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CASE SERIES

Prenatal Ultrasound and Genetic Diagnosis of *EFTUD2* Haploinsufficiency in Two Fetuses: A Case Series

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Abstract: Mandibulofacial dysostosis with microcephaly (MFDM) is caused by haploinsufficiency of *EFTUD2* gene. This syndrome is characterized by microcephaly, malar and mandibular hypoplasia, ear abnormalities, developmental delay, and intellectual disability. In this study, we report two cases of fetuses presenting a phenotype consistent with MFDM and confirmed *EFTUD2* gene variants. The patients were referred following abnormal ultrasound findings. Genetic diagnostics in both cases revealed heterozygous variants in the *EFTUD2* gene that had not been previously reported prenatally. In the first patient, exome sequencing identified a c.2698_2701del p.(Val865Serfs*34), while in the second a novel large deletion involving multiple genes, including the entire *EFTUD2* gene, was detected by microarray analysis. Prenatal diagnosis of MFDM requires precise ultrasound assessment. Therefore, consideration of characteristic features observed in fetuses with MFDM is essential for differential diagnosis and guiding targeted genetic testing.

Keywords: facial dysostoses, prenatal testing, exome sequencing, microarray

Introduction

Human facial dysostoses are a group of rare and heterogenous congenital disorders characterized by abnormalities of the bones and soft tissues of the craniofacial region. In these conditions, the development of the first and second branchial arches is disturbed.[1–3] The formation of craniofacial structures from pluripotent neural crest cells is a complex and tightly regulated process that occurs during both embryonic and fetal stages of the development. Some abnormalities are caused by deregulation of cellular signaling pathways, which may have either genetic or environmental origins.[4]

In general facial dysostoses are categorized into two main groups: mandibulofacial dysostoses (MFDs) and acrofacial dysostoses (AFDs), the latter of which are associated with limb defects.[1] One of the mandibulofacial dysostoses is mandibulofacial dysostosis with microcephaly (MFDM, OMIM # 610536) also known as mandibulofacial dysostosis, Guion-Almeida type (MFDGA). MFDM is a rare autosomal dominant multiple malformation syndrome with high penetrance and variable expressivity, with an estimated incidence at about 1 in 1,000,000 individuals. It is characterized by malar and mandibular dysplasia, microcephaly, dysplastic ears with conductive hearing loss, distinctive facial features and asymmetry, cleft palate, choanal and esophageal atresia, heart defects, and short stature. Intellectual disability and developmental delay are also prominent features of this condition.[5–7] In the differential diagnosis of mandibulofacial dysostosis with microcephaly (MFDM), several conditions should be considered, including CHARGE syndrome, Goldenhar syndrome, Feingold syndrome, and other mandibulofacial dysostoses (MFDs). Numerous overlapping phenotypic features exist between MFDM and CHARGE syndrome, such as choanal atresia, dysplastic ears, facial asymmetry, congenital heart defects, and intellectual disability. Distinguishing between these syndromes relies on careful evaluation of characteristic facial dysmorphisms, as well as the presence or absence of intrauterine growth restriction

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(IUGR) and microcephaly, which is a defining feature of MFDM. Regarding Goldenhar syndrome and MFDM, shared phenotypic features include ear dysplasia, preauricular tags, and facial asymmetry. However, microcephaly is relatively uncommon in Goldenhar syndrome and can serve as a differentiating feature. Feingold syndrome and MFDM also exhibit partially overlapping phenotypes, including microcephaly, intellectual disability, esophageal atresia, and limb anomalies. Differentiation can be aided by the identification of brachymesophalangy of the second finger, which is highly characteristic of Feingold syndrome.[8,9]

MFDM is caused by heterozygous variants or deletions of the elongation factor Tu GTP binding domain containing 2 gene (*EFTUD2*, OMIM * 603892, located on chromosome 17. This gene encodes U5-116 kDa GTPase, a crucial component of the spliceosome.[10] The spliceosome is a complex composed of small nuclear ribonucleoproteins and its primary function is to remove intronic sequences from pre mRNA, enabling proper mRNA processing and gene expression.[11]

Significantly, more than 160 cases of MFDM associated with pathogenic variants in the *EFTUD2* gene have been documented in the HGMD database. As of the present date, the LOVD database reports 171 variants in the *EFTUD2* gene, of which 114 have been classified as pathogenic or likely pathogenic. Prenatal diagnoses of haploinsufficiency involving this gene remain exceedingly rare. According to available literature, only a few publications have emphasized the uniqueness of cases such as ours. Moreover, approximately 75%, of these variants are sporadic, while the remaining cases involve variants inherited from a parent with milder phenotype or arising due to germline mosaicism. In the latter case, even if the variant is confirmed as de novo, a small risk of recurrence in a subsequent pregnancy remains due to the possibility of parental germline mosaicism. Most of the identified variants are single nucleotide variants, including small deletions/duplications or insertions, splice-site mutations, as well as nonsense and missense variants. Importantly those involving the coding sequence of the gene predominate. Copy number variants (CNVs), such as deletions of the entire *EFTUD2* gene, are much less common.[12,13]

