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CASE REPORT

Genotype–phenotype spectrum and correlations in Xia-Gibbs syndrome: Report of five novel cases and literature review

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Abstract

Background: Xia-Gibbs syndrome (XGS) is a rare neurodevelopmental disorder caused by pathogenic variants in the AT-hook DNA-binding motif-containing 1 gene (*AHDC1*), encoding a protein with a crucial role in transcription and epigenetic regulation, axonogenesis, brain function, and neurodevelopment. *AHDC1* variants possibly act through a dominant-negative mechanism and may interfere with DNA repair processes, leading to genome

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instability and impaired DNA translesion repair. Variants affecting residues closer to the N-terminal are thought to determine a milder phenotype with better cognitive performances. However, clean-cut genotype–phenotype correlations are still lacking.

Cases: In this study, we investigated five subjects with XGS in whom exome sequencing led to the identification of five novel de novo pathogenic variants in *AHDC1*. All variants were extremely rare and predicted to cause a loss of protein function. The phenotype of the reported patients included developmental delay, hypotonia, and distinctive facial dysmorphisms. Additionally, uncommon clinical features were observed, including congenital hypothyroidism and peculiar skeletal abnormalities.

Conclusions: In this study, we report uncommon XGS features associated with five novel truncating variants in *AHDC*, thus expanding the genotype and phenotypic spectrum of this complex condition. We also compared our cases to previously reported cases, discussing the current status of genotype–phenotype correlations in XGS.

KEYWORDS

AHDC1, DNA repair, genotype–phenotype correlations, loss-of-function variant, neurodevelopmental syndrome, Xia-Gibbs syndrome

1 | INTRODUCTION

Xia-Gibbs syndrome (XGS) is a rare neurodevelopmental disorder characterized by global developmental delay (DD), intellectual disability (ID), speech delay with limited or absent language, hypotonia, and sleep abnormalities/sleep apnoea. Other, less frequent clinical findings include movement disorders, ataxia (that often become apparent in childhood or adolescence), short stature, seizures, strabismus, behavioral concerns, or autism spectrum disorders (ASD), and variable neuroimaging structural anomalies. This condition, which is caused by de novo truncating mutations in *AHDC1*, was first described in 2014 by Xia et al., who identified a de novo truncating variant in a patient with DD, hypotonia, mild dysmorphic features, and sleep apnoea (Chander et al., 2021; Cheng et al., 2019; Díaz-Ordoñez et al., 2019; Xia et al., 2014). Since then, around 280 cases of XGS have been reported (Bosch et al., 2016; Chander et al., 2021; Park et al., 2017; Miller et al., 2017; Popp et al., 2017).

The AT-hook DNA-binding motif-containing 1 (AHDC1) gene is located on the short arm of chromosome 1 within the cytogenetic band 1p36.11–35.3. This region is proximal to the chromosomal region causing partial or complete 1p36 deletion syndrome (Gajecka et al., 2007) and *AHDC1* lies nearby the *AT-rich interaction domain 1A (ARID1A)* gene, whose mutations cause autosomal-dominant Coffin-Siris syndrome (Tsurusaki et al., 2012). *AHDC1* consists of seven exons with a single coding exon and encodes the 1,603-amino acid protein AT-hook DNA-binding motif-containing protein 1 (Xia et al., 2014). AHDC1 has two AT-hook DNA-binding motifs located at codons 396–408 and 544–556, which are DNA-binding motifs acting to fasten proteins to AT-rich sequences in DNA (Karlson et al., 1989). However, the physiological function of the AHDC1 protein and the mechanisms underlying XGS remain elusive (Chander et al., 2021).

In this study, we provide a detailed molecular and phenotypic description of five novel patients with XGS harboring de novo variants in *AHDC1* and review previously reported cases focusing on the possible genotype–phenotype correlations in this complex condition.

