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Nondisjunction in Trisomy 21: Origin and Mechanisms

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Down's Syndrome is a chromosomal abnormality with a gain of a third copy of chromosome 21, characterized by craniofacial abnormalities, intellectual developmental delay and growth retardation. In the present study, one-day-old neonate and a patient with an aborted fetus were presented to rule out genetic aberrations via CMA screening. DNA was extracted using from the case samples and evaluated by CMA Affymetrix platform. Chromosomal abnormalities in the fetus such as trisomy emerges due to several factors including maternal age, genetic mutational events, non-disjunction of chromosomes and epigenetic changes. Genetic Cause of trisomy 21 is chromosomal aneuploidy and gain of three copies of chromosome 21. The Trisomy 21 can occur due to Robertsonian translocation, Mosaicisms or duplication of critical region of chromosome 21. The trisomy 21 is the result of nondisjunction of homologous chromosomes 21 during gametogenesis at the time of embryo development. CMA is an effective molecular diagnostic tool for predicting and diagnosing chromosomal abnormalities which needs to be promoted in India for

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healthy pregnancy outcomes. We report two cases of confirmed post-natal Trisomy 21. The main aim of this study is to focus on genetic diagnosis and its awareness which is still lacking with 100% coverage, in developing countries to reduce the burden of genetic diseases and associated emotional and economic consequences.

Keywords: Chromosomal microarray; down's syndrome; maternal age; chromosome 21; diagnosis; trisomy; non- disjunction.

1. INTRODUCTION

Chromosomal abnormalities in the fetus such as trisomy's are very common due to several factors including maternal age, anatomy, and environment. Down's Syndrome (DS) is a chromosomal abnormality characterized by mental retardation and reduced growth. It is a genetically abnormal condition/disorder with a full or partial gain of a third copy of chromosome 21 (chr.21). Advanced maternal age remains one of the major risk factors for the nondisjunction of chromosomes leading to the development of chromosomal aneuploidy. Cytogenetic techniques, chromosomal microarray assay (CMA), and next-generation sequencing have been in use efficiently for the diagnosis of chromosomal aneuploidies including deletions, and duplications. Trisomy 21 is a common chromosomal anomaly and about 80% of cases result in miscarriage [1,2]. T21 is the most common autosomal disorder which occurs due to the non-disjunction of chr.21 during the first or second meiotic division of maternal or paternal gametogenesis [3,4]. The disease was described by John Langdon Down first of all, as "Mongolian Idiocy" in 1866 [5]. However, the term "Mongolism" was justifiably criticized for its racial implications [5]. Therefore, it is also known as T21 or T21 or Down Syndrome (DS). According to a study, 95% of DS cases are due to T21 [6]. The remaining 5% comprise the translocation and mosaic types of DS.

Three types of changes may occur at chromosomal level causing DS: i) *Complete Trisomy* - the presence of 3 chromosomes formed (or gain of one extra chromosome) during fertilization in all the cells of an individual, ii) *Mosaic Trisomy* (less than 5% of DS cases) - some of the cells in the body have extra chromosome 21 and not all and iii) *Translocation Trisomy* - when a chr.21 is attached to another chr e.g., chr. 21 (21;21), chr 14, 13, 15, or 22. 21. Approximately 3% of cases of DS occur due to Robertsonian translocation, most commonly t (14; 21). Robertsonian translocation t (21; 21), can occur from a balanced carrier parent, because of ovarian mosaicism for Robertsonian translocation or may appear de novo [7]. The ovarian mosaicism may predispose a person to DS risk.

The annual birth rate of DS babies is around 40,000 accounting for 1.4/1000 live birth in India. DS may result in recurrent pregnancy loss. Recurrent pregnancy loss is a challenging reproductive health concern that impacts a significant percentage of couples, approximately ranging from 2% to 5%, cytogenetic abnormalities being one of the leading causes in addition to various other epigenetic factors [8].

