Journal of Genetic Syndromes & Gene Therapy

Gómez-Manjón et al., J Genet Syndr Gene Ther
2016, 7:3
DOI: 10.4172/2157-7412.1000297

Case Report Open Access

Noninvasive Prenatal Detection of a Partial Trisomy 4 Using Whole Genome Semiconductor Sequencing

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Received date: May 02, 2016; **Accepted date:** May 13, 2016; **Published date:** May 20, 2016

Abstract

Massively parallel sequencing of cell free fetal DNA (cffDNA) obtained from maternal plasma is used to detect fetal trisomies and selected sex chromosomal aneuploidies. Different technologies can be used to detect fetal chromosomopathies noninvasively, such as Next Generation sequencing and microarrays. In this case report, we show a procedure for detecting chromosomal imbalances as a result of balanced translocations inherited from parents, using noninvasive prenatal detection of common aneuploidies based protocol. This case study illustrates the potential power of whole-genome semiconductor sequencing when used to augment the diagnostic spectrum of noninvasive prenatal testing to detection of copy number variants.

Keywords: Cell-free fetal DNA; Noninvasive prenatal testing; Chromosomal imbalances; Massively parallel sequencing; Prenatal diagnosis

Introduction

Karyotyping of cultured fetal/placental cells obtained by invasive testing is the gold standard for prenatal diagnosis of chromosomopathies, its diagnostic accuracy range to 97.5-99.8% [1]. It allows the detection of fetal aneuploidies, polyploidies, balanced and unbalanced rearrangements, large microdeletions and duplications [2]. However, it needs fetal tissue obtained through invasive procedures such as chorionic villous sampling (CVS) or amniocentesis. CVS can be performed between 10 and 13 weeks of gestation. Amniocentesis is usually offered after 15 weeks of gestation. Both methods are associated with higher pregnancy loss rates, quoted by the American College of Obstetricians and Gynecologists as one miscarriage in every 300-500 procedures for amniocentesis and about the same for chorionic villus sampling [3].

In 1997, Lo et al. [4], discovered the cell-free fetal DNA circulating in maternal blood, but at that moment, they did not have the appropriate technology for its analysis. The introduction of next-generation sequencing has revolutionized prenatal diagnosis, this technique provides the sensitivity and accuracy required to analyze cffDNA. Noninvasive prenatal testing (NIPT) of trisomies 13, 18 and 21 have a positive predictive value (PPV) of 98 to 100%, and a false-positive rate of 0.0 to 0.1% [5]. In comparison, the positive predictive value for detecting sex chromosome aneuploidies (SCA) has been reported as 48%, while the false-positive rate for detection of SCAs is approximately 0.3% [5,6]. This lower accuracy in diagnosis of SCA has been attributed to confined placental mosaicism or abnormal maternal karyotype [7].

The actual impact of this becomes clear if the test is assessed in terms of its positive predictive value. NIPT has a PPV 10 times better than current first trimester screening in a similar population but this is still far below the near 100% required for a diagnosis of trisomy [8]. However, if NIPT is offered to pregnant women with a higher a priori risk, the PPV increases. Thus, different approaches have described to implement NIPT in prenatal care, as an intermediate step between serum screening and invasive diagnostic testing [9-12] or as a replacement for serum screening [8,13,14].

Non-invasive prenatal testing (NIPT) procedures have been recently expanded to panels that include screening for common microdeletion syndromes. In this way, sensitivities greater than 85% have been reported if the deletion might be detected on a G-banded karyotype and 60- 85% for deletions shorter than 7 Mb, with increasing sensitivity as fetal fraction increases [15,16]. Depending on the resolution used for expanded NIPT, more of the recently identified smaller microdeletion (and duplication) syndromes may also be detected.

Selected microdeletion syndromes are candidate conditions for broader NIPT screening scenarios that in the coming years may be considered.

Case Report

This report concerns a 34 years old patient, gravida 3, parity 0, abortion 2. In the first pregnancy, the combined risk for trisomy 18 and 21 was higher than 1: 50 because of a nuchal translucency of 3.8 mm and low levels of biochemical serum markers. In our center the cut- off value in combined screening for aneuploidy suspicion is higher than 1: 270. So the patient was referred to our department for invasive testing by amniocentesis.

The fetal karyotype from cultured amniocytes showed the derivative chromosome 46,XY,der(8)t(4;8)(q21.3p23.1). Subsequent parental studies showed a normal karyotype in the mother and a balanced translocation between chromosomes 4 and 8 in the father. The second

pregnancy resulted in a spontaneous first trimester abortion. No genetic study was performed in fetal tissues.

