

## Cytogenetic Analysis in Down Syndrome

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**ABSTRACT** Clinically diagnosed Down syndrome cases are referred for karyotyping and counseling. Data on incidence patterns of the 3 cytogenetic types of Down syndrome from the cases seen during the duration of 35 years is presented. Chromosomes were examined after G-banded technique of peripheral lymphocyte cultures. For each patient, in addition to the detailed case history in the proforma 15 metaphases were examined and in cases of mosaicism, the count was increased to 25 to 50 metaphases for analysis. A total of 874 cases were confirmed to have the karyotype of Down syndrome. 509 were male probands (58.24%) and 365 (41.76%) were female cases. The most common was free trisomy 21 in 759 (86.9%), translocation in 77 (8.8%) and mosaicism in 38 (4.3%) cases. Robertsonian translocation 14;21 (48;62.34%) was prevalent among the 77 cases with translocation and the remaining 29 cases had the translocation of chromosome 21 either to chromosomes 1 (1) or 15(2) or 21 (26). The sex ratio has indicated the prevalence of the males for the total sample (1.41:1) (509: 365) as well as for the 3 basic cytogenetic types of DS. Included in the male trisomy 21 are the 2 cases with Klinefelter syndrome (KFS)(48,XXY+21) and one with *de novo* Robertsonian translocation between chromosomes 13 and 14.

### INTRODUCTION

Trisomy 21, the Down syndrome (DS), is the commonest autosomal chromosomal abnormality in the newborns and its incidence is 1 in 826 live births. Around 25% of DS conceptions survive to birth and the post birth data indicate greatly improved life expectancy in 80%. Even though implications exist for providing the health care to DS individuals, there seemed to be decline in the birth prevalence, due to the parental screening. In India, the reported incidence of DS is around 1 in 1250 live births (Verma 2006).

Karyotyping or chromosomal analysis, is necessary for each case suspected to have the trisomy 21 status, so that the genetic implications may be assessed.

Based on the cytogenetic diagnosis, the presence of the extra 21 in DS has been classified as free trisomy 21, translocation DS and trisomy 21 mosaicism.

In 95% of DS, the extra 21 is in a free state, because of the parental meiotic non-disjunction phenomenon during gametogenesis. 90 to 95% of the trisomy 21 condition in DS is because of

the maternal meiotic error; out of which, 1<sup>st</sup> meiotic error is the reason in 75% and 2<sup>nd</sup> meiotic error in 25%. In case of paternal origin of the extra 21 in 3 to 5% of trisomy 21, the 1<sup>st</sup> meiotic error is 25% and 2<sup>nd</sup> meiotic error is reported in 75% (Gardner and Sutherland 1996).

Translocation DS seen in 4% of DS has the extra 21 translocated to other chromosomes or to the acrocentric chromosomes of D and G group that is, 13,14,15,21 and 22. The individuals are known as carriers or translocation heterozygotes and its occurrence is 1 in 500 live births. The mechanisms of the formation are the fusion at the centromere or union (because of the breakage between the short and long arms of union) following the breakage in the short arms. The fusion of the homologous or non-homologous acrocentric chromosomes is also known as whole-arm exchange or Robertsonian translocation. Even though the arrangement has the centromeres from both the involved chromosomes, one of the centromeres is suppressed and appears as the monocentric chromosome (Earle et al. 1992; Han et al. 1994). The fused short arms containing the repetitive genes for ribosomal RNA may be lost, during the cell division, but do not affect the phenotype.

Non-homologous Robertsonian translocation (rob t) is frequent; out of which the prevalence of the balanced rob (14q;21q) is around 1 in 10,000 and rob(21q21q) is about 1/3<sup>rd</sup> as frequent. About 1/3<sup>rd</sup> of translocation DS cases

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are inherited from a parent and usually mother is the balanced carrier. *De novo* rob (14q21q) also originates most often in maternal germ cells and is due to the 2<sup>nd</sup> meiotic non disjunction event with an unusual pattern of genetic recombination in 1<sup>st</sup> meiosis preceding the 2<sup>nd</sup> meiosis, rob (14q21q) in *de novo* status, is originating mostly in maternal meiosis, because of an unusual recombination in 1<sup>st</sup> meiosis between the chromatids 14 and 21, followed by non-disjunction in 2<sup>nd</sup> meiosis. Homologous rob (21q21q) are mostly isochromosomes and are also formed as the true translocation in the post conception phase, by the fusion of the maternal and paternal chromosomes. Only around 7% of DS with rob (21q21q) or rob(21q22q) may have a carrier parent and mother usually is the carrier (Petersen et al. 1991; Schaffer et al. 1992; Tolmie and MacFayden 2007)

