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DE NOVO APPARENTLY BALANCED TRANSLOCATION WITH A NOVEL ABNORMAL PHENOTYPE: REVIEW AND CASE PRESENTATION

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Abstract: Orofacial clefts (OFC) are congenital malformations of great complexity, syndromic or non-syndromic, isolated or associated with other congenital anomalies, in whose etiology, the genetic factor plays a crucial role, genetic mutations and chromosomal abnormalities being undisputed etiological mechanisms involved in the appearance of OFC. We report the first case in the literature of de novo apparently balanced reciprocal chromosomal translocation t(7;16)(p14;p11.1) between two non-homologous autosomal chromosomes, phenotypically associated with unilateral cheilognathopalatoschisis and clubfoot. This new phenotypic association was successfully diagnosed prenatally in a fetus of 23 weeks, illustrating not only the undeniable role of cytogenetics in the early identification of fetal chromosomal abnormalities but also the accuracy of the ultrasonographic prenatal diagnosis as well as the need and the recommendation that classical cytogenetic investigations should be completed, as appropriate, with high-resolution genetic investigations in all cases in which de novo apparently balanced chromosomal rearrangements with abnormal phenotype are identified. Both regions we studied, 7p14 and 16p11.1, represent an important field for future research on the identification of new and potential genes and genetic disorders involved in the complex etiological spectrum of OFC.

Keywords: orofacial clefts, chromosomal translocation, cheilognathopalatoschisis, prenatal diagnosis, ultrasound examination, karyotype.

INTRODUCTION

Orofacial clefts (OFC) are congenital malformations of great complexity, with variable extent and severity, syndromic or non-syndromic, isolated or associated with other congenital anomalies, resulting from the complex interactions between genetic, epigenetic and environmental factors [1-3].

According to recent World Health Organization data, the overall prevalence of OFC is on average 1.42‰ live births, with ethnic, racial, geographical and socio-economic variations [4-6].

Population studies have shown that the highest prevalence of OFC is among American Indians, registering a value of 2.62‰ of live newborns, while among the Caucasian population the prevalence of OFC is only 1.55‰ of live newborns [7].

In relation to the OFC distribution among the world's continents, in Asia the prevalence of OFC is the highest, with a value of approximately 1.57‰ of live newborns. In Europe, the incidence of OFC is slightly lower, registering a value of 1.55‰ of live newborns and Africa is the continent with the lowest prevalence of OFC, registering an average value of only 0.57‰ of live newborns [8, 9].

Gender segregation studies have indicated that cleft lip and palate predominantly affect males, while cleft palates are more frequent in females [10-12].

Family studies calculating the recurrence risk of OFC in offsprings indicated that both the recurrence risk of cleft lip and the recurrence risk of cleft lip and palate are significantly increased, being double in males

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compared to females [13, 14].

Family aggregation studies, the increased recurrence risk of OFC in offsprings as well as twin studies, which show the high concordance of OFC in monozygotic twins compared to dizygotic twins, demonstrate the crucial role of the genetic factor in the etiology of OFC, genetic mutations and chromosomal abnormalities being unquestionable etiological mechanisms involved in the development of OFC [15]. Until now, there have been identified more than 500 genetic syndromes and chromosomal abnormalities whose phenotype include OFC [16, 17].

We present the first case in the literature of de novo apparently balanced chromosomal translocation t(7;16)(p14;p11.1) between two non-homologous autosomal chromosomes phenotypically associated with unilateral cheilognathopalatoschisis and clubfoot, diagnosed prenatally in a 23 weeks fetus.

Case presentation

A 26-year-old Caucasian patient, pregnant for the first time, without significant personal or family medical history, coming from the urban environment, is sent by her attending obstetrician to Alco San Medical Center in Bucharest, Romania, for complex fetal morphology ultrasound scan.

The patient was presented and explained in plain words, the content of the consent form regarding the clinical, paraclinical and ultrasound examination, the procedural steps to be followed in order to carry out specialized investigations, as well as the content of the consent form regarding the protection of personal data. Ethics approval was obtained from the local Ethics Committee of Alco San Medical Center, from Bucharest, Romania, and written informed consent for participation was obtained from the parents.

After the patient gave her written consent for further investigations and research, the ultrasound scan was performed in the Department of Maternal-Fetal Medicine of the Alco San Medical Center by an expert in obstetrics-gynecology, maternal-fetal medicine and medical genetics.

The examination was performed transabdominally, using a Voluson E10 ultrasound machine, BT18 produced by General Electric, GE Healthcare division (Wauwatosa, WI, USA) using the RM6C three-dimensional/four-dimensional (3D/4D) volumetric probe.

