CHAPTER II

CYTOGENETIC STUDIES IN MENTALLY RETARDED INDIVIDUALS WITH SPECIAL REFERENCE TO DOWN SYNDROME FAMILIES
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INTRODUCTION

(A) Cytogenetics of Mental Retardation

The mentally retarded child is in a state of arrested or incomplete development of the brain. Generally, in mild retardation the IQ is 50-70, while in severe retardation the IQ is anything below 50. But in practice the boundaries cannot be precisely defined. The American Association of Mental Deficiency defines Mental Retardation (MR) as "sub-average general intellectual functioning which originates during the developmental period and is associated with impairment in adaptive behaviour". Although a host of biochemical, chromosomal and environmental factors have been recognised gradually as etiological agents, the medical diagnosis remains in doubt in 50% of cases.

Mental retardation is an important health problem all over the world. Various epidemiologic studies indicate that 2-3% of children in India suffer from mental retardation (Gupta and Sethi, 1970). Although there have been several studies on mentally retarded children in India, yet relative contribution of genetic factors and spectrum of genetic diseases causing mental retardation in our country is not well known (Rama Rao et al., 1986). This study was therefore undertaken to determine the chromosomal anomaly if any as a cause of mental retardation in the patients referred
from regions of Western India.

Down Syndrome (DS) is the most common human genetic abnormality associated with mental retardation (Patterson, 1987). Its frequency at live birth is 1 in 700. The majority of DS conceptuses do not survive to term and 75 to 90% of them die in utero, thus its frequency at conception is much higher - about 1 in 100 conceptions (Kola et al., 1993). Down Syndrome was first reported by John Langdon Down (1866) who published the first comprehensive description of the disorder. Later, the association of DS with trisomy 21 by Lejeune et al. (1959) was a landmark in the understanding of mental retardation and human malformations.

As mentioned earlier, trisomy of chromosome 21 arises through meiotic non-disjunction and accounts for 96% of DS cases (Hamers et al., 1990). The remaining 4% arise from either mitotic non-disjunction in early embryo resulting in mosaicism or, more commonly, from chromosomal translocation of the whole (in the vast majority) or part of chromosome 21 to other chromosomes, most frequently to the acrocentric chromosomes. Consequently, more than 99% of individuals with DS will have trisomy of all the genes on human chromosome 21 in some or all of their cells. The study of rare partial translocation cases has led to the definition of a particular region of chromosome 21, known as the minimum critical or obligatory DS region, which is responsible for a large subset of the DS phenotype. This region is localized to band 21q22 in the distal portion of the long arm of chromosome 21. Patterson (1987) has estimated that the chromosome 21 has approximately 1,500 genes. A number of them have now been mapped to the obligatory DS and surrounding regions of chromosome 21 including superoxide dismutase 1, amyloid precursor protein, E26 specific proto-oncogene and the interferon α/β receptor genes. It is generally believed
that the individual characteristics which comprise the Down Syndrome phenotype are a function of an increased gene dosage effect of specific genes, and it is of great interest to determine exactly which genes or combination of genes are responsible for particular characteristics (Kola et al., 1993).

Children born with this disorder suffer from a wide variety of abnormalities, both anatomical and biochemical, many of them severe. IQs generally range between 25 and 50. Serious congenital anomalies are commonly associated with this syndrome. Congenital heart disease is seen in at least 40% of patients. The patients age prematurely and individuals with DS appear to be at a significantly increased risk of developing Alzheimer’s disease (Patterson, 1987). Biochemically they suffer from elevated levels of purines - a condition that by itself can lead to neurological impairment, mental retardation and immune system deficiencies. Additional complications include susceptibility to infection and a twenty to fifty fold increase in the risk of developing leukaemia (Patterson, 1987).

It is not surprising, therefore, that individuals with DS typically have shortened life spans. In 1929 their estimated life expectancy was only nine years. Since 1980s improved medical care had increased that average to more than 30 years, and now 25% of individuals with DS live to the age of 50 (Patterson, 1987). Lejeune (1990) has rightly said that "with all the progress accumulated during the last 30 years, the destiny of the trisomy 21 affected persons has not yet been substantially ameliorated". He further added that "no simple management, short of chromosome turning-off, can be predicted for trisomy 21".
The term chromosome heteromorphism or variant is recommended to refer to variations detected in the normal population (Paris Conference, 1971; Supplement, 1975). Significance of such variants if any, is not yet known, although some studies do indicate their role in non disjunction. Large variations in the size of the heterochromatin (C-bands) of chromosome 1, 9, 16 and Y are commonly observed. Individuals with extreme variation from the average are categorised as C-band heteromorphism.

Polymorphism of the C band of chromosome 9 is one of the commonest normal variations in the human karyotype, and apart from the variations in size, there are frequent variations in the position of the C band. Its normal position is in the proximal region of the long arm (Paris Conference, 1971), but when sometimes part or all of the C-band appears in the proximal region of the short arm, these cases are described as partial or total inversion. Mutton and Daker (1973) observed that chromosome 9 shows a high susceptibility for structural rearrangements and particularly pericentric inversions. Ghosh and Singh (1976) and Craig Holmes (1977) made similar observations and reported that among the chromosome variants chromosome number 9 is most frequently represented because of the high variability in length of its paracentromeric heterochromatin block. Total inversion of 9qh are found in approximately 1% of normal population (Kaiser, 1980).

Another common variant found in the general population is the pericentric inversion of the heterochromatin region of chromosome Y. Earlier study by Fredrich and Nielsen (1974) reported that incidence of pericentric inversion of the Y chromosome in general population is approximately 1 per 1,000.
The acrocentric chromosomes, are often involved in non-disjunction and translocations. Moreover, Schmid et al. (1976) found that the acrocentric chromosomes are preferentially attracted to the centric heterochromatin of chromosome 1, 9 and 16 and the larger the C band, larger is the attraction.

The extensive data of Carothers et al. (1982) have revealed no detectable impairment of reproductive fitness in unselected carriers of heterochromatic variants. In a large prenatal study, Hsu et al. (1987) recorded major polymorphisms of constitutive regions of chromosome 1, 9, 16 and Y in the New York city population. They did not associate these chromosomal polymorphisms with any deleterious phenotypic or clinical effect nor with fetal wastage.

Howard-Peebles and Stoddard (1979) have suggested that for individuals with inversion 9, the reproductive possibilities appear to be five fold:

1. no effect;
2. either reduced fertility or complete reproductive failure;
3. duplication/deletion of chromosomes resulting in either abnormal offspring or spontaneous abortion;
4. chromosome interactions resulting in offspring with other chromosome abnormalities as proposed by Lejeune (1963) and others.
5. disturbances of some RNA synthesis during meiosis resulting in metabolic variations during early embryogenesis and ultimately malformation (Boue et al., 1975).

There have been reports suggesting a greater risk of chromosome aberrations in carriers of C-band heteromorphisms (Holbeck et al., 1974; Nielsen et al., 1974). Halbrecht and Shabtay (1976) reported a greater rate of chromosome breakage in cells
of individuals with C-band variants. Boue et al. (1975) have suggested that both 9qh+ and inversion 9 carrier couples may have a higher risk for producing chromosomally abnormal and/or malformed offspring and may also be associated with a tendency of spontaneous abortion. Some other authors have also described an increased tendency to early spontaneous abortions in familial pericentric inversions (Simpson et al., 1977; Osztovics et al., 1982; Stetten and Rock, 1983). Subfertility and sterility of male inversion carriers have also been reported (Kaiser, 1984; Groupe de Cytogeneticiens Francais, 1986). Such findings suggest that the variant chromosome plays a role in the failure of gametogenesis in both males and females, perhaps by altering meiosis or mitosis.

Ford and Lester (1978) reported increased number of hyperploid cells in lymphocyte cultures in individuals with 9qh+ and pericentric inversion 9qh. They suggested that these variants cause, or increase the likelihood of mitotic non-disjunction in vitro. Moreover, the mitotic non-disjunction is most likely to be increased by factors that influence spindle structure and function, possibly by affecting centromeric attachment to the spindle fibres. They further said that cellular conditions leading to mitotic instability may predispose to more significant changes in meiosis.

