

Prenatal diagnosis of CHARGE syndrome in a fetus with a novel CHD7 splicing mutation: A case report

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Received November 18, 2025; Accepted June 25, 2026

DOI: 10.3892/etm.2026.13331

Abstract. CHARGE syndrome is a rare genetic, multisystem disorder with a number of malformations associated with coloboma, heart defects, choanal atresia, retardation of growth and/or development, genitourinary malformation and ear abnormalities. The primary causes of CHARGE syndrome are mutations in the chromodomain helicase DNA binding protein 7 (*CHD7*) gene. Therefore, the present report outlines the case of a fetus with a variety of ultrasound findings, including persistent left superior vena cava (LSVC), delay in the fusion of the cerebellar vermis, inferior vermis hypoplasia and borderline mega cisterna magna (MCM). Fetal exome sequencing revealed the presence of the novel c.2836-3C>G splicing mutation in *CHD7*, which was classified to be likely pathogenic. Genetic testing of the parents showed that neither were carriers of the mutation, suggesting a *de novo* origin. Due to the poor prognosis resulting from the combination of clinical findings and the identification of the novel, likely pathogenic mutation, the couple opted for pregnancy termination following genetic counseling. The present report includes the description of the fetal sonographic features and MRI findings. To the best of our knowledge, this is the first documented case of persistent LSVC and borderline MCM observed in a fetus with CHARGE syndrome. Since prenatal diagnosis of

CHARGE syndrome is rare, the present report may serve as a valuable reference for evaluating similar findings in other prenatal cases.

Introduction

CHARGE syndrome is a rare genetic, multisystem disorder with numerous malformations and distinctive diagnostic criteria. The term 'CHARGE' describes a group of clinical features that include coloboma, heart defects, choanal atresia, retardation of growth and/or development, genitourinary malformation and ear abnormalities (1). A renewed definition of the diagnostic criteria for CHARGE syndrome was proposed by Blake *et al* (2) and later updated by Verloes (3). According to the most recent criteria, the major diagnostic features are coloboma, choanal atresia and hypoplasia of the semicircular canals. Minor diagnostic features include rhombencephalic dysfunction (brainstem dysfunction and cranial nerve III-XII anomalies, including neurosensory deafness), hypothalamo-hypophyseal dysfunction (including growth hormone and gonadotrophin defects), ear malformation (internal or external), malformation of mediastinal organs (such as the heart and esophagus) and mental retardation.

The primary causes of CHARGE syndrome are mutations in the chromodomain helicase DNA binding protein 7 (*CHD7*) gene (accession no. NM_017780.4), which encodes a protein that is part of the chromodomain helicase DNA-binding (CHD) protein family (4). The CHD protein family is a highly conserved group of nuclear proteins that consists of nine members in vertebrates. The functions of this protein family are diverse; however, all proteins in this family serve a role in transcriptional regulation (5-7). *CHD7* in particular functions as an ATP-dependent nucleosome remodeler (8). In order to remodel nucleosomes, *CHD7* functions as part of a multiprotein complex, binding to <10,000 sites across the mammalian

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Key words: fetal exome sequencing, next-generation sequencing, prenatal diagnosis, CHARGE syndrome, case report

genome. CHD7 is also an important regulator of developmental transcription factor genes and signaling pathways (such as *p53* and *SOX2*) (9,10).

Therefore, the present report outlines the case of a fetus who exhibited a number of ultrasound findings, including persistent left superior vena cava (LSVC), delay in the fusion of the cerebellar vermis, inferior vermis hypoplasia and borderline mega cisterna magna (MCM). To further investigate these findings, fetal exome sequencing was performed in the present study, revealing a novel mutation in *CHD7*.

Case report

In January 2024, a 30-year-old pregnant woman (gravida: 1 and parity: 0) was referred to Access to Genome P.C. (Athens, Greece) following the detection of increased nuchal translucency at 12 + 1 weeks (3.13 mm). The personal and family medical history of the patient was unremarkable.