The 3<sup>rd</sup> chromosomal arrangement in 1% of DS is the mosaicism for 21. There may be 2 cell lines with 46 as well as 47,+21 chromosomal complements. It is opined that there is the tendency for the proportion of trisomic cells to be higher in fibroblasts and in early life. In mosaicism, the typical DS features may be less prominent, depending on the percentage of the normal to the trisomy 21 cell lines. The origin of the extra 21 may be from the zygote with 46 or 47 cell lines. In the former, non-disjunction phenomenon leads to the mosaic cell lines of 45/ 46/ 47 chromosomes where the cell lines with 45 chromosomes – 21, become non-viable. On the other hand, in cell lines with 47 anaphase lag of the extra 21 results in 46 and 47 cell lines and is associated with the maternal age as in free trisomy 21 (Tolmie and MacFayden 2007).

We report the percentage occurrence of the 3 cytogenetic types of 21 in the chromosomally confirmed individuals with DS.

### MATERIAL AND METHOD

The present study being retrospective, since 1975, has the information in detail on 900

consecutively referred and confirmed individuals with DS. Data of about 35 years is being presented.

Karyotyping was from the GTG banded peripheral lymphocyte cultures. For the translocation case, family study was carried out for determining the parental carrier status.

### RESULTS

Based on the chromosomal findings (Table 1), trisomy 21 was observed in 759 cases; mosaicism in 38 and translocation in 77 cases. Included in the male trisomy 21 are 2 cases with Klinefelter syndrome (KFS) (48,XXY+21) and one with *de novo* Robertsonian translocation between chromosomes 13 and 14. Parents showed normal karyotype.

In mosaicism, the normal to trisomy 21 cell lines ranged from 4% to 96%. There was the case of mosaicism in a male proband for trisomy 18 and 21 (47,XY, +18 (84%)/47,XY,+21(16%). Parents had normal karyotype. Another case had mosaicism for a fragment (47,XX+21/48,XX+21+frag(pat), which was paternal in origin. Both in the female proband and in her father the fragment was observed with the application of silver nitrate stain, was determined to be the fused nucleolar organizing regions of the acrocentrics.

The sex ratio has indicated the prevalence of the male index case (1.41:1) (509:365) for the total sample as well as for the 3 basic cytogenetic types of DS.

It is translocation 14;21 (62.34%) (Table 2) which seemed to be prevalent among the 77 DS with translocation of 1 or 15 or 21 either to 1 or 21. Translocation 1;21 was detected as derivative 21 (47, XX+der(21), mat t(1;21)(p11;p11), del (1p11 ->ter). Proband had normal 1 and its partner from 1qter ->1p11. The 1p11 -> pter has joined the 21 at 21p11. The arrangement of 21s were as 2 normal 21s and derivative 21 (21qter->21 p11::1p11->1pter). For the chromosome 1 it was a balanced arrangement whereas for 21 it was 3 copies of 21. Mother's karyotype was 46,

**Table 1: Cytogenetic types and sex ratio in DS.**

Types of DS	Male		Female		Total		Sex-ratio
	No.	%	No.	%	No.	%	
Trisomy 21	447		312		759	86.9	1.43:1
Mosaic DS	21		17		38	4.3	1.2:1
Translocation DS	41		36		77	8.8	1.1:1
Total	509	58.24%	365	41.76%	874	100.0	1.41:1

**Table 2: Translocation DS and its subdivisions.**

Types of <i>tDS</i>	Male	Female	Total	
			No.	%
1;21	-	01	01	1.3
14;21	25	23	48	62.34
15;21	-	02	02	2.6
21;21	16	10	26	33.76
Total	41	36	77	-

XX,t(1;21) (p11;p11) (1qter->1p11::21p11->21pter;21qter->21p11::1p11->1pter).

