


CASE REPORT

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Inherited unbalanced reciprocal translocation with 3q duplication and 5p deletion in a foetus revealed by cell-free foetal DNA (cffDNA) testing: a case report

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Abstract

Background: Since 2011, screening maternal blood for cell-free foetal DNA (cffDNA) fragments has offered a robust clinical tool to classify pregnancy as low or high-risk for Down, Edwards, and Patau syndromes. With recent advances in molecular biology and improvements in data analysis algorithms, the screening's scope of analysis continues to expand. Indeed, screening now encompasses additional conditions, including aneuploidies for sex chromosomes, microdeletions and microduplications, rare autosomal trisomies, and, more recently, segmental deletions and duplications called copy number variations (CNVs). Yet, the ability to detect CNVs creates a new challenge for cffDNA analysis in couples in which one member carries a structural rearrangement such as a translocation or inversion.

Case presentation: We report a segmental duplication of the long arm of chromosome 3 and a segmental deletion of the short arm of chromosome 5 detected by cffDNA analysis in a 25-year-old pregnant woman. The blood sample was sequenced on a NextSeq 550 (Illumina) using the VeriSeq NIPT Solution v1 assay. G-band karyotyping in amniotic fluid only detected an abnormality in chromosome 5. Next-generation sequencing in amniocytes confirmed both abnormalities and identified breakpoints in 3q26.32q29 and 5p13.3p15. The foetus died at 21 weeks of gestation due to multiple abnormalities, and later G-band karyotyping in the parents revealed that the father was a carrier of a balanced reciprocal translocation [46,XY,t(3;5)(q26.2;p13)]. Maternal karyotype appeared normal.

Conclusion: This case provides evidence that extended cffDNA can detect, in addition to aneuploidies for whole chromosomes, large segmental aneuploidies. In some cases, this may indicate the presence of chromosomal rearrangements in a parent. Such abnormalities are outside the scope of standard cffDNA analysis targeting chromosomes 13, 18, 21, X, and Y, potentially leading to undiagnosed congenital conditions.

Keywords: Cell-free DNA, Copy number variations, Reciprocal translocation

Background

Analysis of cell-free foetal DNA (cffDNA) in the maternal bloodstream is a non-invasive prenatal approach that enables clinical screening for aneuploidies, minimising risks and the need for invasive procedures [1–4]. Presence of cffDNA in the maternal bloodstream was initially

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described by Lo et al. [5] in 1997. cffDNA consists of 150–200-base pair (bp) fragments that originate mainly from the placenta and are released into the mother's blood, detectable at about 4–5 weeks of gestation [6]. At about 10 weeks, the concentration of cffDNA reaches a sufficient level to provide adequate specificity and sensitivity to perform screening in prenatal care [7].

Since 2011, providers have used clinical cffDNA screening in maternal blood to detect the most frequently observed foetal chromosome aneuploidies, including Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), Patau syndrome (trisomy 13), and sex chromosome aneuploidies like Turner syndrome (45,X) and Klinefelter syndrome (47,XXY) [8–10]. cffDNA analysis can also determine foetal rhesus D status [11] and detect some microdeletion syndromes such as 22q11 deletion syndrome, cri-du-chat syndrome (5p deletion), Wolf–Hirschhorn syndrome (4p deletion), Prader Willi or Angelman syndrome (15q deletion), Jacobsen syndrome (11q deletion), Langer–Giedion syndrome (8q deletion), and 1p36 deletion syndrome [12–14].

With new advances in next-generation sequencing (NGS) and bioinformatic analysis, the number of genetic conditions analysable by cffDNA screening is expanding. For instance, analysis of all 24 chromosomes [15–18] and even detection of some monogenic conditions [19] are currently available in the portfolio of some laboratories offering cffDNA analysis worldwide. One of the most interesting advances in analysis of cffDNA in maternal blood is detection of placental copy number variations (CNVs) of a relatively long size without increasing sequencing depth [20–25].