In the present pregnancy, the nuchal translucency´s (NT) measure was 6.3 mm, which behaved as a generalized subcutaneous edema covering head, thorax and abdomen. It is well established that using a cutoff value of NT ≥ 3.5 mm, the detection rate of chromosomal abnormalities is 48.8% [17]. Because of sonographical markers and her past medical history, the patient was referred to our department and it was decided to carry out a CVS at 12 weeks gestation. Before the invasive procedure, the woman accepted to participate in a blinded study for NIPT, and a blood sample was collected. Ethics committee approval and informant consent from the patient was obtained for this test.

Both QF-PCR and karyotyping were performed according to our standard protocol [18]. QF- PCR showed normal results for chromosomes 13, 18, 21, X e Y. Fetal karyotype from trophoblastic cells showed the same derivative chromosome, involving chromosomes 4 and 8, detected in her first pregnancy (Figure 1).

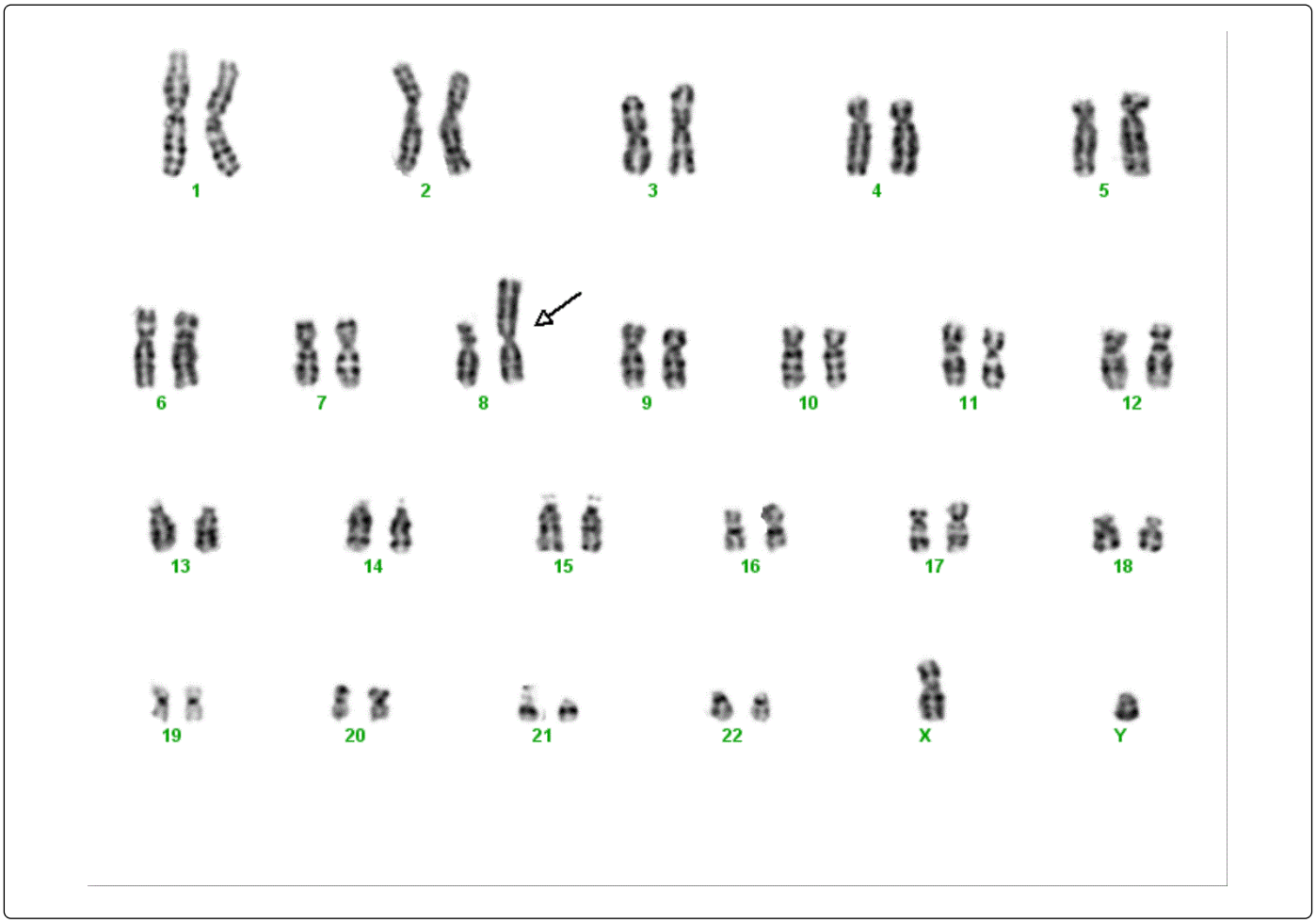

Figure 1: Fetal karyotype from trophoblastic cells showed a karyotype 46,XY,der(8)t(4;8)(q21.3p23.1).