Pardue and Hening (1990) reported that heterochromatin has significant genetic effects and it can markedly influence the level of activity of genes which, by chromosome rearrangement, are transferred into, or close to heterochromatic regions (position effect variegation). In addition, a number of euchromatic genes have been identified whose expression could be modified when extra copies of certain regions of heterochromatin are present in the genome.

Trisomy 21 has been described in association with other aneuploidies as well
as structural rearrangements or both, on rare occasion. Faed et al. (1978) found evidence of a tendency to non-disjunction in a family with inversion 9 carriers and they hypothesized that the striking preponderance of inversion 9 carrier males in this family may also imply an interference with sex chromosome segregation at meiosis.

Examples of familial pericentric inversion and 21 trisomy offspring have been reported by Hamerton et al. (1975) and Prieto et al. (1981). Wang and Hamerton (1979) again detected a higher frequency of 9qh+ in a trisomic group than in normal newborns. Moreover, in a large study by French authors (Groupe de Cytogeneticiens Francais, 1986), numerous examples of trisomies 18 and 21 as well as sex chromosome aneuploidies due to pericentric inversions have been reported. It seems that in pericentric inversion carriers, non-disjunction of chromosome 21 could be due to an interchromosomal effect (Lejeune, 1963).

(C) Acrocentric Chromosome Association in Down Syndrome

In humans, the nucleolus organizer regions (NORs) are located on the short arms of the 5 pairs of acrocentric chromosomes (13, 14, 15, 21 and 22). NORs are composed of tandem copies of genes encoding for ribosomal RNA (rRNA) (Schwartzacher and Wachtler, 1983; Sommerville, 1985). During interphase, the NORs are nucleolus associated and more than one chromosome may participate in the formation of a single nucleolus whereby the DNA from their NORs uncoils and extends deep into the substance of the nucleolus (Busch and Smetana, 1970). They may be observed in metaphase chromosome preparations by using silver staining technique in which silver is thought to react specifically with a non-histone chromosomal protein associated with rDNA (Goodpasture and Bloom, 1975;
An important feature of the NORs is that they exhibit polymorphism. The number of silver grains found over these regions using \textit{in situ} hybridization (Evans et al., 1974), and the size of the region stained using the Ag-As technique (Tantravahi et al., 1976), vary between homologous and nonhomologous acrocentric chromosomes. Some acrocentrics are completely negative for these properties.

It has been suggested that the level of NOR activity generally parallels to the size of NOR band obtained by silver staining and the number of rRNA genes (Warburton et al., 1976; Miller et al., 1977). On the other hand, Mikelsaar et al. (1977) and Markovic et al. (1978) concluded that the NOR activity is a fixed characteristic of a chromosome and constitutes a hereditary property. Further, Jotterand Bellomo and Van Melle (1981) showed that inactive NORs remain inactive while active NORs vary over generations. Perez Castillo et al. (1986) suggested:

(a) The existence of an interindividual and intercellular variation of NOR activity in man, and

(b) that there is an optional threshold NOR activity, which when exceeded could result in the disturbances at meiotic level.

It has already been mentioned that during interphase the NORs are associated with nucleolus. During mitosis, the nucleoli disappear, but the chromosomes may still be seen at metaphase grouped together with their short arms in close proximity and such interactions of the NORs are called ‘satellite associations’ (Miller et al., 1977). This observation led to the speculation that interaction of NORs may be etiologically involved in the process of nondisjunction of acrocentric chromosomes leading to aneuploidy (Anneren et al., 1984; Schmickel et al., 1985). Other investigations had
suggested that certain NOR variants particularly the presence of a duplication of the NORs called as "double NOR" (dNOR) are strong risk factors for Down Syndrome (Jackson-Cook et al., 1985; Jackson-Cook and Brown, 1987).

In the present study, the frequency of satellite associations and dNORs were determined in the controls and DS and ascertained whether the results could be associated with DS.

(D) **Telomeric Association in Down Syndrome**

Early cytological and genetic studies demonstrated that chromosomes with broken ends were unstable (Blackburn and Szostak, 1984; Zakian, 1989) and the broken ends were able to fuse end to end, leading to dicentric, ring or other unstable chromosome forms. The instability of chromosomes with broken ends was in contrast to the stability of normal chromosomal ends and this led to the concept of the telomere as being the specialized structure at the natural end of a eukaryotic chromosome, without which the chromosome is unstable.

Telomeres are highly conserved among all well characterized eukaryotic nuclear chromosomes and are quite different from those of linear viral, non-nuclear plasmid or mitochondrial genomes. Hence, in otherwise divergent eukaryotes, the telomeric DNA sequences and structure are similar and essentially consists of a stretch of very simple, tandemly repeated sequence. Examples of telomeric repeat units include AGGGTT for humans as well as other vertebrates (Blackburn, 1991). A terminal tract of this simple DNA sequences, typically of a few hundred base pairs in yeast or ciliates, and thousands of base pairs in vertebrates, seems to be sufficient to maintain a stable telomere (Blackburn, 1991).
The "telomere hypothesis" has been supported by numerous reports. Since long it has been known that telomeres play an important role in chromosome structure and function. Early studies in *Drosophila* (Müller, 1938) and maize (McClintock, 1941) showed that under normal conditions, aberrations involving telomeres were extremely rare, and that broken chromosomes lacking telomeres are highly unstable, producing translocations, fusions, and other aberrations (Blackburn and Szostak, 1984; Zakian, 1989). Thus, telomere deletion could have severe consequences on cell function.

One effect on the cell is occurrence of replicative senescence (or loss of proliferative ability in normal somatic cells) and its possible link to cellular aging was first described in the classic study of cultured human fibroblasts by Hayflick and Moorhead (1961). Later on, the loss of proliferative ability in fibroblasts *in vitro* was confirmed in other somatic cells and in other species (Harley, 1991). Further, a number of authors have reported increase in the frequency of cells with chromosomal aberrations, especially the telomeric fusions (associations) with increased number of cell cycles (Saksela and Moorhead, 1963; Benn, 1976; Sherwood et al., 1989).

Replicate senescence occurs due to telomere loss after each cell cycle. Watson (1972) and Olovnikov (1973) independently described the "end replication" problem, i.e. the inability of DNA polymerase to fully replicate the ends of a linear DNA molecule. All known DNA polymerases replicate DNA in a 5' to 3' direction from a primer. At the end of a molecule there is no DNA to which the primer could attach, and hence the 3' end of the molecule cannot be replicated. Organisms with linear chromosomes therefore require a specialized replication mechanism. In the absence of such a mechanism, a gradual loss of DNA will occur, which will eventually result in loss of an essential gene. A more immediate and catastrophic consequence of telomere
loss is probably chromosome instability caused by the fusion of nontelomeric DNA ends. This, in turn, would establish breakage fusion bridge cycles because of the resulting dicentric chromosomes (Broccoli and Cooke, 1993).

Micro-organisms and viral genomes being circular have obviously evolved mechanisms to overcome the end-replication problem. All of these mechanisms in prokaryotes and viruses prevent incomplete replication of the ends of chromosomes (Harley, 1991).

However, immortal eukaryotic cells, evolved a novel solution to overcome incomplete replication of their linear chromosomes as these cells could balance incomplete replication by de novo synthesis of telomeres (Blackburn and Szostak, 1984). Support for this dynamic equilibrium model of end replication in eukaryotes came from the characterization of telomere terminal transferase (telomerase) in Tetrahymena by Greider and Blackburn (1989). Telomerase activity in human cells was first identified by Morin (1989) in extracts of immortal cervical carcinoma cell line, HeLa.