The ultrasound examination revealed the presence of a monofetal pregnancy, in evolution, with the fetus in a cephalic presentation and recorded the following biometric parameters: biparietal diameter 53.9 mm, fronto-occipital diameter 71.6 mm and cranial circumference 198.3 mm.

The ultrasound examination of the viscerocranium indicated: visible and normally configured eyeballs, with transsonic lens, nasal cavities with nasal bone: 7.4 mm, normal appearance of the right nasal pyramid, left nasal pyramid modified basally by left cheiloschisis and oral cavity with left cheilognathopalatoschisis (which also includes damage to the hard palate) normal tongue, normognathia and present swallowing movements (Figs 1-4).

The neck is normally configured, with a 2.1 mm thickness of the nuchal fold and the spine is apparently normally structured, without anomalies.

The ultrasound morphological examination of the fetal thorax indicated: antero-posterior thoracic diameter 52.8 mm, transverse thoracic diameter 48.7 mm, with apparently normal lungs, a four chamber heart, with long axis oriented to the left, normally developed septum, cardiac valves of normal appearance, apparently normal disposition of the large vessels at the base of the heart and a fetal heart rate of 156 beats/min.

The biometric assessment of the abdomen registered the following values: antero-posterior abdominal diameter 63.9 mm, transverse abdominal diameter 61.2 mm and abdominal circumference 196.7 mm, with normally developed internal organs.

The ultrasound examination of the limbs revealed trisegmental upper limbs, with humerus length 36.7 mm, ulna length 34.9 mm, radius length 33.8 mm, five-fingered hand, normally structured and trisegmental lower limbs, with femur length 36.8 mm, tibia length 34.6 mm, fibula length 34.6 mm, feet with five toes, normally structured in a plane perpendicular to the frontal plane of the calf and left foot with valgus position (Figs 5-10).

The fetal morphology assessment continued with the ultrasonographic investigation of the umbilical cord, the amniotic fluid, the placenta and the Doppler velocimetry in the uterine arteries, elements where no particularly abnormal aspects were identified.

After thorough ultrasonographic examination of the fetal morphology, the following prenatal diagnosis was established: Monofetal pregnancy in evolution 23.1 weeks (chronological) and 23.2 weeks (biometric); Fetal congenital malformation: left cheilognathopalatoschisis and left clubfoot; Estimated fetal weight 556 g.

Considering the congenital malformations highlighted at the ultrasound scan, further prenatal investigations were recommended. Thus, during the genetic consultation the patient was informed that her

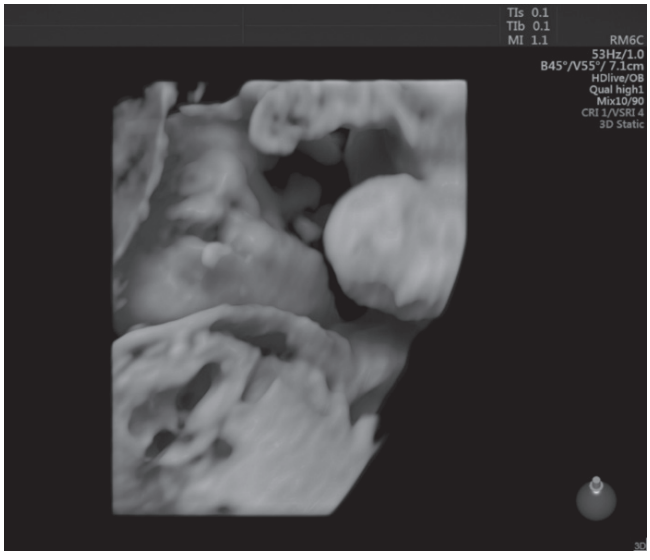

Figure 1. Three-dimensional ultrasound image of the fetal viscerocranium indicated: nasal cavities with normal appearance of the right nasal pyramid, left nasal pyramid modified basally by left cheiloschisis and oral cavity with left cheilognathopalatoschisis.