The use of high-resolution microarrays in prenatal testing has allowed the detection of significantly smaller chromosomal imbalances compared to conventional cytogenetic methods. While classical cytogenetics methods can provide a diagnosis in about 30% of cases, the application of microarrays increases the detection rate by approximately 6%. A significant increase in the diagnostic yield of microarrays was observed in cases where congenital anomalies are detected on prenatal ultrasound and the result of karyotype are normal. For this reason, aCGH testing is currently considered the first-tier test in the genetic evaluation of fetuses with structural defects and is regarded as the gold standard for detecting copy number variations.[14]

Notably, despite the great importance of aCGH in prenatal diagnosis, more than half of fetuses with birth defects remain undiagnosed. Therefore, it is often necessary to extend the diagnostic process using sequencing techniques. While whole genome sequencing (WGS) provides information on single nucleotide variants across the entire genomic sequence, including intronic and intergenic regions, whole exome sequencing (WES) refers to the protein-coding sequences, which account for the majority of known disease-causing variants. WGS is not routinely used in clinical diagnostics primarily due to high cost and longer analysis time. The implementation of WES for postnatal patients with neurodevelopmental disorders and congenital defects has significantly improved diagnostic yield. Approximately 30% of such patients receive a definitive genetic diagnosis following exome sequencing. This success highlights the potential benefit of applying exome sequencing in the prenatal diagnostic setting as well.[15,16]

MFDM can be diagnosed prenatally through a combination of detailed ultrasound examination and appropriately selected genetic testing methods. Prenatal diagnosis of *EFTUD2* variants reported in the literature has been achieved using exome sequencing[17,18] as well as chromosomal microarray analysis followed by targeted panel sequencing.[5] In one case, exome sequencing identified the likely pathogenic variant *EFTUD2*:c.2340_2341del,[17] whereas another report described the detection of *EFTUD2*:c.1058+1G>A.[18] In a case with a normal prenatal microarray result, subsequent panel sequencing revealed the heterozygous splice-site variant *EFTUD2*:c.2046-1G>T. Our cases demonstrate the importance of using diagnostic methods that detect both CNVs and SNVs and present unique variants: *EFTUD2*: c.2698_2701del not previously reported prenatally and a novel 17q21.31 deletion not previously described in MFDM patients.

Materials and Methods

In this study, we present two cases of fetuses in whom variants in the *EFTUD2* gene were detected. The Caucasian patients were referred to the Genetics Clinic at the Polish Mother's Memorial Hospital Institute for genetic evaluation following abnormal findings on prenatal imaging. Genetic diagnostics were conducted both at our center and at the Institute of Mother and Child. In both cases, patients decided to terminate the pregnancy after receiving the genetic diagnosis.

Ethical Approval

The study was performed in accordance with the principles of the Declaration of Helsinki and received positive evaluations from the Bioethics Committees of the Institute of Mother and Child (approval number: 6/2022) and Polish Mother's Memorial Hospital Research Institute (approval number: KB-89/2025). Institutional approval covered the study protocol. Separate institutional approval was not required for publication of anonymized case details in accordance with institutional regulations.

Patient 1

The first case involved a 28-year-old primigravida with a non-contributory anamnesis. She was referred for genetic consultation due to suspected micrognathia and choroid plexus cysts identified via ultrasound at external facility. After detecting these abnormalities of craniofacial and central nervous system, amniocentesis at 18 weeks of gestation was performed. A chromosomal microarray (aCGH) performed at the referring center yielded normal results. To better define the fetal phenotype, a detailed ultrasound examination was conducted using the GE Voluson E6 machine with RAB 4-8D, C1-5D, and RIC 5-9D probes. The following findings were noted:

- Bilateral, asymmetrical, hypoechogenic lesions protruding into the ventricles at the level of the thalamic eminences, described as cavitations of the thalamic eminences. Differential diagnosis included striatal cysts. These lesions had previously been misidentified as choroid plexus cysts at the referring center.
- A complete Pierre-Robin sequence, characterized by:
  1. Glossoptosis: The fetal tongue was markedly retracted posteriorly relative to the alveolar ridge.
  2. A V-shaped cleft of the posterior palate, with the nasal septum visible at the cranial border of the oral cavity and a large communication between the nasal and oral cavities.
  3. Severe micrognathia.
- Although the cerebellar anatomy appeared normal, its transverse diameter measured below the 5th percentile for gestational age.
- Head circumference measured at the 6th percentile for gestational age according to Hadlock. Fetal head circumference (HC) centiles were calculated according to the reference charts, which remain the standard biometric reference for second-trimester fetal cranial measurements.[19]
- Based on the ultrasound findings, the fetal phenotype was deemed abnormal, presenting with a complete Pierre-Robin sequence, bilateral cavitations of the thalamic eminences, and reduced head and cerebellum measurements. Due to the high suspicion of an underlying genetic disorder, chromosomal microarray, and/or exome sequencing was recommended for further investigation. Additionally, TORCH screening was conducted and yielded negative results. TORCH is a maternal screening for intrauterine infections included TORCH testing, referring to *Toxoplasma gondii*, Other (including syphilis), Rubella virus, *Cytomegalovirus*, and Herpes simplex virus, which represent the classic group of congenital infections associated with fetal growth restriction, microcephaly, and central nervous system abnormalities. To further characterize the brain lesions, fetal MRI was also advised. The identified abnormalities were assessed as severe, indicating a high risk of an irreversible condition. Some of the anomalies are shown in Figure 1.

