

CASE REPORT

Prenatally diagnosed distal 16p11.2 microdeletion with a novel association with congenital diaphragmatic hernia: a case report

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Introduction

Congenital diaphragmatic hernia (CDH) is a fetal abnormality affecting diaphragm and lung development, which causes a herniation of abdominal organs into the thorax. It is a life-threatening severe condition affecting ~ one of 3000 newborns with a survival rate of ~67% [1]. Approximately 50–80% of CDHs are diagnosed in the prenatal period through ultrasonographic findings of abdominal organs in the thorax, shift of the heart on the right and shift of mediastinum. Cystic adenomatoid malformation of the lung, pulmonary sequestration, bronchogenic cysts, and pulmonary hypoplasia/agenesis needs to be considered in differential diagnosis.

Congenital diaphragmatic hernia may occur as an isolated defect, but ~40% of CDH cases are nonisolated and have at least one additional anomaly, such as intrauterine growth retardation (IUGR) and/or other malformations

Key Clinical Message

A prenatal case presenting with congenital diaphragmatic hernia (CDH) and distal 16p11.2 microdeletion suggests two possible causative hypotheses: (1) a functional effect of chromatin loopings between the distal and the proximal 16p11.2 microdeletion traits, associated with CHD; (2) a possible role of *ATP2A1*, a deleted gene involved in diaphragm development.

Keywords

16p11.2 deletion syndrome, array-CGH analysis, congenital diaphragmatic hernia, prenatal diagnosis, ultrasound fetal anomalies.

[2]. CDH is etiologically heterogeneous; however, more than 50 different genetic causes have been associated with CDH. Chromosome aneuploidies, large chromosome deletions/duplications, and complex chromosome rearrangements identifiable by karyotype are present in 10–35% of CDH cases and occur at greatest frequency in nonisolated, prenatally diagnosed cases [3]. An additional 3.5–13% of cases without identifiable karyotype abnormalities have copy number variations (CNVs) detectable by chromosome microarray analysis [4]. A CNV associated with CDH maps to 16p11.2 (from ~29.5 Mb to ~30.1 Mb [hg18]), spans approximately 600 kb and causes the proximal 16p11.2 microdeletion syndrome, characterized by developmental delay, autism spectrum disorder, epilepsy, and obesity [5]. This deletion is functionally different from the more distal 16p11.2 220 kb-microdeletion, which may cause developmental delay, behavioral problems, and mild facial dysmorphisms [6].

We here report a case of isolated CDH in a fetus at 18 gestational weeks carrying the distal 16p11.2 microdeletion, diagnosed by high-resolution Comparative Genomic Hybridization array (a-CGH).

Case Report

A 27-year-old woman was referred to our unit at 18 weeks of gestation for fetal malformation. Her husband was 29 years old. The couple had a 3-year-old healthy daughter, and there was no family history of congenital malformations. Ultrasound examination showed an isolated left CDH. The stomach was located in left hemithorax with the hearth in dextroposition but keeping levocardia (Fig. 1). No other malformations were detected.

High-resolution a-CGH analysis was performed on DNA extracted from cultured amniocytes using CGX™-HD v1 4x180K chip (PerkinElmer, Waltham, MA), following manufacturer's protocols. This analysis revealed a *de novo* microdeletion at 16p11.2, spanning from 28,833,437 bp to 29,046,252 bp (Fig. 2A and B). Genoglyphix® software (Signature Genomics, Spokane, WA), referring to GRCh37/hg19 Genome Assembly, was used to analyze the data.

The microdeletion was confirmed by FISH analysis with the 16p11.2 ATXN2L-LAT probe (Agilent Technologies Inc. Santa Clara, CA) mapping to the region (Fig. 2C). CEP16 (green labeled) was used as control probe. The analysis also revealed that the parents were unaffected. Conventional cytogenetics revealed a normal male karyotype in the proband and his parents.

After genetic counseling the couple, informed about the increased risk of intellectual disability and developmental delay, opted for continuing the pregnancy. Unfortunately, the baby died shortly after birth from



Figure 1. Ultrasound examination at 18 gestational weeks. The stomach (ST) was herniated in left hemithorax with the hearth (H) in dextroposition. A little of small bowel (*) herniated through the defect also was present in thorax.

respiratory distress. For this reason, no postnatal follow-up was possible.