In yeast, the average number of terminal repeats present at a telomere is constant, suggesting a regulated process (Zakian et al., 1990). Mutations in a number of loci in yeast affect telomere length, which suggests that many factors may be involved in telomere maintenance. A mutation in one of these loci, est 1, causes the gradual loss of telomeric repeats together with aneuploidy and senescence (Lundblad and Szostak, 1989). Similar phenotypes have been generated by incorporation of altered sequence of telomeres in Tetrahymena (Yu et al., 1990). Loss of telomerase activity has been suggested to result in these phenotypes.
In man, the length of terminal repeat arrays is shorter in somatic tissues (≈10 kb) than in sperm (≈15 kb) (de Lange et al., 1990). The telomere length is maintained in normal human sperm over the age range of 18-68 years (Harley, 1991). Moreover, the telomere is observed to decrease in length with increasing age of the donor, at least in some somatic tissues (Hastie et al., 1990). Harley (1991) proposed that somatic cells lacked telomerase activity which results in gradual loss of telomeric DNA due to incomplete replication or degradation of ends, while germ line telomeres would be maintained with age. In plants also it is observed that the broken chromosomes are healed in zygotic, but not in the terminally differentiated tissues (McClintock, 1941), suggesting a developmentally regulated repression of telomerase in somatic cells of higher eukaryotes.

The first direct evidence for telomere loss during cellular aging was obtained by analysis of cultured human fibroblasts (Harley et al., 1990). From various experiments, the rate of telomere loss has been calculated in vitro and in vivo, and a good correlation between the measured rate of telomere loss in vitro (≈50 bp/cell doubling) and the estimated rate of loss in vivo (≈70 bp/cell doubling) has been obtained (Harley, 1991).

Hastie et al. (1990) reported a significantly lower rate of telomere loss in human blood cells during aging in vivo. It is also possible, that telomerase is expressed at low levels in the stem cell population of tissues with very high proliferative capacity. A small rate of loss of telomeric repeats with age in vivo would reflect the imbalance between telomerase activity and incomplete replication or degradation (Harley, 1991). It has been demonstrated that the overall length of telomeric arrays in fibroblasts could be used to predict the number of cell divisions which a culture is
capable of undergoing before senescence (Harley et al., 1990).

In view of the available data for telomere hypothesis, Harley (1991) suggested that telomere loss could act as a mitotic clock, reflecting the replicative history of normal somatic cells, and as a genetic time bomb, contributing to chromosomal abnormalities in cell aging and transformation.

The normal ends of human chromosomes (telomeres) sometimes appear to be fused end to end, and such arrangements are called as telomeric associations (Fitzgerald and Morris, 1984). Telomeric association has been described in SV40 virus-infected cells (Moorhead and Saksela, 1963), senescent fibroblast cell lines (Benn et al., 1976) and also in patients with ataxia telangiectasia and Thibierge-Weissenbach syndrome (Hayaschi and Schmid, 1975; Oxford et al., 1975; Dutrillaux et al., 1978; Taylor et al., 1981), in a variety of leukaemias (Fitzgerald and Morris, 1984; Morgan et al., 1986; Raimondi et al., 1987; Saltman et al., 1989) as well as solid tumors (Mandahl et al., 1985; Dewald et al., 1987; Kovacs et al., 1987; Pathak et al., 1988; Hastie et al., 1990).

The incidence of acute leukaemia in children with DS was found to be substantially greater than in normal children (Holland et al., 1962; Patterson, 1987). Rapid ageing has also been reported for DS patients (Sinet, 1982; Franceschi et al., 1986).

In the present study, the frequency of telomeric associations was determined in the DS patients and age matched control children as it would be interesting to determine these associations in view of the fact that DS patients are prone to leukaemia and rapid ageing.
MATERIALS AND METHODS

Patients

The Department of Zoology, Gujarat University has an ongoing Clinic with three medical Consultants, a Paediatrician, Gynaecologist and a Radiologist.

The patients were referred from various hospitals and clinics of Gujarat and the adjoining states of Rajasthan, Maharashtra and Madhya Pradesh to the Departmental Clinic for chromosomal analysis.

Out of total of 605 patients referred to the Clinic, 222 were Mentally retarded (MR) cases. The patients were examined by the Consultant Paediatrician. Clinical features, family history and detailed family pedigree were recorded in a proforma. Clinical photographs were taken for cases with abnormal clinical features.

Parents of Down Syndrome (DS)

Patients with features of DS were first confirmed by carrying out chromosome analysis. The studies on the parents of DS confirmed cytogenetically to have free trisomy 21 were then carried out with their consent. The families who did not agree to participate in the study were excluded.

Laboratory Methodology

Blood Lymphocyte Culture

Peripheral blood is the most frequently used tissue for post natal chromosome studies or diagnosis primarily because it is easy to obtain and simple to culture. The standard procedure of Hungerford (1965) was followed with slight modifications for metaphase chromosome preparations.
About 2-3 ml of peripheral blood was collected by vein puncture in a sterile heparinized syringe and was gently mixed to avoid clotting. Whole blood culture of 0.5 ml were set up in duplicate in a sterile tissue culture tube containing 7 ml of RPMI-1640 medium presupplemented with 10% fetal calf serum, 0.1 ml of Phytohaemagglutinin (PHA, Sigma, USA) and 3 drops of Heparin. Cultures were incubated at 37°C for 69 hours and were also gently mixed every 24 hours to avoid clumping and to stabilize the pH of the medium. At 69th hour colchicine, (0.8 µg/ml) (Sigma, USA) was added to the cultures and they were kept for 1 hour.

Harvesting

After the colchicine treatment, the culture tubes were centrifuged at 800-1000 rpm for 12 minutes. The supernatant was discarded by pipetting off the media. The pellet was resuspended in 5 ml of prewarmed hypotonic solution (0.075M KCl, pH 7.0). The culture tubes were allowed to incubate in a water-bath preadjusted at 37°C for 45 minutes. After incubation, few drops of freshly prepared chilled 3:1 methanol-acetic acid fixative was added, and mixed gently. Separate pipettes were used for each culture tube to avoid cross contamination. The culture tubes were then centrifuged at 1000 rpm for 12 minutes. The supernatant was discarded, and the cells were resuspended in 5 ml of fresh fixative and mixed thoroughly. The tubes were centrifuged again. Atleast three changes of fresh fixative was given. After the final centrifugation, the cells were suspended in a small volume of fixative (approximately 1 ml, depending on the size of the pellet) to give a slightly opaque suspension.
Slide preparation

The slides were previously cleaned by passing through chromic acid followed by thorough washing in running tap water. Slides thus cleaned were kept in distilled water at 4°C. Then 3 to 4 drops of the cell suspension was dropped evenly on a chilled wet slide from the Pasteur pipette and dried on a hot plate at 60°C. About 7 to 9 slides were prepared for each case after examining the preparations. The remaining cell suspension was conserved in fixative at 4°C to prepare the slides as and when required.

Giemsa Trypsin Giemsa Banding

The technique of Sun-Chu-Chang (1973) was followed with slight modifications as the routine banding method for all the cases studied. One to five days old slides were heated on a slide warmer at 55-60°C for 5 minutes and then cooled to room temperature. The slides were treated with trypsin-EDTA solution (20 mg Trypsin and 10 mg EDTA dissolved in 50 ml of Sorenson’s buffer pH 7.0 and stirred for 15 minutes on a magnetic stirrer) for about 10-20 seconds. The slides were rinsed thoroughly in 3 changes of distilled water and stained in 4% Giemsa in phosphate buffer for 5 minutes. The slides were again rinsed in distilled water, allowed to dry and then observed under microscope.

Centromeric Banding

C-banding was done following the method of Sumner et al. (1971). 1-2 week old slides were treated with 0.2N HCl for 30 minutes at room temperature. The slides were rinsed thoroughly in distilled water and then immersed in freshly prepared 5% Ba(OH)$_2$ solution kept previously in a water bath at 60°C for 15 minutes and rinsed
thoroughly in distilled water. The slides were then dipped in 0.02N HCl if precipitates persisted and rinsed thoroughly in distilled water. The slides were incubated in 2XSSC solution (saline sodium citrate solution) for 30 minutes maintained at 56°C in a water bath. The slides were then rinsed thoroughly in distilled water and stained in 4% buffered Giemsa for 15-20 minutes.

NOR-Banding

NOR staining was done following the method of Goodpasture and Bloom (1975) with slight modifications in the present study. Two days to two week old slides were used for NOR localisation. Four drops of filtered 50% silver nitrate solution and 2 drops of 2% gelatin were placed onto a slide and mixed gently. The slide was then covered with a cover glass and placed onto a hot plate maintained at 60°C until the solution turned golden brown. The cover glass was removed with stream of distilled water and the slide rinsed thoroughly in distilled water. The slide was dipped in 2% buffered Giemsa stain for a few seconds, rinsed in distilled water, dried and observed under the microscope.