The ability to detect CNVs entails a new challenge for cffDNA analysis, enabling detection of new genetic conditions that have gone unnoticed in couples in which one member is a carrier of a structural rearrangement such as a translocation or inversion [26]. Here, we describe a case of two segmental aneuploidies initially detected by extended cffDNA analysis and confirmed in the foetus by invasive procedures. Karyotype of the parents revealed that the father carried a balanced reciprocal translocation.

Case presentation

Clinical report

A 25-year-old, G1P0, healthy pregnant woman was referred to the Clínica Nanu in Sao Paulo, Brazil, at a gestational age of 16 weeks. As routine practice, an ultrasound examination was performed to monitor the foetus's developmental state. Ultrasonograms revealed a nuchal translucency (NT) of 7.12 mm and generalised oedema. The pregnant woman was 167 cm tall and weighed 59 kg and exhibited typical developmental

milestones. She and her 29-year-old husband were not consanguineous. There was no family history of genetic disease, but her husband's mother and half-sister had a history of miscarriage without a defined aetiology. Despite the abnormalities observed by ultrasound, the patient refused amniocentesis.

Screening for aneuploidies by cffDNA analysis

Extended cffDNA analysis in maternal blood was offered to screen for aneuploidies. The analysis was performed at Igenomix Brazil with the VeriSeq NIPT Solution v1 assay (Illumina). Briefly, cffDNA was obtained from 1-mL plasma with a modified protocol of the QIAamp DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany). Sample indexing and library preparation for sequencing were performed using TruSeqNano DNA Sample Prep Kits (Illumina Inc., San Diego, CA, USA). Libraries of all samples of a run were pooled, and paired-end reads of 36 bp were sequenced in a NextSeq 500 system (Illumina Inc.). VeriSeqNIPT Assay Software v1 was used for the analysis of the aneuploidy status and foetal fraction. To detect partial abnormalities, WisecondorX (v1.1.5) was run following the default workflow [27], and visualisation was generated using ggplot2 [28] and ggbio [29] R packages. Results of the cffDNA analysis with WisecondorX showed two calls ($|z\text{-score}| > 5$) on chromosomes 3 and 5, compatible with a duplication of approximately 20 Mb ($z\text{-score} = 13.5$) in chromosome 3q (Fig. 1A) and a deletion of approximately 30 Mb in chromosome 5p ($z\text{-score} = -13.95$) (Fig. 1B). To rule out the possibility of a sequencing artefact, cffDNA analysis was performed on a second blood draw, and the results were concordant. Results were discussed with the obstetrician, and the couple received genetic counselling.

Chromosome analysis on amniotic fluid by karyotype

A subsequent ultrasound indicated spina bifida, severe cystic hygroma, and hydrops. Given the nature of both the ultrasound and cffDNA results, the patient agreed to have an amniocentesis to confirm the findings. Amniocentesis was performed of ultrasound at the Centro de Ultrasonografia, Medicina e Cirurgia Fetal FMFLA. Two samples of amniotic fluid were collected; one was sent to an external laboratory for karyotype analysis. Amniocytes were cultured in appropriate culture media, and 25 metaphases were analysed (G-banding with a resolution of 400 bands). Cytogenetic analysis of amniocytes revealed an abnormal chromosome set corresponding to a cytogenetically male foetus showing an alteration in chromosome 5 but not in chromosome 3 (46,XY,der(5)) (Fig. 2A).