Several meiotic chromosome segregation pathways can result in oocyte aneuploidy. During chromosome segregation there is an increased risk of errors during meiosis. During the development of oocyte, these errors can result in incorrect number of chromosomes and may occur at: (i) primordial germ cell mitotic proliferation for oogonia generation (ii) oocyte meiotic division and (iii) early embryo mitosis after fertilization. Meiosis involves two cell divisions (MI and MII) produces haploid gametes. During prophase I, which occurs in the ovary events such as chromosome pairing, synapsis, and crossing over (recombination) facilitate the exchange of genetic material between maternal and paternal chromosomes. Crossover creates chiasmata, physical links between homologous chromosomes, ensuring their proper segregation during metaphase I. At birth, oocytes arrest at the diplotene or dictyotene stage of prophase I and remain arrested until ovulation, surrounded by follicular cells in the primordial follicle. In each menstrual cycle, luteinizing hormone triggers oocytes to resume meiosis I. Homologous chromosomes, segregate during MI, and the oocyte enters meiosis II, where sister chromatids separate. Oocytes remain arrested at metaphase II until fertilization. Different chromosome segregation patterns contribute to oocyte aneuploidy [9]. Nondisjunction occurs when the sister chromatids fail to separate properly.

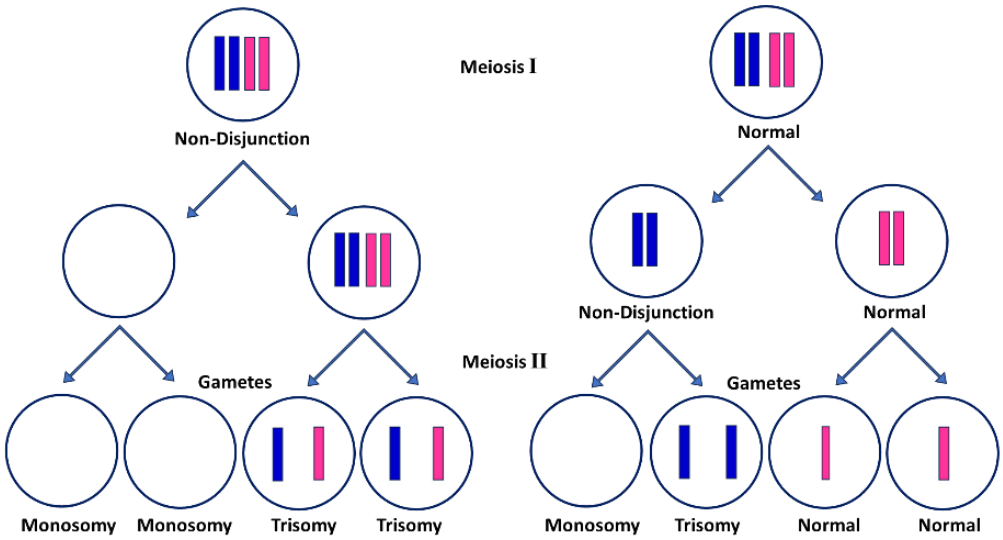

Fig. 1. Trisomy due to non-disjunction of chromosomes during Meiosis I & II

Premature sister chromatid separation and reverse segregation are other patterns observed. PSCS involves early separation of one homolog's sister chromatids, while RS results in sister chromatids of both homologs separating at meiosis I. These patterns can lead to aneuploidy, but some oocytes still have the correct number of chromosomes at the end of meiosis. Maternal age plays a significant role in oocyte aneuploidy, with increased risk in older women and miscarriages. Several mechanisms have been proposed to explain the higher incidence of oocyte aneuploidy in older females [10]. These include meiotic recombination failure, deterioration of chromosome cohesion, defects in the spindle assembly checkpoint mechanism, alterations in histone and tubulin modifications, and mitochondrial dysfunction leading to oocyte aneuploidy [11].

To rule out any copy number variation, deletion, or duplication CMA is in practice since 2010 in clinical diagnosis and decision-making and is a gold standard technique performed either using an array of comparative genomic hybridization (aCGH) or SNP array to detect copy number variations (CNVs) [12]. A CNV is defined as a segment of DNA at least 1 kb in size that differs in copy number compared to the reference genome [13]. In repro-genetic screening tests, it gives a clear picture of all chromosomes when compared to cytogenetics tests such as karyotyping and FISH and also it shows sub-microscopic deletion or duplications of specific loci on the chromosome of a specific gene linked with the clinical condition [12]. Identification of sub-microscopic imbalances or CNVs which are not detected in other cytogenetic tests. It is a reliable technique to identify chromosomal abnormalities and imbalances in miscarried fetuses and stillbirths [14]. In the present study, we report two cases of post-natal T21 confirmed via CMA in a day-old preterm neonate and another in an aborted fetus (Table 1, Table 2, Table 3 and Fig. 2 and Fig. 3).