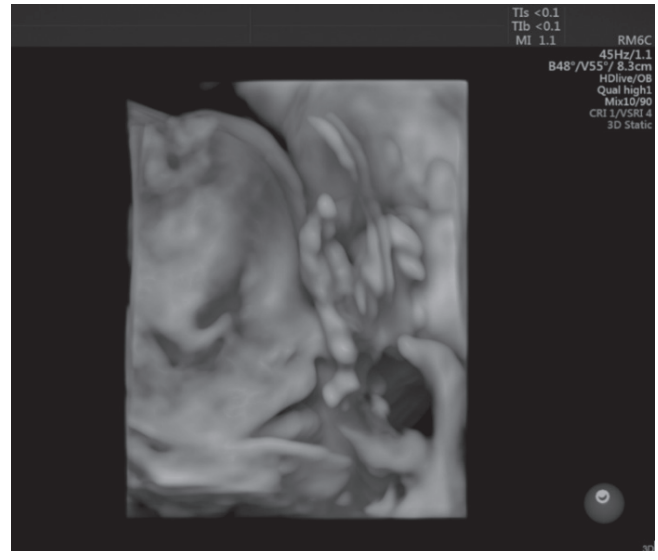

Figure 2. Three-dimensional ultrasound evaluation of fetal profile indicated: normal appearance of the right nasal pyramid and left cheiloschisis.

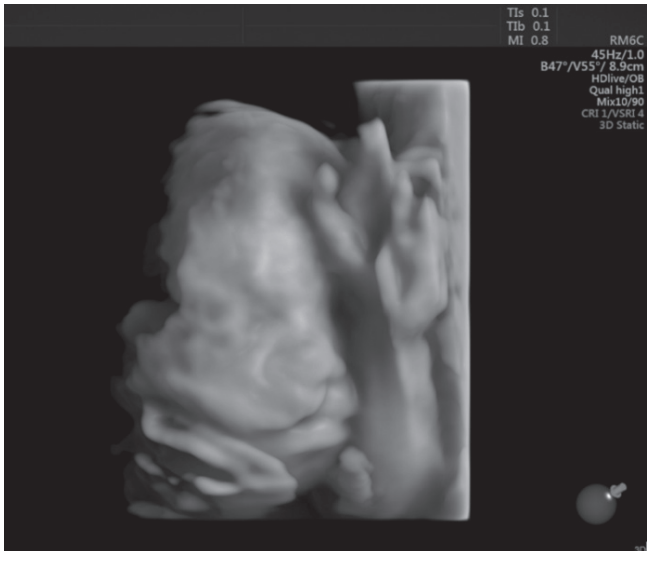

Figure 3. Three-dimensional ultrasound evaluation of fetal profile indicated: visible and normally configured eyeballs, normal appearance of the right nasal pyramid, left nasal pyramid modified basally by left cheiloschisis, and normognathia.

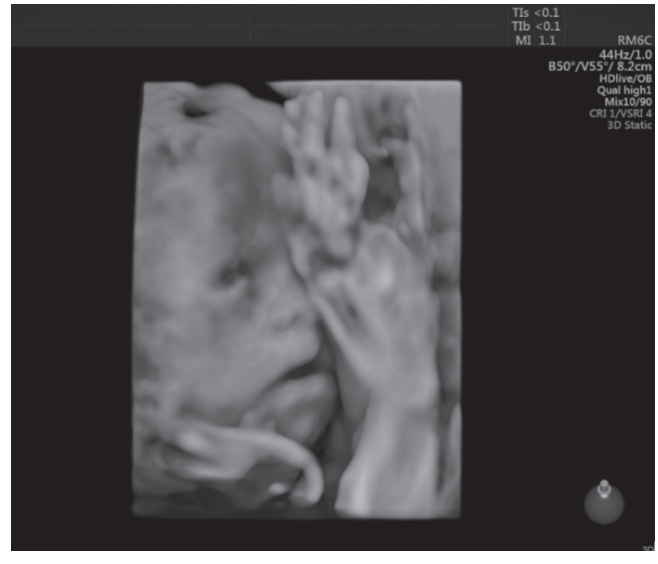

Figure 4. Three-dimensional ultrasound evaluation of fetal profile indicated: normal appearance of the right eyeball, with transsonic lens, visible and normally configured right nasal pyramid, left cheiloschisis, and normognathia.

fetus is malformed, the fetal congenital anomalies were described and the need for additional investigations was explained, namely amniocentesis accompanied by amniotic fluid sampling and determination of fetal karyotype.

Amniocentesis was performed with the consent of the pregnant woman, following the standard protocol.