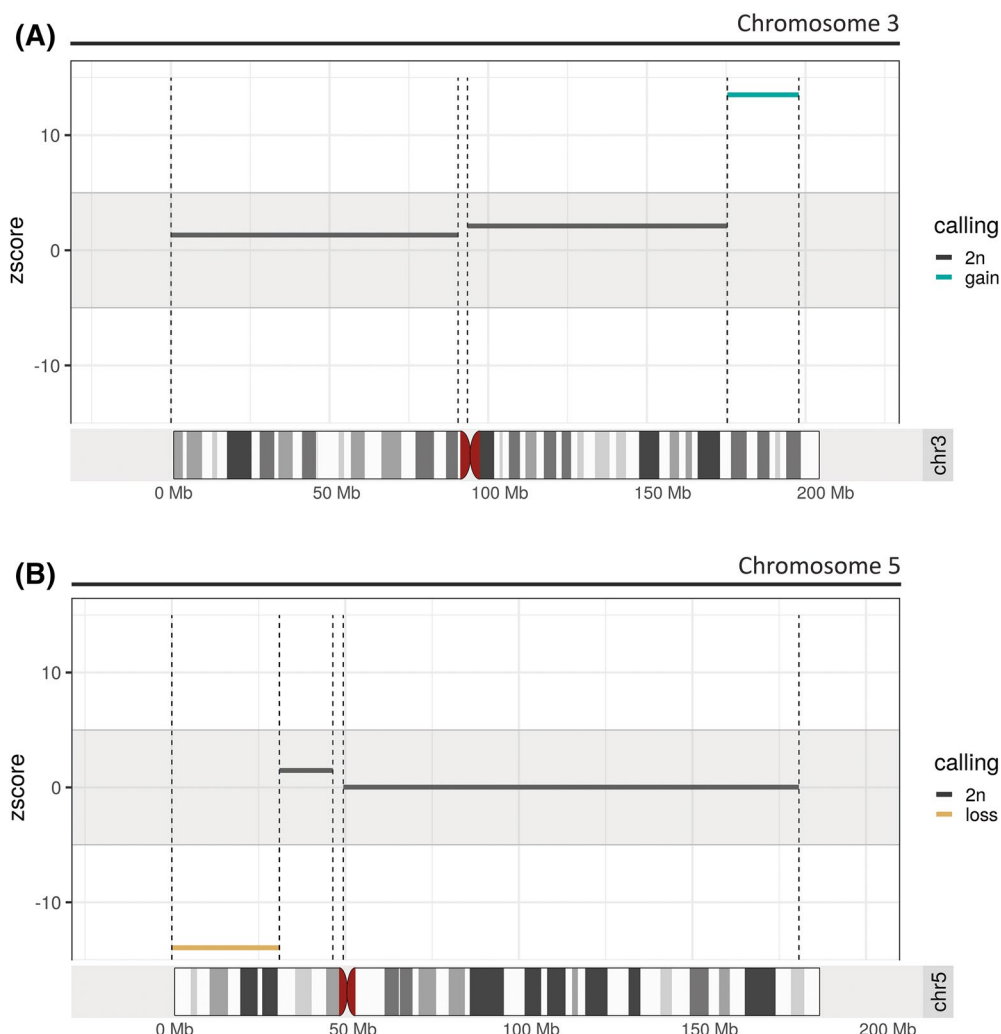


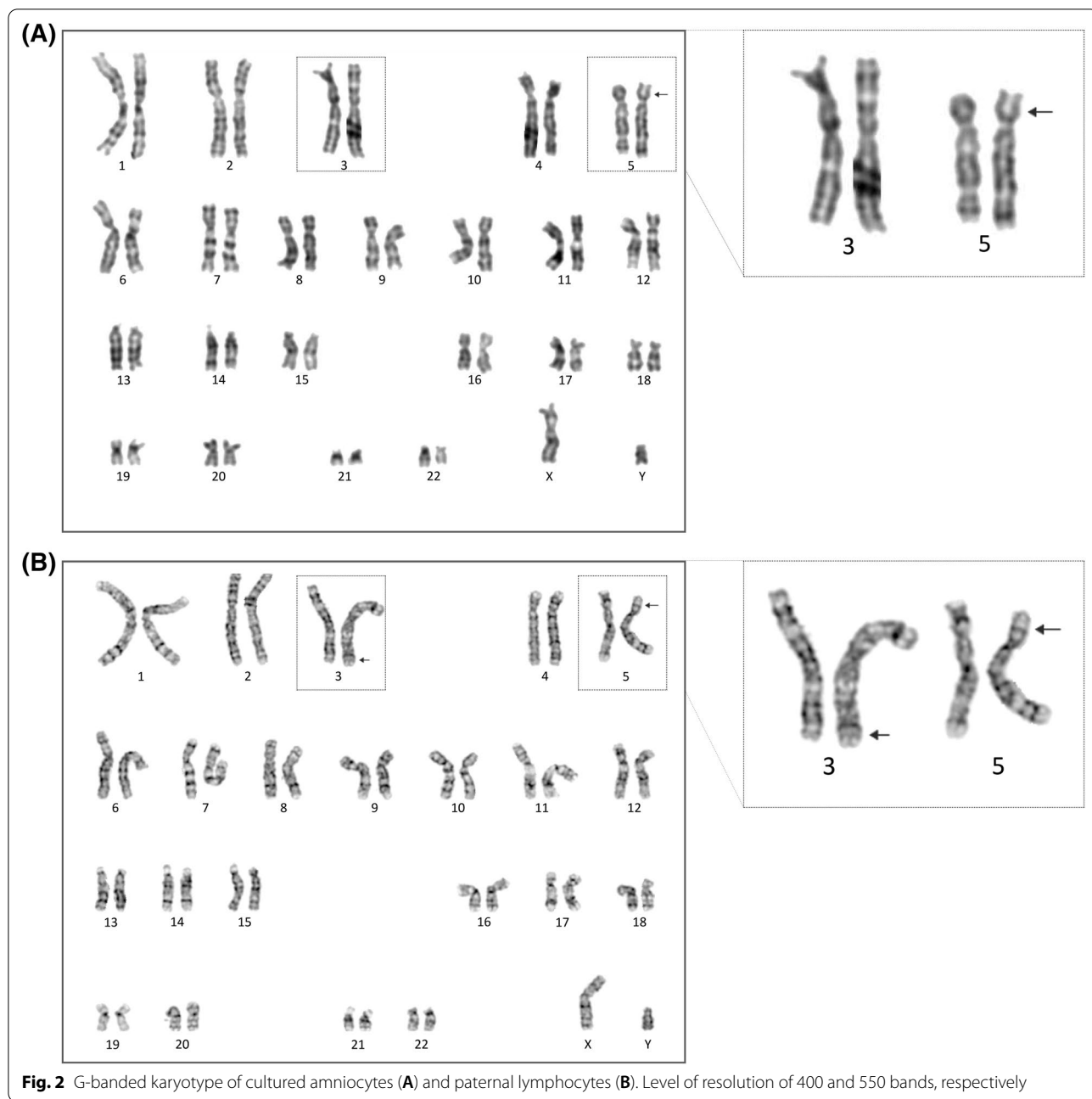
Fig. 1 Results of partial aneuploidy screening performed with the VeriSeq NIPT Solution v1 assay and the WisecondorX software, using cffDNA from the maternal bloodstream. Z-score variations (vertical axis) for the different cytogenetic positions (horizontal axis) are shown. Note that the distances between the cytogenetic bands are presented in megabases (Mb). **A** Results showing a gain of approximately 25 Mb in the long arm of chromosome 3 (light blue bar) with a mean Z-score value of 13.5. **B** Results showing a loss of approximately 30 Mb in the short arm of chromosome 5 (orange bar) with a mean Z-score value of -13.95

Chromosome analysis on amniotic fluid by NGS

A second aliquot of amniotic fluid was sent to Igenomix Brazil for rapid prenatal diagnosis by qfPCR, which detected no anomaly for chromosomes 13, 18, 21, or sex chromosomes. Given that the karyotype did not detect the alteration observed by cffDNA analysis in chromosome 3, we used genomic DNA from uncultured amniocytes to perform NGS to identify single-copy losses and gains of whole or partial segments of chromosomes. NGS was performed according to the protocol published by García-Pascual et al. [30]. With our procedure validated to detect aneuploidies > 10 Mb, we determined chromosomal breakpoints. Analysis revealed both alterations and

chromosomal breakpoints at 3q26.32 and 5p13.3. These involved a segmental gain of 22.1 Mb of genetic material in chromosome 3 [(chr3:175865635_197962430) × 3; 3q26.32q29] and a segmental loss of 32.0 Mb in chromosome 5 [(chr5:10000_31993149) × 1; 5p15.33p13.3] (Fig. 3). According to data in UCSC, ClinVar, and Decipher databases, there are 230 genes in this chromosome 3 fragment, 44 of which are described in the OMIM database as related to a pathology. In the chromosome 5 fragment, 128 genes are described, 16 of which are related to a pathology.

