ORIGINAL ARTICLE

A novel Xp11.22–22.33 deletion suggesting a possible mechanism of congenital cervical spinal muscular atrophy

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Abstract

Background: Congenital cervical spinal muscular atrophy (CCSMA) is a rare, nonprogressive, neurogenic disorder characterized by symmetric arthrogryposis and motor deficits mainly confined to upper extremities. Since its first proposal by Darwish et al. 39 years ago, only few cases have ever been reported. Vascular insult to the anterior horn of cervical spinal cord during fetal development was speculated to be the cause, however, the exact pathogenesis is still not well understood.

Methods: In this study, whole-exome sequencing (WES) and copy number variation (CNV) analysis were conducted on a definitive CCSMA patient, confirmed by the clinical manifestations and other supplementary examinations.

Results: On physical examination, the patient was mainly characterized by symmetric, congenital, nonprogressive contractures, hypotonia, and muscle weakness mainly confined to the upper limbs, which were further supported by MRI and electromyography. Neuromuscular biopsy of the deltoid muscle demonstrated the type 1 myofiber predominance without any infiltration of inflammatory cells. The WES and CNV analysis unveiled a de novo Xp11.22–22.33 deletion. On further examination of the genes contained within this segment, we recognize *UBA1* gene as the most likely pathogenic gene. Ubiquitin-like modifier activating enzyme 1 is encoded by *UBA1* gene (MIM 314370) located in Xp11.3 and is a critical protein that plays a vital role in ubiquitin-proteasome system and autophagy. It is well documented that *UBA1* gene mutation causes X-linked infantile spinal muscular atrophy (XL-SMA), which manifests phenotypes of arthrogryposis, hypotonia, and myopathic face. Type 2 XL-SMA, which follows a nonprogressive and nonlethal course is very similar to the presentations of CCSMA.

Conclusion: The phenotypic similarities between this CCSMA case and XL-SMA prompt us to hypothesize a possible connection between *UBA1* gene deficit and the pathogenesis of CCSMA. Our study is the first to demonstrate that CCSMA might have a genetic etiology, thus, expanding our insights into the underlying cause of CCSMA.

1 | BACKGROUND

Spinal muscular atrophies (SMAs) are a group of hereditary disorders characterized by progressive weakness and atrophy of muscles, due to the degeneration of motor neurons at anterior horn of the spinal cord and motor bulbar nuclei in the brain stem (Darras, 2015). The majority of spinal muscular atrophies are caused by homozygous mutations or deletions of the *SMN1* gene (MIM 600354). However, not all forms of SMA are caused by the *SMN1* gene defects (Darras, 2011). A good example is the *ubiquitin-like modifier activating enzyme 1 gene* (*UBA1*, MIM 314370), mutations of which can also give rise to a rare form of SMA known as X-linked SMA (XL-SMA) (Dlamini et al., 2013; Jędrzejowska et al., 2015; Ramser et al., 2008). *UBA1* encodes the primary enzyme, UBA1, responsible for activating ubiquitin as the first step in the ubiquitin-conjugation pathway, subsequently marking proteins for degradation by the proteasome (Groen & Gillingwater, 2015). XL-SMA is clinically similar to SMA and is characterized by loss of lower motor neurons, muscle weakness, hypotonia, and a lack of reflexes. In addition, congenital contractures and fractures are also commonly associated with XL-SMA (Dlamini et al., 2013; Jędrzejowska et al., 2015; Ramser et al., 2008).

Moreover, cases have also been reported of segmental spinal muscular atrophy with involvement of mere upper or lower extremities. In 1981, Darwish et al. (Darwish et al., 1981) reported three cases of nonprogressive symmetric muscular atrophy and joint contractures, confined to the upper limbs. The term "congenital cervical spinal muscular atrophy" was employed to describe the condition that the cervical myotomes were specifically affected. Thereafter, additional cases have been described globally (Hageman et al., 1993; Kaiboriboon & Hayat, 2001; Savas et al., 2015; Yalaz et al., 1992). All patients in those cases had symptoms of muscle wasting, hypotonia with flaccid paresis, and congenital contractures, predominantly affecting the upper limbs. The clinical manifestations of all those previously reported patients had the common characteristics of non-progression, de novo onset and mere cervical cord involvement, and hence intrauterine ischemic attack to the anterior horn cells was postulated as an underlying mechanism of CCSMA (Kaiboriboon & Hayat, 2001). However, after 30 years since then, whether there might be a genetic etiology is still unknown.