Chorionic villi sampling (CVS) was performed at 12 + 1 weeks. DNA was extracted from the CVS sample and high-resolution chromosomal microarray analysis was performed using the SurePrint G3 CGH ISCA v2 Microarray Kit, 8x60K (cat. no. G5955A; Agilent Technologies). The analysis resolution of this platform is ~500 kb along the genome and ~50 kb in ~240 regions of known clinical importance which are associated with ~512 genetic diseases, according to the International Standard Cytogenetic Consortium (11). This analysis showed a normal male genomic pattern with no imbalances.

During a routine ultrasound examination performed at 18 + 1 weeks, the presence of persistent LSVC was observed (Fig. 1). At 21 + 6 weeks, a fetal brain MRI was performed, exhibiting borderline enlargement of the cisterna magna (essentially, MCM; 10.5 mm; Fig. 2A). Delay in the fusion of the cerebellar vermis was also observed (Fig. 2A). Marked upward rotation of the cerebellar vermis axis was not observed. The fourth ventricle appeared to have lost its triangular shape. The normal communication of the 4th ventricle with the cisterna magna was occluded. Elevation of the cerebellar tentorium was not observed. In a sagittal section, the primary fissure of the cerebellar vermis was recognized, while the ratio of the anterior to the posterior lobe of the vermis appeared to be disturbed and equalized. The inferior part of the cerebellar vermis appeared to be thin and small. Cerebellar vermis biometry was 12 mm in height and 5 mm in anteroposterior diameter (normal values for this gestational age are 8.5-11.5 and 5.5-8 mm, respectively) (12). Inferior vermis hypoplasia (Dandy-Walker variant) was observed without ruling out atypical vermis hypoplasia of Joubert Syndrome (Fig. 2A). Examination of other anatomical structures of the fetus revealed the presence of a small-sized stomach, as well as mild prenasal and nuchal edema (Fig. 2B). At 23 + 5 weeks, a level II ultrasound was performed, which further demonstrated the MRI findings. Specifically, it demonstrated the borderline MCM and inferior vermis hypoplasia with delay in the fusion of the cerebellar vermis (Fig. 3A), the mild prenasal edema (Fig. 3B) and the small-sized stomach (Fig. 3C).

Although microarray analysis, performed following the detection of increased nuchal translucency, appeared normal, the presence of multiple sonographic and MRI findings raised

concern for an underlying genetic condition and fetal exome sequencing was therefore performed on fetal DNA from the CVS sample. DNA was extracted from the CVS sample using the NucleoSpin Tissue kit (Macherey-Nagel GmbH & Co. KG), according to the manufacturer's instructions. Exome amplification and library preparation was performed using the VAHTS Universal Plus DNA Library Prep Kit for Illumina V2 (cat. no. ND627-01; Illumina Inc.), according to the manufacturer's instructions. The samples were qualified using the Qubit Fluorometric Quantification system (Thermo Fisher Scientific, Inc.). Next-generation sequencing was performed using the DNBSEQ-T7 MGI Tech genetic analyzer (MGI Tech Co., Ltd.) according to the manufacturer's instructions, using paired-end 150 bp reads (2x150 bp). The loading concentration of the final library was 10 pM. Data analysis was performed using the VarSome Clinical (Saphetor SA) bioinformatics platform (v11.18.0; <https://clinical.varsome.com/>) (13) and SpliceAI (version 1.3.1; <https://spliceailookup.broadinstitute.org/>) (14), and was performed for all genes that are associated with known genetic diseases and syndromes, according to the Online Mendelian Inheritance in Man database (<https://www.omim.org/>). All findings from the aforementioned analysis were evaluated in accordance with the international literature and the American College of Medical Genetics and Genomics (ACMG) guidelines (15). The reference genome was UCSC hg19. Bioinformatics analysis revealed the presence of the novel c.2836-3C>G splicing mutation in *CHD7*. According to the ACMG guidelines, this mutation is categorized as likely pathogenic based on the PP3 (multiple lines of computational evidence support a deleterious effect on the gene or gene product), PM2 (absent from controls or at extremely low frequency in the gnomAD database), and PS2 (*de novo* occurrence in a patient with the disease and no family history with both maternity and paternity confirmed) criteria (15).