Under sterile conditions, local anesthesia of the abdomino-pelvic wall was accomplished and under ultrasound guidance, approximately 8 ml of amniotic fluid was drawn, using a puncture needle with a length between 18-22 cm and a thickness of 0.8 mm, inserted transabdominally in the amniotic cavity. Subsequent, the amniotic fluid extracted was centrifuged for 5 minutes at a speed of 1000 RPM. From the supernatant we performed biochemical analysis and from the sediment represented by amniocytes, the amniocyte culture was obtained. This was developed under strict aseptic conditions, at 37 degrees Celsius, for a period of

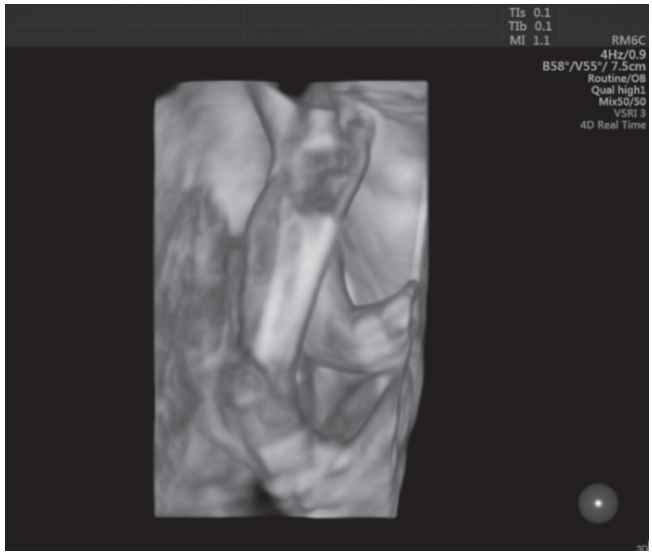

Figure 5. Three-dimensional ultrasound evaluation of the fetal limbs revealed normal appearance of the right lower limb and left foot with valgus position.

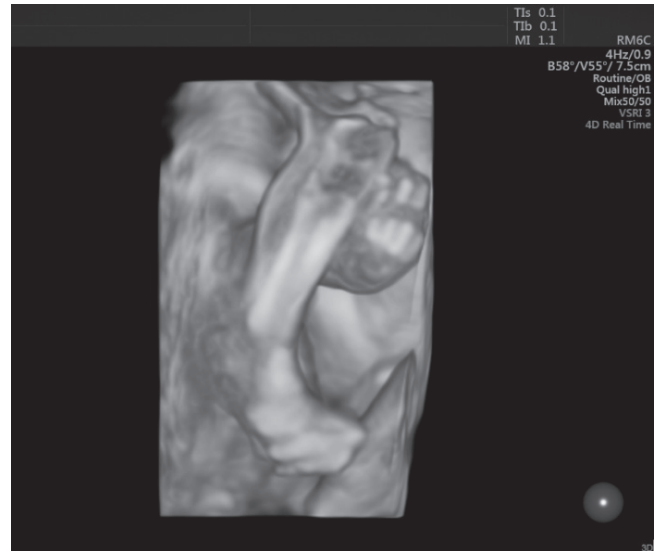

Figure 6. Three-dimensional ultrasound evaluation of the fetal limbs revealed trisegmental lower limbs, feet with five toes, normally structured, and left foot with valgus position.

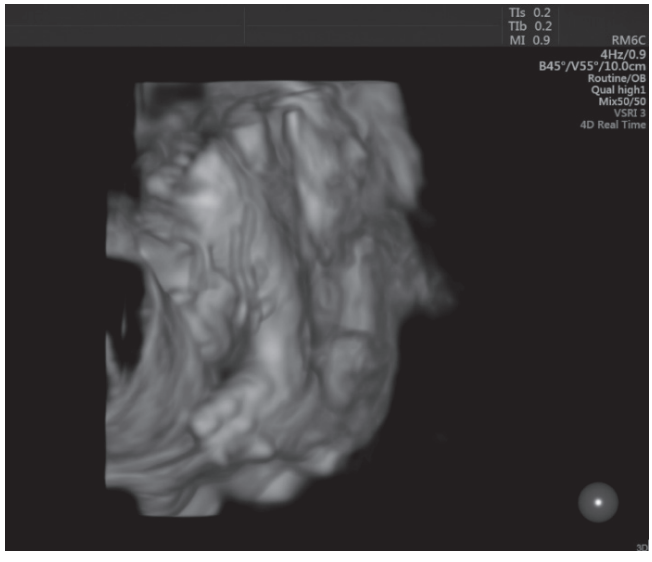

Figure 7. Three-dimensional ultrasound evaluation of the fetal lower limbs revealed left clubfoot.