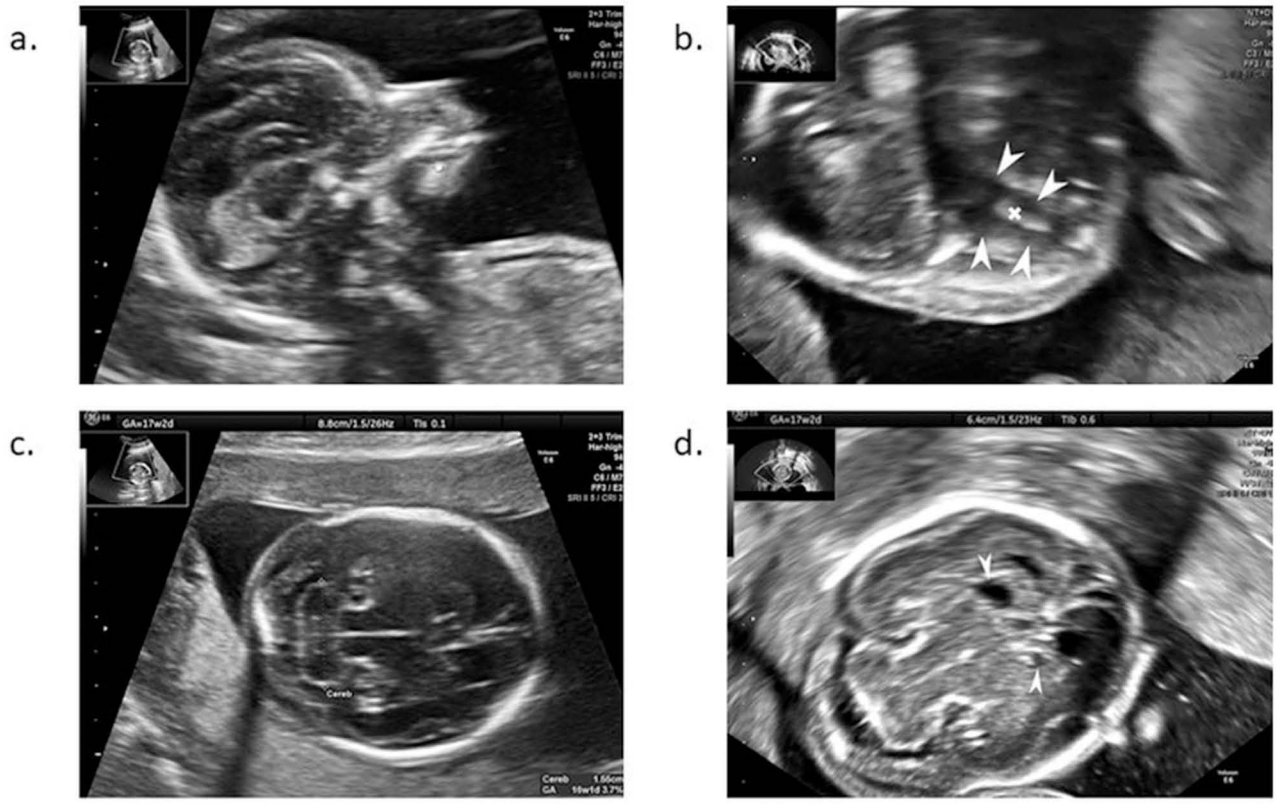

Figure 1 Ultrasound scans showing abnormalities of Fetus 1: (**a**) severe micrognathia with glossoptosis – the position of the tip of the tongue is indicated by a "hand icon", (**b**) cleft "arrowheads" of posterior palate with caudal part of nasal septum - vomer "x" - visible at the roof of the oral cavity, (**c**) measurement of the transverse cerebellar diameter, (**d**) thalamic eminence cavitations "arrowheads".

A fetal MRI, performed at 19 weeks of gestation revealed the following findings:

- Hyperintense foci in the caudate-thalamic sulci, measuring approximately 7 mm on the right and 5 mm on the left, with a low-signal outline suggesting potential post-stroke or post-hemorrhage changes in the germinal matrix or underlying basal nuclei (a more detailed assessment of basal nuclei anatomy would be feasible in a more advanced gestational age).
- A mildly heterogeneous signal of cerebrospinal fluid in the lateral ventricles, possibly indicating post-hemorrhagic changes.