Sanger sequencing was performed in the fetus and the parents in order to determine the presence of the c.2836-3C>G mutation and to establish whether the mutation was inherited or whether it occurred *de novo*. For the fetal sample, Sanger sequencing was performed using the DNA already isolated for exome sequencing. For the parental samples, DNA was extracted from peripheral blood samples using the NucleoSpin Blood kit (Macherey-Nagel GmbH & Co. KG), according to the manufacturer's instructions. DNA amplification was performed via PCR using GoTaq DNA Polymerase (Promega Corporation). The PCR thermocycling conditions were as follows: Initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 30 sec, with a final extension at 72°C for 5 min. Following amplification, part of the *CHD7* gene was sequenced and visualized using the ABI 3500 Genetic Analyzer (Thermo Fisher Scientific, Inc.), and was then compared with the control sequence (NM_017780.4). The sequences of the primers used were: 5'-CCGGAACA GAGCGTGTGGTAA-3' (forward primer) and 5'-TGTACC CACAAATGCATACCCAAAG-3' (reverse primer). Results confirmed the presence of the novel mutation in the fetus, while it was not detected in either of the parents, indicating that this was a *de novo* mutation (Fig. 4).

In the present study, *in silico* analysis was performed using SpliceAI using genome version hg38 and default maximum

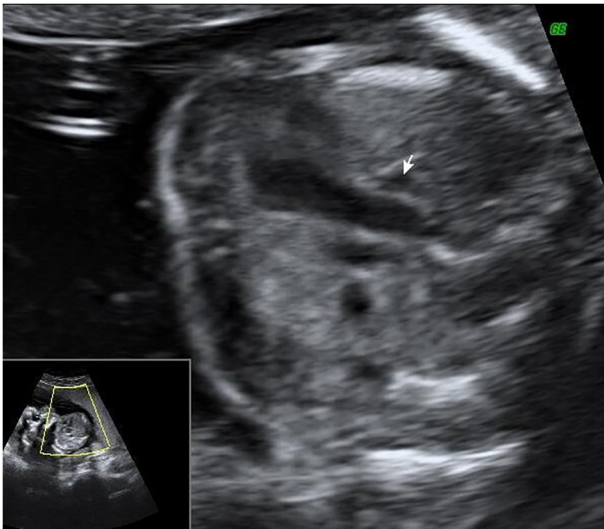


Figure 1. Ultrasound examination revealing the presence of persistent left superior vena cava (white arrow).

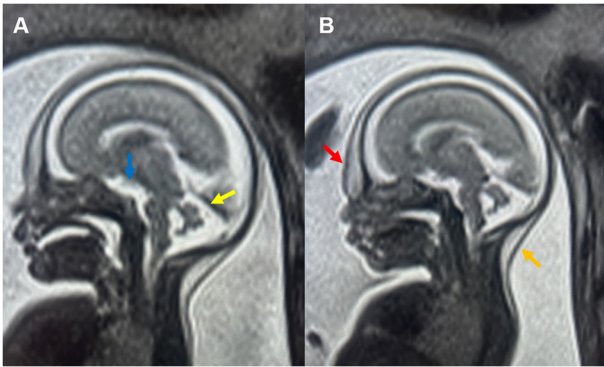


Figure 2. MRI findings of the fetus. (A) Borderline enlargement of the cisterna magna (blue arrow) and inferior vermis hypoplasia (Dandy-Walker variant) with delay in the fusion of the cerebellar vermis (yellow arrow). (B) Mild prenatal edema (red arrow) and nuchal edema (orange).

distance window of 500 bp. The SpliceAI Δ score range is 0-1, indicating the probability of a splicing effect, with high probability defined as >0.5 and highly confident defined as >0.8 . The results showed a high probability of disruption of the canonical splice acceptor site (Δ score: 0.81) and the potential activation of a nearby cryptic splice acceptor site (Δ score: 0.68). The scSNV-ADA database (v1.1, hg38) was also used via the VarSome Clinical platform. The scSNV-ADA score range is 0-1, indicating the probability of altered splicing, with supporting pathogenic defined as >0.957813 . The analysis yielded a high probability of altered splicing, with a score of 0.995936.