Whole genome sequencing was used for the NIPT. Cell-free fetal DNA obtained from maternal plasma was extracted using QIAamp DSP Virus Kit® using a manufacturer´s modified protocol. The library for sequencing was prepared using Ion Plus Fragment Library Kit® according a homemade protocol. The sample was sequenced in the Ion Proton platform® in an 8-plex run using counting-technology for the analysis. We obtained 47,801,931 reads after filtering of which, 6,723,356 reads were from the target sample. The medium read length was 153 nucleotides.

Sequencing data was processed following a bioinformatics algorithm based on LifeTechnologies® pipeline, TMAP. For each sequencing run, the multiplexed sequence reads were subject to a classification step, in which barcoded 5´adapters were identified and matched against a predefined set, in order to split the multiplex into individual samples. In a subsequent filtering step, very short reads were discarded. Thus, the chromosomal origin of each sequenced read was identified by comparison with the reference human genome hg19, GRCh37 (UCSC Genome Browser). Finally, duplicated reads were removed and unique aligned reads were counted by autosome.

Z-score was calculated for each autosomal chromosome in the case and control samples by subtracting the mean percentage autosomal chromosome of a reference set of two euploid pregnancies from the percentage autosomal chromosome of the test case and divided by the standard deviation of the value for percentage autosomal chromosome among the reference sample set [19].

$$\%chrN = \frac{\text{Unique count for chrN}}{\text{Total unique count}}$$

$$chrN\ \ z-score\ \ for\ \ test\ \ sample = \frac{(\ \%\text{chrNsample} - \text{mean}\ \%\ \text{chrNreference}\ )}{\text{SD}\ \%\ \text{chrNreference}}$$

A subset of 78 samples from singleton pregnancies were analyzed in order to establish reference data from euploid pregnancies. Simultaneously, read counts in window bins were used to detect fetal CNVs. Fetal aneuploidy was defined by an absolute Z-score above 3. Partial trisomy 4 was detected due to a Z-score of 6.76 obtained for chromosome 4 after the analysis described above (Figure 2).