2 | PATIENTS AND METHODS

The study was conducted in accordance with the Declaration of Helsinki and approved by the local Institutional Ethics Committees. Patients were enrolled at different Italian Institutions (Istituto Giannina Gaslini, Genoa; Federico II University, Naples; AORN San Pio, PO Gaetano Rummo, Benevento; Bambino Gesù Children's Hospital, Rome; Arcispedale S. Maria Nuova, Reggio Emilia) and clinically evaluated by pediatric geneticists and neurologists. Informed consent was obtained by the parents or legal guardians according to Telethon Undiagnosed Program (TUDP) guidelines, trio-WES was performed in the three families on genomic DNA extracted from

peripheral blood. Agilent Sure Select QXT Clinical Research Exome (Agilent Technologies, Santa Clara, CA, USA) was used and Sequencing data were processed with in-house software for the GATK Best Practices pipeline for WES variant analysis execution.[14]After filtering for allele frequency (≤0.01% in public databases, including GnomAD v2.1.1; https://gnomad.broadinstitute.org/), family segregation, conservation (GERP score), and predicted impact on protein function through in silico tools (including SIFT, PolyPhen-2, Mutation Taster), the best candidates were de novo variants in *AHDC1* in all probands. These variants were eventually validated through Sanger sequencing.

3 | CLINICAL REPORTS

3.1 | Patient1

Patient 1 was born to unrelated healthy parents of Italian descent, after a pregnancy complicated by intrauterine growth retardation (IUGR). Family history was remarkable for epilepsy and neurodevelopmental delay. Growth parameters at birth showed short length (1st pc), while weight and head circumference were normal. The patient presented with neonatal jaundice, congenital hypothyroidism, and facial dysmorphisms. In the first 2 years of life, motor clumsiness and DD were diagnosed, associated with short stature (<3rd pc) and obstructive sleep apnea (OSA). Griffiths developmental scales II at 40 months revealed a competence corresponding to 13 months. He started to walk with a wide-based gait at the age of 3 years, his speech was limited to a single word, and bowel control was only achieved at age 6. At the age of 7 yearsASD and attentive deficit-hyperactive disorder (ADHD) were also diagnosed. Dysmorphic craniofacial features included a prominent forehead, small nose, wide philtrum, thin lips, thin hair in temporal regions, and low-set ears (Figure 1a). Other dysmorphic features were fetal pads, brittle nails, short neck, pectus excavatum, and small feet with hammerfirst fingers. He had unilateral hearing loss and ophthalmologic evaluation revealed exotropia and hypermetropic astigmatism. Brain magnetic resonance imaging (MRI) at the age of 1 year showed a reduced volume of centrum semiovale and mildly delayed myelination, which disappeared at the age of 3 years (Figure 1b).

3.2 | Patient 2

The proband is the only son of a non-consanguineous couple with a negative family history. Prenatal ultrasounds were unremarkable and he was born at 42 weeks after an unremarkable pregnancy. Apgar score was 9–9. Birth weight was 3,080 g (15th pc) and length 50 cm (33rd pc). The neonatal period was uneventful. He was able to sit at 10 months of age and started to walk independently at 18 months. He pronounced the first words at 18 months but the language showed a very slow progression, as he was able to produce sentences of 2–3 words at the age of 11 years and 3 months. He also suffered from febrile seizures until 7 years of age. Neurological examination showed hand flapping triggered by excitement with sufficient social skills. The patient was also diagnosed with cryptorchidism requiring surgical correction. At 11 years of age, his weight was 20 Kg (20th pc), height 128.2 cm (first pc), and occipito-frontal circumference (OFC) 54 cm (37th pc). He had sparse hair and a prominent nasal bridge. Brain MRI showed two hyperintense signals in the temporo-mesial region. Brainstem auditory evoked potentials and visually evoked potential were normal. The heart ultrasound was normal.