Since mutations in *DYNC1H1* (MIM 600112) and *BICD2* gene (MIM 609797) have been identified as a cause for spinal muscular atrophy, lower extremity predominant type (Rossor et al., 2015), it is also reasonable to suppose an inheritable etiology for this upper extremity predominant type of SMA. However, no genetic testing has been performed in any of those precedent studies.

To explore this, we present a girl whose clinical manifestations met the diagnostic criteria for CCSMA. The patient was featured by symmetric congenital contractures of multiple joints confined to her upper extremities. The diagnosis of congenital cervical spinal muscular atrophy was later confirmed by a combination of her clinical manifestations and a series of supplementary exams. Eventually, whole-exome sequencing (WES) and copy number variation (CNV) analysis were performed to examine any possible molecular explanation for this disease.

2 | METHODS

2.1 | Ethical compliance

This study has been formally approved by the institutional ethics committee. Informed consent for presentation of the results has also been obtained from the patient and her legal guardians. Clinical data were extracted including clinical manifestations and supplementary exams.

2.2 | Exome analysis

Genomic DNA from peripheral blood samples was extracted following standard procedures. The proband's DNA was initially sequenced using the Next-Generation Sequencing (NGS) technique. DNA was isolated from peripheral blood with CWE9600 Automated Nucleic Acid Extraction System using CWE2100 Blood DNA Kit V2 (CoWin Biosciences, China, CW2553). And after fragmentation, purification, amplification, quantification, quantified DNA was sequenced with 150-bp paired-end reads on Illumina NovaSeq 6000 platform (Illumina, Inc.) according to the standard manual. The raw data produced on NovaSeq platform were filtered and aligned against the human reference genome (hg19) using the BWA Aligner (http://bio-bwa.sourceforge.net/) after evaluated according to Illumina Sequence Control Software (SCS). The single-nucleotide polymorphisms (SNPs) were called by using GATK software (Genome Analysis Toolkit) (www.broadinstitute.org/gatk). Variants were annotated using ANNOVAR (annovar.openbioinformatics.org/en/latest/). Effects of

single-nucleotide variants (SNVs) were predicted by SIFT, PolyPhen-2, and MutationTaster programs. All variants were interpreted according to ACMG standards and categorized to be pathogenic, likely pathogenic, variants of unknown clinical significance (VUS), likely benign and benign.

2.3 | Copy number variation analysis

Copy number variation analysis (CNV) was later performed on the proband to verify the Xp deletion suggested by NGS. A 750 ng of genomic DNA was fragmented to an average size of 200–300 bp, and DNA libraries were constructed by KAPA Library Preparation Kit. A DNA library was constructed using T reagent. The constructed DNA library samples were taken for high-throughput sequencing of Illumina NovaSeq. High-quality double-ended sequencing reads were aligned to the human reference genome sequence of the UCSC database using the Burrows–Wheeler Aligner tool. The preset window is 50 Kb, and the adjustment amount is 5 Kb each time. Each window performs two-step calibration of GC and population model. After removing the abnormal window, the standard deviation between the copy ratio and the reference set of each window is calculated. The standard deviation is less than 0.15, which is considered to pass the quality control, and then, determined by the software. The break point gives the final CNV segment size and its copy ratio value. Identified and mapped CNVs were interrogated against publicly available databases, including Decipher, Database of Genomic Variants (DGV), 1000 genomes, and Online Mendelian Inheritance in Man (OMIM).