The foetus died in utero at 21 weeks due to multiple malformations.



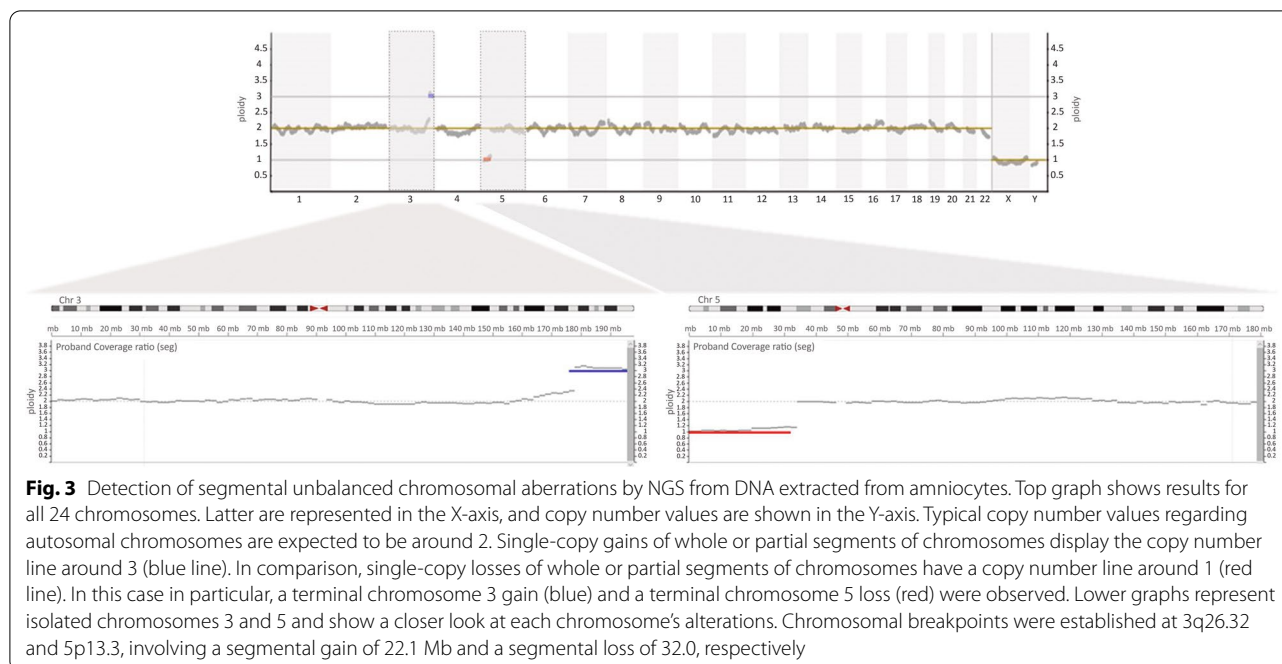
Cytogenetic assessment of parents by karyotype

After confirmation of abnormalities in amniotic fluid, the couple received further genetic counselling. Karyotyping of peripheral blood (at a resolution of 550 bands) was performed to rule out a balanced rearrangement in either parent. The paternal karyotype showed a balanced translocation between chromosomes 3 and 5 [46, XY,t(3;5)(q26.2;p13)] (Fig. 2B), while the maternal karyotype revealed no apparent abnormality.

Taking into account all these findings, we established the result of the complete cytogenomic analysis for the foetus as:

46,XY,der(5)t(3;5)(q26.2;p13)dpat.seq[GRCh37]
der(5)t(3;5)(q26.32;5p13.3)dpat.

NC_000005.10:g.pter_31993149delins[NC_000003.1
2:g.175865635_qter].



Discussion and conclusion

Prenatal diagnosis of large subchromosomal CNVs in clinical practice still relies on invasive testing, such as karyotyping of placental or foetal genetic material through chorionic villus sampling (CVS) or amniocentesis [31]. Chromosomal microarray analysis (CMA) is a robust tool for detecting invisible small chromosomal deletions or duplications and is recommended as a first-tier diagnostic tool for some patients with well-defined syndromes [32]. During the prenatal period, CMA is strongly recommended for high-risk pregnancies, especially those with abnormal ultrasound with no alterations detected in the karyotype obtained from amniotic fluid or CVS [33]. In general, there is no recommendation for CMA in low-risk pregnancies with no ultrasound findings. Since abnormal CNVs can occur even in pregnancies without any structural abnormality observed in ultrasound [33], these genetic changes may constitute the first available evidence to detect a future newborn with congenital conditions or intellectual disabilities.

