Mandibulofacial dysostosis with microcephaly syndrome diagnosed by prenatal exome sequencing: a case report

Sílvia Feliu, Gabriela Palacios, Elisabet Clua, Pilar Prats, Gerard Albaigés, Alberto Rodríguez, Bernat Serra

Department of Obstetrics, Gynecology and Assisted Reproduction, Hospital Universitari Dexeus, Barcelona, Spain

ABSTRACT

Mandibulofacial dysostosis with microcephaly (MFDM) is a rare gene deletion syndrome caused by a heterozygous pathogenic variant or deletion in the *EFTUD2* gene. Typical features described in affected patients include developmental delay, microcephaly, micrognathia, malar hypoplasia, esophageal atresia and congenital heart disease, among others. We report a case that was diagnosed in a fetus at 34 weeks of pregnancy. Obstetric ultrasounds performed in a 42-year-old patient revealed multiple anomalies suggestive of a genetic disorder from the first weeks of the second trimester, but prenatal genetic tests (quantitative fluorescent polymerase chain reaction, karyotype and microarray) were normal. Sequencing of a prenatal exome from a sample obtained by amniocentesis revealed the *c.146_168del* variant in *EFTUD2*, a finding compatible with MFDM. After receiving a post-test genetic counseling session, the patient requested termination of the pregnancy. In addition to describing this case, we review current literature regarding prenatal exome sequencing.

KEYWORDS

Genetic disorder, prenatal diagnosis, pregnancy termination, genetic counselling.

Introduction

Mandibulofacial dysostosis with microcephaly (MFDM, OMIM 610536) is a multiple malformation syndrome caused by a heterozygous pathogenic variant or deletion in the *EFTUD2* gene, located on the large arm of chromosome 17 (17q21.31). MFDM is inherited in an autosomal dominant manner. It has high penetrance with variable expressivity. Although most of the described variants are de novo, familial recurrence has been described secondary to germline mosaicism or inheritance from a parent with a milder phenotype [1].

MFDM is characterized by a wide range of clinical findings. Highly penetrant features include developmental delay or intellectual disability, microcephaly, micrognathia, malar hypoplasia, small or dysplastic pinnae, facial asymmetry and hearing loss (typically conductive, but occasionally sensorineural or mixed). Less frequent manifestations, but clinically useful, are preauricular tags, auditory canal atresia or stenosis, esophageal atresia or stenosis, congenital heart disease, thumb abnormalities and choanal atresia or stenosis. Other anomalies, less frequently associated with this syndrome, are: renal anomalies, cryptorchidism and/or small scrotum, central nervous system abnormalities, spine anomalies, strabismus, epibulbar dermoid, branchial cleft and/or remnant, laryngeal cleft, midline mandibular defect and gastric malrotation. The diagnosis is suspected in individuals that meet clinical criteria, and is confirmed by the identification of a pathogenic variant or deletion in *EFTUD2* [1-4].

MFDM is a pan-ethnic disorder with no recognized racial or ethnic predisposition. More than 100 cases have been reported to date in various ethnic groups, but its exact prevalence has not been established [5-11]. Thus far, we are not aware that any case has been diagnosed prenatally [3].

Our study reports the first prenatal case of MFDM diagnosed at 34 gestational weeks. We describe the ultrasound findings and the protocol followed in our clinical center to achieve the diagnosis.

Article history

Received 7 Jun 2021 - Accepted 26 Aug 2021

Contact

Sílvia Feliu; silfel@dexeus.com

Hospital Universitari Dexeus, Barcelona, Spain.

C/Sabino de Arana, 5-19, Barcelona, Spain.

Department of Obstetrics, Gynecology and Assisted Reproduction.

Phone number: +34619030032

Case report

During a routine, annual gynecological checkup at our Obstetrics, Gynecology and Reproductive Medicine Department at the Hospital Universitari Dexeus, Barcelona, a 42-year-old woman expressed her desire to become pregnant. She was referred to the Assisted Reproduction Unit for complementary tests and discussion of her reproductive options. At the time of consultation, the patient did not have a partner or previous sexual intercourse. In addition, the patient had a low ovarian reserve. Taking all these facts into account, double gamete donation or embryo donation were offered. After medical coun-

seling, the patient opted for embryo donation. Preparation and transfer were performed without incident.