A thorough literature search was performed using PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) and Google Scholar (<https://scholar.google.com/>) databases to evaluate whether the phenotypic findings observed in the present case have been previously reported in other prenatal cases of CHARGE syndrome. Keywords used included 'CHD7', 'CHARGE syndrome', 'persistent left superior vena cava', 'mega cisterna magna', 'prenatal diagnosis' and other related terms. The

literature search demonstrated that the observed combination of sonographic, MRI, and genetic findings was consistent with CHARGE syndrome. Additionally, it demonstrated that, even though persistent LSVC and borderline MCM are related to CHARGE syndrome, they have not been reported in a fetus with CHARGE syndrome thus far. Due to the combination of all aforementioned clinical findings and the presence of the novel, likely pathogenic mutation in *CHD7*, the prognosis was poor due to the high likelihood of a multisystem disorder consistent with CHARGE syndrome, which is associated with a number of malformations, encompassing the major and minor diagnostic criteria detailed previously. The aforementioned literature search findings were incorporated into the genetic counseling provided to the couple; they were informed of the molecular diagnosis of CHARGE syndrome, the associated poor prognosis, the *de novo* nature of the detected mutation, and the low recurrence risk for future pregnancies. Following genetic counseling, the couple opted for termination of pregnancy, which was performed at 24+5 weeks. The couple also received post-termination follow-up care 2 weeks later, including physical evaluation, psychological support and counseling regarding future reproductive planning.

Discussion

In the present report, the case of a fetus with persistent LSVC, delay in the fusion of the cerebellar vermis, a borderline MCM, inferior vermis hypoplasia, small-sized stomach, as well as mild prenatal and nuchal edema, is outlined. Prenatal whole exome sequencing revealed the presence of the novel, splice site c.2836-3C>G mutation in *CHD7* of *de novo* origin, suggesting a CHARGE syndrome diagnosis.

As aforementioned, CHARGE syndrome is a rare multi-system disorder with a number of clinical characteristics. The majority of cases are diagnosed in the neonatal or childhood period. So far, prenatally diagnosed cases of CHARGE syndrome are limited (16). This low number of prenatally diagnosed cases may be attributed to the fact that the majority of abnormalities, especially the primary defects such as coloboma, choanal atresia and auditory deficit, cannot be visualized through prenatal ultrasound (16). Furthermore, abnormalities that may be observed prenatally may be subtle or may seem as an isolated abnormality or phenotypic diversity (16). In a retrospective study of 13 cases with CHARGE syndrome, it was observed that 2 of 13 cases (15.4%) exhibited normal ultrasound scans during pregnancy, 1 of 13 (7.7%) exhibited cystic hygroma in the first trimester and presented intrauterine fetal demise at 16 weeks and the remaining 10 of 13 cases (76.9%) exhibited abnormal ultrasound features *in utero*. Among these 10, 1 case showed increased nuchal translucency in the first trimester and 5 cases exhibited abnormal ultrasound features in the second trimester (including micrognathia, cardiac defects and facial defects). A further 4 cases showed abnormal ultrasound findings in the third trimester, which included micrognathia, isolated fetal growth restriction and polyhydramnios (17).