Interpretation of the MRI was limited by significant motion artifacts, particularly affecting the craniofacial region, which hindered a complete evaluation. Given the normal aCGH result and the high risk of a genetic etiology, exome sequencing was strongly recommended.

Patient 2

The second case involved a 33-year-old woman, gravida 2, para 2, who was referred for genetic consultation due to fetal micrognathia. Based on first-trimester ultrasound, the gestational age was estimated at 22 weeks. Exactly at that time, following the progression of structural abnormalities and the confirmation of suspected craniofacial dysmorphism, amniocentesis was performed.

The patient's (pregnancy, family, and environmental) anamnesis was unremarkable. Ultrasound examination revealed the following abnormalities:

- A heart defect, specifically the presence of an additional left superior vena cava draining into a dilated coronary sinus.
- Brachycephaly with a retracted forehead, suggestive of early-onset microcephaly.

- Corpus callosum agenesis or severe dysplasia with absent septum pellucidum, and dysmorphic subcortical nuclei (striatum and caudate nucleus).
- Low-set, dysplastic external ears, and hypoplastic middle ears.
- An abnormal hard palate, indicating either a V-shaped cleft or a gothic palate.
- Micrognathia, likely indicating Pierre-Robin sequence.
- Polyhydramnios.

Several of these abnormalities are shown in Figure 2.

The fetal phenotype was assessed as severely abnormal, with a high likelihood of a serious, irreversible condition leading to significant postnatal disability. In order to determine the underlying genetic cause and to provide accurate

