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ORIGINAL ARTICLE

Recurrence of Trisomic Pregnancies in Four Families: A Cytogenetic and Molecular Study

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Abstract The risk for recurrence of non disjunction trisomy 21 is conventionally considered to be less than 1%. Within a span of 3 years, we observed recurrence of non disjunction trisomies in four families. The objective of the present study was to determine low level mosaicism in either of the couple and to identify the parental origin of additional chromosome 21/18. The four couples who had recurrent trisomic conceptions were investigated for the underlying mosaicism by analysis of 100 cells from peripheral blood of the couple and the parental origin of supernumerary chromosome 21/18 were identified using microsatellite markers. Low level mosaicisms in peripheral lymphocytes of couple were ruled out for all four families. Microsatellite markers have shown maternal origin of chromosomal nondisjunction for all the families and defective first meiotic division as the most common mechanism for nondisjunction. This observation raises the need for discussing the option of invasive testing while counseling the couple with an affected child with non-disjunction trisomy as the risk for recurrence of trisomies in subsequent pregnancy might not be as low as 1%.

Keywords Nondisjunction · Trisomy 21 · Trisomy 18 · Recurrence · Meiosis I · Meiosis II

Introduction

Trisomy 21 occurs in 0.45% of all clinically recognized pregnancies [1]. Most of them occur due to meiotic non-disjunction. Recurrence of non disjunction (NDJ) trisomy 21 in two or more offsprings of healthy and phenotypically normal young parents occurs extremely rarely. The risk of recurrence of NDJ trisomy 21 is estimated to be less than

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1% [2, 3]. The two possible explanations suggested for the recurrence include low level mosaicism in either of the couple or a genetic predisposition to nondisjunction. The theory of genetic predisposition was not established by a study of 22 families with recurrent trisomy 21 [4]. The only well established risk factors for trisomy 21 conceptions are parental mosaicism for a trisomy 21 cell line and advanced maternal age, which increases the predisposition to meiotic nondisjunction [5]. Gonadal mosaicism may be the most plausible explanation for the great majority of women who have more than one trisomic pregnancy at a younger age, while multiple trisomy 21 pregnancies in older women are more likely to be due to occurrence by chance [6].

Materials and Methods

The four couples who had recurrent trisomic conceptions (three families with trisomy 21 and one family with recurrence of trisomy 18) were investigated for the underlying mosaicism by analysis of 100 cells from peripheral blood of the couple and the parental origin of additional chromosome 21/18 was identified using microsatellite markers. Quantitative fluorescent PCR (QF-PCR) was used for the analysis of DNA from probands, fetuses and parents.

Family 1

A non-consanguineous couple had attended the genetic clinic for prenatal diagnosis. Mother was 30-years-old. The first child of this couple had NDJ trisomy 21 and was born when the mother was 27-years-old. There was no history of miscarriages. Grand-maternal age at the time of conception of the mother was 24 years. The couple decided to go ahead with amniocentesis considering around 1% risk for having an affected baby with trisomy 21. Analysis of 100 cells from the peripheral blood of the couple was normal. The fetal karyotype showed NDJ type of trisomy 21. The couple had a normal offspring in the subsequent pregnancy following prenatal study.

Family 2

A 27-years-old woman had come for prenatal diagnosis as she already had a 5 year old boy with NDJ type of trisomy 21 whom she conceived at 21 years. This was her second pregnancy and there was no history of miscarriages. An ultrasound scan done at 12 weeks of gestation showed nuchal translucency of 1.8 mm. Subsequent scan at 17 weeks had shown unossified nasal bones, nuchal fold thickness (5.1 mm), hypoechoic liver and bilateral clinodactyly. The couple was counseled to have a diagnostic test for ruling out recurrence of trisomy. The fetus was found to be affected with NDJ trisomy 21. Analysis of 100 cells from the peripheral blood of the couple for mosaicism of chromosome 21 was normal.

Family 3

A couple had come with a child with NDJ trisomy 21. The mother was 38 years old. There was no history of miscarriages. This couple had a previous pregnancy terminated at 20 weeks following the confirmation of nondisjunction trisomy 21 at a maternal age of 37 years. Grand-maternal age at the time of conception of mother was 32 years. Invasive testing for chromosomal anomaly was not carried out in the current pregnancy according to parental decision. Analysis of 100 cells from the peripheral blood of the couple was normal. Sample of the previously affected fetus was not available.