In the present case, there were numerous prenatal sonographic features and brain MRI findings that were consistent with CHARGE syndrome. For instance, persistent LSVC was observed at 18 + 1 weeks, a characteristic that is often observed

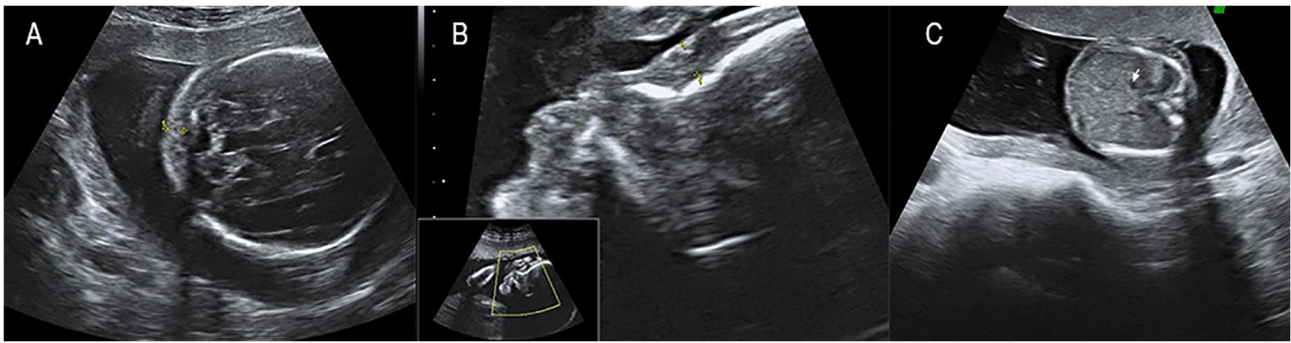


Figure 3. Sonographic features of the fetus. (A) Borderline enlargement of the cisterna magna and inferior vermis hypoplasia (Dandy-Walker variant) with delay in the fusion of the cerebellar vermis. (B) Mild prenatal edema. (C) Small-sized stomach (white arrow).

in patients with CHARGE syndrome. More specifically, in a retrospective study by Goldenberg *et al* (18) involving 8 patients with CHARGE syndrome, persistent LSVC was observed in 50% of the patients. Persistent LSVC is a congenital heart anomaly that is found in 0.3-0.5% of the general population and it can be associated with congenital heart diseases in up to 8% of cases globally (19). However, to the best of our knowledge, it has not yet been detected prenatally in fetuses with CHARGE syndrome. In addition, vermis hypoplasia was observed in the brain MRI at 21 + 6 weeks. Although it is not included in the diagnostic criteria, cerebellar vermis hypoplasia may be a clinical diagnostic feature of CHARGE syndrome (20). In a retrospective study by Sohn *et al* (20) 29.4% of the patients had cerebellar vermis hypoplasia (5 of 17 patients). There is also 1 prenatal case report of cerebellar vermis hypoplasia in a fetus with CHARGE syndrome in the international literature (21). An additional feature observed in the prenatal brain MRI at 21 + 6 weeks is borderline MCM. In a study by Shiohama *et al* (22), the brain morphology of 10 patients with CHARGE syndrome was investigated and, in 1 case, MCM was observed. The present report therefore appears to be the first case of prenatal detection of borderline MCM in a fetus with CHARGE syndrome.

As aforementioned, prenatal diagnosis of CHARGE syndrome is challenging as the characteristic features of the syndrome cannot be visualized by ultrasound. However, certain prenatal findings may serve as early indicators. Increased nuchal translucency in the first trimester may represent an early non-specific marker associated with increased risk of genetic abnormalities and warrants careful follow-up (17,23). During the second and third trimester, detailed anatomical ultrasound and, when indicated, fetal MRI, may reveal structural anomalies, including congenital heart defects and brain abnormalities (16,17). The identification of numerous anomalies, even when subtle, should raise suspicion for syndromic conditions, such as CHARGE syndrome and advanced genetic testing should be considered.

Within this context, the clinical importance of prenatal findings such as persistent LSVC or MCM should be carefully interpreted. When LSVC or MCM is prenatally detected as an isolated finding, it is often considered a benign variant when no other anomalies are present (24,25). However, when such findings co-occur or are accompanied by other subtle abnormalities, the index of suspicion for an underlying syndromic condition

should increase. In such cases, consideration of advanced genetic testing, such as exome sequencing, may be warranted.