The patient did not have any relevant previous medical or surgical history. The first trimester of pregnancy was uneventful. Screening tests both for chromosomal abnormalities and for preeclampsia indicated low risk. At the beginning of the second trimester, she was diagnosed with gestational diabetes.

Morphological ultrasound performed at 21.3 weeks' gestation revealed a slightly dilated umbilical vein, so a fetal echocardiogram was indicated. In addition, the stomach appeared poorly filled. Echocardiogram confirmed dilatation of the intrahepatic portion of the umbilical vein. The remaining fetal vessels seemed to be structurally and functionally normal (Fig. 1).

Ultrasound at 27.0 weeks' gestation showed an estimated fetal weight (EFW) at the 10th percentile (907g) and an amniotic fluid index (AFI) above normal values (35 cm). The umbilical vein and the stomach remained stable (Fig. 2).

Due to the ultrasound findings, maternal serologies for toxoplasma, rubella, cytomegalovirus (CMV), herpes simplex, parvovirus B19, Epstein-Barr and varicella zoster were requested. An amniocentesis was performed for genetic testing as well as an amnio drainage of 1700cc, leaving a residual AFI of 18 cm. All maternal serologies were negative except for positive IgM and IgG CMV antibodies with high IgG avidity. As regards genetic tests, quantitative fluorescent polymerase chain reaction, karyotype and microarray comparative genomic hybridization were normal.

Our Maternal-Fetal Medicine Committee (MFMC) recommended periodic obstetric ultrasound evaluations. At the 30.1-week ultrasound, novel dysmorphic features were observed: retrognathia, anteverted nostrils and dysplastic ears. Small stomach bubble associated with polyhydramnios suggested a

Figure 1 Fetal echocardiogram performed at 24.3 weeks' gestation. Dilatation of the intrahepatic portion of the umbilical vein. A: Maximum expansion diameter: 7.5mm. B: Slight narrowing (2.2mm) detected C: Acceleration of the flow to 50mmHg through the narrowing.

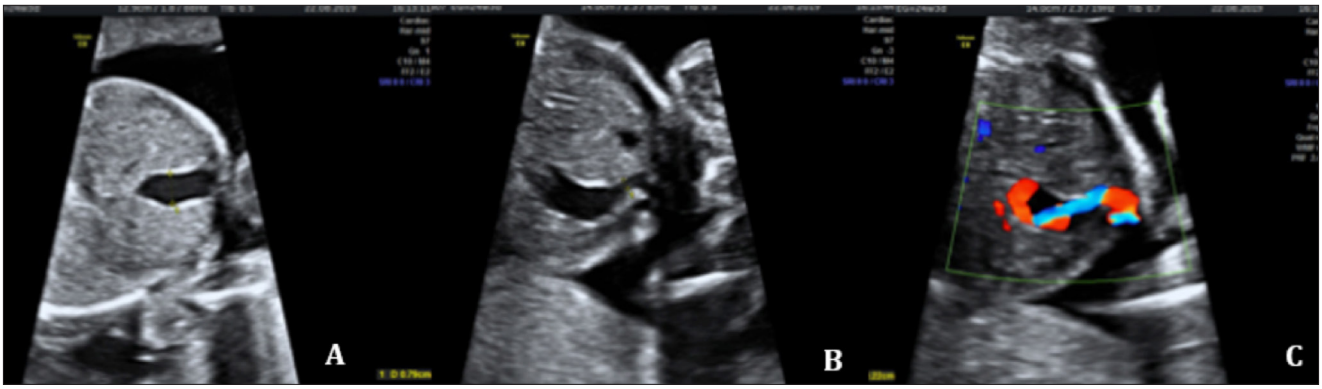
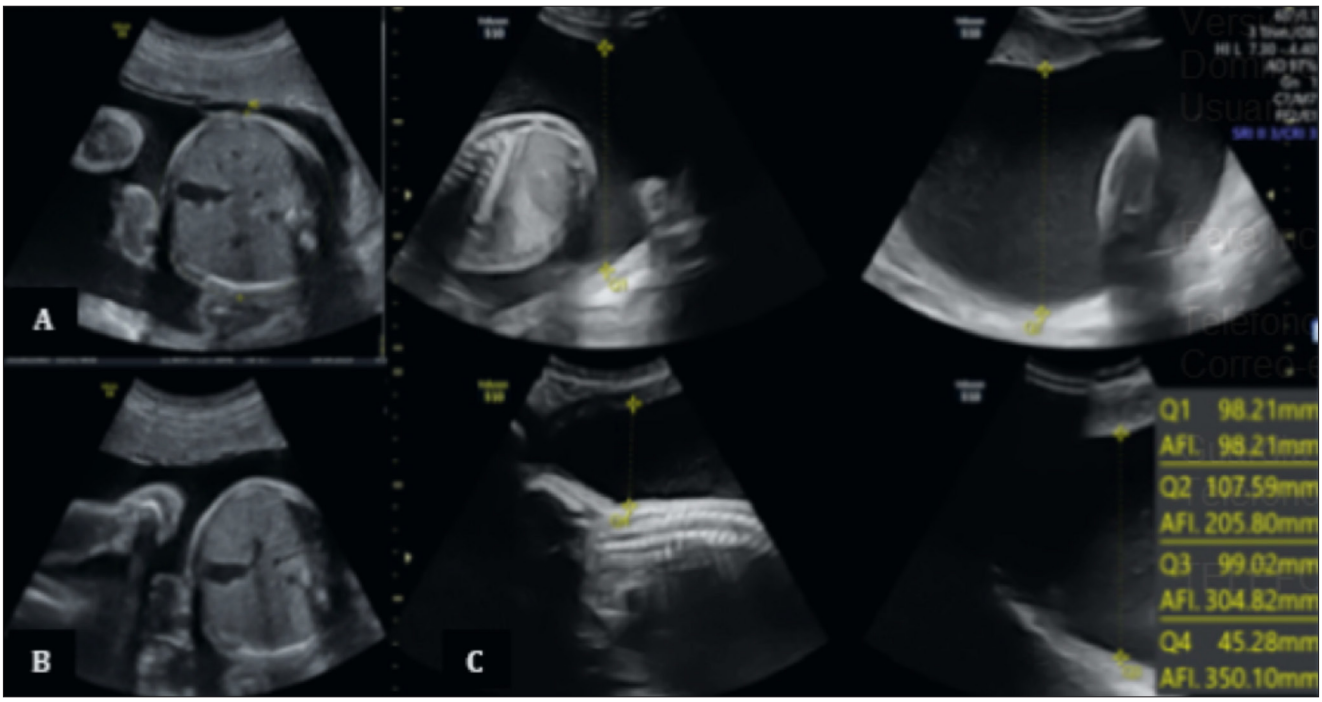