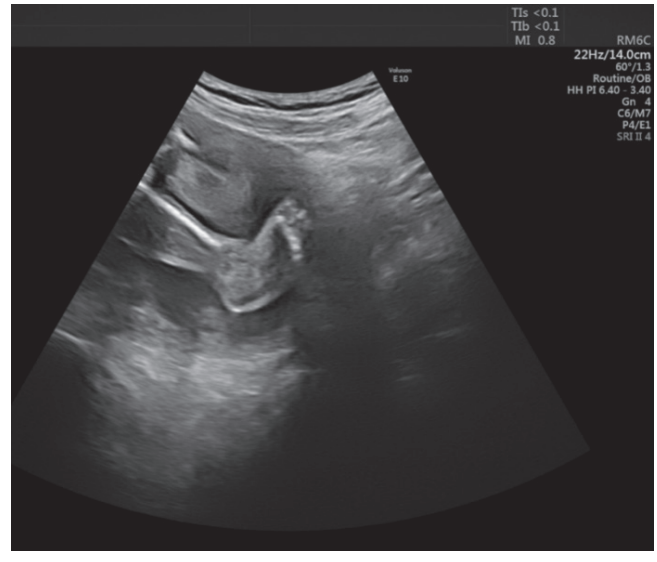

Figure 8. Two-dimensional ultrasound evaluation of the fetal left lower limb revealed left foot in valgus position, with five toes.

10-14 days, along with the necessary nutritional support, afterwards, the culture was processed by blocking the division of amniocytes with colchicine. Next, the culture was examined, followed by the microscopic and computerized analysis of 20 amniocytes, previously stained with 2% Giemsa solution. After completing all the work steps provided in the standard protocol, we obtained the fetal karyotype, which clearly indicated the presence of an abnormal fetal karyotype, respectively 46, XY, t(7;16)(p14;p11.1) (Figs 11 and 12).

Given the diagnosed fetal chromosomal abnormality, during a new genetic consult, we presented and explained the couple the result of the fetal cytogenetic diagnosis and also emphasized the need to perform the cytogenetic analysis of the chromosomes in the peripheral maternal blood and paternal blood, respectively, in order to identify the origin of the fetal chromosomal abnormality.

The parental karyotype performed from peripheral blood, according to standard procedures, did not register the presence of chromosomal abnormalities.

Gathering the results of non-invasive and invasive prenatal investigations, as well as the results of all cytogenetic examinations, the parents decided the termination of pregnancy, due to the genetic diagnosis.

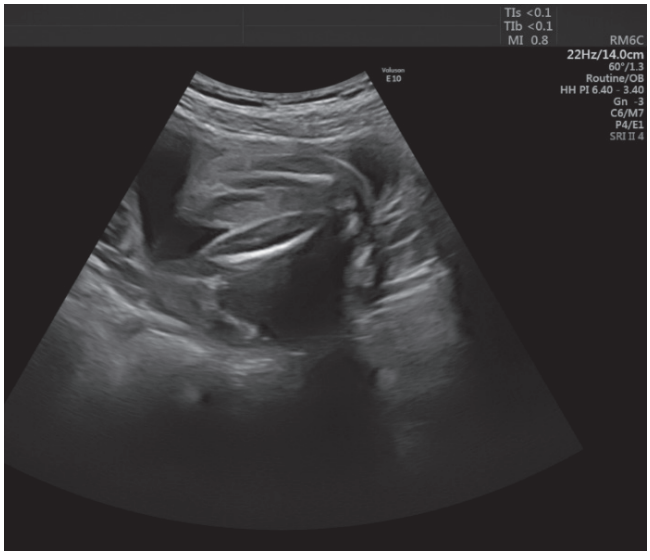

Figure 9. Two-dimensional ultrasound evaluation of the limbs revealed trisegmental left lower limb and left clubfoot.

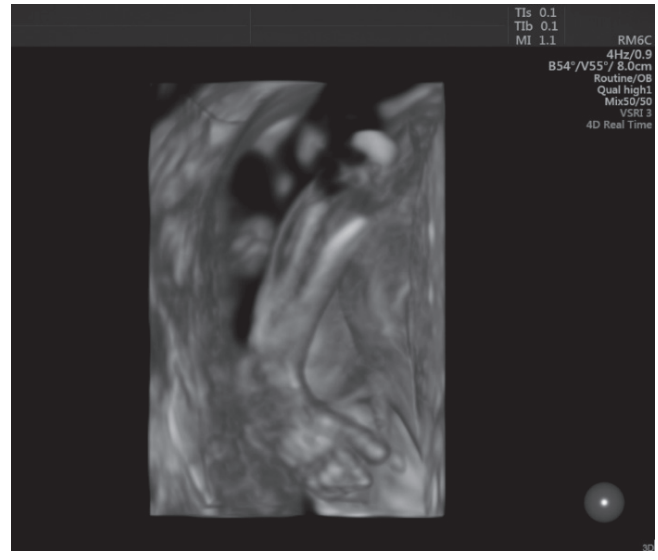

Figure 10. Three-dimensional ultrasound evaluation of the fetal left lower limb revealed trisegmental limb normally structured and left foot with five toes in valgus position.