2. METHODS

Genomic DNA was extracted from blood lymphocytes (blood samples collected in EDTA vacutainers) and the product of conception (POC) using Qiagen DNA blood mini kit. After checking the DNA concentration using nanodrop, and gel electrophoresis, subjected to chromosomal microarray. The 250 ng of genomic DNA was processed for both the samples using CytoScan™ kit modules involving restriction, digestion, amplification, fragmentation, labelling and hybridization followed by loading to array according to the manufacturer`s guidelines.

The CytoScan™ HD array enables the detection of high-resolution copy numbers across the genome as well as providing allelic imbalance information from single nucleotide polymorphisms (SNPs). The results were analysed with the Chromosome Analysis Suite (ChAS, Affymetrix) software and interpreted according to the recommended ACMG guidelines [15] and clinical databases available.

The detected variants are classified as follows:

i) Pathogenic – CNV with sufficient disease related evidence to be classified as pathogenic

ii) Likely pathogenic – CNV with strong disease related evidence in favour of pathogenicity
iii) Benign – CNV with sufficient non-disease related evidence to classify as benign
iv) Likely benign – CNV with non-disease related strong evidence against pathogenicity
v) Uncertain significance – CNV with limiting and/or conflicting evidence regarding pathogenicity

3. RESULTS

Pathogenic copy number variants have been detected by Chromosomal Microarray Analysis in both the cases in Table 2 and Fig. 2 and Table 3 and Fig. 3 respectively for Case I and Case II. Clinical and demographic features have been detailed in Table 1.

3.1 Case I

Two pathogenic CNVs have been found to encompass chr.21: loci q11.2-22.3 and q22.3-q22.3, together, measuring nearly 3.3Mb (Table 2 and Fig. 2). The OMIM genes: SOD1 (147450), TIAM1 (600687), SYNJ1 (604297), MRAP (609196), DONSON (611428), DSCR4 (604829), CFAP410 (603191), TRAPPC10 (602103), ADARB1(601218), and LSS (600909) are responsible for neurodevelopmental disorder, microcephaly, short stature, and speech delay, developmental and epileptic encephalopathy, limb abnormalities, intellectual disability, dysmorphic features, hypotonia and short stature characteristic of T21.

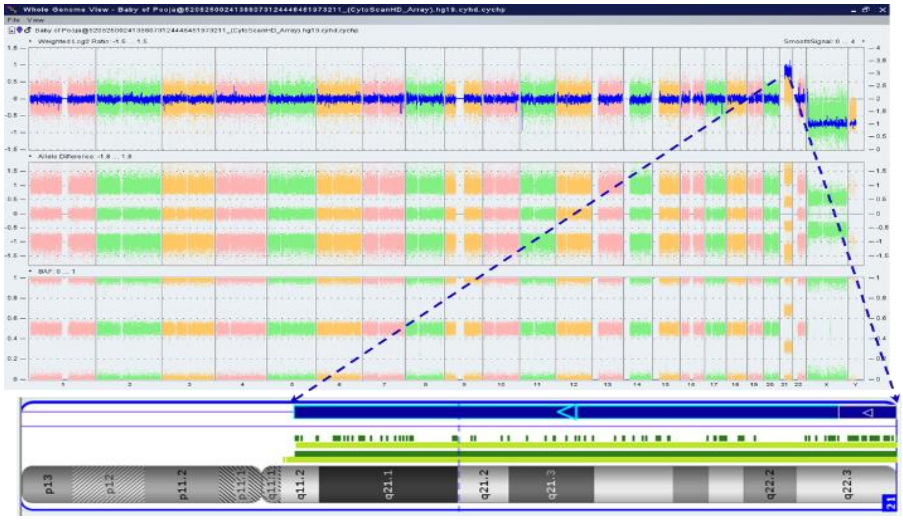

Fig. 2. ChAS suite karyoview and IGV profile showing T21 in case I (neonate) (47, XY,+21), gain ▲