3.3 | Patient 3

The proband is the only son of unrelated healthy parents. Prenatal ultrasound detected possible shortness of lower limbs. He was born at term after a normal pregnancy. Birth weight was 2,550 g, length 44 cm, and OFC 33 cm. Apgar score was 8–10. During the neonatal period, he was diagnosed with hypotonia, physiological jaundice, and posterior left plagiocephaly. Brain and heart ultrasounds were normal. He had global psychomotor delay: walked independently at 2 years and pronounced his first words at 18 months. At the age of 3 years, he was diagnosed with celiac disease and eosinophilic esophagitis, successfully treated with a gluten-free diet. Ocular investigation revealed hypermetropia and astigmatism. A skeletal X-ray survey revealed scoliosis, decreased physiological curves, iliac crest asymmetry, and abnormalities of the right femoral head. Auditory brain response and EEG were normal. At the age of 5.5 years, he presented a single episode of febrile seizures with normal EEG. A follow-up EEG during sleep at 6.6 years detected isolated anomalies in the left temporal area. Brain MRI at the age of 5 years was normal. Rhinolaringoscopic examination showed adenoidal hypertrophy and laryngeal marks of gastric reflux. At 6 years and 8 months, his weight was 27 Kg (75th pc), height 111 cm(third pc), OFC 51.5 cm (25–50th pc), arm-span 108.5 cm, and upper segment/lower segment ratio 0.95. He had a high forehead, brachycephaly, hypertelorism, long and flat philtrum, thin upper lip, high and arched palate, retrognathia, clinodactyly of the fifth finger of hands,

