Diagnosis of De Novo Mosaic Balanced Translocation t(1;3)(q42;q25) in a Fetus Conceived Using Pre-implantation Diagnosis Due to Presence in the Father of a Different Balanced Translocation

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Research

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Abstract

Introduction

Preimplantation genetic testing (PGT) had widely been applied in reciprocal translocation carriers to improve the clinical outcome of assisted reproduction. De novo mosaicism balanced reciprocal translocations in fetus conceived using PGT from a balanced translocation carrier parent has been rarely reported, and the driving mechanism is not clearly.

Methods

Chromosomal microarray analysis (CMA) , karyotype analysis and fluorescent in situ hybridization (FISH) were performed to verify the type and heredity of the rearrangement. STR analysis was used to identify potential contamination as well as kinship verification and identification.

Results

A rare *de novo* mosaicism balanced reciprocal translocation t(1,3)(q42;q25) in fetus conceived using PGT-SR from a t(12;14)(q22;q13) balanced translocation carrier father was been diagnosed by multiplatform genetic techniques. At 31 weeks and 2 days of gestation, premature delivery was caused by uncontrollable uterine contractions. At the 21-months follow up, infant has achieved all psychomotor development milestones as well as growth within the normal reference range.

Conclusion

PGT cases still need close observation in prenatal diagnosis and long-term follow-up.

1. Introduction

Balanced reciprocal translocations (BRT) are common structural chromosomal rearrangement in humans, with an incidence rate of approximately 1/500 ~ 1/625 in newborns[1]. Most BRT carriers have the normal phenotype but a high risk of abortion, infertility, or birth defects in offspring. Most reciprocal translocations are unique. A few to several percent of translocations disrupt haploinsufficient genes or their regulatory regions and result in clinical phenotypes. Balanced translocations from patients with clinical phenotypes have been valuable in mapping disease genes and in illuminating cis-regulatory regions [2]. Couples in whom one partner has a balanced translocation or inversion may have an overall miscarriage rate as high as 49% resulting from unbalanced gametes [3]. Mosaicism for BRT (BRTM) has been rarely reported, most of the previously reported cases had been diagnosed by cytogenetic analysis investigation prescribed by infertility, miscarriages, and/or unbalanced chromosome rearrangement in the offspring [4]. Furthermore,most of BRTM demonstrated in lymphocyte cultures, have been described [5].

Currently, although there are insufficient data indicating that preimplantation genetic testing (PGT) improves the live birth rate in couples with recurrent miscarriage (RM) carrying a structural chromosome

abnormality [3], PGT is still an established alternative to invasive prenatal diagnosis and as such may avoid adverse pregnancy in couples with structural chromosome abnormalities, and also for a high risk of transmitting genetic disorders[6–8]. Although an accurate projection of the anticipated number of unbalanced embryos is not possible at present, confirmation of normal/balanced status results in high pregnancy rates and diagnostic accuracy[9].

It is well known that PGT for structural rearrangements (PGT-SR) can hardly further distinguish between the balanced translocation and structurally normal embryo, so the embryos may still be carriers of BRT inherited from their parents. However here we report a very rare case of mosaic de novo BRT t(1,3)(q42;q25) in fetus conceived using PGT-SR in a t(12;14)(q22;q13) BRT carrier father

2. Materials

2.1 Statement

Patients and their families agree to donate remaining samples and data to scientific research, technical innovation and clinical application after removing the identifiable personal information, and the informed consent was gained from the patient.

2.2 Sample information

A 31-year-old pregnant woman, gravida 1 para0, and her husband 32 years old, suffered from primary infertility for several years. The husband with karyotype 46,XY,t(12;14)(q22;q13) was diagnosed as asthenospermia, while the pregnant woman's karyotype was normal. Subsequently, the couple underwent in vitro fertilization and embryo transfer (IVF-ET) and PGT-SR in other hospitals. In routine protocols, a chromosome karyotype "balanced" embryo was transferred and resulted in a successful pregnancy.

At 11 weeks of pregnancy, the pregnant woman came to our hospital for registration and agreed to accept interventional prenatal diagnosis (amniocentesis) in the second trimester of pregnancy after informed consent. Both karyotype and SNP-array analysis were performed at 19 weeks of gestation in order to evaluate. Short tandem repeat (STR) markers were used for rough identifying relationship testing and potential maternal contamination.