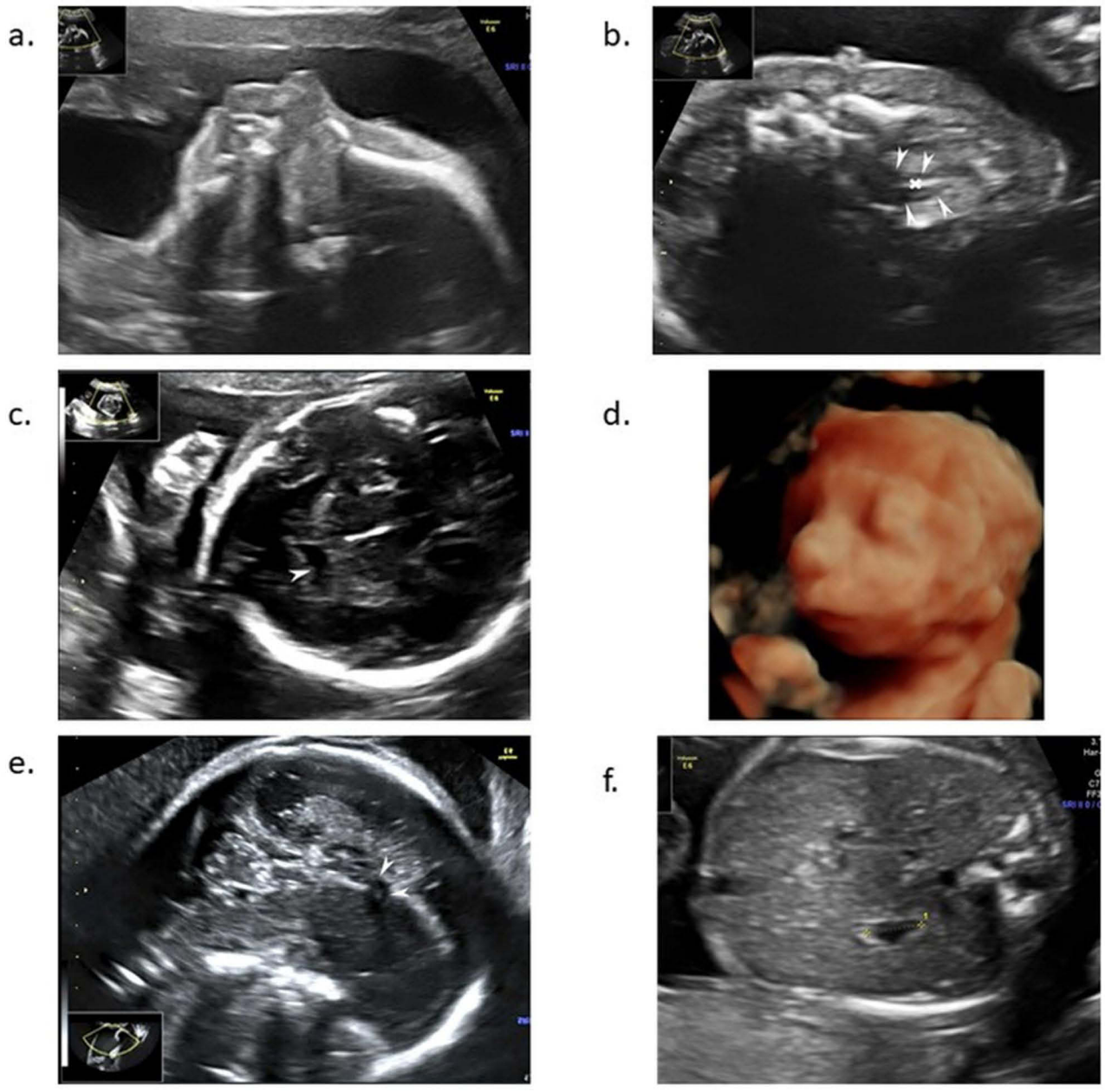

Figure 2 Ultrasound scans showing abnormalities in Fetus 2: (**a**) severe micrognathia, receding forehead, (**b**) V-shape cleft of posterior palate "arrowheads", vomer "x" visible at the roof of the oral cavity (**c**), abnormal cavum septi pellucidi "arrowhead" (**d**) severe micrognathia, low set dysplastic ear (**e**) dysplastic corpus callosum "arrowheads" (**f**) small fetal stomach, suggestive of impaired swallowing.

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genetic counseling, genetic testing was recommended. As a first-line investigation, a chromosomal microarray was initiated.

In both cases amniotic fluid was obtained during amniocentesis procedures, performed with the informed consent of the patients. DNA was extracted using the Sherlock AX (A&A Biotechnology, Poland). The concentration and purity of the DNA were assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific™, Waltham, MA, USA). DNA samples were subsequently documented and registered in the internal database.

DNA analysis was performed at an external laboratory using exome sequencing method. The Twist Human Core Exome Plus Kit (Twist Bioscience) was used for library preparation. Sequencing was carried out on a NovaSeq6000 platform (2×100bp Illumina) at CeGaT GmbH, Tübingen, Germany.

Mean coverage in the sample was 181.0, and 99.4% of the targets were covered > 30X. The variants were filtered according to population frequency, biological effect and clinical correlation with the phenotype associated with the given gene. No automatic prioritization software was used.

After bioinformatic filtering, the remaining variants were classified according to ACMG criteria[20] and subjected to a clinical evaluation including: variants described as disease-associated based on HGMD, ClinVar and in-house databases, potentially causative variants with very low allele frequencies in control populations database (GnomAD), variants in disease-associated genes that can be predicted to have a functional effect, gains or losses of disease-associated genes. The clinical interpretation of relevant variants was carried out by a specialized team; physician with specialization in clinical genetics and specialist in laboratory medical genetics.

The presence and extent of CNVs were examined using the whole-genome microarray CytoSure 8x60K (Oxford Gene Technology, UK) according to the manufacturer's protocol with a commercial male genomic DNA as a control. The results were analyzed with CytoSure Interpret Software, v.4.10, with the human genomic sequence GRCh37 (hg19) used as a reference. The average resolution of the used microarray was 120 kb. CNVs were called using thresholds + 0.3 for duplications and – 0.5 for deletions, respectively.

Detected variants were interpreted and classified using the following databases: DGV (Database of Genome Variants), DECIPHER (Database of Genomic Variation and Phenotype in Humans using Ensembl Resources), OMIM (Online Mendelian Inheritance in Man), ISCA (ClinGen Dosage Sensitivity Curation Page), ClinVar-NCBI and PubMed.

Results

As a result of the diagnostic investigations pathogenic variants involving the *EFTUD2* gene were identified in both fetuses. These variants were considered responsible for the phenotype observed on prenatal ultrasound.

In patient 1, following a normal microarray result, exome sequencing was performed. This analysis revealed a heterozygous deletion of four nucleotides in the exon 25 of the *EFTUD2* gene NM_001142605:c.[2698_2701del];[2698_2701=] (Figure 3). In accordance with clinical guidelines, Sanger sequencing was conducted in the fetus and both parents to confirm the variant and determine its origin. The analysis showed that the variant originated *de novo*. This deletion results in a frameshift. According to the ACMG classification, the variant was assessed as pathogenic and had been previously reported as such in both the ClinVar and HGMD databases. The variant described above has been submitted to the ClinVar database under accession number VCV000265116.4.

In Patient 2, microarray analysis revealed a heterozygous interstitial deletion of a 779.18 kb fragment of the long arm of chromosome 17, as shown in Figure 4. This deletion was located in the 17q21.31 region and encompassed 19 coding genes, involving the whole *EFTUD2* gene: arr[GRCh37] 17q21.31(42927777-43706954)x1. The deletion was not reported in the Database of Genomic Variants (DGV). The exact same deletion was also not reported in the DECIPHER and ClinVar. Based on data from available databases and literature reports, the deletion was classified as pathogenic and considered consistent with the abnormalities observed on prenatal ultrasound. A comparative analysis using molecular cytogenetic techniques was recommended in both parents of the fetus to assess the inheritance pattern of the deletion. The detected deletion has been submitted to ClinVar and is available under the accession number VCV003341110.1.