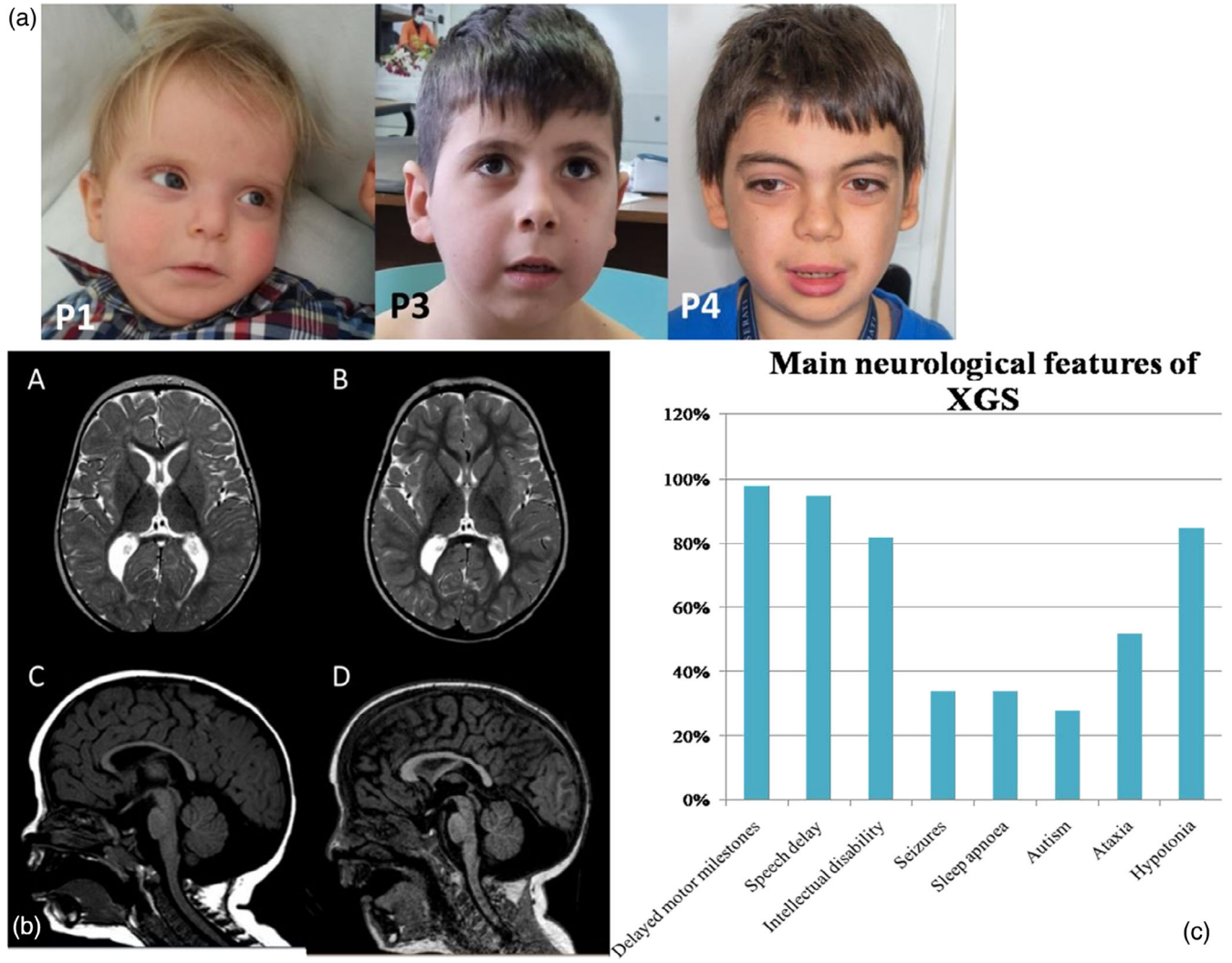

FIGURE 1 (a) Dysmorphic features of the patients. P1: prominent forehead, small nose, wide philtrum, thin lips, and low set ears; P3: brachycephaly, hypertelorism, long and flat philtrum, thin upper lip, high and arched palate, retrognathia; P4: synophrys, arched eyebrows, long eyelashes, ptosis on the left, short nose, anteverted nares, flat philtrum, thin upper lip, thick everted lower lip, small teeth, mild retrognathia, dysmorphic ears with anteverted up-lifted lobes. (b) P1: brain MRI, axial T2-weighted images at 10 months (a) and 3 years old (b). Sagittal 3D-T1-weighted images at 3 months (c) and 3 years old (d). (c) Summary of the main neurological features of XGS (adapted from Della Vecchia) (Della Vecchia et al., 2021). P = patient

single palmar crease on the right hand, short fourth and fifth metacarpal bones, coxa vara, joint hyperlaxity, and mild hypotonia.

3.4 | Patient 4

The proband is the first child of healthy non-consanguineous parents with an unaffected son. Family history was unremarkable. The patient was born on the 40th week after an uneventful pregnancy. Birth weight was 3,220 g (25th pc), length 52 cm (85th pc), OFC 37 cm (97th pc). Apgar score was 9–10. Brain ultrasound was normal. Cardiac ultrasonography revealed a patent foramen ovale. Feeding difficulties, gastroesophageal reflux, dysphagia, and severe constipation occurred since the first months of life. Abdominal ultrasound showed mild bilateral hydronephrosis. At 8 months, a kidney stone with ureterocele was diagnosed and the patient underwent surgical intervention. Brain-stem audiometry showed mild bilateral conductive deafness. Developmental milestones were delayed and neurological examination showed generalized hypotonia and atypical social skills, being very sociable and attached to other children and adults. He was also diagnosed with autism. The child suffered from sleep apnea, which dramatically improved after tonsillectomy and adenoidectomy were performed at 4 years of age. At 8 years of age, he started to suffer

from partial seizures, which were treated with Carbamazepine. EEG showed: slow high-voltage complexes in the temporal-occipital regions in sleep, presence of spindles, and hints of K-complexes. Brain MRI at 7 years revealed asymmetric lateral ventricles. Clinical evaluation revealed dolichocephaly, facial asymmetry, high forehead, synophrys, arched eyebrows, long eyelashes, ptosis on the left, upslanting palpebral fissures, short nose, anteverted nares, flat philtrum, thin upper lip, thick everted lower lip, small teeth, mild retrognathia, dysmorphic ears with anteverted up-lifted lobes, anterior and posterior low hairline, telethelia, mild general hypertrichosis, thin toes, hallux valgus. Weight was 24 kg (10–25th pc), height 131 cm (50th pc), and OFC 55 cm (90–97th pc). X-ray of the feet showed asymmetry of the ossification nucleus in the first toes with cone-shaped epiphysis of proximal phalanx of the second and third toes.