3 | RESULTS

3.1 | Clinical report

A 12-year-old female patient was admitted to the pediatric surgery department of Qilu Hospital for the surgical interventions for her arthrogryposis multiplex congenita. She was the second born child of healthy non-consanguineous parents and had a healthy older sister. There was no documented family history of joint contracture or any neuromuscular diseases. Fetal movements were evidently experienced in the gestation of this patient as well as in that of her older sister, according to her mother's description. There was no recorded history of intrauterine growth restriction, contamination of amniotic fluid, nuchal cord, or birth asphyxia. The patient presented, at birth, with "congenital arthrogryposis multiplex" restricted to her upper limbs and there were web-like structures at her bilateral shoulder and elbow joints. Severe symmetric shoulder girdle, arm, and hand weakness was noticed since birth. The extent of the deformities has not progressed since birth (Figure 1).

On neurological examination, severe bilateral hypotonia and muscle weakness were manifest in both arms. Besides, both upper arms and forearms were internally rotated with apparently restricted range of external rotation. Hypoplasia of bilateral deltoid muscle, biceps brachii and triceps brachii was apparent (Figure 1a–c) and webbed skin folds could also be noticed at her bilateral axillae, volar aspects of elbow joints and the base of interphalangeal space (Figure 1c,d). Both hands were ulnar deviated at wrist joint. Disrupted dermatoglyphic patterns were conspicuous with total absence of distal phalangeal flexion creases (Figure 1e). The voluntary movements of her bilateral upper arms were severely compromised and only confined to slight shoulder abduction, adduction and flexion, slight elbow extension, and limited forearm supination. Her hand functions were also seriously vitiated due to severely restricted range of motion in both wrist joints and limited extension and adduction of all digits. No tendon reflexes (biceps, triceps, and brachioradialis) could be induced. Deep and superficial sensation remained intact. Besides, the patient was apparently short in statue (121.4 cm; −4.54 SDS) and each of her developmental milestones was delayed according to her parents. She also presented a myopathic facial appearance. However, she had as normal intelligence quotient as her peers. All her vital signs were normal during her hospitalization.

3.2 | Radiologic findings

X-rays of bilateral upper arms revealed an enlarged epiphysis of greater tuberosity. A full-length spine plain film showed loss of normal cervical lordosis and upper thoracic kyphosis. Mild thoracic scoliosis was also detected with the Cobb angle of 18 degrees. An enlargement of intervertebral foramen was also apparent. Osteoporosis was evident on all her X-ray films (Figure 2a).

MRI of her upper limbs illustrated a generalized replacement of myofibers with fibroadipose tissue in her bilateral upper arms (Figure 2c,d). And the cervical spine MRI also showed herniation of intervertebral disc C4-7 with impingement of the spinal cord of related segment (Figure 2b). However, no apparent change in signal intensity of the cord was noticeable at related level on T2WI images. The MRI also found a retention cyst of the sphenoid sinus, but currently no evidence would support its relevance to her upper limb deformities.