Since the initial clinical application of *cffDNA* analysis, the main objective has been to classify pregnant women with low- or high-risk for whole chromosome anomalies, especially chromosomes 13, 18, 21, X, and Y. However, improvement of the algorithms for data analysis used in the *cffDNA* screening now enables detection of other genetic changes. That includes CNVs, which in many cases are associated with maternal pathological processes such as gynaecological and haematologic tumours [34–37]. Correspondingly, increasing studies

describe CNV alterations detected by *cffDNA* analysis during pregnancy and confirmed by invasive procedures. Wang et al. [37] described detection of subchromosomal abnormalities in chromosomes 13 and 21 derived from the mother with a balanced translocation [46,XX,inv(9)(p12q13),t(13;21)(q31.3;q21.3)]; Mei et al. [38] described a 10p15.3p13 duplication inherited from the father with a balanced translocation [46,XY,t(5;10)(q35.1;p13)]; Chen et al. [39] described a duplication in 18q11.32q21.2 and a deletion in Xp22.33p11 derived from a maternal reciprocal translocation; Zheng et al. [40] found a deletion in chromosome 21 of a foetus whose karyotype was 46,XN,del(21)(q11.2q22.1); and finally, Liu et al. [41] described the concomitant occurrence of 8q duplication and 13q deletion in a foetus derived from a maternal balanced translocation. Similar cases have been reported for patients undergoing *cffDNA* analysis in maternal blood. Recently, Yin et al. [42] described detection of CNVs by *cffDNA* testing with 32 of 48 suspicious cases confirmed with amniocentesis karyotyping.

Here, we describe a case in which *cffDNA* analysis classified the pregnancy as high-risk for foetal chromosomal abnormalities (a 3q segmental duplication and 5p segmental deletion). Initially, only the abnormality in chromosome 5 was confirmed by karyotyping of amniotic fluid. The recommended resolution in routine karyotyping when analysing amniocytes is 400 bands [43], with a theoretical resolution of 5–10 Mb. However, the actual resolution depends on different factors (e.g., type of bands affected, efficiency in chromosome preparation).

In many cases, large CNVs could sometimes be missed [33, 44, 45]. This case highlights that, in the absence of a CMA, optimal resolution for the karyotype should have been 550 bands [46]. A subsequent analysis using NGS in DNA from uncultured amniocytes confirmed the cffDNA findings, setting the chromosomal breakpoints at 3q26.32 and 5p13.3. These imbalances were later traced to a balanced reciprocal translocation in the father (Fig. 2B), establishing the karyotype for the foetus as 46,XY,der(5)t(3;5)(q26.2;p13)pat.

The 3q duplication [dup(3q)] syndrome is rare, usually diagnosed after birth [47, 48], and is characterised by anomalies in the limbs and possible association with internal malformations, growth deficiency, dysmorphic face, and cognitive deficits [49]. Pasińska et al. [50] reported a prenatal diagnosis of 3q duplication syndrome in a foetus with enlarged skin oedema up to the sacral and pericranial regions with a NT of 8 mm. Interestingly, we observed oedema and a similar NT value in the ultrasound examination. The critical region responsible for the typical phenotype of dup(3q) syndrome was mapped to region 3q26.3q27.7, which contains many genes (e.g., *NLGN1*, *BCHE*, *TNIK*, *SOX2*, *Map6D1*) characterised by high expression during foetal brain development [51, 52]. The CNV we report here involves the 3q29 region associated with microcephaly, moderate cognitive deficits, and other abnormalities [53] that characterise 3q29 microduplication syndrome (MIM 611936). As reported by Abreu-González et al. [54], some patients with dup(3q) syndrome also have duplicated 3q29, so this region also could contribute to the phenotype.