One of the male probands who had double 14;21 (46,XY, rob(14;21)(q10;q10)pat; rob(14;21)(q10;q10)mat) arrangement had the familial transmission of the 14;21 translocation from both his carrier but non-consanguinous parents. Proband's maternal grandmother and younger female sib also had rob(14q;21q) (Fig. 1).

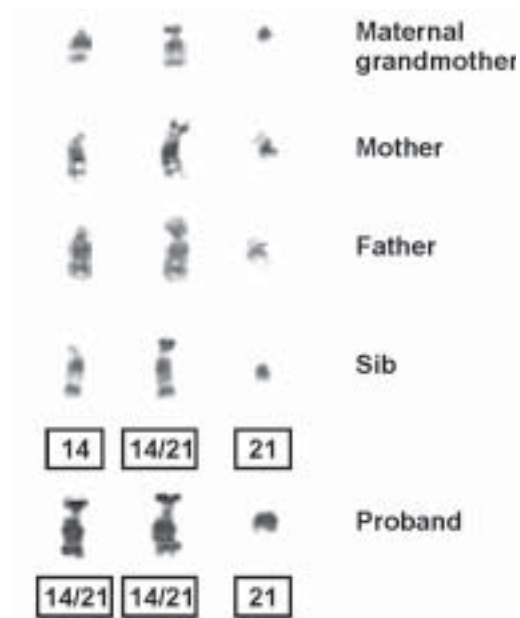
The parental origin was confirmed in 12 carrier parents of translocation DS (7.8%) (Table 3). Ninety eight partners had normal karyotype (Table 4). Maternal origin was found to be frequent and that too for the female translocation DS patients. Karyotyping could not be done in 22 parents (28.65%), because, in spite of the request they neither consented nor volunteered.

### DISCUSSION

In the present study, among the 3 cytogenetic types of DS (Table 1), the occurrence of trisomy 21 in 86.9% cases is lower than the reported 95% incidence in the population. Likewise are the increase in the percentage frequency of mosaicism and translocation, 4.3% and 8.8%, in comparison to the 1% and 4% incidence, reported in the literature. The differences may be because of the sample and methodology. In India, early marriage and early reproductive life are customary, because of which the occurrence of trisomy 21 associated to older maternal age may be

**Table 3: Parental origin of translocation in DS.**

<i>tDS</i> Parent	Paternal		Maternal	
	Male	Female	Male	Female
1;21 n 01 Parent	-	-	-	01
14;21 n 48 Carrier	01	02	02	04
15;21 n 02 Normal	-	-	-	-
21;21 n 23 Carrier	02	-	-	-
Total n 77	03	02	02	05
	5/12	41.67%	7/12	58.33%

**Fig. 1. Partial karyotype-GTG double heterozygote for translocation 14;21 familial transmission**

decreased, followed by the increase in the translocation DS, in younger parents. In case of mosaicism, with more number of metaphase spreads analyzed, it is confirmed.

**Table 4: Parental origin of translocation in DS.**

<i>tDS</i>	Carrier		Normal karyotype		Not done		Total
	Father	Mother	Father	Mother	Father	Mother	
1/21 n 1	01	-	01	-	-	-	02
14/21 n 48	03	06	29	31	13	13	96
15/21 n 2	-	-	02	02	-	-	04
21/21 n 26	02	-	15	17	09	09	52
Total n	05	07	47	51	22	22	154 partners
77 %	12	7.8%	98	63.6%	44	28.6%	Mother carrier 6/7 85.7%

The incidence of KFS and DS karyotype in the same individual is estimated to be less than 0.1%. The presence of the double aneuploidy may be because of either a double event of non-disjunction resulting in one abnormal gamete or less likely, separate events in gametogenesis in both the parents (Ford et al. 1959, Al-Awadi et al. 1998, Cyril et al. 2005). In the present study, the percentage occurrence of the 2 cases with 48,XXY+21, in 759 trisomy 21 cases, is 0.26%.

The higher male sex ratio may be the inherent tendency of Y belonging to the G group chromosome to be closer to its other members, 21 and 22, especially, the smallest acrocentric, the 21. The reasons for the excess of male DS associated to the paternal errors are not yet clearly known (Petersen et al. 1993) (Table 1).