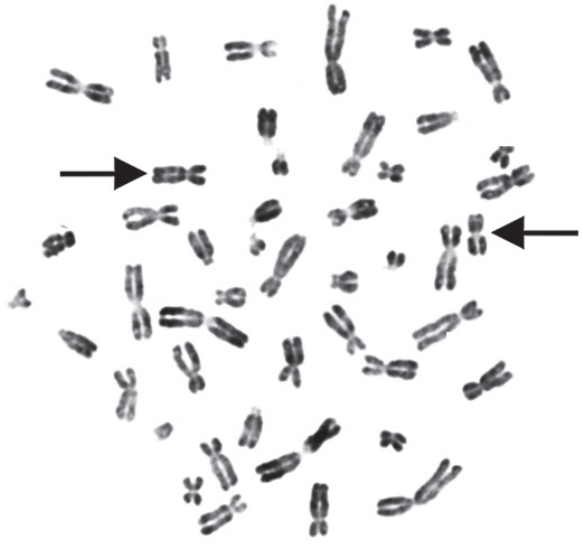

Figure 11. Metaphase plate obtained from amniotic cell culture. Cytogenetic analysis identifies: Reciprocal translocations between chromosomes t(7;16).

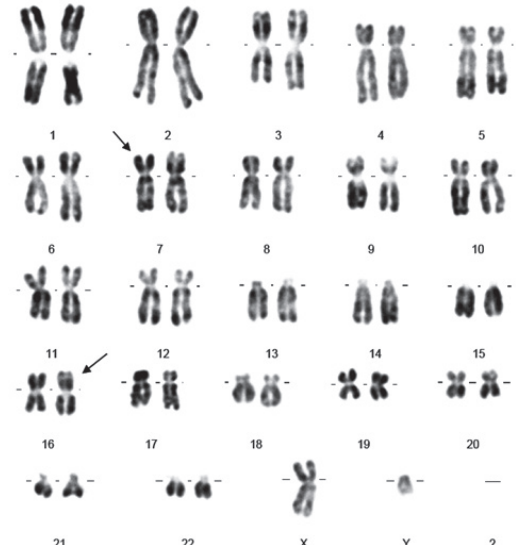

Figure 12. Fetal karyotype: 46, XY, t(7; 16)(p14; p11.1)

DISCUSSION

During the fetal morphology ultrasound examination, the most common craniofacial malformations encountered are OFC [18, 19].

From 2011 till 2018 the Commission of European Communities reported in Europe an average global prevalence of OFC of 1.27 ‰ live births, of which, the average global prevalence of cleft lip is 0.74 ‰ live births and the average global prevalence of cleft palate is 0.53 ‰ live births, the value being higher in northern european countries, compared to southern European countries [20, 21].

In Romania, 350-400 children with different forms of OFC are born annually, their estimated average prevalence, in the absence of a national rigorous recording and reporting, being about 1.25 ‰ live births.

A recent study, dating from 2019, indicates a prevalence for OFC in south-eastern Romania of 1.18 ‰ live births, the most common being unilateral cleft lip and complete bilateral clefts of the soft palate and hard palate [22].

Given the overwhelming social impact of OFC,

both for the patients and their families, an appropriate multidisciplinary approach is compulsory, established early and conducted with great professionalism, to allow a better assessment of the diagnosis and adequate counseling of the family [23, 24].

Currently, the prenatal diagnosis of OFC is possible by systematic visualization of the fetal face during routine prenatal ultrasound investigation, completed with complex and complete ultrasonographic examinations performed in centers of excellence, starting from the 20th week of pregnancy [25].

For the fetal ultrasound examination, we used the most advanced technology dedicated to obstetrics, by General Electric (Wauwatosa, WI, USA). The Voluson E10 ultrasound machine we used, is a product from the Expert series, in one of the latest versions BT18, is currently the most advanced and powerful ultrasound examination system developed for advanced, complex diagnosis in maternal-fetal medicine and the RM6C 3D/4D volumetric probe (GE Medical Systems, Zipf, Austria) we used, offers the best resolution based on Radiance System Architecture, allowing us the prenatal diagnosis of OFC with high accuracy [26-31].