3. Method

3.1Cell culture and karyotype analysis

The karyotype of this family was reanalyzed in our laboratory. Cell culture and G-band karyotype analysis were performed according to standard cytogenetic methods. Fetal cells obtained from amniotic fluid and cord blood were cultured with double-line using standard methodologies. At least twenty metaphases chromosomes were counted and three metaphases chromosomes were analyzed in each line by two independent laboratory technicians[10].

3.2 CMA

Affymetrix CytoScan 750K array platforms were used to detect copy number variants was performed as described in previous research[10],

3.3 FISH

The balanced reciprocal translocation was confirmed by pter (1,3,12 pter Subtelomere FISH Probe) green and qter (1,3,12,14 qter Subtelomere FISH Probe) red probes, according to the BlueFish Probes Labeling Protocol (BlueGnome, Cambridge, UK).

3.4 STR

STR analysis was used to exclude maternal contamination and to identify family affinities by using a 5-dye fluorescent technology and a co-amplification method to detect 21 loci (20 STR loci and Amelogenin, Supplemental Table 1) (Microreader™ 21(Direct) ID System, Microread Genetics, China) according to the operating procedure. Among 21 STR loci, heterogenic contamination was judged when at least 3 loci have more than two alleles.

4. Result

A routine cytogenetic analysis of the fetus suggested a mosaic de novo BRT with the karyotype mos 46,XY,t(1,3)(q42;q25)[40]/46,XY[39], whereas the father's karyotype was 46,XY,t(12;14)(q22;q13), and only the pregnant woman had normal karyotypes 46,XX(Fig.1A).The results of FISH showed that the fetus was the carrier of translocation between the subtelomere of chromosome 1 and chromosome 3, with the mosaic rate of 40%. However, no translocation was found in the subtelomeres probes of chromosome 12 and 14 (Fig.1B). Genome wide SNP-array analysis detected no copy number variants (CNVs) in the genomic DNAs of fetal amniotic fluid cells, excluding cryptic genomic imbalances at translocation breakpoints (Fig.2). According to all methods used, the fetal karyotype could be written as 46,XY,t(1,3)(q42;q25)[40]/46,XY[39].ish t(1;3)(1p+,3q+;3p+,1q+),12p13q24.3(12p×2,12q×2),14p13q32(14q×2)[8]/1p36.3q44

(1p×2,1q×2),3p26q29(3p×2,3q×2),12p13q24.3(12p×2,12q×2),14p13q32(14q×2)[12].arr[GRCh37](1-22)×2,(XY)×1

At the same time, the STR results excluded the possibility of exogenous contamination, and all the tested STR loci were inherited by either parent indicating genetic compatibility of father and son.

Ultrasonography at 30 weeks of gestation showed no abnormal fetal development. At 31 weeks and 2 days of gestation, premature delivery was caused by uncontrollable uterine contractions. The birth weight of the newborn was 1680g and the length was 42cm. The Apgar scores of 10 at 1 minute, 5 minutes and 10 minutes. Neonatal echocardiography was normal except for patent foramen ovale. The results of

chromosome karyotype in neonatal umbilical cord blood were mos 46,XY,t(1;3) [50]/46,XY[50], which was basically consistent with amniotic fluid. Placental pathology showed no idiopathic abnormality.

At the 21-months follow up, the growth and development of the infant were normal, he raised his head, turned over and sitted on schedule. He walked steadily when 18 months old. Now he can walk, run and jump freely, and have better language ability than his peers, according to his parents' description.

Discussion

Chromosomal BRT was found in 3.2% of couples with recurrent implantation failure [11]. PGT had widely been applied to improve the assisted reproduction outcome of reciprocal translocation carriers. However, current techniques have the same limitation, and therefore, it is hard to distinguish between the balanced translocation and structurally normal embryo. To solve this problem, Hu et al[12] established a clinical applicable approach to identify precise breakpoint of reciprocal translocation and to further distinguish normal embryos in PGT. Recently, Zhang et al demonstrate a highly efficient approach for preimplantation genetic haplotyping in clinical application of balanced translocation carriers. Unfortunately, these techniques have to be performed based on the carrier's locus information, so they do not appear to solve the *de novo* BRTM as our case. Our case as one of the few *de novo* BRTM carriers detected by prenatal analysis and confirmed after birth.