In literature, it has been shown that 25% instances of rob translocation may be familial and the *de novo* in 75% and in both the types the maternal origin may be predominant (Schaffer et al. 1992). In the present study rob (14q21q) has occurred in 62.34% of cases (n=48) and the origin was found to be maternal in 6 out of the 9 confirmed cases (or out of the 16 karyotyped parents) and paternal origin in 3 cases. It has also been stated that in male, the presence of rob translocation may lead to spermatogenic arrest and male infertility.

The percentage of occurrence of true rob (21q21q) may be around 0.5% and chromosome 21 as isochromosome 46,i(21)(q10) in translocation DS arises mostly *de novo* (Robinson et al. 1994). In the present study, in 33.76% (n=26) of cases rob(21q21q) has occurred and in 2 out of the 12 parents karyotyped, the origin was determined to be paternal (Tolmie and Mac Fayden 2007).

DS with translocation may also be due to the presence of reciprocal translocation of 21 with other chromosomes in one of the carrier parents and seen in the DS patient as the derivative. Here, DS is due to the 3 copies of the DS critical region in 21q arm and the incidence may be less than 0.1% in DS (Gardner and Sutherland 1995). In the present study one female proband had DS features because of the derivative arrangement between 1 and 21, whose mother had reciprocal translocation between 1 and 21. Proband's mother's next conception was medically terminated upon the detection of the presence of reciprocal translocation 1 and 21 in the male conceptus, which was subjected to prenatal diagnosis.

The presence of 2 times the rob(14q21q) and the non-consanguinous parents as the carrier including the maternal grandmother and the male probands younger female sib may be considered as a rare coincidental complexity.

The frequency was in confirmation with the findings in literature.

### Molecular Correlation to 21

The smallest human chromosome 21 belonging to G group was the 2<sup>nd</sup> chromosome to be sequenced. The estimated number of genes varies from 200 to 400 which spans around 47 million nucleotides representing 1.5% of the total DNA in cells. In the 23 locations, 49 loci have been described. In DS critical region in 21q22->qter are the 40 loci considered to be the most influential genes for the typical DS phenotype (Antonarakis et al. 2004). The gene dosage effect has been demonstrated for amyloid precursor protein, superoxide dismutase -1, purine synthetic enzymes, human oncogene ETS-2, alpha-A crystalline. The genes on the 21q are of particular interest because DS individuals develop Alzheimer's disease early in life, have increased risk to leukaemias and higher frequency to cataracts and other systemic anomalies (Gelehrter et al. 1998). It is surmised that the extra copies of the genes may disrupt the normal development resulting in DS phenotype and its associated increased risk to the medical problems. There also exists the proposal on 'developmental disability' that states that DS features may be because of the features arising from the specific disruptions of genetic homeostasis rather than from the direct dosage effects. The concerned genes and their effects may be inconspicuous solitary effects but do contribute to the overall phenotype in combination with other genes.

### Counseling

The parents who have had the child with DS and KFS have been informed that the child may manifest the features of KFS and follow up was emphasized (after Harper 2005).

The proband with DS and trisomy 18 has manifested the DS features. Being mosaic, there exists the chance for the variability between trisomy 18 trisomy 21 cell lines in the various tissues and systems.

A multi specialty team is the mode to manage the affected DS individuals and their families.

Fundamentally, may be under emotional circumstances, the existence of the condition in the affected children, is the fact to be conveyed to the parents. Then over a period of counseling sessions, the confirmation of the diagnosis, identification of the risk, medical management education, list of special schools and organizations, vocational training, career and issues on marriage may be provided.

The affected parents may be informed that the recurrence risk for trisomy 21 or mosaicism may be low (<1 %), but maternal age has to be considered. Translocation depends on the sex of the carrier parent. A carrier parent may give rise to conceptions with trisomy condition for 21 or carrier or chromosomally imbalanced with 33% risk for the 3 categories. In practice, female carrier has 10% chances of getting a conception with chromosomal abnormality and male carrier less than 2.5% except for the 21;21 translocation where carrier parent has 100% chance of risk to get affected offspring.

The adequate support as per the social-educational-economical-regional-financial needs and requirements of the family and as per the specific needs of the DS individuals are well appreciated.

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