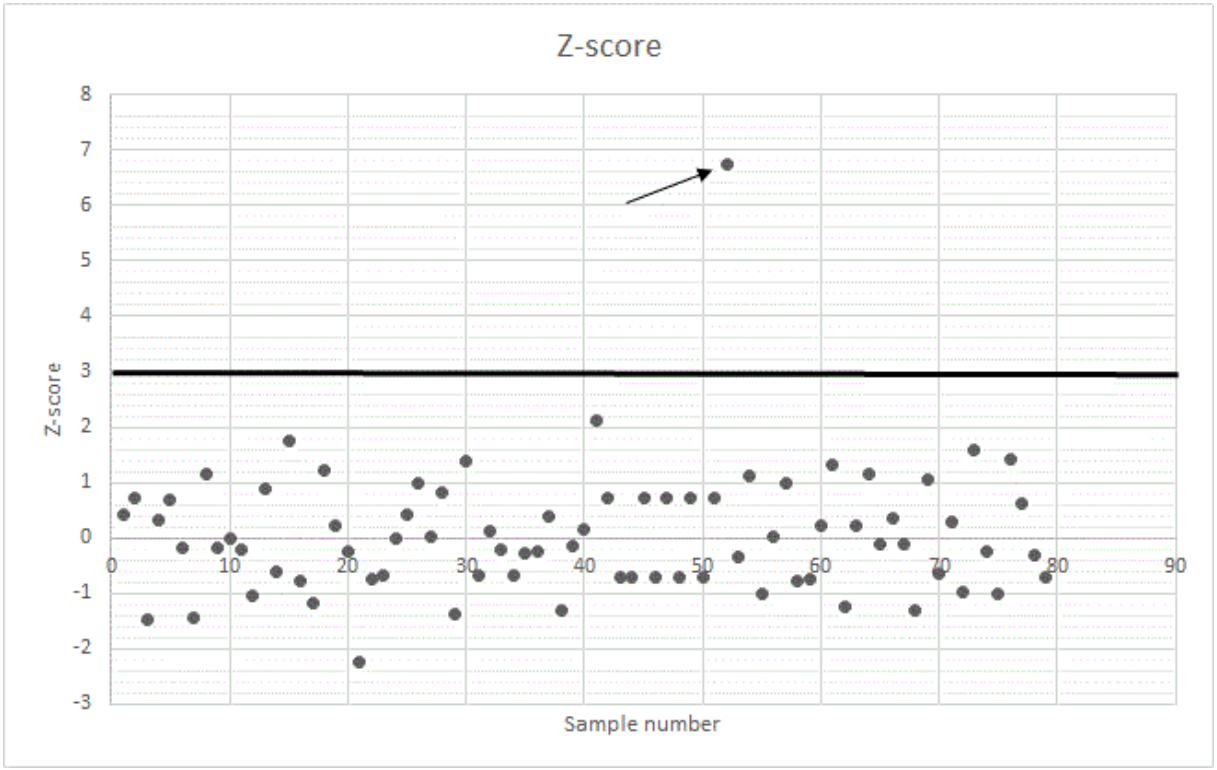

Figure 2: Z-score obtained for chromosome 4 on the set of samples analyzed. Partial trisomy 4 detected after the analysis of the sample 46,XY,der(8)t(4;8)(q21.3p23.1) is pointed by an arrow.

Discussion

Noninvasive prenatal testing of common fetal aneuploidies such as Down syndrome, trisomy 18 and 13 by sequencing of cffDNA present in maternal plasma has been clinically implemented since 2011 in both the USA and China and its implementation is spreading in Europe. Recent advances in noninvasive prenatal diagnosis have enabled the detection of structural and functional abnormalities associated with deletion/duplication syndromes such as Cri du Chat, Di George and Angelman syndrome [16].

Theoretically, unbalanced translocations should be identifiable by NIPT. In this kind of chromosomal abnormality, a partial trisomy or monosomy is present [20]. A case of a parental inherited unbalanced translocation detected by NIPT is described in this paper, using a basic reads counting strategy for each chromosome and simple statistics such as Z-score.

The major advantage of this approach is the reduction in the required invasive tests to detecting these kinds of chromosomal abnormalities. There are some limitations, until now only segmental imbalances larger than 5 Mb can be identified [21]. On the other hand, in order to decrease false positive results, the detection of unbalanced translocations should only be applied when the carrier status of a parent is previously known.

Even if cffDNA testing is designed to ascertain risk of chromosomal abnormality in the fetus, maternal imbalances are more readily detected than fetal. Although this might seem a disadvantage, identification of these maternal events is clinically relevant, because women who carry a microdeletion have a 50% chance of passing on the chromosomal abnormality to the fetus [15]. The routine detection of de-novo rearrangements using cffDNA in all pregnant women would not seem to be cost-effective at this time, although it is technically possible.

Although scientific, medical, and ethical issues should be evaluated carefully, this strategy, can detect genomic alterations that may change the obstetrical course and outcome, providing a basis for decisions regarding termination, fetal therapy, mode of delivery, and postnatal referral to a tertiary-care centre with advanced expertise in management.

To our knowledge, only one paper has shown the use of next generation sequencing based on semiconductor technology for detection of unbalanced chromosome translocations for preimplantational genetic diagnosis application [22]. There are no

studies that use this technology for a noninvasive detection of copy number variants in cell-free fetal DNA.

Conclusion

Our study suggests that innovations in genome sequencing aimed specifically at detecting structural variations can offer a rapid adjunct to cytogenetic techniques. Sequencing enables precise definition of individual disrupted genes, thereby adding to the information available for outcome prediction, medical planning, and genetic counselling. We have demonstrated that employing the same approach for detect Down syndrome, a basic reads counting, is possible to detect large unbalanced translocations. More studies are needed to determine the minimum size of the translocation detectable using this basic strategy.

Funding Source

IGM is supported by a strategic grant from the Biomedical research institute Hospital 12 de Octubre (I+12). This work was co-funded by FEDER and Instituto de Salud Carlos III (ISCIII), grant P12/0639, from Ministerio de Industria y Competitividad (MINECO), Spain.

Original Publication

This manuscript contains original unpublished work and is not being submitted for publication elsewhere at the same time.

Conflict of Interest

The authors declare no conflicts of interest.

Ethics

The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 1983. We do not use patients' names, initials or hospital numbers, especially in illustrative material. Ethics committee approval and informant consent from the patient was obtained for this test.

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