De novo apparently BRT are detected in approximately 1/800~1/1000 prenatal tests[13, 14], a preferential involvement of chromosomes 22, 7, 21, 3, 9 and 11 and a less involvement of chromosomes X, 19, 12, 6 and 1 was observed. Nonrandom distribution of the breakpoints across chromosomes was noticed. Association in the location of recurrent breakpoints and fragile sites was observed for chromosomes 11, 7, 10 and 22, while it was not recorded for chromosome 3[14]. In the present case, we confirmed the break points at 1q34 and 3q25, this finding partly coincides with the involved chromosome as above literature described, but with different breakpoints.

BRT mosaicism had been rarely reported[4] mainly observed in subjects with a normal phenotype accompanied by reproductive failure. Estimated frequencies in the postnatal and prenatal populations examined were calculated to be 5.7×10^{-5} and 4.1×10^{-5} by Opheim et al[15]. Recently, as described by Garzo et al [4], just 25 cases had been reported in previous research, and they described 10 new cases of balanced reciprocal translocation mosaicism, and suggest that carrier individuals might be more frequent than expected. To date the incidence of BRTM is poorly defined, may be explained by that there are few reported cases, which are not convenient for statistics. Another reason is due to the missed or inaccurate diagnosis of BRTM in the detection process, such as low proportion mosaicism, lack of technical means to detect micro-abnormal of chromosome. Finally, the size of the recombination fragment, the resolution of chromosome bands and the number of cell counts were all related to the accurate diagnostic of BRT mosaicism. Indeed, mosaicisms must be confirmed in at least two different cultures or in different tissues to exclude the possibility of in-vitro origin of the chromosomal

rearrangement[16]. In this study, fetal chromosomal abnormalities were observed in two separate initial cultures of both amniotic fluid and cord blood, with the similar mosaicisms rate.

The origin of BRTM is still obscure. The plausible driving mechanism has been postulated to be either postzygotic[17] or to occur in the prezygotic[18]. There are two hypothetical mechanisms for postzygotic events: mosaicisms (which occurs in mitosis of single zygotes) and chimerism[19] (fusion of two zygotes). Chimerism can be distinguished from mosaicism by evaluation of the extent of genotypic differences, such as STR. Indeed, in mosaicism one paternal allele and one maternal allele should be found at all loci, whereas in chimerism two alleles for one or both parental contribution(s) should be observed at least at one locus[19]. As indicated in Figure 3, an apparently STR result showed that one paternal allele and one maternal allele were found at all loci. Considering that the mosaicisms proportion of our case was close to 50% in both amniotic fluid cells and umbilical cord blood, the mechanism of BRTM in our case was plausible due to a *de novo* mitotic error may originate in a zygote within the first or early cell divisions, which results in a mosaic embryo with the variant present in a half proportion of cells, and this mosaicism can affect somatic and/or gonadal tissues[20].However, due to the growth deviation of different cell types in the process of cells culture, the mosaic ratio of different fetal tissues may be different.

At present, the relationship between phenotype and BRT mosaicism/chimerism (including tissue-specific mosaicism) was not clear. A long-term follow-up study suggested that children with prenatally diagnosed de novo apparently BRT have similar long-term health and developmental outcomes as children of the same age in the general population [21]. However, a *de novo* apparently balanced translocation still may lead to the disruption of a gene and therefore be causative of abnormal phenotypic consequences [22, 23]. Except for premature delivery and low birth weight, there was no significant abnormality in prenatal ultrasound and postpartum physical examination in our case. And according to the 21-months follow up, infant has achieved all psychomotor developmental milestones as well as growth within the normal reference range. Certainly, long-term health and developmental follow-up is needed for this infant, when he reaches child-bearing age, sperm karyotype analysis can be used to determine the rate of gonadal mosaicism in order to guide his fertility, assisted reproductive technology will be recommended to avoid adverse pregnancy if necessary.