CHARGE syndrome is primarily caused by mutations in *CHD7*, a gene that is involved in transcriptional activation and is an important regulator of developmental transcription factor genes and signaling pathways (9,10). The role of *CHD7* has also been demonstrated using animal models. Mouse mutants have been shown to develop similar abnormalities as described in CHARGE syndrome, such as inner ear, choanal, heart, eye, genital and palate defects (26). It has also been demonstrated that as in humans, normal eye development in mice is sensitive to *Chd7* haploinsufficiency (27). Further studies in zebrafish have shown that *chd7* mutants display craniofacial, cardiac, visual, auditory and locomotive defects (28).

In the present case, the novel c.2836-3C>G mutation that was detected, is located in the splicing region of intron 10 of the *CHD7* gene and, according to bioinformatic analysis tools, it is expected to affect the correct mRNA splicing. Mutations that affect the splice site region are expected to impair normal splice-site recognition and lead to aberrant splicing, such as exon skipping or activation of a cryptic acceptor site (29). For instance, *in silico* analysis using SpliceAI predicted a high probability of disruption of the canonical splice acceptor site and the potential activation of a nearby cryptic splice acceptor site. These would result in a frameshift and the introduction of a premature termination codon, potentially leading to the production of a truncated protein or triggering non-sense-mediated mRNA decay. Therefore, the detected mutation is expected to impact the form and function of the produced *CHD7* protein. Although RNA studies would be required to further demonstrate the exact splicing outcome, these predictions support a loss-of-function mechanism and, consequently, haploinsufficiency, which is consistent with the established pathogenicity mechanism for *CHD7* (30,31). Notably, to the best of our knowledge, the c.2836-3C>G mutation in *CHD7* has not been reported in the Genome Aggregation Database (<https://gnomad.broadinstitute.org/>), ClinVar database (<https://www.ncbi.nlm.nih.gov/clinvar/>) or the international scientific literature. As aforementioned, according to the ACMG guidelines, this mutation is classified as likely pathogenic based on the PP3, PM2 and PS2 criteria (15). Collectively, this suggested that the detected novel splicing mutation in *CHD7* was responsible for the sonographic and MRI findings in the present fetus.