3.5 | Patient 5

The proband is the second child of healthy non-consanguineous parents. His brother had a speech delay. The patient was born 36 weeks after a normal pregnancy. Birth weight was 3,020 g (77th pc), length 50 cm (88th pc), OFC 34.5 cm (84th pc). Apgar score was 9–10. Clinical examination at 9 years showed scalp aplasia cutis at the vertex, narrow palpebral fissures, arched upper lip, cup-shaped ears, retroauricular hypertrichosis, strabismus, bilateral single palmar crease, wide-set asymmetric and inverted nipples, flat feet. Developmental milestones were delayed (sitting at 13 months, walking alone at 3 years, first words at 2.5 years). Mild cognitive impairment was diagnosed in association with stereotypic movements, ataxic gait, and motor coordination problems. Brain MRI showed cystic-like dilatation of the left retrocerebellar space. Ophthalmological examination revealed right eye strabismus. EEG, echocardiography, abdominal ultrasound, and brain-stem audiometry were normal. At 9.3 years, his weight was 31 kg (75th pc), height 131 cm (25–50th pc), and OFC 53 cm (50th pc).

3.6 | Genetic analysis

In family 1, ES led to the identification of the de novo variant c.2192dupT (p.Asp732Argfs*36) in *AHDC1* (NM_001029882.3). This null variant is absent in GnomAD, is not reported in ClinVar, and is classified as likely pathogenic. In family 2 trio-ES identified the de novo variant c.2188 G > T (p.Glu730*). This nonsense variant is absent in gnomAD, HGMD, and ClinVar. It is predicted pathogenic based on in silico tools (BayesDel_addAF, DANN, EIGEN, FATHMM-MKL, and MutationTaster). In Family 3, the de novo c.4289dup (p.Ala1432Glyfs*49) was identified. This frameshift variant is absent in gnomAD and it is classified pathogenic by in silico tools. In family 4, the de novo frameshift variant c.1446delC (p.Val483Tyrfs*16) was detected. This variant is absent in gnomAD and is predicted to introduce a premature stop codon. ClinVar classifies this variant as pathogenic and it is associated with XGS. In family 5, the de novo frameshift variant c.1102_1114del (p.Cys368Alafs*80) was identified. All variants are predicted to result in a premature protein termination or nonsense-mediated mRNA decay (NMD), leading to loss of function, and have never been reported in the literature.

4 | DISCUSSION

The AT-hook DNA-binding motif-containing protein 1 has a predicted DNA binding domain and in vitro studies demonstrated that AHDC1 plays an important role in transcription and epigenetic regulation (Quintero-Rivera et al., 2015; Xia et al., 2014). It also interacts with nuclear proteins and is implicated in axonogenesis (Chatr-Aryamontri et al., 2017; Vandamme et al., 2011). The reported *AHDC1* variants involve codons 151–1,499 and likely act through a dominant-negative mechanism, with most of them resulting in a truncated protein or NMD (ClinVar, 2021; Quintero-Rivera et al., 2015). These variants affect AHDC1 function, altering its interaction with other proteins important for brain function and development, thus explaining the neurobehavioral manifestations observed in almost all cases (Yang et al., 2015). Of note, similar AHDC1 functional domains are present in REV3L, the catalytic subunit of the DNA polymerase zeta (Gan et al., 2008). This is a key protein involved in the replication of damaged DNA, genome stability, and DNA translesion repair (Gan et al., 2008). This finding leads to speculation that *AHDC1* variants may interfere with DNA repair processes.

The percentage of patients in whom a pathogenic variant in *AHDC1* is detected by sequence analysis is around 97–98%, whereas no data is available on the detection rate of gene-targeted deletion/duplication analysis (Chander et al., 2021; Jiang et al., 2018; Khayat et al., 2021; Ritter et al., 2018). Large deletions of 1p36.11 including *AHDC1* associated with XGS-overlapping features account instead for up to 2–3% of affected individuals. However, these rearrangements include adjacent genes which can independently contribute to neurodevelopmental phenotypes (Chander et al., 2021; Jiang et al., 2018; Park et al., 2017; Ritter et al., 2018; Wang et al., 2020).