3.3 | Laboratory evaluations and other findings

Biochemical blood test revealed normal serum levels for electrolytes, liver transaminases, ALP, CK, lactic acid, ammonia,
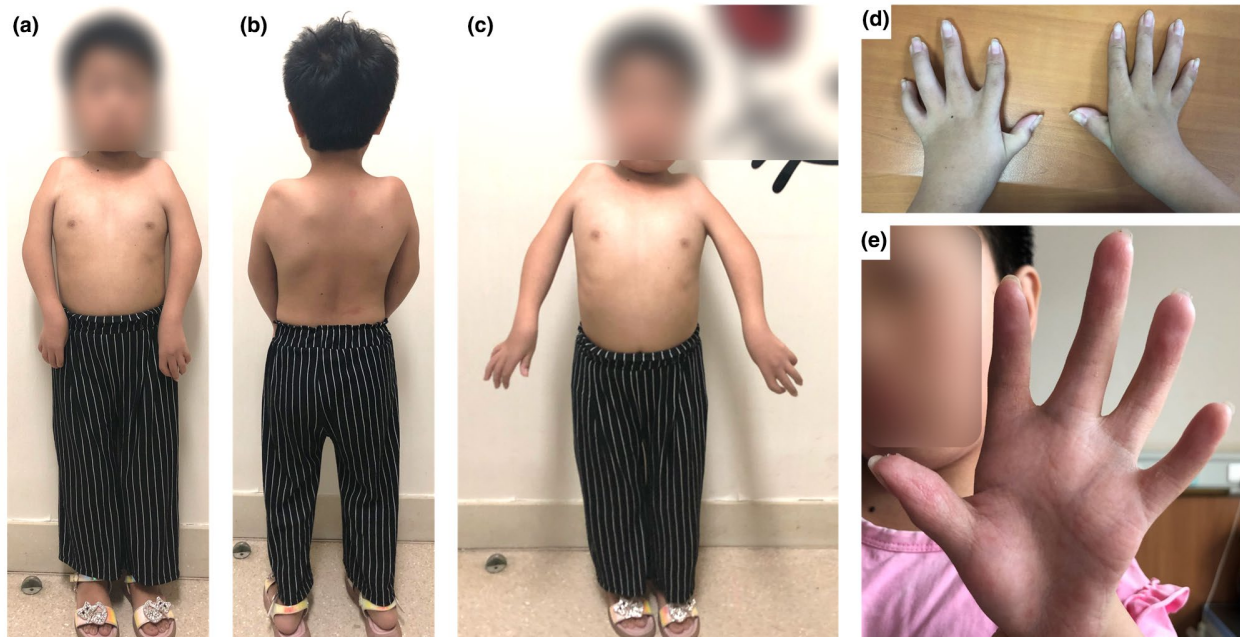
FIGURE 1 (a and b) The gross appearance of the patient at the age of 12. Note the bilateral internal rotation and muscle wasting of the upper arms and forearms, apparent hypoplasia of bilateral deltoid muscle, biceps brachialis and triceps brachialis. Both hands were ulnar deviated at wrist joint. (c) The patient was requested to elevate her upper extremities. Note the restricted ROM of her shoulder and elbow joint. Also, the web-like structures at her bilateral axillae and volar aspects of elbow joints were manifest. (d) Note the web-like structures at the base of interphalangeal space. (e) Disrupted palmar creases with total absence of distal phalangeal flexion creases

and vitamins. CBC test and coagulation were also normal. Her preoperative electrocardiogram supported the diagnosis of sinus arrhythmia, but a cardiac ultrasound performed later excluded any detectable structural abnormalities in her heart. Additionally, no positive findings had been reported in her gynecologic or upper abdominal ultrasonography. Finally, she had normal vital signs including blood pressure, heart rate, respiratory rate, and oxygen saturation.

3.4 | Electromyography and neuromuscular biopsy

The electromyography was performed on the left upper extremity. MUAPs of the left deltoid (innervated by axillary nerve derived from C5, 6) had a significant increase in percentage of polyphasic potentials, amplitudes, and duration (55.6%; 2609 μV; 21.5 ms). And nerve conduction studies of left MCN, AN, and RN showed decreased motor amplitude. Neuromuscular pathology of her deltoid muscle demonstrated fiber size variability without any infiltration of inflammatory cells or signs of myofiber necrosis using hematoxylin and eosin (HE) staining. Extensive infiltration of adipose tissue was also noticeable in the sample (Figure 3a,b). Staining with adenosine triphosphatse (ATPase, pH=4.3) showed type 1 myofiber predominance in the absence of apparent type 2 myofibers. And all type 1 myofibers were similar in size (Figure 3c,d). These findings are consistent with neurogenic muscular atrophy.

3.5 | Whole-exome sequencing (WES) and copy number variation (CNV) analysis

Whole-exome sequencing (WES) was performed for this patient and it implied suspicious heterozygous deletion mutation in chromosome X 2670202–52240626. And the deletion in between contained 315 genes, some of which were pathogenic in different diseases. But considering the limitation of WES in detecting large genetic segment deletion, copy number variation (CNV) sequencing was ordered. CNV-seq confirmed the heterozygous Xp11.22–Xp22.33 deletion, 49.93 Mb in length, which was in conformity with the WES result (Figure 4). Paternity and maternity were confirmed in this patient. The deletion was absent in both parents with confirmed maternity and paternity.