In the event of an ultrasound diagnosis clearly indicating the existence of fetal OFC, genetic investigations must be carried out, taking into account that OFC are often part of the clinical phenotype of a chromosomal genetic syndrome.

For accurate fetal cytogenetic diagnosis, provided by the fetal karyotype, amniotic cells were cultured in two independent cultures (two flasks) and the cytogenetic analysis was performed by two different investigators, using Karyotyping System Image Analysis Software.

Thus, 20 metaphases were analyzed, 10 of which were karyotyped.

The cytogenetic analysis indicated an apparently balanced reciprocal translocation between two non-homologous autosomal chromosomes, namely a chromosome 7 and a chromosome 16. The cleavage occurred at the p14 band located on the short arm of the chromosome 7 and at the centromere of the chromosome 16, respectively, more precisely at the p11.1 band located on the short arm of the chromosome 16. The dislocated segments at the level of these bands were exchanged, thus achieving a reciprocal exchange of genetic material between the two non-homologous chromosomes.

In human populations, reciprocal translocations, and more precisely balanced reciprocal translocations are the most common structural chromosomal abnormalities encountered [32]. Thus, the incidence of balanced chromosomal translocations, in which there is no loss or excess of genetic material detectable on cytogenetic examination, is 2 ‰ live births [33].

If in the case of balanced chromosomal translocations, the risk of their association with different phenotypic abnormalities is reduced, in case of apparently balanced chromosomal translocations, the risk increases significantly [34].

Often, the carriers of apparently balanced reciprocal translocations are asymptomatic, but in some cases a disruption of the DNA sequence can occur at the cleavage point, which can lead to altered gene expression [35]. Thus, it is estimated that 60 % of cases with apparently balanced chromosomal translocations have phenotypically different congenital malformations [36].

Any deviation from the normal number of chromosomes or their normal structure is considered a chromosomal aberration, which may occur spontaneously, de novo, as in the present case, or may be inherited from a parent.

De novo abnormalities are occasional genetic errors that can occur during cell division and can lead to genetically abnormal gametes in terms of structure or number of chromosomes [32]. As a result, if a parent is the carrier of a balanced chromosomal rearrangement, even though he has no clinical signs, he can produce a number of genetically abnormal gametes. The fusion of a gamete with abnormal chromosomes with a normal gamete results in abnormal zygotes which can lead to fetal loss, perinatal death, birth defects, growth retardation, mental retardation and infertility [37].

De novo apparently balanced chromosomal translocations are atypical, rare cases, more often associated with various phenotypic abnormalities [38]. They appear spontaneously, in their etiology being involved several mechanisms, such as cryptic deletion, impairment of gene function following alteration of their structure at the level of the chromosomal breakpoints or uniparental disomy [39].

It is estimated that at conception, 30-40% of embryos have chromosomal abnormalities and the incidence of chromosomal abnormalities in spontaneous abortions during the first trimester of pregnancy is 50-60%. In case of miscarriages during the second trimester of pregnancy, the incidence of fetal chromosomal abnormalities is 1.5 % for mothers aged 35-36 years and increases to 5.5 % for mothers aged 43-44 years. Regarding newborns, about 1 % of them are

chromosomally abnormal.

Broadly speaking, about 0.2 % of the population is a carrier of a chromosomal rearrangement [40]. This percentage increases to 7-9 % in case of couples who previously had repeated miscarriages [41]. Moreover, in infertile men, for example, the incidence of chromosomal rearrangements is 2.15 % and increases in azoospermic men to 15.38 % [42].

Balanced chromosomal rearrangements appear to have a significantly increased rate, suggesting that certain chromosomal aberrations, such as translocations, deletions or inversions, involve genes, so-called candidate genes, which act as susceptible factors for various congenital anomalies and genetic disorders, which include in their clinical phenotype different forms of OFC [43, 44].

Recent studies have confirmed that MTHFR (1p36), LPHN2 (1p31), IRF6 (1q32), TGFA (2p13), SATB2 (2p32), PVRL3 (3q13.3), MSX1 (4p26), ACOD4 (4q21), COL21A1 (6p12.1), 6p23, PVRL1 (11q23), FOXG1 and HECTD1 (14q12), TGFB3 (14q24), CLPTM1 (19q13) and TBX22 (Xq12) are certainly candidate genes involved in the development of non-syndromic cleft lip and palate [45-47]. To all these can be added the proto-oncogenes BCL3 (19q13.1-q13.2) and SKI (1q22-q24), along with the RARA (17q21) gene [48].