Conclusion

To the best of our knowledge, similar to our patient, only Kim et al[24] reported the first case of a de novo BRT conceived using PGT from a balanced translocation carrier mother. So our case is the second and unique case reported in the literature for prenatal diagnosis of a *de novo* BRT mosaicism t(1,3)(q42;q25) in fetus conceived using PGT-SR from a t(12;14)(q22;q13) balanced translocation carrier father. The most reasonable driving mechanism for BRT mosaicism in our case was that a *de novo* mitotic error may originate in a zygote within the first or early cell divisions, which results in a mosaic embryo with the

variant present in a half proportion of cells. Therefore, was the *de novo* BRT an accidental event or was PGT induced cell damage leading to new translocation? This needs to be studied.

Declarations

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Statement of Ethics

This report is a retrospective analysis of the patient's clinical test results and does not have an impact on the patient's clinical decision and management strategy. So ethics approval was not required.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Written informed Consent for Publication of case:

Written informed consent was obtained from the patient for publication of the case details and accompanying images.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

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Author contribution

Shaoqin Zhang and Jianjiang Zhu clinically reviewed the patient and wrote the manuscript. LiRong Cai and Guodong Tang performed the experiments and interpreted the data. Limei Xu and Ran Meng carried out genetic counseling and interventional prenatal diagnosis for pregnant women. Hong Qi jointly conceived this studies and critically revised the manuscript. All authors approved the final version of the manuscript.

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References

1. Mackie Ogilvie C, Scriven PN. Meiotic outcomes in reciprocal translocation carriers ascertained in 3-day human embryos. European journal of human genetics : EJHG. 2002;10(12):801-806.

2. Wilch ES, Morton CC. Historical and Clinical Perspectives on Chromosomal Translocations. Adv Exp Med Biol. 2018;1044:1-14.

3. Franssen MT, Musters AM, van der Veen F, et al. Reproductive outcome after PGD in couples with recurrent miscarriage carrying a structural chromosome abnormality: a systematic review. Human reproduction update. 2011;17(4):467-475.

4. Garzo M, Catusi I, Colombo DM, et al. Ten new cases of Balanced Reciprocal Translocation Mosaicism (BRTM): Reproductive implications, frequency and mechanism. Eur J Med Genet. 2020;63(2):103639.

5. Leegte B, Sikkema-Raddatz B, Hordijk R, et al. Three cases of mosaicism for balanced reciprocal translocations. Am J Med Genet. 1998;79(5):362-365.

6. Otani T, Roche M, Mizuike M, et al. Preimplantation genetic diagnosis significantly improves the pregnancy outcome of translocation carriers with a history of recurrent miscarriage and unsuccessful pregnancies. Reproductive biomedicine online. 2006;13(6):869-874.

7. Ye Z, Hu W, Wu B, et al. Predictive Prenatal Diagnosis for Infantile-Onset Inflammatory Bowel Disease due to Interleukin-10 Signalling Defects. J Pediatr Gastroenterol Nutr. 2020.

8. De Rycke M, Berckmoes V. Preimplantation Genetic Testing for Monogenic Disorders. Genes (Basel). 2020;11(8).

9. Morin SJ, Eccles J, Iturriaga A, et al. Translocations, inversions and other chromosome rearrangements. Fertil Steril. 2017;107(1):19-26.

10. Zhu JJ, Qi H, Cai LR, et al. C-banding and AgNOR-staining were still effective complementary methods to indentify chromosomal heteromorphisms and some structural abnormalities in prenatal diagnosis. Mol Cytogenet. 2019;12:41.

11. Stern C, Pertile M, Norris H, et al. Chromosome translocations in couples with in-vitro fertilization implantation failure. Hum Reprod. 1999;14(8):2097-2101.

12. Hu L, Cheng D, Gong F, et al. Reciprocal Translocation Carrier Diagnosis in Preimplantation Human Embryos. EBioMedicine. 2016;14:139-147.

13. Peng HH, Chao AS, Wang TH, et al. Prenatally diagnosed balanced chromosome rearrangements: eight years' experience. J Reprod Med. 2006;51(9):699-703.

14. Giardino D, Corti C, Ballarati L, et al. De novo balanced chromosome rearrangements in prenatal diagnosis. Prenatal diagnosis. 2009;29(3):257-265.