Figure 2 Ultrasound performed at 27.0 weeks' gestation. A: Umbilical vein: stable. B: Stomach: stable. C: AFI: 35 cm. AFI: amniotic fluid index.



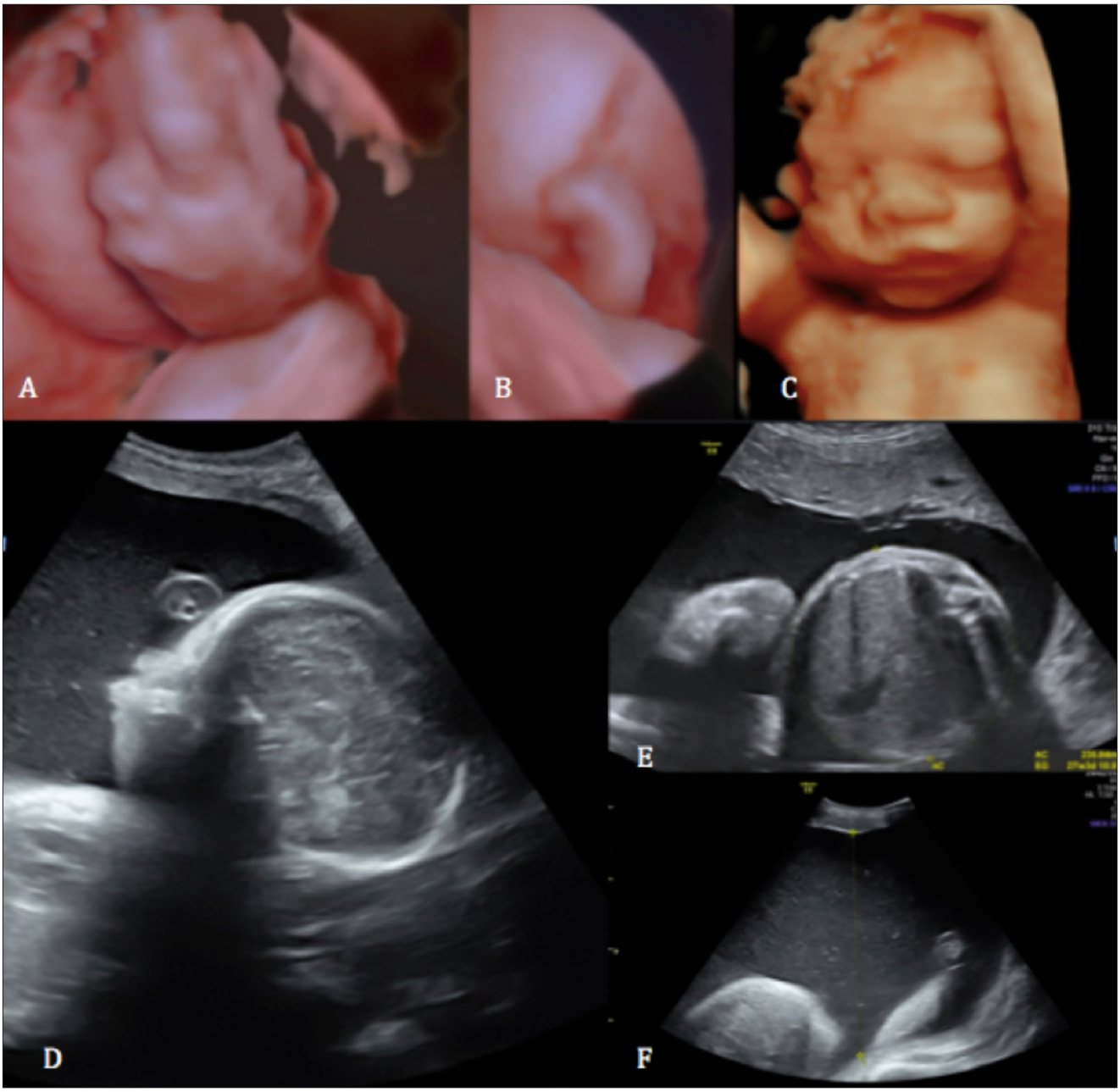
possible esophageal atresia. The EFW was at the 2nd percentile and the umbilical vein remained unchanged (Fig. 3).

Given that the prenatal genetic tests performed had failed to determine a diagnosis in a fetus with multiple anomalies suggestive of a genetic disorder, the MFMC agreed to offer the patient additional genetic testing by means of prenatal exome sequencing (ES). After an exhaustive genetic counseling session by a well-trained genetic counselor, the patient decided to perform the study. Fetal DNA was obtained from the amniocentesis previously performed. A fetal MRI examination was also performed to complete an exhaustive morphological evaluation of the fetus. MRI confirmed the ultrasound findings (esophageal atresia – as suggested by the small stomach bubble and polyhydramnios –, retrognathia and choanal atresia).

At 34.1 weeks, amnio drainage was performed to alleviate maternal symptoms caused by polyhydramnios (3000cc of amniotic fluid was removed). A few hours later, the patient started experiencing painful uterine contractions. Her cervical length was 33 mm. Although she had an immediate response to tocolytics, she required hospital admission for two days.

During her hospital stay, the result of the prenatal ES was obtained. The *c.146_168del* variant in the *EFTUD2* gene was found, compatible with MFDM (OMIM 610536). The variant was confirmed with Sanger sequencing. After receiving a post-test genetic counseling session in which the results were explained in detail, the patient requested termination of the pregnancy. A late termination of pregnancy was approved by an external committee.

Figure 3 A, B, C and D: Dysmorphic features (retrognathia, anteverted nostrils and dysplastic ears). E: Small stomach bubble and dilatation of the umbilical vein. F: Polyhydramnios.



Fetal autopsy (Fig. 4) revealed mandibular malformation with micrognathia, malar hypoplasia, small nose and facial asymmetry, esophageal atresia with distal tracheoesophageal fistula (type III or C) and aberrant right subclavian artery (not seen prenatally). Morphological alterations of the ears were not observed and the nostrils were permeable.

For the purpose of studying the inheritance of the variant, it was decided to contact the couple who had donated the embryo. They had performed a cycle of IVF with oocyte donation and had donated six embryos for reproductive use (Fig. 5). The outcomes of the embryo donation are shown in figure 5.