Family 4

A 27-years-old G4 P1 A2 L0 was referred to genetic clinic as her second trimester screening had shown a 1:50 risk for trisomy 18. Her first child was conceived at 22 years of age and the baby had NDJ trisomy 18. Following this pregnancy, she had two first trimester miscarriages. Amniocentesis at 16 weeks had revealed NDJ trisomy 18 in the current pregnancy. Karyotype analysis of the couple did not show any mosaicism in peripheral lymphocytes. Sample of the previous child with trisomy 18 was not available as the baby had expired in the neonatal period.

Results

Family 1

We used quantitative fluorescent PCR (QF-PCR) for the analysis of DNA isolated from the fetal cells obtained from the amniotic fluid (Panels A and E), the peripheral lymphocytes of maternal blood (Panels B and F), and paternal blood sample (Panels C and G); and the DNA isolated from the blood sample from the proband (panels D and H) using two STR markers in chromosome 21 (D21S11 and D21S1437). The D21S11 and D21S1437 markers showing 1:2 triallelic peak ratio demonstrates the trisomy 21 in the fetus (Panels A and E of Fig. 1). The comparison of PCR fragments in father and mother has revealed maternal origin of the extra chromosome 21 in the fetus (Panels E, F and G of Fig. 1). The heterozygosity of the maternal alleles in the fetus and the heterozygosity in the mother for the marker D21S11 (Panels A and B, Fig. 1) indicates the origin of the extra chromosome may be attributed to the

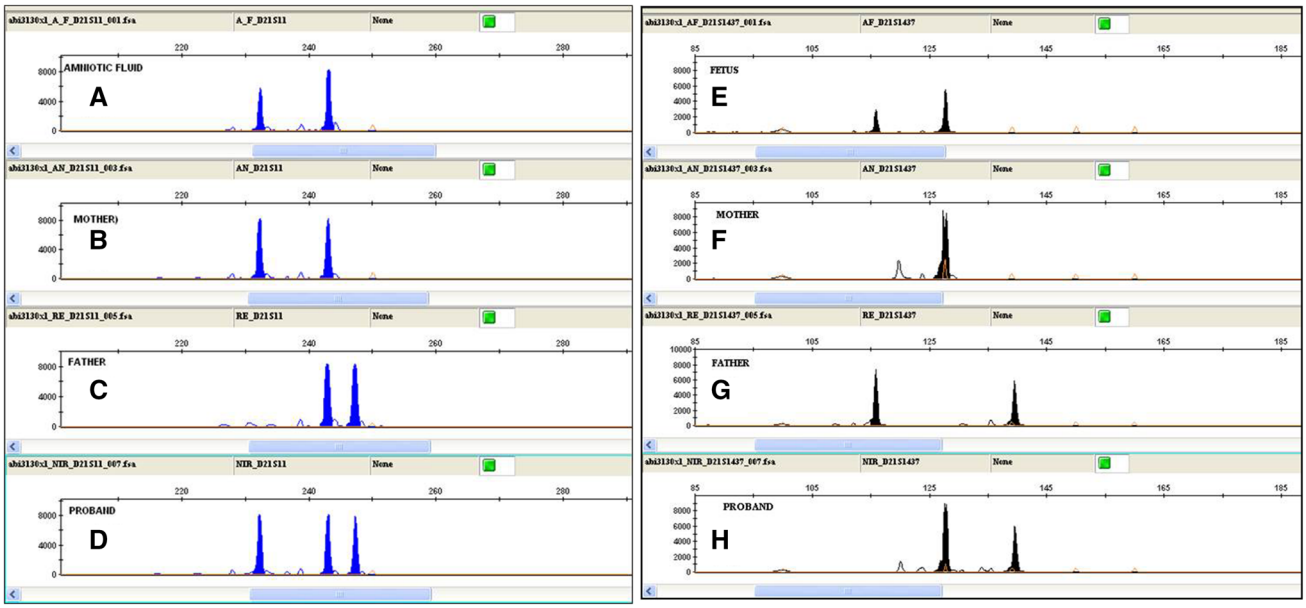

Fig. 1 Detection of trisomy 21 by QF-PCR with D21S11 STR marker (Panels **A**, **B**, **C** and **D**) and determination of parental origin of trisomy D21S1437 STR marker (Panels **E**, **F**, **G** and **H**) in family 1