TABLE 1 Summary of the main clinical features in XGS patients

Clinical feature	Xia et al. (2014)	Yang et al. (2015)	Bosch et al. (2016)	Garcia-Acero and Acosta (2017)	Jiang et al. (2018)	Ritter et al. (2018)	Gumus (2020)	Cardoso-Dos-Santos et al. (2020)	Khayat et al. (2021)	Ellis et al. (2021)	Total*	P1	P2	P3	P4	P5
Common features																
Intellectual disability	4/4	7/8	NA	+	NA	4/5	+	+	30/34	+	49/55	+	+	+	+	+
Speech delay	4/4	7/8	+	+	NA	5/5	+	+	32/34	+	53/55	+	+	+	+	+
Motor delay	4/4	6/8	+	+	NA	5/5	+	+	32/34	+	52/55	+	+	+	+	+
Hearing deficit	NA	NA	NA	NA	NA	1/5	NA	NA	NA	+	36/40	+	−	−	+	−
Structural brain abnormality	4/4	6/8	NA	+	12/20	3/5	+	NA	NA	−	27/40	−	+	−	+	+
Ataxia	NA	NA	NA	NA	13/20	NA	NA	+	23/34	−	36/55	−	−	−	−	+
Sleep apnoea	3/4	2/8	+	+	9/20	3/5	NA	+	17/34	+	38/75	+	−	−	+	−
Autism	NA	NA	NA	NA	5/20	NA	NA	NA	10/34	+	16/55	+	−	−	+	−
Hypotonia at diagnosis	4/4	NA	NA	NA	18/20	4/5	+	NA	30/34	+	58/66	−	−	−	+	−
Seizures	NA	NA	NA	NA	6/20	NA	+	+	15/34	+	24/57	−	−	+	+	−
Scoliosis	−	1/8	NA	−	4/20	−	−	+	9/34	−	12/74	−	−	+	−	−
Strabismus	−	3/8	NA	−	8/20	−	−	−	14/34	−	25/74	+	−	−	−	+
Short stature	−	2/8	NA	−	NA	NA		−	17/34	−	19/50	+	−	−	−	−
Uncommon features																
Laryngomalacia	2/4	−	NA	+	−	−	−	−	6/34	+	10/74	−	−	+	−	−
Tracheomalacia	1/4	NA	NA	−	NA	−	−	−	3/34	−	4/46	−	−	−	−	−
Osteo-articular anomalies	−	3/8	NA	−	−	4/5	−	−	1/10?	+	9/51	+	−	+	+	+
Hypothyroidism	−	−	NA	−	−	−	−	−	−	−	−	+	−	−	−	−
Aplasia cutis	−	−	NA	−	−	1/5	−	−		+	2/51	−	−	−	−	+

Abbreviations: P, patient; NA, not available.

XGS is characterized by highly variable phenotypes, but cognitive impairment, motor delay, language delay, neonatal hypotonia, and sleep apnoea are considered "cardinal" signs (Table 1). Other common features include seizures, scoliosis, ataxia, autism, and strabismus (Khayat et al., 2021) (Figure 1c). The complexity of this condition requires multidisciplinary medical evaluations. In addition to the core phenotypic signs, Khayat recently identified other minor clinical manifestations. Scoliosis, seizures with EEG abnormalities, altered brain MRI (He et al., 2020) and ocular problems/strabism were reported in at least two individuals although they were not shared by at least 80% of the reported individuals. These features have been classified as "secondary phenotypes" (Khayat et al., 2021).