After obtaining informed consent, Sanger sequencing of

Figure 4 Fetal autopsy. A, B, C: Mandibular malformation with micrognathia and retrognathia, malar hypoplasia, small nose and facial asymmetry. D: Esophageal atresia.

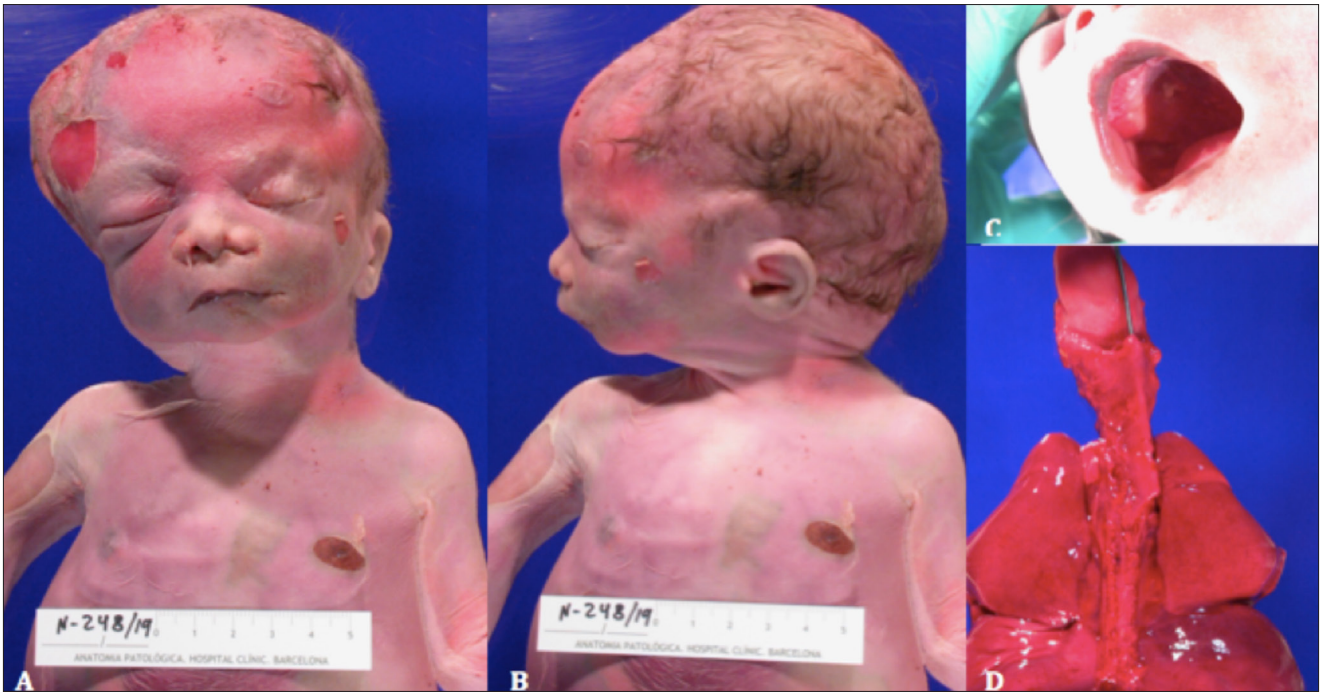
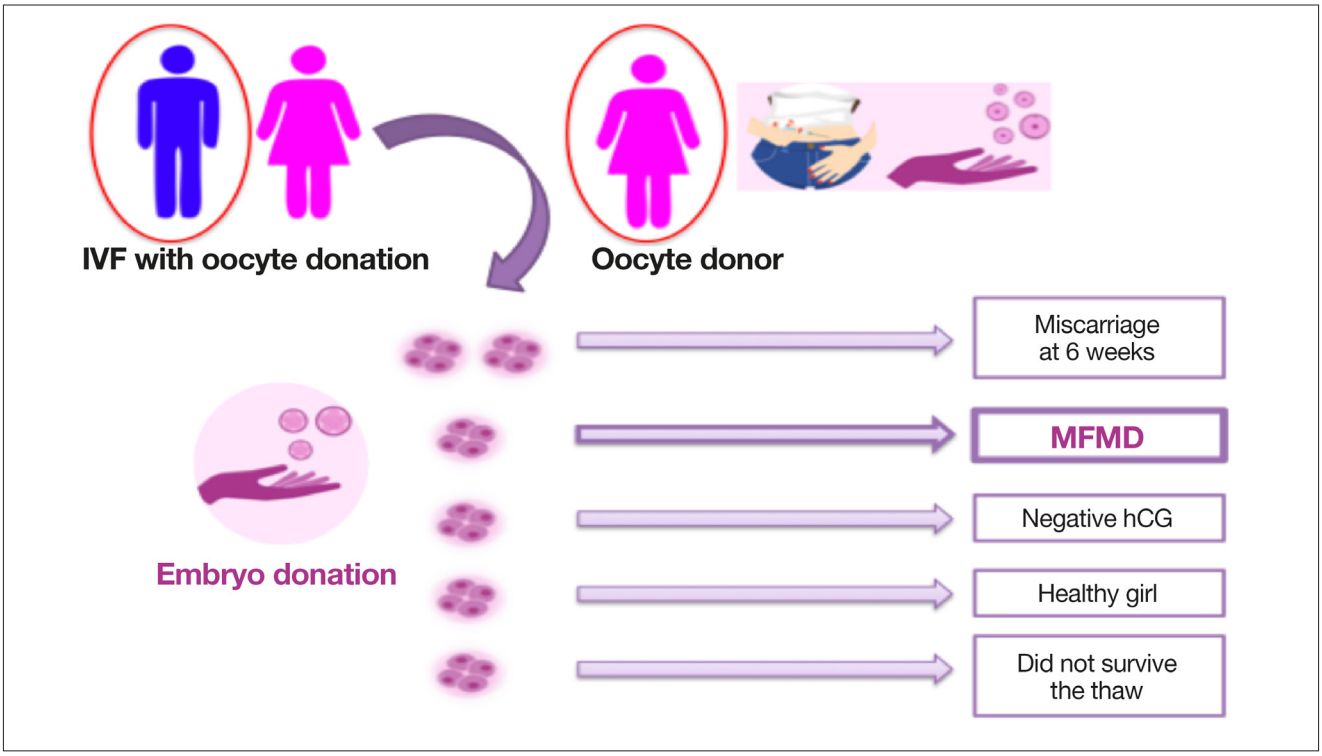