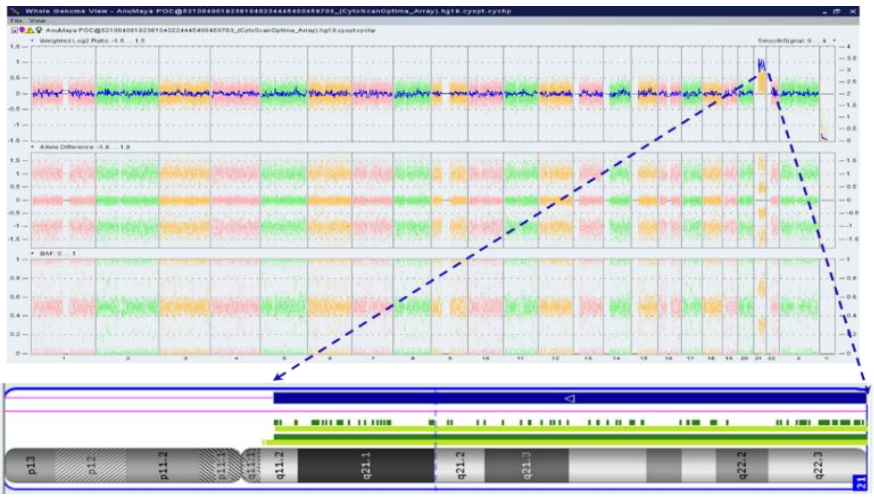

Fig. 3. ChAS suite karyoview and IGV profile showing T21 in case II (46, XX,+21), gain ▲

Table 1. Demographic and clinical details of two cases

Case	Sample	Status	History	Clinical Indications
I	Blood	One day old Preterm neonate (35 weeks)	a) Advanced maternal age (35 yrs. 4 mo.) b) Antenatal scan diagnosis of Ascitis with thickened pulmonary valve c) Severe pulmonary stenosis with right dilated atrium & ventricles d) Dysfunctional right ventricle with hydrops	2D echo showed dilated right ventricle, hypotonia, dysmorphic facial features with upward slanting eyelids, serpentine tongue, short single palmer crease, short neck with congenital heart disease.
II	POC	Aborted fetus	a) Age (30 yrs.) b) Previous history of miscarriage	Ultrasound report showed a single gestational sac with a weak decidual reaction, cardiac activity was absent.

Table 2. T21 CMA analysis using ChAS suite – Case I

Chr.	Cyto band	Size (kbp)	Type	CN State	OMIM Genes	Microarray Nomenclature	Interpretation
21	q11.2-q22.3	29962	Gain	3.0	129	arr[GRCh37] 21q11.2q22.3(15006458_44968648)x3	Pathogenic
21	q22.3-q22.3	3123	Gain	3.0	39	arr[GRCh37] 21q22.3(44974018_48097372)x3	Pathogenic

Table 3. T21 CMA analysis using ChAS suite – Case II

Chr.	Cyto band	Size (kbp)	Type	CN State	OMIM Genes	Microarray Nomenclature	Interpretation
21	q11.2-q22.3	33090.915	Gain	3.0	167	arr[GRCh37] 21q11.2q22.3(15006458_48097372)x3	Pathogenic

3.2 Case II

A pathogenic gain of over 3.3Mb was found on chr. 21q11.2- 22.3 (Table 3 & Fig. 3) and has been reported in the OMIM database to be associated with Down's syndrome. This region comprises of several pathogenic genes, TRAPPC10(602103), SYNJ1(604297), SIK1 (605705), ADARB1 (601218), DONSON (611428), PCNT (605925), TIAM1 (600687), and WDR4 (605924) found to be related to neuro developmental disorder, intellectual disability, dysmorphic features, hypotonia and short stature after detailed analysis. All these phenotypic features are T21 specific to the Down's Syndrome.