Additional rare or novel clinical features were observed in our cohort. Patient 1 lacked neonatal hypotonia and had unusual clinical manifestations, such as congenital hypothyroidism and unilateral hearing loss. The latter was also observed in patient 4 and has been previously described in a single patient with otitis media and cholesteatoma, carrying the c.4494dupG (p.C1499Vfs*89) variant (Ritter et al., 2018). Patient 2 also lacked neonatal hypotonia, specific neuroimaging abnormalities, and ataxia. However, he presented with seizures, which are reported in 34% of XGS patients (Della Vecchia et al., 2021). Atypical osteoarticular manifestations and scoliosis were observed in 4/5 of our patients: hammer-first fingers in patient 1; joint laxity, reduction of physiological spinal curvatures, coxa vara and phalangeal abnormalities in patient 3; hallux valgus with cone-shaped epiphysis of the proximal phalanx of toes in patient 4; flat feet in patient 5. Abnormal ophthalmologic findings were present in 3/5 subjects (patients 1, 4, and 5), consisting of strabismus. Gastrointestinal manifestations, such as feeding and chewing problems, constipation, and G-tube feeding, have been reported in XGS (Chander et al., 2021; Gumus, 2020; Jiang et al., 2018; Ritter et al., 2018; Yang et al., 2015). In our cohort, they were present in 2/5 patients: celiac disease and eosinophilic esophagitis in patient 3; gastroesophageal reflux in patient 4. Even though there is increasing evidence of poor growth in XGS patients (Chander et al., 2021; Yang et al., 2015), this aspect was not present in our cohort. Also, no signs of structural airway abnormalities were observed in our patients, despite tracheomalacia or laryngomalacia being relevant manifestations in XGS (Chander et al., 2021). Interestingly, patient 5 presented with congenital aplasia cutis and had a first clinical diagnosis of Ear-Scalp-Nipple syndrome (Marneros et al., 2013), due to the association of aplasia cutis of the scalp with cup-shaped ears, asymmetric nipples, and developmental delay. Aplasia cutis has been occasionally described in previous studies (Chander et al., 2021; Ellis et al., 2021; Murdock et al., 2019; Ritter et al., 2018) and could be considered as a rare specific marker for the syndrome.

Genotype–phenotype correlations in XGS remain elusive. It has been speculated that truncating pathogenic variants affecting residue closer to the N-terminal may determine a milder clinical phenotype with better cognitive performances. This observation is supported by the results of 20 Modified Checklist for Autism in Toddlers (M-CHAT) performed in children with XGS, which revealed that patients bearing variants closer to the C-terminus of the protein (e.g., p.Ser1258*, p.Gln1270Argfs*75, and p.Ser1330*) are nonverbal and with high M-CHAT scores (Jiang et al., 2018; Mubungu et al., 2020). Of note, a single patient carrying an early stop-gain variant (p.Gln262*) was found to have a low M-CHAT score and could produce sentences (Jiang et al., 2018; Mubungu et al., 2020). In a recent work by Khayat et al., truncating mutations closer to the N-terminus were more likely associated with seizures or scoliosis (Khayat et al., 2021). However, phenotypic heterogeneity is not uncommon in individuals harboring the same variant, suggesting that clear-cut genotype–phenotype correlations are difficult to establish (Jiang et al., 2018; Mubungu et al., 2020). In fact, two unrelated children carrying the c.2849del (p.Pro950Argfs*192) variant have been recently reported to show significantly different phenotypes (Faergeman et al., 2021). Most XGS patients harbor truncating *AHDC1* variants while likely pathogenic missense variants have been reported in ten individuals (Chander et al., 2021; Khayat et al., 2021). However, possible correlations between the type of the variant and the clinical phenotype remain unclear.

In conclusion, our study expands the molecular and phenotypic spectrum of XGS, highlighting the relevance of uncommon clinical manifestations, such as congenital hypothyroidism and skeletal abnormalities. These findings suggest that affected individuals may show a highly variable phenotype, supporting the importance of proper genetic counseling and multidisciplinary evaluation in the management of this syndrome. Although associations between certain variant types and clinical manifestations are plausible based on the available literature, definite genotype–phenotype correlations remain difficult to establish. The report of additional large cohorts will play a fundamental role in the identification of less elusive correlations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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