Figure 5 In this scheme, we can see the origin of the embryo that was transferred to our patient. Parents are surrounded by red. Other embryos from the same parents that were given to other women are also shown.



variant *c.146_168del* in *EFTUD2* was performed in the male partner (DNA obtained from peripheral blood), confirming absence of the variant.

We also tried to contact the oocyte donor to study the inheritance of the variant, but we could not reach her. One of the six embryos donated for reproductive use resulted in a healthy girl. We did not contact this family, since the variant in the *EFTUD2* gene seems to be de novo.

The patient is currently undergoing a new cycle of assisted reproduction. This time, with double gamete donation.

Discussion

Ultrasound-detected fetal sonographic abnormalities are identified in 2-3% of pregnancies. In these cases, an invasive test (amniocentesis *vs* chorionic villus sampling) to study genetic anomalies (karyotype and microarray) is routinely offered nowadays [12].

An underlying genetic etiology can be identified in up 40% of dysmorphic fetuses when using these tests in combination, still leaving most of cases undiagnosed [13]. This is a common scenario, where the information is limited to ultrasound findings alone. Counseling these families is very challenging due to the broad differential diagnosis and large range of prognoses and expectations [12].

Genome sequencing (GS) and ES are technologies that interrogate the genome at a nucleotide level. GS involves assessing both the coding and the non-coding regions of the genome; however, it is difficult to achieve complete sequencing of the genome due to difficulty of sequencing and analysis in certain regions. ES is limited to the protein-coding regions of more than 20,000 genes, comprising about 1-2% of the total genome, where >85% of disease-causing genetic variants are located [13]. Although GS may be more informative due to its scope, it requires greater data analytics and is not routinely utilized in clinical testing at this time. By contrast, ES is a current option for the evaluation of fetuses with structural anomalies. Its utilization is increasing in prenatal care, especially during the last five years [14].

Diagnostic rates of prenatal ES range from 6.2 to 80% in published articles [15-23]. The diagnostic rate is dependent on the indication for ES and varies based on multiple factors including the following: single *vs* multiple organ system affected, specific organ system affected, and proband *vs* trio sequencing [4]. The lowest reported rate was obtained when performing ES on all fetuses with any anomaly visualized on ultrasound [22], while the highest rates were in small studies with carefully chosen cases [18].

