Rfs*75)	walking at 4 yrs, 9 mo	yes	no verbal speech	bitemporal hollowing, brachycephaly, plagiocephaly, short nose, narrow mouth, high-arched palate, tented upper lip, and high, prominent forehead with prominent glabella	unknown	hypomyelination, thin CC, small brain stem
	10	M	5 yrs	c.2373_ 2374delTG (p.C791Wfs*57)	walking at 2 yrs	yes	current 2-word sentences	bitemporal hollowing, brachycephaly, plagiocephaly, short nose, narrow mouth, high-arched palate, tented upper lip, and high, prominent forehead with prominent glabella	unknown	prominent CSF, small arachnoid cyst vs. abnormal cisterna magna
	11	F	16 yrs	c.1480 A>T (p.K494X)	unknown	yes	no verbal speech	bitemporal hollowing, brachycephaly, plagiocephaly, short nose, narrow mouth, high-arched palate, tented upper lip, and high, prominent forehead with prominent glabella	unknown	demyelination, involving subcortical U fibers, retrcerebellar cyst

Table 1 (continued)

Reference	Case	Gender	Age	Variant in *AHDC1*	Developmental delay	Cognitive delay	Speech delay	Dysmorphic facial features	Sleep disturbance	Brain abnormality
Bosch et al., [2016]	12	F	22 yrs	c.1402dupT p.Cys468Leufs*49	delayed	unknown	first words at 4 yrs	long asymmetric face, proptosis, broad nose, full lips, broad mouth, wide-spaced teeth, small high palate, simple left ear, overfolding of the right superior helices, hyperlordosis, soft skin, 5th finger clinodactyly, fetal finger pads, pes equines, many moles	snoring	asymmetric wide ventricles, thin corpus callosum
Present case		F	8 yrs	c.2030_2030del (p.G677Afs*55)	sitting at 12 mo, walking at 28 mo	severe	currently speaking single words	midfacial hypoplasia, hypertelorism, micrognathia, epicanthic fold, prominent teeth, upslanting palpebral fissures	sleep apnea	frontal and temporal cortical atrophy, loss of posterior ventricular white matter

CC, corpus callosum; CSF, cerebrospinal fluid; mo, months; N/A, not applicable; yrs, years.

lacia requiring 8 surgical interventions due to glottal stenosis. Her family history was unremarkable

Brain MRI revealed frontal and temporal cortical atrophy with loss of posterior ventricular white matter (Fig. 1). The girl's karyotype, microarray, and metabolic tests were normal.

Molecular Analysis

WES was performed using Illumina HiSeq platform (overall performance of 90% exome coverage at 40× depth) identifying a new mutation in *AHDC1* (c.2030_2030delG; p.Gly677AlafsX52) verified by Sanger sequencing and absent in both maternal and paternal DNA. It was therefore interpreted as a de novo event that results in a frameshift of the *AHDC1* open reading frame and is predicted to cause a premature termination codon after 52 amino acids. Predicted truncating mutations in *AHDC1* are absent in the genome mutation databases dbSNP and ExAC. The pathogenic effect of the described mutation in *AHDC1* was predicted by the bioinformatic tools PolyPhen-2 (prediction "probably damaging" – score of 0.958) [Adzhubei et al., 2010], MutationTaster (prediction "disease causing") [Schwarz et al., 2014], and SNPs&GO (RI = 8 Effect = neutral) [Calabrese et al., 2009]. Furthermore, glycine is a conserved amino acid between species; therefore, any change in this amino acid can generate alterations of the protein structure.

Discussion

Here, we report a proband with DD and dysmorphic facial features due to a frameshift mutation in *AHDC1*, which was discovered by WES. To date, 12 individuals with *AHDC1* mutations have been reported in the literature. Developmental histories of patients evidenced that all had delayed speech and psychomotor delay; most individuals had mild dysmorphic facial features that could be seen at a young age and had a history of sleep disturbance because of anomalies of the upper airway structure.

The independent occurrence of 12 de novo mutational events at this locus in unrelated individuals with similar phenotypes is highly unlikely, suggesting that mutations in *AHDC1* cause this complex disorder, which has not been previously reported in the literature since Xia et al. [2014]. It is remarkable that all cases were detected by WES after several genetic tests. Clinical features of the reported cases of Xia et al. [2014], Yang et al. [2015], and Bosch et al. [2016] compared with the clinical presentation of our case are summarized in Table 1.

Xia et al. [2014] proposed that *AHDC1*-associated ID is due to a dominant-negative mechanism, given the autosomal-dominant inheritance and the single coding exon of this gene [Xia et al., 2014]. This results in an insufficient dosage of encoded protein which would sever the link between DNA damage response and proper brain development [Quintero-Rivera et al., 2015]. This is based

on a model in which the protein encoded by the gene with the gene dosage imbalance or mutation affects the bindings to other protein(s) to form a functional complex which may lead to the formation of inactive complexes [Poot et al., 2011].

Although there are no functional or computational studies of these proteins, we speculate that *AHDC1* variants may interrupt protein translation, possibly disrupting the interaction with other proteins important for brain development and function.

WES has proven to be an important tool to improve our ability to diagnose patients with heterogeneous diseases; especially, the expanded use of WES has led us to find new genes causing diseases and new diseases caused by genes. Variants in *AHDC1* should be validated with in vivo and in vitro assays to understand the mechanisms of the genotype/phenotype relationship.

Acknowledgments

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Statement of Ethics

Written informed consent was obtained from our patient's legal guardian for publication of this case report. The study was approved by the ethics committee of the Roosevelt Pediatric Orthopedic Institute, Bogotá, Colombia.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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