4. DISCUSSION

Case I was a one day infant sample and the probable reason might be the advanced mother's age, and there was no history and parents were normal, therefore the T21 might have generated due to nondisjunction and de novo. The siblings were normal. Advanced maternal age remains one of the major risk factors of nondisjunction [16-18], However, the biological mechanisms of this phenomenon are still not clear. The majority of cytogenetic studies indicated that the origin of the extra chromosome was maternal in about 80% of the informative cases and paternal in about 20% [1,19]. Nondisjunction in maternal meiosis I has been reported to be associated with reduced recombination between the non-disjoined chromosomes 21 [20], suggesting an important role for pairing/recombination failure or reduced recombination in the aetiology of T21. Several clinical studies in India and other countries reported infants born with Trisomy's [21]. Aging affects female fertility resulting in reduced oocyte quality and implantation rates, and increased spontaneous abortions. The occurrence of fetal miscarriage gradually increases from 5.3% in women aged ≤30 years to 7.6% in women aged 31–34 years, 12.8% in women aged 35–39 years and 22.2% in women aged ≥40 years [22]. Also, the incidence of pregnancy losses affected by aneuploidy is lower in women aged <40 years (65%) compared with women aged >40 years (82%). Trisomy cases occur at a low frequency of ~2% among women under the age of 25 years after which the frequency increases to over 40% at ages >40 years [23].

Case II was a product of conception sample detected with T21. Miscarriage is a multifactorial clinical condition and may occur due to Trisomy's, chromosomal abnormalities, or unfavorable epigenetic changes. Women with a previous history of miscarriage due to trisomy's have been reported to be at higher risk of further conception with trisomy's/aneuploidies, which are the result of nondisjunction during meiosis [22]. DS remains the most common chromosomal condition, occurring in 1 of every 733 births. According to estimates only 80% of T21 pregnancies end in a miscarriage or intrauterine fetal demise, and only 20% may progress to term delivery [24]. Down syndrome survivors have to face a number of clinical conditions ranging from intellectual disabilities, craniofacial features, congenital heart defects and leukemia. We report two cases of confirmed T21 with gain on chromosome 21 [25].

CNVs detected in both the samples in our study were pathogenic and pathologically significant with respect to T21.

In case I, the reason for trisomy, might be advanced maternal age which is the most common factor influencing the disjunction of chromosomes leading to T21. However, In the second case, T21 is suspected to be the pre-eminent cause of fetal demise. The patient also had a clinical history of recurrent miscarriages. The couple was not aware of genetic testing or prenatal screening with a lack of proper follow-up until the subsequent miscarriage. Detailed analysis using different software [26] showed the genes responsible for down syndrome on chromosome 21 and have been mentioned in following subsection:

4.1 Chromosome 21 and DS Genes

Various genes present on the q-arm of chr.21 have been implicated in DS and related phenotypic features observed after analysis. The *Superoxide Dismutase 1* (*SOD1*:147450) gene encodes superoxide dismutase, an enzyme associated with amyotrophic lateral sclerosis (ALS). In T21 (DS), elevated levels of this enzyme have been reported. *T-Cell Lymphoma Invasion and Metastasis 1* (*TIAM1*:600687) is linked to a neurodevelopmental disorder characterized by language delay and seizures. Another gene, *Synaptojanin 1* (*SYNJ1*:604297) encodes synaptojanin-1, which is involved in synaptic vesicle dynamics. Changes in its activity can lead to developmental and epileptic encephalopathy and Parkinson's disease, while altered enzyme activity can be associated with