15. Opheim KE, Brittingham A, Chapman D, et al. Balanced reciprocal translocation mosaicism: how frequent? Am J Med Genet. 1995;57(4):601-604.

16. Fryns JP, Kleczkowska A. Reciprocal translocation mosaicism in man. Am J Med Genet. 1986;25(1):175-176.

17. Kleczkowska A, Fryns JP, Van den Berghe H. On the variable effect of mosaic normal/balanced chromosomal rearrangements in man. Journal of medical genetics. 1990;27(8):505-507.

18. Cantu JM, Ruiz C. On a prezygotic origin of normal/balanced translocation mosaics. Ann Genet. 1986;29(4):221-222.

19. Malan V, Vekemans M, Turleau C. Chimera and other fertilization errors. Clinical genetics. 2006;70(5):363-373.

20. Lannoy N, Hermans C. Genetic mosaicism in haemophilia: A practical review to help evaluate the risk of transmitting the disease. Haemophilia. 2020;26(3):375-383.

21. Sinnerbrink IB, Sherwen A, Meiser B, et al. Long-term health and development of children diagnosed prenatally with a de novo apparently balanced chromosomal rearrangement. Prenatal diagnosis. 2013;33(9):831-838.

22. Fukushi D, Yamada K, Suzuki K, et al. Clinical and genetic characterization of a patient with SOX5 haploinsufficiency caused by a de novo balanced reciprocal translocation. Gene. 2018;655:65-70.

23. Pesz K, Pienkowski VM, Pollak A, et al. Phenotypic consequences of gene disruption by a balanced de novo translocation involving SLC6A1 and NAA15. Eur J Med Genet. 2018;61(10):596-601.

24. Kim JW, Shim SH, Lee WS. De novo balanced reciprocal translocation t(2;3)(q31;q27) in a fetus conceived using PGD in a t(2;14)(q35;q32.1) balanced reciprocal translocation carrier mother. Clin Case Rep. 2017;5(6):841-844.