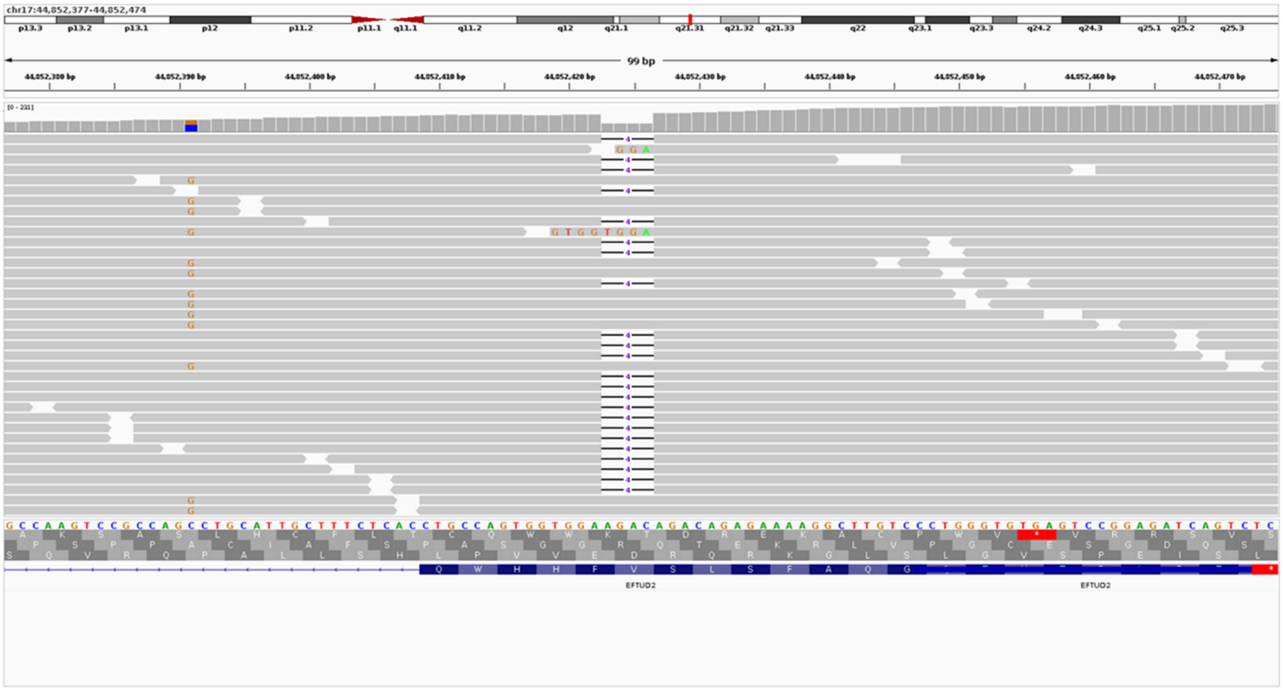

Figure 3 Exome sequencing revealed a heterozygous deletion of four nucleotides in *EFTUD2* (NM_004247.4:c.2698_2701del).

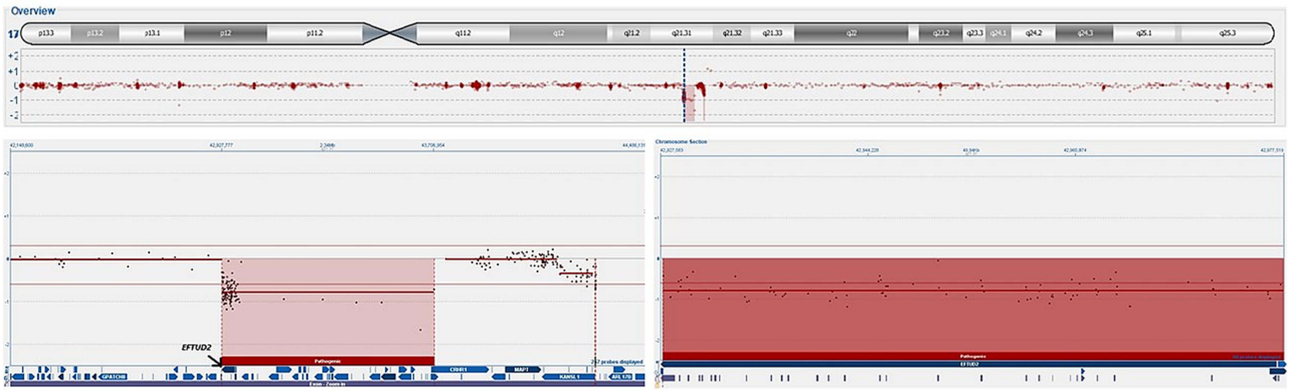

Figure 4 Array CGH profile of 17q21.31 deletion involving the *EFTUD2* gene. The entire chromosome 17 is visible with the deletion marked, a smaller fragment showing the genes included in the deletion with *EFTUD2* highlighted, and a close-up of the entire *EFTUD2* gene.