DS. *Melanocortin 2 Receptor Accessory Protein* (*MRAP*:609196) is associated with glucocorticoid deficiency 2, a disorder resulting from resistance to adrenocorticotropin in the adrenal cortex. Affected individuals have cortisol deficiency and are at risk of hypoglycemia or severe infections if left untreated but not directly related to T21. Microcephaly, short stature, and limb abnormalities may be caused by mutations in *Downstream Neighbor of SON* (*DONSON*: 611428). The critical locus of *DSCR4*: 604829, is part of the Down syndrome critical region (*DSCR*) on chr. 21 and spans a 1.6-Mb region on chromosome 21q22.2, between the DNA marker LA68 and ERG (Dunn et al., 2006). The *Cilia- and Flagella- Associated Protein 410* (*CFAP410*:603191) is involved in ciliogenesis and DNA damage repair, but its relationship to trisomy 21 or Down syndrome is unclear. The *Trafficking Protein Particle Complex, Subunit 10* (*TRAPPC10*:602103) is associated with neurodevelopmental disorder characterized by microcephaly, short stature, and speech delay. Mutations in the *Adenosine Deaminase, RNA-Specific, B1* (*ADARB1*:601218) mutations are associated with a neurodevelopmental disorder characterized by hypotonia, microcephaly, and seizures. Variations in *Lanosterol Synthase* (*LSS*:600909) can lead to a syndrome involving alopecia (hair loss), intellectual disability, and cataracts. While LSS is not directly associated with trisomy 21, it plays a role in cholesterol biosynthesis, which may have broader implications in genetic conditions. In human fetal neurons *Salt-Inducible Kinase 1* (*SIK1*:605925) mutations are related to developmental and epileptic encephalopathy and abnormal neuronal morphology whereas, changes in *Pericentrin* (*PCNT*:605925) is associated with microcephalic osteodysplastic primordial dwarfism, type II. Another important gene *WD Repeat-Containing Protein 4* (*WDR4*:605924) plays a role in tRNA modification, which is important for protein synthesis and mutations may lead to microcephaly, growth deficiency, seizures, and brain malformations.

In both the cases, unfortunately, the couple belonged to a remote area and were unaware of critical genetic screening and diagnostic methods like CMA for ruling out chromosomal anomalies after anomalous ultrasonography (USG) findings. These methodologies are much more prevalent in the urban areas to rule out chromosomal birth defects to be managed throughout life as. The couples were genetically counselled in both the cases and are under the follow up. The clinical interventions for 'Case I' are getting reviewed to devise a management plan for the Down's Syndrome affected neonate.

Focusing on the awareness of genetic diseases in developing countries like India, there are several challenges in India, regarding the use of genetic diagnosis using Chromosomal microarray (CMA) and other methods as diagnostic tools for detecting abnormalities in fetus. The primary challenge being the limited availability and accessibility of such facilities across the country. Many parts of the country, especially the transitioning rural areas, and remote locations lack the necessary infrastructure and expertise to deploy CMA effectively restricting it to the developed cities.

Another roadblock is the affordability, as high cost makes it inaccessible the lower socioeconomic diaspora. Which in turn limits their access to accurate and comprehensive genetic testing as well as limiting the doctors to recommend it.

Moreover, the complicated paradigm of ethical considerations and awareness towards adoption of CMA in India is far from easy to navigate. Many individuals are unaware of the benefits and capabilities of this latest diagnostic advancement, leading to underutilization and delayed diagnosis. To overcome these difficulties, efforts are needed to improve the accessibility of CMA testing in an economic manner. Moreover, awareness campaigns and educational health camps across the country need to be undertaken to enhance the public comprehension for promoting its utilization. Crucial focus should be placed on encouraging safe pregnancy management and ethical practices in prenatal care in accordance to the regional regulations safeguard at the same time.

5. CONCLUSION

CMA is a reliable technique and has become the 1st tier diagnostic tool to detect chromosomal aneuploidies in prenatal as well as in post-natal samples especially in case of evident phenotypic neurological deficits and growth delays such as in Down's Syndrome. The use of CMA to understand and diagnose sudden/recurrent fetal demise or missed abortion helps at the genomic level in terms of appropriate decision-making and clinical management for further planning of a healthy pregnancy. Essentially, prenatal diagnosis should be made mandatory for females

having advanced maternal age and in case of a miscarriage to rule out any major genetic anomaly. It can help for better planning of a future pregnancy to lessen the medical as well as emotional burden on families. The government, medical and diagnostic institutions need to come together and devise effective ethical strategies for healthy pregnancy and maternal welfare for healthier children affordable to all economic groups and in remote areas of developing countries. Our study will add to the growing body of literature in genomic diagnosis and research so that a better approach can be devised with mandatory genomic diagnosis in prenatal cases for certain patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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