Quinacrine Banding

Q-banding was done following the method of Pearson (1972). The slides were immersed in McIlvaine’s buffer pH 5.6 for 10 minutes. Four drops of 0.5% Quinacrine dihydrochloride were placed onto the slide and covered with a cover glass in the dark for 20 minutes. The slides were then rinsed in McIlvaine’s buffer pH 5.6 and mounted in buffered glycerol (McIlvaine’s buffer and Glycerine, 1:1). The excess mounting solution was blotted off.
The cultures were set up in sterile conditions. The culture room was facilitated with Laminar hood flow, U.V. lamp and the surface of the work area was cleaned with alcohol and fumigated once a month to keep the place in aseptic conditions. Chromic acid treated glasswares and other tissue culture materials were wrapped in non-toxic paper and covered with aluminium foil and then sterilized in an autoclave at 15 lbs pressure for 25 minutes.

**Source of Chemicals**

Phytohaemagglutinin (PHA), Colchicine, Cytochalasin B were obtained from Sigma, USA. Tissue culture media (RPMI-1640), Giemsa and Serum used were from Hi-media (India).

**Acrocentric chromosome associations in DS**

G-banded and silver nitrate stained metaphase preparations were used for analysis of acrocentric chromosome association. Hundred metaphase plates having all the acrocentric chromosomes were analysed for acrocentric associations in 20 DS patients and 20 age matched control children. The presence of two or more acrocentric chromosomes in close proximity at their satellite end was considered to be an association. The slides were scanned to analyse the number of associations and type of association(s) viz. D-D, G-G, D-G and multiple in each plate.

**Telomeric associations in DS**

Telomere to telomere associations were scored in G-banded slides. Hundred metaphase plates were analysed in 20 DS patients and 20 age matched control
children. Telomere associations was considered to be present when single chromatid or double chromatids of two chromosomes were observed to be close to each other and appeared to fuse end to end. Such associations were scored as single or double chromatid associations.

Statistical Analysis

The data was analysed using Student’s ‘t’ test, Chi-square test, Karl Pearson’s Correlation co-efficient and Regression analysis as and when required.

RESULTS

(A) Cytogenetics of Mental Retarded Patients

Cytogenetic findings of the entire group of 222 MR children are summarized in Table 1 and also depicted in Fig.1. In the present study, Down Syndrome has made the greatest contribution to mental handicap due to any chromosomal aberration. 116 (52.3%) of the total cases were confirmed to have DS. The most common abnormality was free trisomy, which accounted for 112 (96.5%) of the DS cases; translocation leading to trisomy 21 was observed in 4 (3.5%) DS cases; out of which two (21;21) were observed to be de novo, while other two cases t(21;21)inv9qh (Case 1) and t(11;21) (Case 2), were detected to be of maternal origin. A G banded metaphase preparation showing t(21;21) in a DS case is shown in Plate A, Fig.1.

The male:female sex ratio for trisomy 21 was 2.2:1 and the incidence of chromosomal variants was 6 (2.7%) in the present study. The most common variant was inv(Y), which was observed in 2 DS cases and 1 MR case. In all the 3 cases of inv(Y), the inverted chromosome was inherited from the father. A G-banded
metaphase preparation showing inversion Y and free trisomy 21 is presented in Plate B, Fig.2. Inv9 in one instance was inherited from father, while 15p+ and 22p+ in the other two cases were inherited from their respective mothers.

Two cases referred as mentally retarded were observed with 46,XX,dup16q?13→q?ter. and 46,XX,der(18pter→18q21.3::15p13→15pter). The analysis of parental karyotypes revealed the origin of 16qdup to be *de novo*, while, the 18qdel was derived from mother who showed a balanced translocation between chromosome 18 and 15 - 46,XX,t (15;18)(18qter→18q21.3::15p13→15qter;18pter→18q21.3::15p13→15pter). Thus, in the present study 4 cases were observed with unique chromosome aberrations (Plates C to I; Figs. 3 to 13).

Case 1 46,XY,t(21:21),inv9qh
Case 2 47,XY,+21;t(11;21)(11pter→11q13.1::21p12→21pter;21qter
→21p12::11q13.1→11qter)
Case 3 46,XX,dup16q?13→q?ter
Case 4 46,XX,der(18pter→18q21.3::15p13→15pter)

The remaining 104 cases were cytogenetically normal.

(B) Cytogenetic Findings in Parents of free Trisomy 21

Parents of 64 children confirmed to have free trisomy 21 were investigated for chromosomal analysis. The karyotypic profile of these 128 parents are summarized in Table 2.

On the whole, 14 couples showed polymorphism in either parent (21.9%), wherein 8 (12.5%) cases of polymorphism were observed in fathers and 6 (9.4%) cases in mothers. Partial karyotypes of paternal inv9 and maternal 15p+ are shown in Plate
In case of paternal karyotypes, 56 showed normal chromosome complement (46,XY), 2 fathers had inv(Y) which was inherited by the trisomic child. In 5 fathers inv(9)(qh) and in 1 father 16qh+ was observed. However, these variants were not inherited by the trisomic child.

In case of maternal karyotypes, 58 mothers showed normal chromosome complement, while 1 mother had inv9qh, 3 mothers showed the presence of an unidentified marker in 2% of the plates. The above variants were not inherited by trisomic offspring in any of the cases. One mother showed 15p+ and another 22p+, both these variants were detected in their respective trisomic child.

Table 3 shows the distribution of Down Syndrome children according to the type of family and the origin of non-disjunction. Details for parental origin of the extra chromosome 21 in the DS child are given in Chapter III. Of the 8 families where variant chromosomes were observed in the paternal karyotypes, the origin of the extra chromosome 21 could be traced in 5 families and 3 families were non-informative. Out of the 5 informative families, paternal non-disjunction had occurred in 2(40%) cases and maternal in 3(60%) cases. This data is different from the observed values for total cases where paternal error accounted for 16.2% cases and maternal error for 83.8% cases (Please see Chapter III for details). Maternal polymorphism was observed in 6 families of which parental origin of extra chromosome 21 could be determined in 5 families and one family was non-informative. Maternal origin of the extra chromosome 21 was observed in all the 5 (100%) cases which is higher than the observed value of total cases where maternal error occurred in 83.8% cases.

Details of spontaneous abortions observed in mothers of Down Syndrome and
controls are given in Chapter V. 7.41% abortions were observed for controls, 17.11% for the total families with free trisomy 21 and 27.5% for the 14 families where one of the parent showed polymorphism.

(C) Study of Acrocentric Chromosome Association in Down Syndrome

Studies on association of acrocentric chromosomes in 20 DS and 20 age matched control children were carried out in G-banded and silver nitrate stained metaphase preparations. Examples of some of the satellite associations observed are shown in Plate K, Fig.15. The detailed results for each case is shown in Table 4. A total of 2000 cells were scored for controls and for DS children. In the control children, 442 associations were observed while 885 associations were observed in the DS children. In both the groups, maximum of D-G associations were observed, followed by D-D and the G-G associations. The multiple acrocentric chromosome associations were the least in both the groups (Fig.II).

The frequency of Acrocentric Chromosome Association (ACA) in the metaphase spreads varied from individual to individual as is depicted in Fig.III. In the control series, ACA varied between 4% to 45% while in the DS children the minimum and the maximum values were 31% and 67% respectively. Increased frequency of ACA was observed in DS children as compared to the controls and the values were highly significant (P<0.001). The distribution of the different types of association in the DS patients and control children is presented in Table 5.

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Telomeric Associations in Down Syndrome

The distribution of telomere association in each individual subject is shown in Table 6. The age distribution in the individuals of both the subject groups is shown in Table 7. Two types of telomeric associations were observed, one involving single chromatids and the other double chromatids. Chromosomes were involved in random manner in the associations and practically all the chromosomes were observed in the associations. Examples of the telomere to telomere associations (TTA) between single chromatids are shown in Plate L, Fig.16 and examples of TTA between double chromatids and where more than two chromosomes are involved are presented in Plate M, Fig.17.