Figures

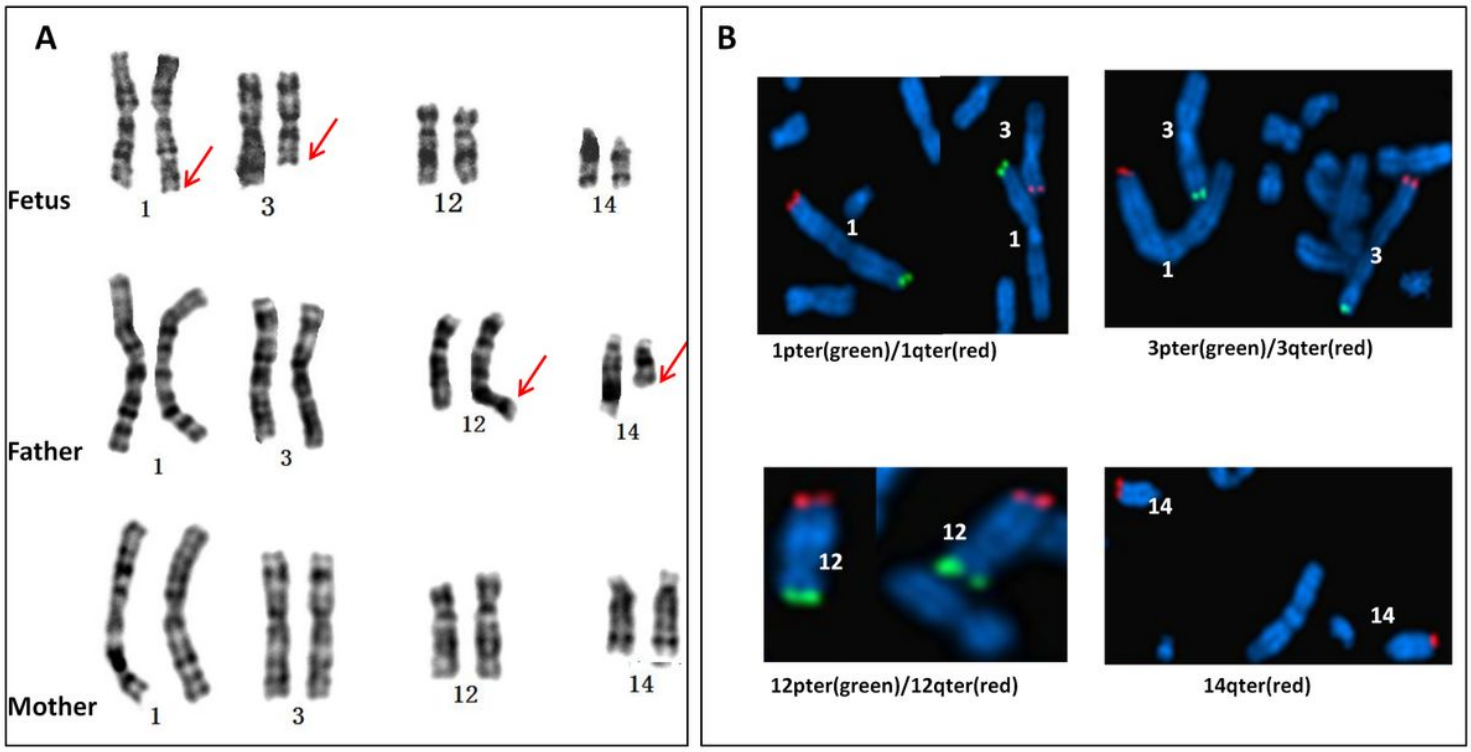

Figure 1

A: Partial chromosomal karyotypes of the family by G-banding. Fetus(above):46,XY,t(1;3)(q42;q25)[40]/46,XN[60]; Father(middle): 46,XY,t(12;14)(q22;q13);Mother(below):46,XX. B: FISH analysis of the Fetal amniotic fluid with 1,3,12,14 subtelomere probes

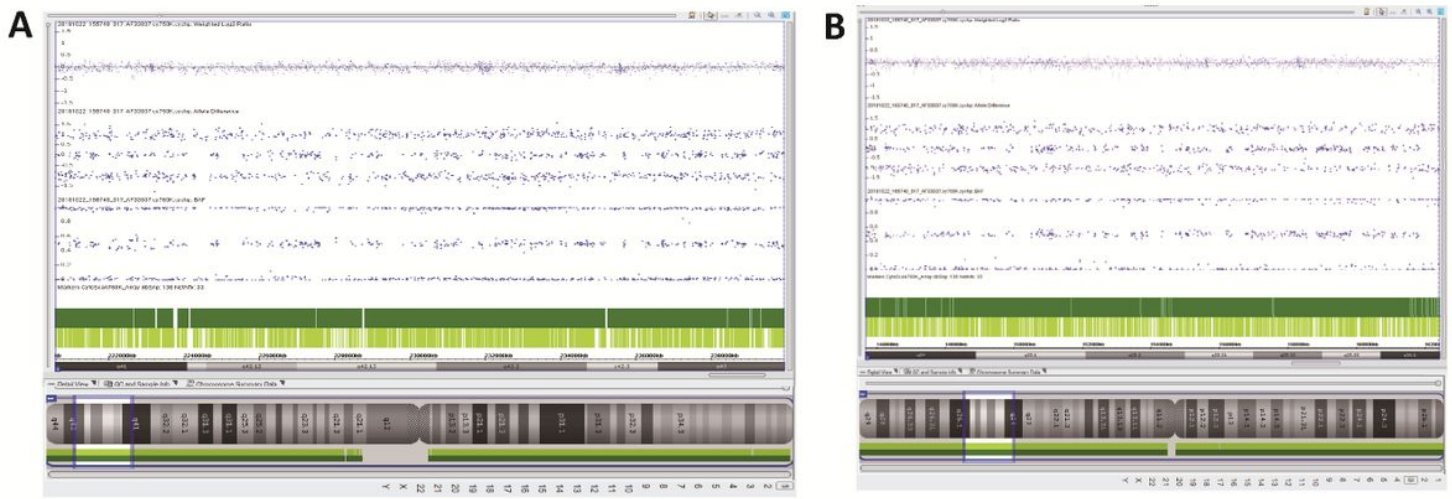

Figure 2

SNP-array profile of chromosome 1q42 (A) and chromosome 3q25 (B) in fetal amniotic fluid cells excluded cryptic genomic imbalances at translocation breakpoints.