4 | DISCUSSION

In this study, we present the clinical findings and supplementary exams of a female proband which strongly support the diagnosis of congenital cervical spinal muscular atrophy. Flaccid paralysis confined to bilateral upper extremities and the electromyography results of chronic neurogenic change is indicative of lower motor neuron lesion confined to the cervical cord. The absence of sensory deficits and the normal motor nerve conduction velocities suggest abnormality at the anterior horn cell level. Eventually, reinnervation and
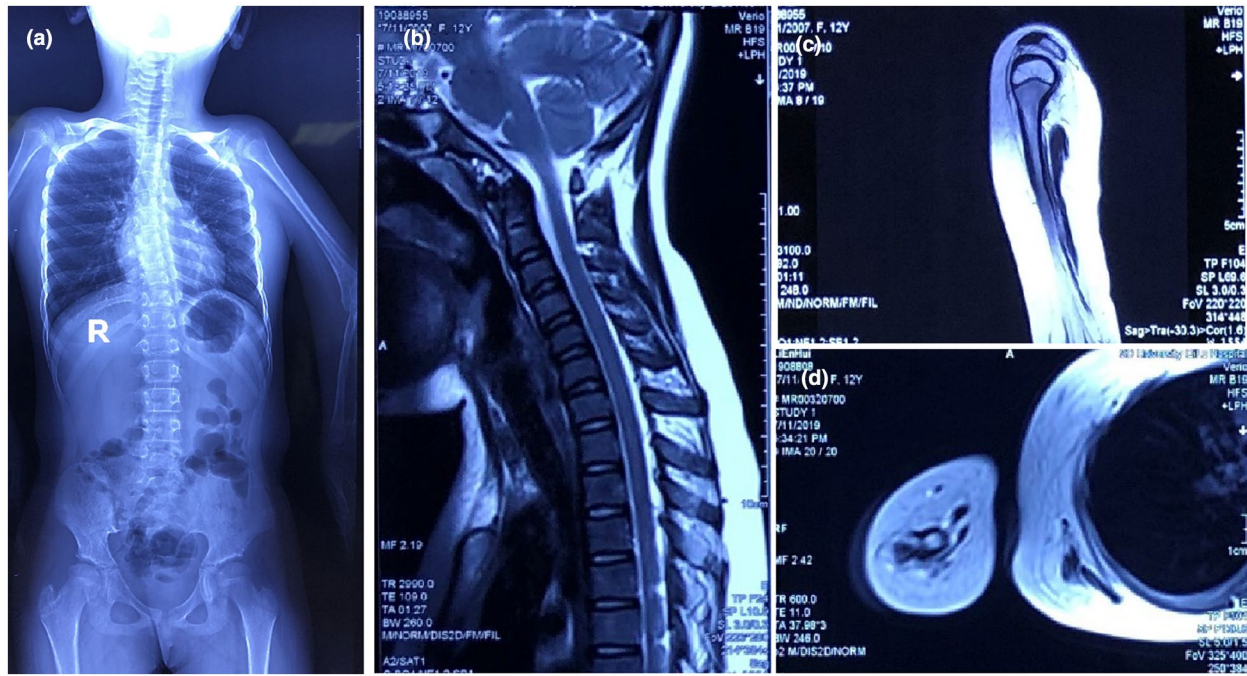
FIGURE 2 (a) Full-length spine plain film showed loss of normal cervical lordosis and upper thoracic kyphosis. Mild thoracic scoliosis was also detected with the Cobb angle of 18 degrees. An enlargement of intervertebral foramen and osteoporosis was also apparent. (b) The cervical spine MRI showed herniation of intervertebral disc C4-7 with impingement of the spinal cord of related segment without apparent change in signal intensity of the cord on T2WI images. A retention cyst of the sphenoid sinus could be noticed. (c and d) MRI of her upper limbs illustrated a generalized replacement of myofibers with fibroadipose tissue in her bilateral upper arms

hypertrophy of muscle fibers took place, which was confirmed by the neuromuscular histology in this case. Thus, came the diagnosis of congenital cervical spinal muscular atrophy (CCSMA).