The number of metaphases with telomeric associations varied amongst individuals as can be seen from the data presented in Fig.V. For the age matched controls, the range of telomeric associations was between 2 and 9 for single chromatid and between 0 and 3 for double chromatid. In the Down Syndrome children the TTA varied between 9 and 23 for single chromatid and between 1 and 5 for double strands.

The data for number of telomeric associations in DS and controls is shown in Fig.IV. Of the total cells with association, TTA involving single chromatid was present in 319 (87.15%) cells for DS children and in 86 (85.14%) cells for the control children. Thus, in both DS and control children the telomeric associations mostly involved single chromatid. The proportion of single chromatid TTA was found to be similar for both DS and controls, which was confirmed by Chi square test.

The frequency of single chromatid TTA was observed to be 15.95% for DS children and 4.3% for the control children, which was highly significant (P<0.001). Similarly, for double chromatid TTA the frequency was observed to be 2.4% for DS
PLATE A

Preparation from Peripheral Blood Lymphocyte Culture of a DS patient with
Karyotype- 46,XY,t(21;21)

Fig.1 G-banded metaphase plate showing free 21 and t(21;21) (arrows).
PLATE B

Preparation from Peripheral Blood Lymphocyte Culture of a DS patient with Karyotype- 47,Xinv(Y),+21

Fig.2 G-banded metaphase plate showing free 21 and inv(Y) (arrows).
PLATE C

Fig.3 Clinical photograph of Case 1.

Fig.4 Clinical photograph of Case 1 showing dental deformities.
PLATE D

Metaphase Preparation from Peripheral Blood Lymphocyte Culture of Case 1 with Karyotype- 46,XY,t(21;21),inv9.

Fig.5 G-banded metaphase plate of case 1 showing chromosomes 21, t(21;21) and inv(9) (arrows).
Fig. 6  Clinical Photograph of Case 2.
PLATE F

Metaphase Preparations from Peripheral Blood Lymphocyte Cultures of Case 2 with Karyotype - 47,XY,+21;t(11;21)(11pter→11q13.1::21p12→21pter;21qter→21p12 ::11q13.1→11qter) and from the mother who had transmitted the translocation to the proband.

Fig.7  G-banded metaphase plate of Case 2 showing der(11), der(21), normal 11 and free 21 chromosomes (arrows)

Fig.8 (a)  G-banded partial karyotype of mother showing normal and derived chromosome 11 and normal and derived chromosome 21.

(b)  G-banded der(21) showing acrocentric chromosome association with a chromosome 21.

(c)  Silver nitrate stained partial karyotypes showing NOR activity in der(21), der(11) and acrocentric chromosome associations involving der(21).
Fig. 9  Clinical photograph of Case 3.
Metaphase preparations from Peripheral Blood Lymphocyte Cultures of Case 3 with karyotype - 46,XX,dup16q?13→?qter, and from the maternal and paternal preparations of the proband.

**Fig.10** (a) Partial karyotype of Case 3 showing normal and duplicated (right) chromosome 16.

(b) Examples of chromosomal aberrations in the mother.

**Fig.11** Paternal metaphase showing the unidentified marker (arrow).
PLATE I

Fig. 12  Clinical photograph of Case 4.

Fig. 13  Metaphase Preparations from Peripheral Blood Lymphocyte Cultures of Case 4 with Karyotype - 46,XX,der(18p→18q21.3::15p13→15p) and from the maternal cultures of the proband with karyotype - 46,XXt(15;18)(18p→18q1.3::15p13→15q;18p→18q21.3::15p 13→15p).

(a)  G-banded partial karyotype showing - two normal chromosomes 15 and normal and deleted (right) chromosomes 18 in case 4.

(b)  Maternal chromosomes normal 15 and t(15;18)(p13;q21.3) and normal and deleted chromosomes 18.

(c)  Silver nitrate stained preparations showing NOR activity in the deleted chromosome 18 and its involvement in acrocentric chromosome associations.
PLATE I

PROBAND

MATERNAL

NOR ACTIVITY
PLATE J

Metaphase preparations from parents of free trisomy 21 children with variants.

Fig. 14

(a) G-banded and C-banded partial karyotype showing the normal and inverted chromosome 9.

(b) G-banded partial karyotype showing a normal chromosome 15 and 15p+ (right).
PLATE J

a  inv 9h  inv 9h

b  14
PLATE K

Fig. 15  G-banded and silver nitrate stained metaphase chromosomes showing acrocentric chromosome associations involving

(a) D-D chromosomes
(b) G-G chromosomes
(c) D-G chromosomes
(d) more than two chromosomes
(e) increased NOR activity
PLATE L

Fig. 16  Showing examples of telomeric associations involving single chromatid.
Fig. 17  Examples of telomeric associations

(a) involving more than 2 chromosomes

(b) involving double chromatids.
PLATE M
Table 1

Chromosome findings in 222 Mentally Retarded cases in the present study

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<th>Total number of cases karyotyped</th>
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<tbody>
<tr>
<td>No. of abnormal cases detected</td>
<td>= 118</td>
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</table>

(A) Down Syndrome Cases

Total No. of DS = 116

(i) Free Trisomy : 112

47,XY,+21 = 74
47,XX,+21 = 34

(ii) Free Trisomy with variants :

47,Xinv(Y),+21 = 2
47,XY,+21,22p+ = 1
47,XX,+21,15p+ = 1

(iii) DS due to translocation :

46,XY,t(21:21) = 1
46,XY,t(21:21) = 1
46,XY,t(21:21)inv9qh = 1
47,XY,+21,t(11;21)(11pter→11q13.1::21p12→21pter;21qter→21p12::11q13.1→11qter = 1

(B) Non-DS MR

(i) Cases without any abnormality = 104

46,XY = 58
46,Xinv(Y) = 1
46,XX = 45

(ii) Cases with abnormality :

46,XX,dup16q?13→q?ter = 1
46,XX,der(18pter→18q21.3::15p13→15pter) = 1
CHROMOSOME FINDINGS IN 222 MR CASES
IN THE PRESENT STUDY

- Normal karyotype: 47%
- Free trisomy: 49%
- Trisomy with translocation variants: 2%
- Other chromosomal anomaly: 1%

Fig I
Table 2

Cytogenetic Findings in 64 parents of patients with free trisomy 21

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<th>A. Paternal Karyotypes</th>
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<td>46,X inv(Y)</td>
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<tr>
<td>46,XY,16qh+</td>
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</tbody>
</table>

| B. Maternal Karyotypes |                |                |
| Total number of individuals = 64 |                |                |
| 46,XX                   | = 58           |                |
| 46,XX,inv9qh            | = 1            |                |
| 46,XX/47,XX,+mar [98:2] | = 3            |                |
| 46,XX,15p+              | = 1            |                |
| 46,XX,22p+              | = 1            |                |
Table 3
Distribution of DS children according to the type of family and origin of non-disjunction

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Table 4: Number of satellite Associations in DS and in controls

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* association of 3 or more than 3 chromosomes
** P<0.001
NO. OF SATELLITE ASSOCIATIONS

Fig II

* P < 0.001
Fig III

TOTAL SATELLITE ASSOCIATIONS

NO. OF ASSOCIATIONS

SUBJECT NO.
Table 5

Distribution of different types of acrocentric chromosome association in DS and controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. of cells scores</th>
<th>Type of Association</th>
<th>Mean No. of satellite association per subject</th>
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<td>G-G</td>
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<td>106 (5.3)</td>
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<td>DS (20)</td>
<td>2000</td>
<td>264 (13.2)</td>
<td>160 (8)</td>
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Figures in parenthesis give the percentage associations in cells scored
* Association of 3 or more than 3 acrocentric chromosomes viz. DGG, DDG, DDGG, DDDG, GGGG.
Table 6
Number of Telomeric Associations in DS and in controls

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<th>TYPE OF TELOMERIC ASSOCIATION</th>
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<th>No. of cells scored</th>
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* P<0.001
Fig IV

NO. OF TELOMERIC ASSOCIATIONS

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<th>Type of Association</th>
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* P < 0.001
Fig V

TOTAL TELOMERIC ASSOCIATIONS

![Graph showing total telomeric associations for control and DS subjects.](image)
Table 7
Age of Control and DS patients at the time of the study

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<th>Age (Years)</th>
<th>Subject DS</th>
<th>Age (Years)</th>
</tr>
</thead>
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<td>DS1</td>
<td>13</td>
</tr>
<tr>
<td>C2</td>
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<td>DS2</td>
<td>7 months</td>
</tr>
<tr>
<td>C3</td>
<td>6</td>
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<td>6 months</td>
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<td>DS4</td>
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<td>DS5</td>
<td>1</td>
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<td>2 months</td>
</tr>
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<td>2</td>
<td>DS7</td>
<td>1</td>
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<tr>
<td>C8</td>
<td>2</td>
<td>DS8</td>
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<td>C9</td>
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<td>DS9</td>
<td>1</td>
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children which was significantly higher than the frequency for control children, 0.75% (P<0.001). The total pooled frequency of TTA observed in DS children (18.3%) was also significantly higher than in control children (5.05%) (P<0.001).