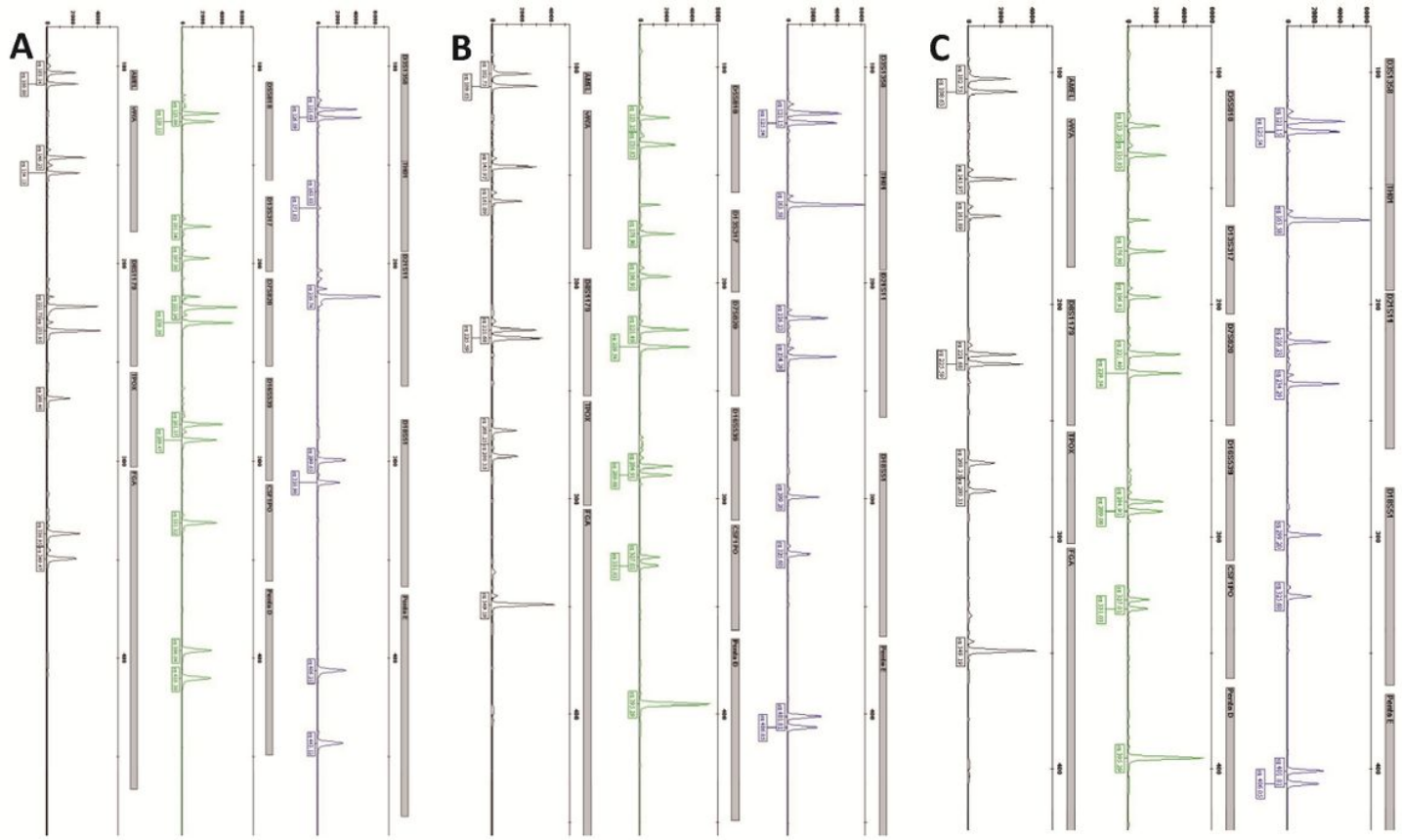

Figure 3

STR showed no heterogenic contamination, and all of fetal STR loci(C) were inherited from one paternal allele (A) and one maternal allele (B).

Supplementary Files

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- SupplementalTab1.docx