Discussion

Human facial dysostoses are a heterogenous group of disorders characterized primarily by craniofacial abnormalities. Haploinsufficiency of the *EFTUD2* gene, located on chromosome 17q21.31, is responsible for MFDM.[21] This syndrome was first described by Guion Almeida et al in 2000 and 2006. They diagnosed six patients who all presented with craniofacial abnormalities such as micrognathia or hypoplasia of the zygomatic arch leading to classification of the condition as a mandibulofacial dysostosis. Additionally, the patients exhibited distinctive features such as microcephaly and trigonocephaly, ear anomalies, cleft palate, and both intellectual and growth delay.[22]

In this study we report two cases of fetuses with congenital anomalies associated with variants in the *EFTUD2* gene. To the best of our knowledge, the prenatal phenotype of MFDM has been described in only a few cases to date. Prenatal diagnosis of MFDM is a major challenge due to the overlap of phenotypic features with other genetic syndromes including Treacher-Collins syndrome or CHARGE syndrome. However, the identification of central nervous system

anomalies may serve as a critical diagnostic clue, facilitating differentiation and guiding clinicians toward the correct diagnosis.[6]

A study on 41 fetuses with micrognathia was performed by Mouthon et al. Genetic diagnosis was established in 21 cases, with monogenic disorders identified in nine of them. A diagnosis of MFDM caused by a variant in the *EFTUD2* gene was made in two patients, although only after birth.[23]

Additional prenatal cases with MFDM features confirmed by genetic testing have been described by Khachnaoui-Zaafrane et al, Dragoi et al, Xu et al, and Khachnaoui-Zaafrane et al reported a 26 year old gravida 2 para 0 (one prior miscarriage) referred for evaluation due to abnormal ultrasound findings. The fetus presented with dysplastic, low-set ears, cleft palate, and retrognathia. Amniocentesis and chromosomal microarray analysis revealed a normal male karyotype. The patient opted for pregnancy termination and further genetic testing was performed post-mortem. Gene panel sequencing revealed a likely pathogenic splice-site variant in the *EFTUD2* gene.[5]

The case of a 31-year-old woman gravida 1 para 1, at 33 weeks of gestation was reported by Dragoi et al. Prenatal ultrasound revealed several abnormalities such as polyhydramnios, a small stomach, micrognathia, low set ears, and increased nuchal thickness. The parents did not opt for genetic diagnosis during pregnancy. Following birth, clinical evaluation was performed, and multiple dysmorphic features were found: abnormal facial shape, abnormality of the outer ear (preauricular skin tags), cleft palate, microretrognathia. Additional findings included oesophageal atresia, choanal stenosis, generalized muscle weakness, and tracheomalacia. Whole-exome sequencing revealed a likely pathogenic variant in the *EFTUD2* gene.[17]

The patient described by Xu et al was a 27-year-old primigravida referred for routine first-trimester screening. During the ultrasound examination, the suspicion of fetal micrognathia was raised. Genetic testing was subsequently performed, revealing a normal karyotype and normal array CGH result. Follow-up ultrasounds in the later stages of pregnancy confirmed micrognathia and additionally revealed polyhydramnios and features suggestive of esophageal atresia. The parents opted for termination of pregnancy. The phenotype of aborted fetus was reevaluated and a cleft palate, dysplastic low set years, and proximally placed thumbs were found. WES trio analysis revealed the presence of a heterozygous variant in the *EFTUD2* gene, that was confirmed to have arisen de novo in the fetus.[18]

A summary of selected prenatal ultrasound findings from the above literature reports, along with our two cases, is presented in Table 1. Only features identified during prenatal ultrasound examinations were included; findings observed postnatally or during fetal autopsy were excluded.

To the best of our knowledge, an identical deletion to the one observed in our patient has not been reported in the literature or in publicly available databases. Notably, smaller deletions encompassing the entire *EFTUD2* gene have been described in patients with the MFDM phenotype. Two such cases were reported by the Gordon et al. Among three groups of patients they described one group with mandibulofacial dysostosis, oesophageal atresia and/or microcephaly with or without additional features. Out of 14 patients in this group, 10 had pathogenic variants in the *EFTUD2* gene, while two had variants of uncertain significance. All diagnoses were made postnatally. The patients showed various phenotypic features, including facial dysmorphism, ear anomalies, microcephaly, and intellectual disability. In two of them, microcephaly was already identified during prenatal ultrasound examinations. The study group also included a fetus in whom genetic diagnosis was made following the termination of the pregnancy. Several patients were initially referred for evaluation with a suspected diagnosis of Goldenhar, Feingold, or CHARGE syndrome reflecting the phenotypic overlap among these conditions. As previously mentioned, two of the reported pathogenic variants were larger deletions encompassing four genes, including *EFTUD2*. Although these deletions were smaller than the one identified in our patient, they were fully contained within the region deleted in our case. Both patients exhibited clinical features such as mandibular hypoplasia and dysplastic external ears, which were also observed in our fetus. In addition, one patient had an ASD heart defect and the other had a cleft palate, both of which were likewise identified in our proband.[8]