DISCUSSION

(A) Cytogenetic Investigations in 222 Mentally Retarded Patients

It is well known that chromosome imbalance, especially of autosomes, could cause complex phenotypes of multiple congenital anomalies (MCA) and mental retardation (MR). However, it is emphasized that chromosome abnormalities account for only a fraction of patients with MR and MCA (Tharapel and Summit, 1977). In some instances the conditions are the result of factors such as teratogenic agents, ionizing radiations or gene mutation. Some of these phenotypes are easily recognized on clinical grounds (example Down Syndrome), while others are not so easily recognized, either due to their rarity or wide spectrum of anomalies. The etiological factors involved in the remaining vast majority of such patients are unknown. Details of the chromosome findings in the 222 Mentally Retarded cases investigated in the present study are given in Table 1. Out of the 222 patients referred as MR, MCA or Down Syndrome for chromosomal analysis, majority were found to have Down Syndrome. 104 cases were found to have normal chromosome complement while two patients were observed to have 46,XX,dup16q?13→q?ter and 46,XX,der(18pter→18q21.3::15p13→15pter) respectively. These two cases are discussed in later part of this chapter. A male child referred for MR showed an inversion Y which was inherited from the father.
In a number of investigations it has been reported that trisomy 21 accounts for 95-96% of Down syndrome cases, 4-5% are due to translocation and about 1% are mosaics (Kola, 1989; Hamers et al., 1990). The frequency of free trisomy 21 was 96.5% and translocation was 3.5% in the present study while no case of mosaicism was observed. Hence, the observations are quite similar to the previous reports.

Four cases of Down Syndrome were observed with translocation, out of which, two cases of translocation were *de novo* in nature, while the other two translocations (Case 1 and 2) were maternal in origin. These two cases will be discussed later.

In the present study, six (2.7%) children were observed to have normal chromosome variants. Of the 138 male children referred, 3 (2.2%) were observed with inversion Y. Pericentric inversion of Y chromosome occurs in 1/1000 new born males (Friedrich and Nielsen, 1974). In comparison, the presently observed high frequency of inversion Y in 3(2.2%) of the total 138 male children investigated is quite interesting. All the 3 children had inherited the inverted Y from their fathers. Most of the males with inv(Y) reported in the literature are in cases of infertility, abnormal sexual development or mental retardation. Jacobs and Ross (1986) suggested that males with inv(Y) might have an increased risk of having sibs with 47,XXY or 45,X. However, it is not known whether inv(Y) might increase the risk of autosomal aneuploidy. Several cases of occurrence of pericentric inversions and other chromosomal aberrations have been reported (Kaiser, 1984; Borgaonkar, 1985). However, very limited cases of inv(Y) with trisomy 21 have been reported (Sparkes et al., 1970; Baheux-Morlier et al., 1970; Berger et al., 1973; Orye and Verhaaren, 1974; Borgaonkar, 1985; Krishnamurthy et al., 1989). The occurrence of inversion and aneuploidy in the same individual might suggest a possible interchromosomal effect.
as a predisposing factor for non-disjunction (Kaiser, 1984) which will be discussed later in this Chapter.

Two trisomy 21 cases were observed to have 22p+ and 15p+. The incidence of D and G variants has been reported to be significantly high in children suspected for chromosomal abnormalities (Krag-Olsen et al., 1980). However, the mechanism and the implications of these variants in non-disjunction has not been established unequivocally.

In the present study, the male:female sex ratio was quite high for trisomy 21 cases (2.22:1) as compared to 1.25:1 for the mentally retarded cases. Test of significance of male:female ratio in DS and MR showed that the ratio of males was significantly higher in DS. There have been several reports on a significant excess of males among individuals with an additional chromosome 21 (Nielsen et al., 1981), but such an excess has not been reported among translocation Down Syndrome individuals (Hassold et al., 1983). A significant number of males among abortuses trisomic for chromosome 21 but not for those involving other chromosomes were observed (Hassold et al., 1983), and they suggested that trisomy 21 has a higher sex ratio than other trisomies and also that the excess of males are likely to be present at conception which is not the result of selection.

Mikkelsen et al. (1990) and Hassold et al. (1984) observed a significant excess of males associated with non-disjunction at paternal meiosis I. Some, if not all, of the excess of males in trisomy 21 are attributed to non-disjunction at paternal meiosis I. Eiben et al. (1990) reported an excess of females in chromosomally normal abortions. In line with the above reports, it was observed that while maternal non-disjunction was not associated with a significant increase in sex ratio in the present study, a significant
excess of males were found to be associated with non-disjunction at paternal meiosis (detailed description is in Chapter III).

Some unique abnormalities detected in the present study are:

1) Case 1 : 46,XX,t(21;21),inv9qh

Case Report

The child was referred for cytogenetic confirmation of Down Syndrome. Detailed family history revealed 9 instances of spontaneous abortions, 1 neonatal death, a child with DS (proband) and 3 phenotypically as well as genotypically normal sisters.

The proband, a 11 year old male was born full term to healthy, normal, non-consanguinous parents. His early milestones were markedly delayed with standing at 7 years and walking at 8 years and mild hypotonia. On examination his height was 101 cm, head circumference 45 cm and weight 15 kg. No investigations were done previously. The clinical photograph of the proband is shown in Plate C, Figs.3 and 4. He had typical features of DS- mongoloid facies, epicanthic folds, upward slant of eyes, depressed nasal bridge, high arched palate, geographical tongue, hypoblastic teeth with caries, clinodactyly, unilateral simian crease in left palm as well as increased distance between the first and second toe. The child was prone to frequent eye, ear and respiratory tract infections.

The proband’s mother has a very poor reproductive history. Of the fourteen known pregnancies, nine ended in spontaneous abortions. Of the five live births, one died within a year after birth, the male child is afflicted with DS and the three females are normal aged 14, 8 and 7 years. The deceased male child reportedly had congenital
abnormalities and is suspected of having DS. The family history was otherwise negative with regard to congenital malformations, recurrent abortions or mental retardation.

Cytogenetics

The peripheral blood lymphocyte cultures of the proband showed 46,XY,t(21;21),inv9qh chromosome complement. A G-banded metaphase preparation showing t(21;21),inv9 and free chromosomes 21 is shown in Plate D, Fig.5. The pattern of inheritance in the proband showed the Robertsonian translocation with maternal transmission and inv9qh of paternal origin. The mother was mosaic with translocation t(21:21) in 2% cells and normal cell line in 98% cells of the peripheral blood. The karyotype of father is 46,XY,inv9qh. The lymphocyte cultures of the sisters of the proband revealed normal chromosome complement.

The translocation seen in the mother in this case could be considered to have originated de novo, as there was no familial history. In general, carriers of balanced Robertsonian translocations are phenotypically normal. The anomaly, however has been found to be associated with repeated abortions (Gracias Espinal et al., 1982). This observation may be attributed to the fact that the carriers of homologous Robertsonian translocations are unable to produce normal children since all the gametes should be either nullisomic or disomic for the chromosome involved in the translocation. In cases of t(13;13) and t(21;21), the trisomic zygotes occasionally survive to term and such carriers may therefore be ascertained both through their abnormal children and multiple abortions.