The concept of CCSMA was initially raised in 1981 by Darwish et al, who reported three patients with nonprogressive, sporadic, severe symmetric lower motor neuron deficit in the upper extremities (Darwish et al., 1981). Since then, only few sporadic cases of similar conditions have been reported all over the world. Consistent with those previously reported cases of CCSMA, in our case, the upper limbs were disproportionately affected and the degree of wasting was often out of proportion to the degree of weakness. The marked degree of upper-limb muscle involvement was also evident on muscle MRI, which showed a characteristic pattern of muscle fat replacement and selective hypertrophy similar to that seen in patients with SMA. Previously, it is speculated that in utero vascular insult to the cervical spinal cord or transient intrauterine hypotension (Kaiboriboon & Hayat, 2001) might be a plausible cause to this disease, since the reported cases are all characterized by a nonprogressive course with no family history and mere localized involvement of cervical spinal cord. But no further direct evidence has been offered to support this vascular injury theory. Besides, since there is always an identifiable pathogenic gene in most non-5q SMA (Savas et al., 2015), for example, *DYNC1H1* (MIM 600112), *IGHMBP2* (MIM 600502),

and *VRK1* (MIM 602168), it is not surprising that we linked CCSMA to a genetic etiology, which has not been reported in any of those previous cases.

Modern genetic sequencing techniques enable us to discover a 49.93 Mb Xp11.22–22.33 deletion containing 315 genes in this patient. Later, by further examining the basic information of all the 315 genes in the database of OMIM and GeneCards, we find that *UBA1* gene is highly in conformity with the phenotypes of this girl. E1 ubiquitin-activating enzyme (UBA1) is a critical protein that is involved in the ubiquitin-proteasome system (UPS) and autophagy, which is vital for maintaining intracellular protein hemostasis (Chang et al., 2013; Moudry et al., 2012). It is encoded by the *UBA1* gene, which is a large 22 Kbp gene with 26 exons and contains an inactivation escape element 1 (INE1) adjacent to exon 15, enabling it to escape lyonization (Hunter et al., 2015).