Discussion

The majority of CDH cases are sporadic, with the recurrence risk for isolated CDH typically quoted as <2% based on a model of multifactorial inheritance. Although CDH is etiologically heterogeneous, more than 50 different genetic causes have been associated with it. Indeed, most of them were described only in few cases. Genome-wide screening for candidate CNVs identified many loci scattered throughout the genome that are recurrently deleted or duplicated in individuals with CDH. Each genomic region associated with this defect is likely to harbor one or more CDH-related genes. For some of these regions, a specific gene has been already proposed [7, 8].

A dosage imbalance (either gain or loss) of the T-box 6 (*TBX6*) gene, which maps within the critical region for proximal 16p11.2 ~600 kb-microdeletion, has been proposed to contribute to the development of CDH [5]. It encodes a transcription factor involved in several developmental processes such as paraxial mesoderm structural differentiation. Accordingly, the proximal 16p11.2 recurrent microdeletion has been associated with CDH [9–11]. This deletion, defined by the breakpoints BP4 and BP5, has a population prevalence of approximately 1/2000 [12]. It is functionally different from the less frequently reported more distal 16p11.2 region, which is defined by the breakpoints BP2–BP3 (~28.74 Mb to ~28.95 Mb [hg18]) and spans approximately 220 kb. The microdeletion of this trait is associated with a highly penetrant form of isolated severe early-onset obesity referred to the *SH2B1* gene as well as with intellectual disability and developmental delay [6]. CDH has never been described so far in such a deletion.

We here report a case of a fetus with CDH in whom a *de novo*, distal 16p11.2 BP2–BP3 microdeletion was detected. No other genome CNVs have been observed. The deletion did not involve *TBX6*, even though it cannot be excluded that a regulatory region mapping to the 16p11.2 distal region may modulate the expression of this gene. This hypothesis is corroborated by the demonstration that gene-rich BP2–BP3 and BP4–BP5 16p11.2 intervals, whose CNVs are linked to overlapping phenotypes, are reciprocally engaged in complex chromatin loopings, which may orchestrate cotranscription of interacting genes [13].

It is also plausible to speculate that a dosage imbalance of genes mapping to this chromosome trait might induce anomalies in the diaphragm development. The deleted region here described encompasses nine known protein-coding genes: *ATXN2L*, *TUFM*, *SH2B1*, *ATP2A1*,