In the case of t(21;21), the offspring are affected and the risk of the carrier
parent having a child with DS may be 100%. The three normal sisters observed in this case are the result of mosaicism observed in the mother. The normal chromosome constitution in the girls could have arisen either from an oocyte with a normal chromosome constitution or by segregation of the normal chromosome 21 in an oocyte carrying the translocation. The mosaicism as observed in the mother could be due to post-zygotic structural rearrangement (Creau-Goldberg et al., 1987). The mother had t(21;21) in only 2% of the cell line while 98% comprised of normal chromosome complement in the peripheral lymphocyte cultures. The high number of abortions observed in this case do not correlate with only 2% of the cell line having the translocation. The large number of abortions suggest that her germ line consists largely of cells with the translocation. The chromosome studies of tissues other than peripheral blood could not be performed to detect percentage mosaicism in other tissues as well.

Wang and Hamerton (1979) and Serra et al. (1990) showed an increase of inv9qh among DS subject and postulated that the inversion predisposed the carrier parent to non-disjunction. Further, inv9qh is also observed in association with congenital abnormalities and reproductive loss (Boue et al., 1975). Hence, the large number of spontaneous abortions observed in the family may be linked to both the structural anomalies in the parents - Robertsonian translocation of chromosome 21 in mother and the inversion 9 observed in the father. The proband is unique in having both the translocations t(21;21) and inv9qh. To the best of our knowledge, this is the first report of a DS case with 21;21 Robertsonian translocation and inv9qh both of which are inherited from the parents.
2) Case 2 : 46,XY,+21;t(11;21)(11pter→11q13.1::21p12→21pter;
21qter→21p12::11q13.1→11qter).

Case Report

The proband was referred at 7 months for cytogenetic confirmation of Down Syndrome. The parents were 32 years and 28 years respectively, were healthy and non-consanguinous. The mother had a history of 3 first trimester abortions, 3 years old phenotypically normal girl and the proband. One paternal uncle of the mother is reported to be mentally retarded. On the paternal side, second brother of the father had severe mental retardation and expired at 2 years of age while the fifth brother was mildly retarded and was 28 years of age.

The proband was born 20 days premature by forcep delivery and cried after 2 days. The child suffered from physiological jaundice after 3 days of birth and has continuous respiratory tract infections since birth. Milestones are delayed with no head holding or turning over and he recognizes only his mother.

The clinical photograph of the proband is shown in Plate E, Fig.6. The proband shows typical features of Down Syndrome - mongoloid facies, upward slant of eyes, epicanthic folds, lowset ears, hypertelomerism, depressed nasal bridge, bilateral clinodactyly and simian crease, increased distance between first and second toe and hypotonia.

Cytogenetics

On the first glance it appeared that the proband was just another case of free trisomy 21. But detailed analysis by G-banding and NOR staining showed that it involved the translocation of chromosome 11 and 21 and was interpreted as:
47,XY,+21;t(11;21)(11pter→11q13.1::21p12→21pter;21qter→21p12::11q13.1→11qter). Plate F, Fig.7 shows the derived chromosomes 11 and 21 together with two chromosome 21s and normal 11 of the proband.

Subsequent cytogenetic analysis in parents revealed that the translocation was maternal in origin and the proband had inherited the der(11) and der(21) from the mother (Plate F, Fig.8a). The paternal karyotype was found to be normal.

Silver nitrate staining in the slides of both the proband and the mother showed the presence of NOR activity on both the chromosomes involved in translocation. Plate F, Fig.8c shows the NOR activity in both the derived chromosomes and their involvement in acrocentric associations.

Absence of familial incidence of abortions or mental retardation in the family suggests that the balanced translocation might have occurred de novo in the mother. Though cytogenetic studies could not be performed in the proband's sister, her normal phenotype and mental status suggests that either she is genotypically normal or has inherited the balanced translocation of her mother. Further, the abortions reported by the mother might have occurred due to chromosomal anomaly in those fetuses as a result of the translocation present in the mother. In view of the increased risk of having congenitally abnormal children, carriers of such balanced translocation should be advised to seek genetic counselling and go for prenatal diagnosis at pregnancy.

3) Case 3 : 46,XX,16q?13→q?ter

Full trisomy 16 is one of the trisomies most frequently observed in spontaneous abortions and is always lethal (Boue et al., 1975; Kajii et al., 1980). Although complete trisomy 16 is non-viable, there are reports of live born infants with
duplication of 16q. The majority of the cases have involved virtually the entire long arm of chromosome 16 and have been associated with dysmorphic features and limited post natal survival (Davison and Beasley, 1984; Nyhan, 1989).

The proband showed a 16q duplication involving the whole of q arm except the heterochromatin and occurred *de novo* in this case.

**Case Report**

The proband, the first child of healthy non-consanguinous parents was born full term following a pregnancy complicated by less amniotic fluid. There was no history of spontaneous abortions and the mother had not taken any drugs during the pregnancy and neither was she exposed to any radiations or infections. The mother was 34 years old and the father was 36 years old at the birth of the affected child.

The proband was referred at 9 months for delayed development and stiff neck. On examination, her weight was 5.6 kg and head circumference 41.5 cms.

Serum Thyroid hormones and TSH levels were normal. Her milestones were delayed with no head holding and sitting with support. The clinical photograph of the proband is shown in Plate G, Fig.9. The infant was noted to have coarse features, with eyes slanting downwards, lowset ears, hypertelorism, flat nose, microcephaly, tongue protruding often, short neck, hypertrichoses, asymmetric skull, mild hypotonia and simian crease in right hand. The child kept her neck stiff and had incomplete flexure of fingers.
Cytogenetics

Two hundred metaphases each were analysed for the proband and her parents. The cultures of the proband showed 46,XX,dup16q?13→q?ter chromosome complement, with 32% aneuploid cells. Heterochromatin region was not involved in the duplication and was confirmed by C-banding. Partial karyotype of normal and duplicated 16 observed in the proband is shown in Plate H, Fig.10a. The lymphocyte cultures of the parents revealed normal chromosome complement. The cells of mother had 26% aneuploid cells and chromatid/chromosomal gaps and breaks in 12% of the cells; while the cells of father showed 22% aneuploid cells and 4% cells with unidentified marker. Plate H, Figs.10b and 10c show the chromosomal aberrations and markers observed in maternal and paternal karyotypes respectively.

Houlston et al. (1994) reported that only 21 cases of infants with duplications involving 16q had been described till then. Of the reports which specified the trisomic region, six cases have involved complete duplication of the long arm (16p11→16qter), five cases of duplication of the long arm except for the proximal hetrochromatic region (q13→qter), three cases involving q21→ter and four cases with a duplication of q22→ter.

In previous reports it has been shown that although the patients with trisomy 16q have similar phenotypes, there are some clinical differences among them. In all these cases a number of other chromosomes have been involved with chromosome 16 in balanced translocations which was inherited from the parents including 9p, 11p, 11q, 15p, 13p, 18p, 18q, 20p, 21q, 22p, 22q, 15q. Although the phenotypic effects of partial trisomy 16q in these cases may be influenced by differing loss of genetic material due to secondary chromosome rearrangements, some trends do emerge.
(Houlston et al., 1994). Infants with the largest duplication have died shortly after birth. This reflects in part the severe internal malformations present when the long arm duplication of chromosome 16 is virtually complete. Houlston et al. (1994) suggested that duplication of 16q22→qter is a critical region for the facial features in partial trisomy 16q syndrome.

The duplication of 16q13→qter has been previously reported (Buckton and Barr, 1981; Davison and Beaslay, 1984). Some of the clinical features observed in the present study are similar to the above cases reported viz., failure to thrive, hypotonia, abnormal skull shape, antimongoloid slant, broad nasal bridge, lowset ears, micrognathia and simian crease. In all the previous cases, chromosome 16 was involved in translocation with another chromosome so that the phenotypic effects of partial trisomy 16q might be influenced by secondary chromosome rearrangements.

The present case is different as it does not involve any chromosomal rearrangements and the duplication occurred de novo in the patient which makes it the first report of such a case. It could be considered that the high number of aberrant cells in the lymphocyte cultures of parents were contributing factors for the chromosomal anomaly in the proband.