meiosis I of the female gametogenesis. The D21S1437 marker is uninformative (Panel F of Fig. 1) in this regard due to the homozygosity of the alleles in the mother. The events leading to trisomy 21 in the proband of the couple also have occurred through an aberrant meiosis I during maternal gametogenesis (Panels F and H of Fig. 1), though the proband has inherited a different paternal allele than that of the fetus (Panels E, G and H of Fig. 1). The 1:1:1 triallelic ratio for the D21S11 marker indicate trisomy 21, however this marker does not predict the parental origin of the supernumerary chromosome (Panels B, C and D; Fig. 1) as both parents share a common allele for the marker. D21S1437 marker showing 1:2 triallelic ratio in the proband indicates trisomy 21 and parental origin of trisomy as maternally derived (Panels F, G and H of Fig. 1). However, the 2:1 height peak ratio of the distal D21S1437 STR marker (Panel H of Fig. 1) does provide little information to confirm the phase of origin of the aneuploidy as it is homozygous in mother.

Family 2

For this family we have analyzed the DNA isolated from the fetal cells from the amniotic fluid (Panels A and E); from the peripheral lymphocytes of maternal blood (Panels B and F); and paternal blood (Panels C and G); and the DNA isolated from the proband, a 4 year old female child (Panels D and H) using two STR markers in chromosome 21 (D21S11 and D21S1412). The D21S11 marker showing 1:2 triallelic peak ratio and D21S1412 marker showing 1:1:1 triallelic peak ratio demonstrates the trisomy 21 in the fetus (Panels A and E of Fig. 2). The comparison of the PCR fragments in father and mother revealed maternal origin of the extra chromosome 21 (Panels E, F and G of Fig. 2). The homozygosity of the maternal alleles in the fetus and their heterozygosity in the mother for the marker D21S11 (Panels A and B, Fig. 2) indicates origin of the extra chromosome be attributed to the aberrant meiosis II of the female gametogenesis or an aberrant post zygotic mitosis. However, the heterozygous D21S1412 (Panels E, F, G and H of Fig. 2) might have occurred due to an event of crossing over of the distal marker.

However the events leading to trisomy 21 in the previous child of the couple (proband) does not provide any evidence for recombination. The 1:1:1 triallelic ratio for the D21S11 marker as well as the D21S1412 marker indicate trisomy 21; and latter marker (D21S1412) does clearly predict the parental origin of the supernumerary chromosome as maternal (Panels F, G and H of Fig. 2) as the parents do not have common allele for this marker. The 1:1:1 triallelic peak ratios indicate prezygotic origin of aneuploidy in the previous child with trisomy 21; likely to have had the phase of origin of the aneuploidy as meiosis I of maternal gametogenesis.

Family 3

We analysed the DNA isolated from the blood sample obtained from the proband who was a two year old male child (Panels A, D and G), from the peripheral lymphocytes of maternal blood (Panels B, E and H) and from the paternal blood sample (Panels C, F and I) using three STR