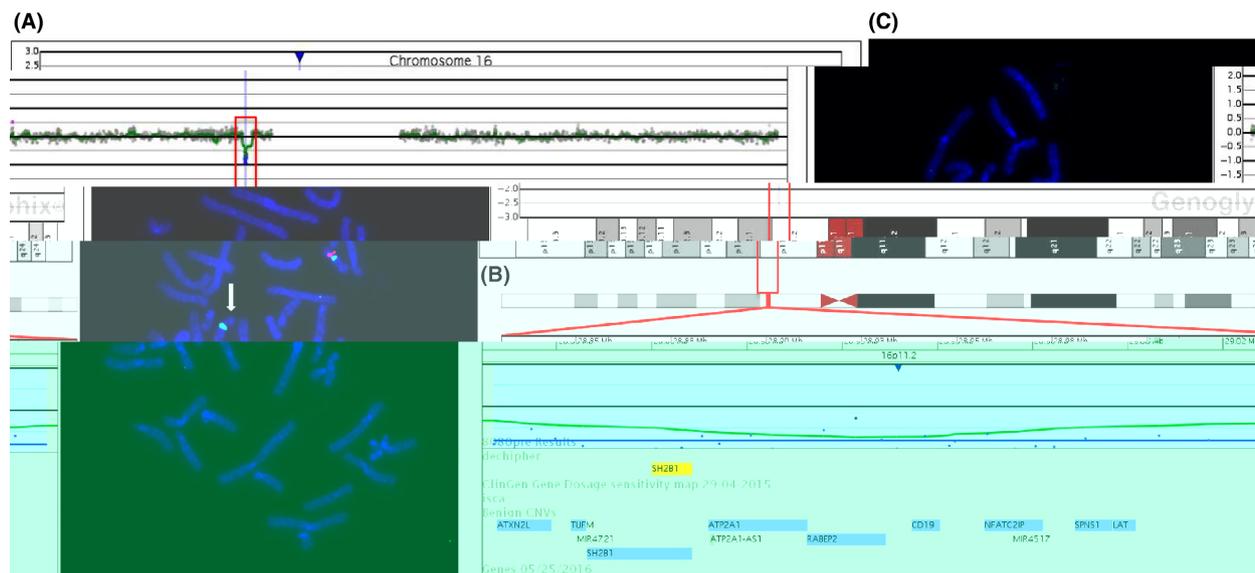


Figure 2. (A) High-resolution array-CGH analysis showing a ~220 kb-microdeletion in the 16p11.2 region from 28,833,437 bp to 29,046,252 bp [hg19]. (B) This region encompasses nine known protein-coding genes: *ATXN2L*, *TUFM*, *SH2B1*, *ATP2A1*, *RABEP2*, *CD19*, *NFATC2IP*, *SPNS1*, *LAT*, and the *hsa-MiR-4517*. Copy number variations were classified according to the Database of Genomic Variants (<http://dgv.tcag.ca/dgv/app/home>), the Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (<http://decipher.sanger.ac.uk/>), and the UCSC Genome Browser (<http://genome.ucsc.edu/cgi-bin/hgGateway>). (C) FISH analysis with the Agilent SureFISH 16p11.2 ATXN2L-LAT probe (Agilent Technologies Inc. Santa Clara, CA) mapping to the deleted 16p11.2 region (red signal). Centromeric probe for chromosome 16 was used as control probe (green signal). Only one red signal was detected in each metaphase of the proband, thus confirming the array-CGH result. White arrow indicates the deleted chromosome.

RABEP2, *CD19*, *NFATC2IP*, *SPNS1*, *LAT*, and the *hsa-MiR-4517*. In particular, we focused on one of these genes, namely *ATP2A1/SERCA1* (OMIM: 108730), as a possible candidate for CDH development. This gene encodes the isoform 1 of the sarcoplasmic reticulum Ca^{2+} -ATPase. Mutations in this gene cause Brody disease, characterized in humans by muscle anomalies which cause exercise-induced contraction of fast twitch (type II) skeletal muscle fibers. Targeted disruption of the *ATP2A1* gene affects diaphragm muscle development in neonatal mice causing a variation in diaphragm fiber size [14]. Moreover, a dramatic increase of *SERCA1b* expression, demonstrated in *in vivo* regenerating muscles, suggests that the expression of this muscle *ATP1A2* isoform may play a role in skeletal and diaphragm muscle growth and development [15].

As already proposed for different microdeletion syndromes [16], this case makes possible to hypothesize that CDH may be a rare phenotypical trait in the spectrum of 16p11.2 distal deletion syndrome and that it is likely not detected postnatally in this syndrome due to high prenatal mortality of CDH-affected fetuses. It is also conceivable that *ATP2A1* haploinsufficiency could be responsible for diaphragm malformations only when combined with allelic variants of other genes involved in diaphragm development. More cases are needed to validate this hypothesis.

Of course, we also take into account the possibility that the presence of this the novo deletion is not the cause of CDH in this fetus but it is just a coincidence.

The systematic use of high-resolution a-CGH is proving helpful in identifying new dosage-sensitive genes involved in human CDH. The identification of a genetic cause for CDH provides important information about prognosis, management, and recurrence risk. This would suggest to recommend, according to guidelines, high-resolution a-CGH analysis in pregnancies with any abnormal ultrasound finding, including isolated CDH. This step together with ultrasound investigations will allow better characterization of individual pathologies in view of possible prenatal or postnatal management interventions.

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Authorship

RG: was the molecular cytogenetics supervisor and drafted the manuscript. GMM, GC, and AS: managed the

pregnancy and ultrasound analyses. AM: carried out the molecular cytogenetics analyses. RC and VS: contributed to cytogenetics analyses. AI: contributed to establish a genotype–phenotype correlation. AC: is the corresponding author. She contributed to draft the manuscript. PM and LN: supervised and critically revised the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

None declared.

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