Mutations in *UBA1* gene are found to cause X-linked infantile spinal muscular atrophy (XL-SMA), which is characterized by congenital muscle hypotonia and arthrogryposis multiplex congenital, dysmorphic facial features like myopathic facies (Dressman et al., 2007; Hunter et al., 2015). Additionally, lower motor neuron lesion that could be traced to the anterior horn is also a pathognomonic feature of most spinal muscular atrophy. These characteristics are highly in accordance with the phenotypes of the girl, thus, making it quite reasonable to suspect the pathogenicity
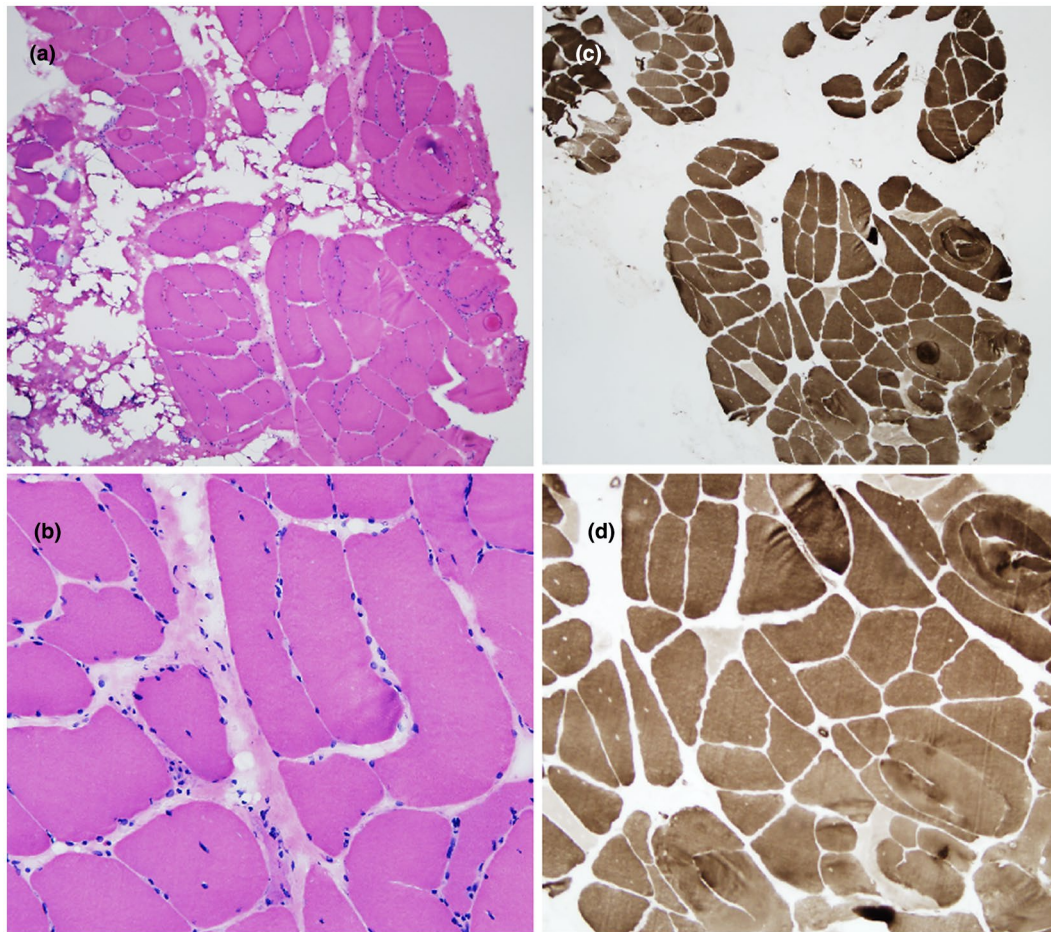
FIGURE 3 Biopsy from the deltoid muscle. (a and b) HE staining showed fiber size variability with extensive infiltration of adipose tissue. No infiltration of inflammatory cells or signs of myofiber necrosis was noted. (c and d) ATPase staining showed predominance of type 1 myofibers of similar size.
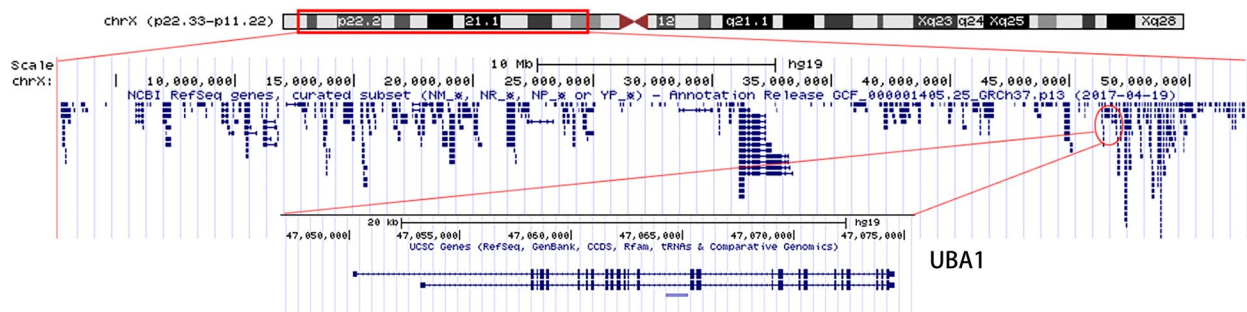

FIGURE 4 Map of del (X)p (11.22-22.33) and the location of UBA1 gene in this reported case

of *UBA1* gene in this case. Besides, by searching Decipher database we also found that some females with Xp deletion containing *UBA1* gene showed features that might be related to dysfunctions of *UBA1* gene, such as muscular hypotonia, pectus carinatum, scoliosis, and short chin (Decipher ID 284367).

The Xp11.22–22.33 deletion corresponds to Xp21 contiguous gene deletion syndrome which is characterized by complex glycerol kinase deficiency (GK), adrenal hypoplasia congenital (NROB1), intellectual disability, and/or Duchenne muscular dystrophy (DMD), mostly seen in males (Baranzini et al., 1997). The clinical features depend on the size of the deletion, as well as on the number and the nature of the encompassed genes (Heide et al., 2015). Among those reported Xp deletions, short stature is the most consistent presentation. However, apart from

her short stature in this case, the patient did not present any characteristics of those listed syndromes. The absence of features in Xp21 contiguous gene deletion allows us to speculate that the normal X chromosome is active, whereas the deleted X chromosome is preferentially inactivated, resulting in skewed X chromosome inactivation (XCI). However, chances are that the X chromosome with the deleted segment is active while its normal counterpart is inactivated. Such a possibility could not be ruled out unless all the missing 315 genes in this segment could be proved to escape XCI, which is currently beyond our ability to verify.