Moreover, the latest research in genetic linkage has indicated that other genes may be considered candidate genes involved in the development of non-syndromic cleft lip and palate, such as FOXP2 (7q22.3-q33) and TOX3 (16q12.1) [49]. Thereby, it has been elucidated that the 16q12 microdeletion is associated with a severe craniofacial dysmorphic syndrome, while the only case cited in the literature, of de novo balanced translocation involving the short arms of chromosomes 7 and 16, namely t(7;16)(p22.1;p11.2) does not associate craniofacial dysmorphic features, but only a set of signs and symptoms that were included in the clinical spectrum of autism [50-52]. Other genetic syndromes caused by the balanced reciprocal chromosomal translocation of the short arms of chromosomes 7 and 16 are not cited in the literature as being associated with OFC.

Taking into account the limitations of the cytogenetic analysis given by its inability to detect submicroscopic genomic alterations < 5Mb, chromosomal mosaicism in low percentage < 30 %, cryptic aberrations and point mutations and corroborating the results of cytogenetic analysis with the fetal ultrasonographic phenotype, we consider that there might have been a disruption of the DNA sequence at the cleavage point 7p14, which altered the gene expression.

We support this hypothesis based on the fact that greater de novo chromosomal deletions may interfere with the normal craniofacial development, producing different clinical forms of OFC and de novo chromosomal deletions 7p14 are considered a risk factor in the appearance of cleft lip with or without cleft palate (CLP), although the basic biological mechanisms are still unelucidated [53]. In addition, the partial monosomy 7p, is phenotypically associated with musculoskeletal abnormalities such as clubfoot (talipes calcaneovalgus), similar to those we illustrated in the ultrasound examination of the fetal morphology [54].
Regarding the 16p11.2 cleavage point, the alteration by deletion at this point, produces the so-called 16p11.2 microdeletion syndrome.

The 16p11.2 microdeletion syndrome, seldom encountered, has been divided into three groups, according to the location and extent of the chromosomal deletion. Therefore, the three groups are the following: group 1 corresponds to the typical 16p11.2 microdeletion, group 2 - extremely rare, which corresponds to the deletion of various sizes, but does not include the typical 16p11.2 microdeletion and group 3 comprises extensive deletion which also includes the typical 16p11.2 microdeletion. Interestingly, from the point of view of the case we presented is the 16p11.2 microdeletion syndrome from group 2, which is extremely rare, including only a few reported cases, in which the deletion corresponds to the loss of the chromosomal fragment between 16p12.1 and 16p12.2, without including the typical microdeletion. The interest lies in the fact that in the literature we found only two interesting cases for our study, respectively, the case of a newborn with CLP and the case of another newborn with clubfoot [55, 56], but in both these cases, the cause was determined by the 16p12.1 - 16p12.2 deletion, without including the typical 16p11.2 microdeletion which corresponds exactly to the splitting point highlighted in the case presented by us. Moreover, up until now, there are no reported cases of patients with 16p11.2 microdeletion syndrome from group 1 or group 3, groups that include the typical 16p11.2 microdeletion and which phenotypically associate OFC or limb abnormalities, which represents additional arguments to consider that in our case the disruption of the DNA sequence took place at the cleavage point 7p14, producing the alteration of gene expression, the phenotypic consequences including cleft lip with cleft

palate and clubfoot.

Our case report is the first case of de novo apparently balanced reciprocal translocation t(7;16) (p14;p11.1) between two non-homologous autosomal chromosomes, phenotypically associated with cheilognathopalatoschisis and clubfoot, diagnosed on prenatal ultrasound at 23 weeks of pregnancy, which illustrates both the crucial role of cytogenetics in the study of de novo apparently balanced chromosomal translocations with abnormal phenotype and the accuracy of non-invasive ultrasonographic prenatal diagnosis.

We consider it is absolutely necessary to perform classical cytogenetic investigations and molecular genetics investigations in all cases where de novo chromosomal rearrangements are identified.

We appreciate that both regions we studied, 7p14 and 16p11.1, remain an important field for future research, which will identify new important genes involved in the etiology of OFC, located in other chromosomal regions for which no abnormalities have been reported so far.

The characterization of different types of chromosomal aberrations and the identification by methods of cytogenetics or high-resolution genetic analysis of new and potential structural chromosomal disorders involved in the etiology of OFC can result in locating relevant genes involved in these abnormalities.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

All authors contributed equally with the first-author, in the preparing, review and editing of the article. All authors read and approved the final version of the manuscript.

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