Moreover, the *EFTUD2* variant identified in our case: NM_004247.4:c.2698_2701del, resulting in frameshift has been previously reported twice in the literature. Matsuo et al described a 6-year-old patient presenting with a range of MFDM symptoms. The boy had microcephaly and hypoplasia of the zygomatic bone and mandible. He suffered from deafness and recurrent seizures. In addition, his development was delayed. Comparative studies in the patient's parents confirmed that the *EFTUD2* variant arose de novo.[24] The same variant in a male patient but inherited from an affected

Table 1 Comparison of Prenatal Ultrasound Findings in Fetuses with MFDM from Our Study and Selected Cases Reported in the Literature

Characteristic Features of MFDM in Prenatal Ultrasound	This Report (Case 1)	This Report (Case 2)	Dragoi et al[17]	Khachnaoui-Zaafrane et al[5]	Xu et al[18]
Head	Microcephaly	Brachycephaly (suggested early microcephaly)			
Craniofacial	Micrognathia, cleft palate, glossoptosis,	Retracted forehead, micrognathia cleft palate or gothic palate	Micrognathia, increased prenasal thickness	Retrognathia, posterior cleft palate	Micrognathia
Ears		Low-set, dysplastic external ears, hypoplastic middle ears	Low-set ears	Low-set, protruding, asymmetrical dysplastic ears with microtia and a left preauricular tag	
Brain	Cavitations of the thalamic eminences, reduced cerebellar measurements, possible post-hemorrhagic changes	Corpus callosum agenesis (or severe dysplasia), absent septum pellucidum, dysmorphic subcortical nuclei		Reduced cerebellar diameter	
Heart		Additional left superior vena cava, dilated coronary sinus			
Additional		Polyhydramnios, small stomach	Polyhydramnios, small stomach	Moderate hydramnios	Polyhydramnios, oesophageal atresia

mother was reported by Huang et al. The boy presented characteristic clinical signs such as microcephaly, micrognathia, cleft palate, and malar hypoplasia as well as ears abnormalities and hearing loss. Developmental delay and cryptorchidism were also noted.[25] In the present study, the prenatal case showed features of dysmorphism and cranial abnormalities consistent with those observed in both previously reported cases.

The cases described above support the pathogenicity of the detected *EFTUD2* variants and demonstrate that haploinsufficiency of this gene can result in a wide spectrum of clinical manifestations. To our knowledge, no similar variants have been reported prenatally in the existing literature. Therefore, the cases presented in our study provide valuable insights into the genetic-phenotypic correlations in fetuses with the MFDM features. The phenotype of patients with MFDM is very broad, and some of the abnormalities like hearing loss and developmental delay may only become apparent postnatally. Consequently, in cases where pregnancies with *EFTUD2* variants are continued, close prenatal monitoring is recommended, followed by postnatal clinical evaluation to allow for appropriate follow-up and early intervention.

Conclusion

Establishing clear prenatal criteria for the diagnosis of MFDM is crucial for targeting genetic diagnostics. Both our cases and previously reported three patients with prenatal suspicion of MFDM and confirmed causative variants highlight the critical role of molecular. The rare variants of *EFTUD2*:c.2698_2701del and the large deletion involving the whole *EFTUD2* gene described in this study further emphasize the importance of precise ultrasound evaluation and the use of

appropriately selected genetic testing methods. It should also be highlighted that in cases of a normal aCGH result accompanied by a phenotype characteristic of mandibulofacial dysostoses, further diagnostic evaluation with exome sequencing is recommended. Given the very limited number of prenatally confirmed cases reported to date, these observations remain preliminary and require validation in larger cohorts. Our study contributes to the further characterization of the clinical manifestation of MFDM in fetuses, a condition that remains rarely detected during the prenatal period. Early identification of a specific genetic syndrome, such as MFDM, is important not only for planning appropriate perinatal and postnatal care but also for providing accurate genetic counseling. A confirmed molecular diagnosis enables estimation of recurrence risk in future pregnancies and supports informed decision-making for affected families.

Abbreviations

MFDM, mandibulofacial dysostosis with microcephaly; MFDs, mandibulofacial dysostoses; AFDs, acrofacial dysostoses; MFDGA, mandibulofacial dysostosis Guion-Almeida type; CNVs, copy number variants; aCGH, array comparative genomic hybridization; WGS, whole genome sequencing; WES, whole exome sequencing.

Consent for Publication

Written informed consent for publication of the medical data, results and images was obtained from the patients following applicable local laws.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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