Since *UBA1* gene is not subject to X chromosome inactivation (Carrel et al., 1996), females have two active copies of the gene. So, theoretically, one would assume that a female with a heterozygous deletion of *UBA1* gene, as is in this case, is not supposed to present any symptoms related to this gene. And it is true that no aberrant phenotype has been observed and reported so far in female carriers of *UBA1* mutations (Ramser et al., 2008). However, it should be noted that those identified mutations were mainly missense mutation or synonymous substitution, resulting in merely partial function loss. None of those reported cases had a complete deletion of *UBA1* gene, as is in this case, in either male or female.

Phenotype controlled by *UBA1* gene is, indeed, sensitive to gene dosage. *UBA1* is an essential and nonredundant gene expressed and required by all cell types from yeast to man, as deletion of the *UBA1* gene has been shown to be lethal in lower eukaryotic species (Groen & Gillingwater, 2015; Kulkarni & Smith, 2008; Lambermon et al., 2002) and, thus, is presumed likewise in humans (Balak et al., 2017). Liu and Pfleger (2013) presented studies in female Drosophilae showing that loss-of-function mutations in mere one allele of the *UBA1* gene was adequate to drastically vitiate their life span. They also reported flies homozygous for null mutations in E1 do not survive, but flies homozygous for hypomorphic *UBA1* mutations can survive to adulthood at a very reduced rate, and these flies show a number of patterning abnormalities and severe motor impairment. Besides, their life span is dramatically reduced compared to heterozygous mutants and wild-type controls. The dosage effect of some genes which can escape X chromosome inactivation has been well demonstrated in some neurological phenotypes such as seizures and Autism Spectrum Disorder (Shoubridge et al., 2019). In conclusion, we speculate that the segment deletion on the inactivated X chromosome caused a significant decrease in the *UBA1* expression level, and thus, eventually, resulted in the characteristic phenotype of this girl. Nevertheless, we should not neglect the other possible scenario, in which the normal, intact X chromosome was inactivated, considering the *UBA1*'s capability to escape XCI. However, no matter which chromosome was inactivated in this case, the dosage reduction of *UBA1* gene caused by the deletion might be a relatively sensible underlying pathogenic mechanism for this patient at present.

Finally, researchers also indicated that some escape genes followed a varied pattern of expression in different part of the brain (Fang et al., 2019). Xu et al. (Fang et al., 2019; Xu et al., 2008) demonstrated that female mice had significantly higher levels of *KDM6A* (MIM 300128) expression in most brain regions except in the amygdala. So, the spatially different expression pattern of *UBA1* might be a plausible explanation for the unique involvement of cervical myotomes in this case.

5 | CONCLUSION

Altogether, these results suggest that insufficient dosage of *UBA1* gene is a promising explanation for this CCSMA scenario. The lack of expression of *UBA1* that normally escapes X-inactivation may compromise anterior horn cells in cervical cord.

In summary, we report a case of congenital cervical spinal muscular atrophy with Xp deletion. We also manage to identify a suspected pathogenic gene, *UBA1*, on this deleted segment that is highly associated with all major clinical features of this patient. Therefore, this study may give us a hint that there might be a genetic cause for cervical spinal muscular atrophy other than vascular etiology. To move a step further, according to this study, there might even be a certain underlying connection between CCSMA and X-linked infantile spinal muscular atrophy that exists in female heterozygous loss of function mutation. On the contrary, further investigation and more cases are required to cast light on the intricate interactions between other genes in this deleted segment and their roles in the phenotype of mere upper-limb involvement and skewed X-inactivation in female with such an X-linked defect.

CONFLICT OF INTEREST
The authors have no conflict of interest.

DATA AVAILABILITY STATEMENT
All data presented above could be provided if the corresponding author receives a formal request. The data are not available publicly due to privacy and ethical restrictions.

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