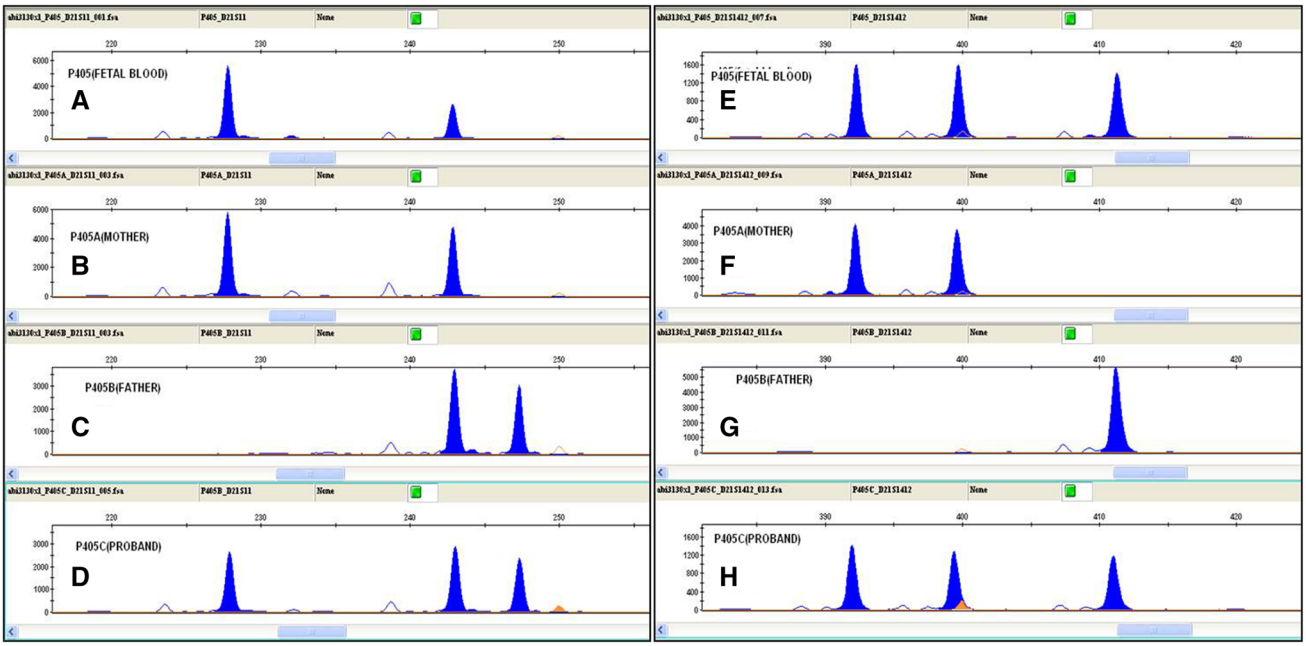

Fig. 2 Detection of trisomy 21 by QF-PCR with D21S11 STR marker (Panels **A**, **B**, **C** and **D**) and determination of parental origin of trisomy D21S1412 STR marker (Panels **E**, **F**, **G** and **H**) in family 2

markers in chromosome 21 (D21S11, D21S1437, D21S1412). The proximal D21S11 or pericentric marker showing 1:1:1 triallelic peak ratio demonstrates the trisomy 21 in the affected child (Panels A, B and C of Fig. 3).The comparison of PCR fragments in father and mother revealed maternal origin of the extra chromosome 21 (Panels D, E and F of Fig. 3). The 1:1:1 triallelic ratio given by D21S11 marker also indicates that the supernumerary chromosome has its probable origin due to the chromosomal nondisjunction in meiosis 1 of maternal gametogenesis or alternately, an early chromatid separation in meiosis 1 followed by the movement of an extra chromatid to a daughter cell formed by meiosis 1.

The 2:1 peak ratio of the more distal D21S1437 STR marker (Panels G, H and I, Fig. 3) accounts for the fact that the sizes of the two alleles of mother and father are of similar size for this marker. The additional chromosome has earlier been ascertained to be of maternal origin (Panels D, E and F of Fig. 3). Though there is no evidence to suggest recombination, use of more number of distal markers would have ruled out the absence of recombination. The D21S1412 was uninformative in this regard due to its homozygosity in mother.

Family 4

We analyzed the DNA isolated from the fetal cells obtained from the amniotic fluid (Panels A and D), from the peripheral lymphocytes of maternal blood (Panel B, E) and from the paternal blood sample (Panels C and F) using D18S391 (Panels A, B and C of Fig. 4) and D18S386 (Panels D, E and F of Fig. 4) microsatellite markers. The 2:1 peak ratio of the STR markers demonstrates the trisomy 18 in the affected fetus. The comparison of the alleles derived from mother and father as determined by the fragment length of the PCR amplicons of the polymorphic microsatellite markers also revealed the maternal origin of the supernumerary chromosome (Panels D, E and F of Fig. 4).

As there is no evidence of crossing over involving the region of the microsatellite loci, it may be assumed that the nondisjunction occurred in meiosis II of the maternal gametogenesis or due to an error in the post zygotic mitosis. The nondisjunction presumably occurred for the maternal origin chromosome containing the STR allele (seen in fetus as double dose) during meiosis II or post zygotic mitosis.

Discussion

Three families with recurrence of aneuploidies resulting in live birth of children/fetuses with trisomy 21 and one family of trisomy 18 are presented in this study. The events leading to the aneuploidy of chromosome 21 have occurred following different events in meiosis in the consecutive pregnancies in at least one of the families studied (family 2). The frequency of parental mosaicism in lymphocyte