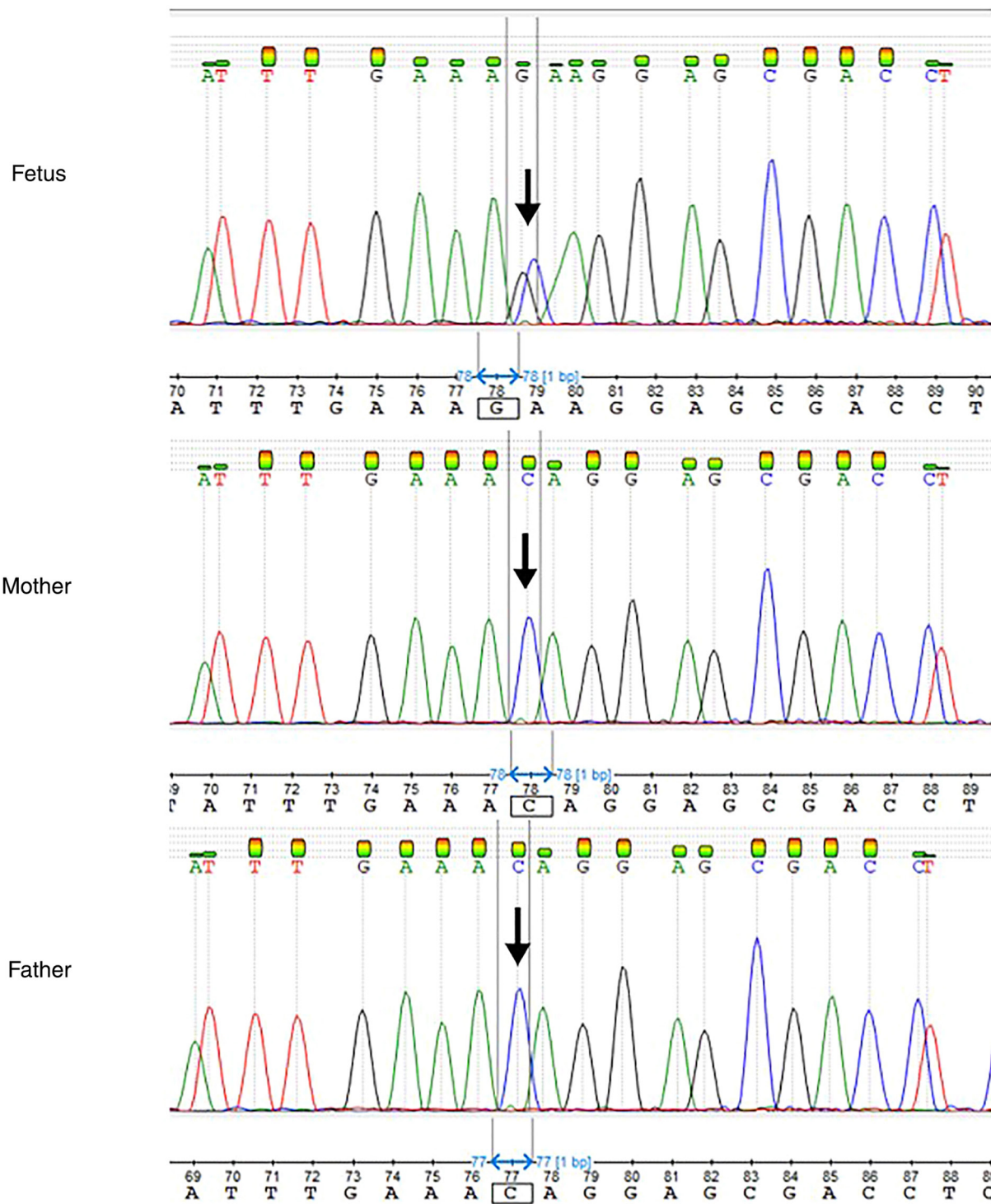


Figure 4. Sanger sequencing results determining the presence of the c.2836-3C>G mutation in the chromodomain helicase DNA binding protein 7 in the fetus. The mutation was not detected in the parents.

Overall, in the present report, the case of a fetus with CHARGE syndrome caused by a novel splicing mutation in *CHD7* is described. To the best of our knowledge, this is the first report of the likely pathogenic c.2836-3C>G mutation in *CHD7*. The present case report therefore adds to the limited number of prenatal CHARGE syndrome diagnoses. In addition, it describes the clinical characteristics and the sonographic and MRI findings of the fetus, which is of marked importance given the rarity of prenatal diagnoses

of CHARGE syndrome. The present report emphasizes the importance of integrating numerous anomalies that may appear minor or borderline into prenatal risk assessment and counseling. The co-occurrence of subtle anomalies should raise suspicion for syndromic conditions (such as CHARGE syndrome) and support comprehensive genetic testing. To the best of our knowledge, this is the first report of prenatal detection of persistent LSVC and borderline MCM in a fetus with CHARGE syndrome. Finally, the present case description

could serve as a valuable reference for evaluating similar findings in other prenatal cases and for providing genetic counseling in cases of prenatal detection of the c.2836-3C>G mutation in *CHD7*.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study may be found in the European Nucleotide Archive under accession number ERR15604719 or at the following URL: (persistent, <https://www.ebi.ac.uk/ena/browser/view/ERR15604719>).

Authors' contributions

EP was primarily responsible for the design of the present study and critically revised the manuscript. CK contributed to the design of the present study and prepared the manuscript. EM and IP were in charge of patient management, project supervision and critically revising the manuscript. ES, CE and SO performed exome sequencing and Sanger sequencing. MR, SS, GKP and MM were responsible for medical treatment and assessment. CC performed the MRI examination. MR performed the ultrasound examination. EM and IP confirm the authenticity of all raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Written informed consent was obtained from the parents for the inclusion of their data as well as the fetal data and images in the present case report. All information revealing the family members identities has been removed. All procedures followed were conducted according to The Declaration of Helsinki, as revised in 2008.

Competing interests

The authors declare that they have no competing interests.

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