Deletions involving the short arm of chromosome 5 are associated with cri-du-chat syndrome (MIM 123450), which is characterised by microcephaly, craniofacial anomalies, catlike crying during the first years of life, psychomotor delay, and possible presence of heart malformations or other anomalies [49]. Gu et al. [55] reported the same subchromosomal deletion (5p13.3p15.33) spanning ~26.22 Mb in two probands with no diagnosis of cri-du-chat syndrome during infancy but presenting developmental delay and dysmorphic and autistic features.

Expanding the scope of cffDNA screening analysis to CNV detection is interesting because many people carry an undetected balanced reciprocal translocation (0.2% of newborns). In other words, 1 in every 250 couples has a high probability of generating embryos with unbalanced rearrangements, some of which may result in children born with congenital abnormalities. Notably, cffDNA analysis is currently not accurate enough to detect all CNVs at any size and at any foetal fraction. However, detection of large subchromosomal

aberrations is feasible [26]; indeed, this approach is described widely in the literature and may identify cases that otherwise go unnoticed. In the case of parents who know they are carriers of balanced translocations and ask for a non-invasive screening approach, some promising data have been published but more research is needed to establish the ability of non-invasive prenatal testing to detect imbalances [56]. According to Srebnik et al. [26], cffDNA screening could be the second-best choice when one parent carries a balanced chromosome aberration and refuses invasive testing because of difficulty achieving a viable pregnancy. Nonetheless, sufficient pre-test counselling and analysis of previous literature on the translocation to determine whether the imbalance is within the platform's detection limits are still required.

In conclusion, the case described here provides further evidence of the possibility of classifying pregnancies by cffDNA analysis as high-risk not only for common trisomies, but also for genetic deletions and duplications. In some cases, this classification may indicate risk of a parent carrying a balanced rearrangement, which would require genetic and reproductive counselling.

Abbreviations

bp: Base pair; cffDNA: Cell-free foetal DNA; CMA: Chromosomal microarray analysis; CNV: Copy number variation; CVS: Chorionic villus sampling; NGS: Next-generation sequencing; NT: Nuchal translucency.

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Authors' contributions

TMA: collection and assembly of data, data analysis and interpretation, and manuscript writing; EM-B: collection and assembly of data, data analysis and interpretation, and manuscript writing; NB: collection and assembly of data, and data analysis and interpretation; CD: cffDNA analysis and NGS in amniotic fluid; HRB: ultrasound and assembly of data; MQGO: sample collection for cffDNA analysis, ultrasound and assembly of data; LR: NGS and karyotype interpretation; IC-G: NGS and karyotype interpretation; RN: bioinformatic and data analysis; MM: collection and assembly of data, data analysis and interpretation, and manuscript writing. All authors read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available from Igenomix Lab S. L.U., but restrictions apply to the availability of these data, which were used under license for the current study and thus are not publicly available. Data are available from the authors upon reasonable request and with permission of Igenomix, S.L.

Declarations

Ethics approval consent to participate

This manuscript is original, has not been published before, and is not currently being considered for publication elsewhere. We affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained. Regarding ethical issues, we verify that the authors had access to relevant aggregated study data and other information (such as study protocol, analytic plan and report, validated data table, and clinical study report) required to understand and report research findings. The authors take responsibility for presentation and publication of the research findings, have been fully involved at all stages of publication and presentation development, and are willing to take public responsibility for all aspects of the work. All individuals included as authors and contributors who made substantial intellectual contributions to the research, data analysis, and publication or presentation development are listed appropriately. The role of the sponsor in the design, execution, analysis, reporting, and funding is fully disclosed. The authors' personal interests, financial or non-financial, relating to this research and its publication have been disclosed.

Consent for publication

We obtained signed consent from the patient involved in this study. These forms are filed with our records and are available as needed.

Competing interests

T.M.A., E.M.-B., N.B., C.D., L.R., I.C.-G., R.N. and M.M. are employed by Igenomix, S.L.; H.R.B. is employed by Clínica HR; and M.Q.G.O. is employed by Clínica Nanu. All authors report no conflicts of interest.

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