The American College of Medical Genetics and Genomics recommends that ES should be considered when specific genetic tests for a phenotype fail to determine a diagnosis in a fetus with multiple anomalies suggestive of a genetic disorder [24]. However, ES is not currently able to reliably detect all types of genetic variation such as copy number changes, low-level mosaicism, aneuploidy, structural chromosome rearrangements or trinucleotide repeat expansions, and it cannot detect variants in non-coding regions.

Therefore, conventional testing options such as microarrays should still be considered before or in parallel to prenatal ES [13].

In our case, obstetric ultrasounds performed in the second trimester of pregnancy revealed dysmorphic features including retrognathia, anteverted nostrils and dysplastic ears, as well as indirect signs suggesting esophageal atresia. It was only after the QF-PCR, karyotype and microarray returned normal results that the MFMC agreed to offer the patient prenatal exome sequencing.

The analysis of ES data is phenotype driven, and is easier to ascertain in the adult or pediatric setting than in the prenatal setting. Given that assessment of the fetal phenotype is indirect, prenatal ES analysis could be limited to the reporting of variants in genes associated with the ultrasound findings [14]. This can be done through a database called Human Phenotype Ontology. For example, in our case, out of a total of 25,000 genes contained in the genome, only 2,500 were studied.

It is important to underline the importance of trio ES: if a variant is found, the parents should be located to study the inheritance. In this way, we will know if the variant had appeared de novo or shows familial recurrence; this aspect is especially important in cases where assisted reproduction has been performed [13].

Conclusions

The outcomes of prenatal ES can have various implications in different areas. To begin with, it requires significant counseling with regard to reproductive decision-making. If the pregnancy is continued, a specific diagnosis can inform pediatricians about the management or possible treatments that may be offered postnatally.

It may also reduce hospital costs by decreasing the number of tests performed postnatally, thereby avoiding the "diagnostic odyssey" and potentially decreasing the length of the hospital stay. In addition, a positive result can have implications for other family members and/or for future pregnancies. For example, in a subsequent pregnancy, preimplantation genetic diagnosis could be performed.

Furthermore, understanding the genetic etiology can also provide closure for the family and help with the grieving process in the case of fetal or neonatal loss. Finally, the use of prenatal ES has the ability to expand phenotypes to the prenatal period and increase our understanding of genes that may be critical to human development [12].

This technology, offered in the prenatal setting, comes with many challenges, including practical, ethical and economic issues [13]. An ethical dilemma inherent in genetic testing is ensuring that genetic information remains confidential. This is confounded by the importance of data sharing in the understanding and classification of variants [25]. Therefore, before ES possibly becomes part of routine prenatal testing, further research is required to improve the evidence base of what is technically possible and what is clinically beneficial. What is clear is that implementation must be accompanied by expert genetic counseling that will be required both before and after testing [13].