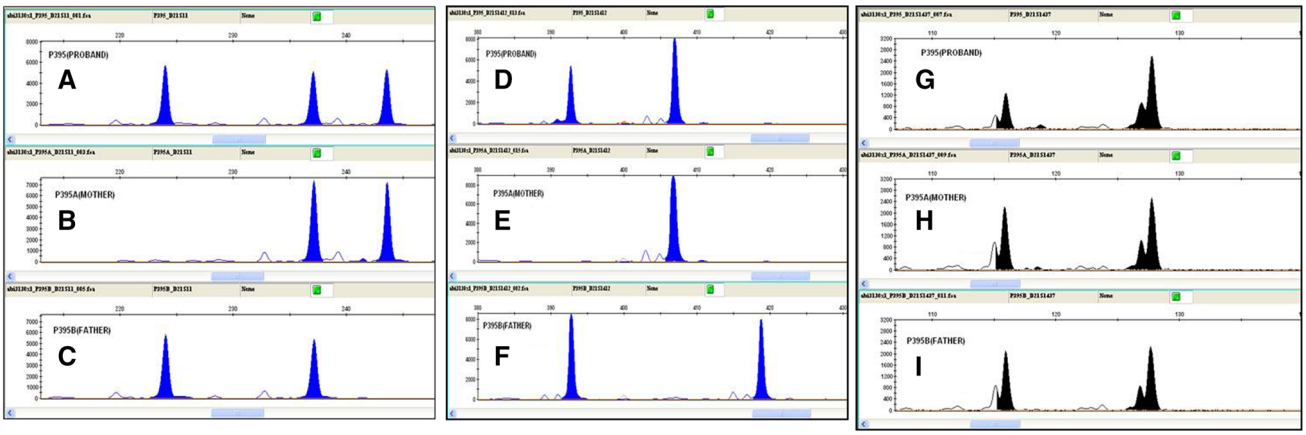

Fig. 3 Detection of trisomy 21 by QF-PCR with D21S11 STR marker (Panels **A**, **B** and **C**) and D21S1437 STR marker (Panels **G**, **H** and **I**) and determination of parental origin of trisomy with D21S1412 STR marker (Panels **D**, **E** and **F**) in family 3

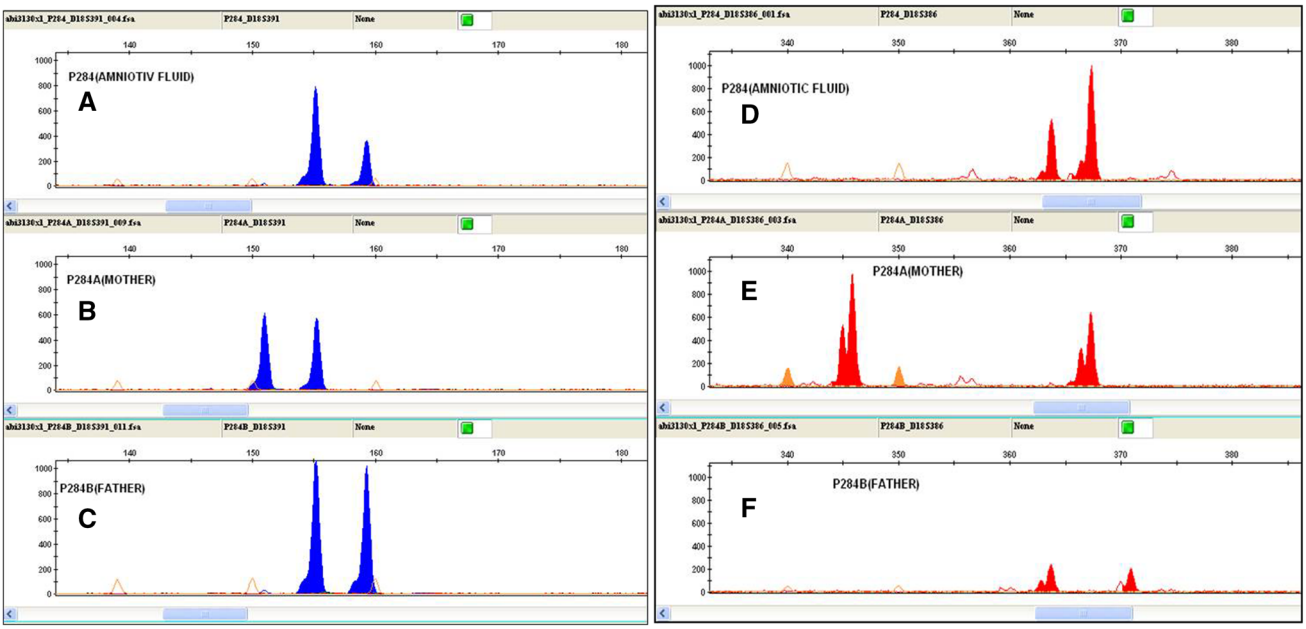

Fig. 4 Prenatal detection of trisomy 18 by QF-PCR with D18S391 STR marker (Panels **A**, **B** and **C**) and determination of parental origin of trisomy with D18S386 STR marker (Panels **D**, **E** and **F**) in family 4

cultures has been estimated to be 2.7–4.3% [7]. PCR based DNA polymorphic markers can reliably detect mosaicism at 5–10% level and are superior to karyotyping [8]. The karyotype analyses from blood or skin are not true representatives of the chromosome constitution of the germline cells. A young family was described by Nielsen et al. [9] with six trisomy 21 conceptions. No trisomic cell line was found following the analysis of blood and skin fibroblasts of the couple, but cytogenetic analysis of ovarian tissue had demonstrated a trisomy 21 cell line in 18% from the ovarian cells thereby explaining the reason for multiple recurrences [9].

A less invasive technique for identification of possible parental gonadal mosaicism is by using highly polymorphic microsatellite sequences. This is possible if a 'novel' allele is detected in the trisomic offspring. If the 'novel' allele is absent in the somatic tissues of both parents, it confirms that the 'novel' allele has originated from the germ cells of either of the parent [4]. Cytogenetic documentation of mosaicism should be present in either of the couple as novel allele could have been derived from either parent [6]. Neither of the offsprings with trisomy 21/18 had a 'new' polymorphic allele nor any of the couple had detectable mosaicism from peripheral lymphocytes.

Table 1 Maternal ages at the time of each trisomic conception and meiotic origin

	Maternal age at first conception	Karyotype at first conception	Maternal age at second conception	Karyotype at second conception	Parental origin	Meiotic origin	
						1	2
1	27	NDJ trisomy 21	30	NDJ trisomy 21	Maternal	MI	MI
2	21	NDJ trisomy 21	27	NDJ trisomy 21	Maternal	MII	MI
3	37	NDJ trisomy 21	38	NDJ trisomy 21	Maternal	–	MI
4	22	NDJ trisomy 18	27	NDJ trisomy 18	Maternal	–	MII

The majority of trisomy 21 conceptions have been shown to be the result of errors during maternal MI associated with advanced maternal age at conception [10]. Determination of meiotic origin is possible by studying the microsatellite markers. The reduction to homozygosity of a given pericentromeric marker was interpreted as an error in meiosis II. The retention of heterozygosity of a given pericentromeric marker is assigned as an error in meiosis I. Maternal origin was confirmed in all four families and maternal meisois 1 error was the most common mechanism for nondisjunction (Table 1). This observation has already been confirmed by studying more than 400 cases of free trisomy 21 [4, 11, 12]. There was no significant difference in the distribution of maternal ages between maternal MI/II error for families 1, 2 and 4 and hence a positive correlation between the particular error at meiotic stage and advancing maternal age could not be drawn from the observation from these families (Table 1). For family three, as the maternal age was 37 and 38 and the error was identified as MI in second pregnancy, the probable explanation is recurrent NDJ due to advanced maternal age occurring by chance.

In addition to three families with recurrence for trisomy 21, we had one family with recurrence of NDJ trisomy 18 which is an extremely rare event as compared to trisomy 21. A retrospective extensive study of the prenatal diagnosis data for 23 years from North America had formulated standardized morbidity ratio (SMR) for prediction of recurrence for trisomies following the birth of an index case of trisomy 21 or trisomy 18 [13]. For trisomy 21, the SMR was 2.4 whereas it was only 1.7 for trisomy 18. This reiterates the observation of extremely low recurrence risk for trisomy 18 when compared to trisomy 21.

During a genetic counseling session, couple with an offspring with NDJ trisomy is usually not offered invasive prenatal diagnosis in subsequent pregnancy on a routine basis considering the very low recurrence risk. The parents are also not offered karyotype analysis as the couple would be expected to have normal karyotype following the birth of a child with NDJ trisomy. This precludes the opportunity to detect possible low level mosaicism for trisomy in the lymphocytes of either of the couple. As NDJ trisomy contributes approximately 95% of the burden of trisomies, this observation of recurrence of trisomies in four families within a period of 3 years is significant from the point of counseling issues. In this scenario, it would be prudent to discuss with the couple the option for prenatal invasive testing in subsequent pregnancies.

Author's contribution SN prepared the manuscript did detailed literature search. AVS did the molecular studies and wrote the interpretation. KMG reviewed the manuscript and had modified the interpretation of molecular results. LP and DY did detailed literature search and had helped in manuscript preparation. MVT and ZH had done the cytogenetic analysis for all four cases and have written the cytogenetic observations. KS has critically evaluated the manuscript and has contributed suggestions. SN had conceived the idea of drafting this paper and has done the final drafting and will act as the act as the guarantor of the manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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