References

1. Huang L, Vanstone MR, Hartley T, et al. Mandibulofacial dysostosis with microcephaly: mutation and database update. Hum Mutat. 2016;37:148-54.
2. Guion-Almeida ML, Zechi-Ceide RM, Vendramini S, Tabith Júnior A. A new syndrome with growth and mental retardation, mandibulofacial dysostosis, microcephaly, and cleft palate. Clinical Dysmorphol. 2006;15:171-4.
3. Lines M, Hartley T, MacDonald SK, Boycott KM. Mandibulofacial dysostosis with microcephaly. 2014 Jul 3 [updated 2020 Nov 12]. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2020: 135-43.
4. Wieczorek D, Gener B, González MJ, et al. Microcephaly, microtia, preauricular tags, choanal atresia and developmental delay in three unrelated patients: a mandibulofacial dysostosis distinct from Treacher Collins syndrome. Am J Med Genet A. 2009;149A:837-43.
5. Bernier FP, Caluseriu O, Ng S, et al. Haploinsufficiency of SF3B4, a component of the pre-mRNA spliceosomal complex, causes Nager syndrome. Am J Hum Genet. 2012;90:925-33.
6. Gandomi SK, Parra M, Reeves D, Yap V, Gau CL. Array-CGH is an effective first-tier diagnostic test for EFTUD2-associated congenital mandibulofacial dysostosis with microcephaly. Clin Genet. 2015;87:80-4.
7. Gordon CT, Petit F, Oufadem M, et al. EFTUD2 haploinsufficiency leads to syndromic oesophageal atresia. J Med Genet. 2012;49:737-46.
8. Lehalle D, Gordon CT, Oufadem M, et al. Delineation of EFTUD2 haploinsufficiency-related phenotypes through a series of 36 patients. Hum Mutat. 2014;35:478-85.
9. Luquetti DV, Hing AV, Rieder MJ, et al. "Mandibulofacial dysostosis with microcephaly" caused by EFTUD2 mutations: expanding the phenotype. Am J Med Genet A. 2013;161A:108-13.
10. Need AC, Shashi V, Hitomi Y, et al. Clinical application of exome sequencing in undiagnosed genetic conditions. J Med Genet. 2012;49:353-61.
11. Voigt C, Mégarbané A, Neveling K, et al. Oto-facial syndrome and esophageal atresia, intellectual disability and zygomatic anomalies - expanding the phenotypes associated with EFTUD2 mutations. Orphanet J Rare Dis. 2013;8:110.
12. Jelin AC, Vora N. Whole exome sequencing: applications in prenatal genetics. Obstet Gynecol Clin North Am. 2018;45:69-81.
13. Best S, Wou K, Vora N, Van der Veyver IB, Wapner R, Chitty LS. Promises, pitfalls and practicalities of prenatal whole exome sequencing. Prenat Diagn. 2018;38:10-9.
14. Monaghan KG, Leach NT, Pekarek D, Prasad P, Rose NC; ACMG Professional Practice and Guidelines Committee. The use of fetal exome sequencing in prenatal diagnosis: a point to consider document of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2020;22:675-80.
15. Alamillo CL, Powis Z, Farwell K, et al. Exome sequencing positively identified relevant alterations in more than half of cases with an indication of prenatal ultrasound anomalies. Prenat Diagn. 2015;35:1073-8.
16. Carss KJ, Hillman SC, Parthiban V, et al. Exome sequencing improves genetic diagnosis of structural fetal abnormalities revealed by ultrasound. Hum Mol Genet. 2014;23:3269-77.
17. Drury S, Williams H, Trump N, et al. Exome sequencing for prenatal diagnosis of fetuses with sonographic abnormalities. Prenat Diagn. 2015;35:1010-7.
18. McMullan DJ, Lord J, Eberhardt R, et al. Exome sequencing of 406 parental/fetal trios with structural abnormalities revealed by ultrasound in the UK Prenatal Assessment of Genomes and Exomes (PAGE) project [abstract C15.5]. European Journal of Human Genetics (2019) 26:3-112. https://doi.org/10.1038/s41431-018-0249-5 - Available at: https://www.nature.com/articles/s41431-018-0249-5.pdf?proof=t%C2%A0.
19. Pangalos C, Hagnefelt B, Lilakos K, Konialis C. First applications of a targeted exome sequencing approach in fetuses with ultrasound abnormalities reveals an important fraction of cases with associated gene defects. PeerJ. 2016;4:e1955.
20. Vora NL, Powell B, Brandt A, et al. Prenatal exome sequencing in anomalous fetuses: new opportunities and challenges. Genet Med. 2017;19:1207-16.
21. Wapner R, Petrovsky S, Brennan K, Bier L, Wou K, Goldstein D. Whole exome sequencing in the evaluation of fetal structural anomalies: a prospective study of sequential patients [abstract]. Am J Obstet Gynecol. 2017;216:S5-S6. Available at: https://www.ajog.org/action/showPdf?pii=S0002-9378%2816%2930988-7.
22. Yadava SM, Ashkinadze E. Whole exome sequencing (WES) in prenatal diagnosis for carefully selected cases [abstract]. Am J Obstet Gynecol. 2017:216:S87-S88. Available at: https://www.ajog.org/action/showPdf?pii=S0002-9378%2816%2931008-0.
23. Yates CL, Monaghan KG, Copenheaver D, et al. Whole-exome sequencing on deceased fetuses with ultrasound anomalies: expanding our knowledge of genetic disease during fetal development. Genet Med. 2017;19:1171-8.
24. ACMG Board of Directors. Points to consider for informed consent for genome/exome sequencing. Genet Med. 2013;15:748-9.
25. Gymrek M, McGuire AL, Golan D, Halperin E, Erlich Y. Identifying personal genomes by surname inference. Science. 2013;339:321-4.