4) Case 4 : 46,XX,der(18pter→18q21.3::15p13→15pter)

Case Report

The child was referred at 10 months for chromosome analysis as a case of delayed development. The proband was born fullterm by breech delivery to non-consanguinous parents aged 35 years and 30 years. The proband was given oxygen after birth and was suspected to have central cyanosis. There was no history of
spontaneous abortions, mental retardation or any other congenital anomaly in both sides of the parents family.

The child had history of recurrent infections since birth with frequent cold and cough and constant temperature. The parents also reported frequent vomiting by the child. The milestones of the proband are delayed with head holding at 5 months and no turning over or sitting, though she can recognize a little. Physical examination revealed height as 64 cm, weight as 4.5 kg and head circumference as 38 cm. She had low birth weight (2.0 kg) and reportedly poor weight gain. The clinical photograph of the proband is shown in Plate I, Fig.12. The child had microcephaly, hypotonia, lowset ears, high arched palate and umbilical hernia.

Cytogenetics

Chromosome analysis of the proband revealed partial deletion of 18q and was interpreted as 46,XX,del(18)(q21.3). Subsequent chromosome analysis carried out in the parents showed that the abnormal chromosome was maternal in origin. The mother carried a balanced translocation 46,XX,t(15;18)(p13;q21.3) interpreted as - 46,XX,t(15;18)(18qter→18q21.3::15p13→15qter;18pter→18q21.3::15p13→15 pter).

Silver nitrate staining in the cultures of mother showed NOR activity in both the chromosomes involved in translocation, although greater activity was observed on the 18q arm than on the 15p, indicating that a large part of the NOR was translocated to the chromosome 18. The chromosome constitution of father was normal (46,XY), while the chromosome analysis in the probands phenotypically normal (5 years old) sister could not be carried out.

Subsequently silver nitrate staining revealed that the proband’s derived 18q also
had NOR activity. Thus, the chromosome constitution of the proband was interpreted as: 46,XX,der(18pter→18q21.3::15p13→15pter). Plate I, Figs. 13a and b shows partial karyotype of the chromosomes 15 and 18 in the proband and mother respectively. The silver nitrate preparations showing NOR activity and satellite associations in the terminal region of 18q is given in Plate I, Fig. 13c.

Partial deletion of the long arm of chromosome 18 was first described by de Grouchy et al. (1964). More than 80 cases are on record (de Grouchy and Turleau, 1991). It is responsible for a distinctive syndrome of facial dysmorphism and mental retardation. Some of the other common features are hypotonia, 'carp shaped' mouth and some ocular anomalies. Majority of the patients have IQ between 30 and 50. Reproduction in these patients is possible, with Subrt and Pokorny (1970) reporting a female patient with six pregnancies.

As the family history is otherwise negative for spontaneous abortions, mental retardation or congenital anomalies, the balanced translocation is thought to have occurred de novo in the mother. Her parents and sibs were not available for confirmation. Though cytogenetic analysis could not be carried out in the proband’s sister, her normal intelligence and phenotype suggests that either she has normal chromosome complement or she has inherited the balanced rearrangement of her mother.

(B) Cytogenetic Findings in Parents of Free Trisomy 21

During the last 20 years attention has frequently been drawn to the association between pericentric inversions and human pathology. Structural aberrations involving chromosomes other than 21 have been observed in Down Syndrome families
(Schinzel, 1984). Investigators who have observed such associations have suggested some mechanistic connection, and the term "interchromosomal effect" has been applied to such a hypothetical interaction which was first proposed by Lejeune (1963). "Interchromosomal effect" implies an interactive disturbance of meiosis, whereby a structural chromosome rearrangement disturbs the normal distribution of the chromosome pairs which are not involved directly in the abnormality and results in aneuploidy or unbalanced gametes and offspring. Such an effect has been observed in Drosophila and in mouse translocations (Ford, 1973). On the basis of his own observations in a series of patients with Down Syndrome, Lejeune (1963) suspected that more patients than would be expected by chance had a balanced familial rearrangement in addition to an extra chromosome 21. He suggested that, parallel to observations in Drosophila, "autosomal rearrangements could increase the probability of abnormal segregation of the sex chromosomes which are themselves not involved in the structural change", and he expanded the hypothesis to autosomes. On the basis of further studies on Down Syndrome cases, this "Interchromosomal effect" has been emphasized by other authors.

Chromosome rearrangements that were unrelated to chromosome 21 were frequently observed in parental karyotypes by several investigators. Inversion 9qh has been reported to occur at a frequency of approximately 1% of normal population (Kaiser, 1980). In the present study, the frequency of families where one parent is heterozygous for inv9qh is 9.4%. If 128 subjects rather than 64 couples are considered, then the frequency of inv9qh heterozygotes becomes 4.7%. On statistical grounds the association can be said to exist if the observed frequency of inv9qh carrier subjects, among the parents of Down Syndrome subjects is significantly higher than in an
unselected sample of normal population.

Serra et al. (1990) compiled the published work on new borns and estimated the frequency prevalence of inv9qh to be 0.95 ± 0.07% (179 in 18,884 individuals). The Chi-Square test for the significance of incidence of inv9qh heterozygotes among parents of DS subjects (6:128) in the present study and that compiled by Serra et al. (1990) (179:18,884) showed that the value is significantly (P<0.001) higher for parents of DS.

Pericentric inversion of the Y chromosome occurs in 1/1000 new born males (Friedrich and Nielsen, 1974). In the present study, out of 64 paternal karyotypes two were observed to have inv(Y) which is significantly higher than in the control population.

Variant 15p+ and 22p+ were observed in one trisomic child each and their respective mothers and is of interest from the point of view of etiology and the significance of inherited variants in causing non-disjunction.

Overall 14 (21.9%) families showed polymorphism where one of the parents was heterozygous for a variant. If 128 subjects are considered instead of 64 couples, then this frequency becomes 10.9%. Chromosome interaction is the simplest hypothesis that could explain the association between the presence of chromosome variants in parents and the birth of a trisomy 21 child. In this case, it would be expected that in DS children, two chromosomes 21 derive from the variant heterozygous parent of a given sex with a frequency higher than that estimated for chromosomally normal parents. Therefore, the parental origin of the extra chromosome 21 was done using cytogenetic heteromorphism of chromosome 21. Details for parental origin studies are given in Chapter III. Out of the total 14 families where one
of the parents was heterozygous for a variant, origin could be traced in 10 informative families, while 4 families were non-informative.

The variant when present in the father, paternal non-disjunction accounted for 40% of the informative cases which is higher than that observed for total cases (16.2%). The variant when present in the mother, origin could be determined in 5 families and maternal error accounted for the extra chromosome in all the 5 families (100%), while for the total cases, maternal error was observed in only 83.8% cases (Chapter III). Thus, non-disjunction occurred in the carrier parent in 7 of the 10 informative families.

In the present study, non-disjunction leading to trisomy 21 occurred in about 85% cases in maternal meiosis (Table 10, Chapter III), so it could be said that the majority of trisomy 21 patients where the mother was a carrier for variant, would nevertheless, have inherited their additional chromosome 21 from the mother. Thus, it would be difficult to ascertain interchromosomal effect in female meiosis. By contrast, when the variant is present in the father, the paternally inherited extra chromosome 21 would make it relatively easy to confirm or to exclude such an effect. As about 16% of trisomy 21 cases had derived their extra chromosome 21 from father in the present study (Table 10), repeated paternal inheritance of the extra chromosome would be a strong indication of such an effect. The results of the present investigation show that the extra chromosome 21 was derived from the father in 40% of the cases when the father was a carrier as compared to 16.2% for the total cases. An interchromosomal effect during paternal meiosis is likely to be responsible for the trisomy 21 in these instances. Thus, the observations made in the present investigation indicate an interchromosomal effect.
According to Chandley et al. (1987), ascertainment of pericentric inversions in man in general is made by the birth of a child with congenital malformations or spontaneous abortions resulting from a duplication/deletion product from the carrier parent. There have been reports that inversions and variants might be associated with a tendency for spontaneous abortion (Boue et al., 1975; Simpson et al., 1993; Osztovics et al., 1982; Stetten and Rock, 1983). Considering the frequency of spontaneous abortions, 27.5% pregnancies ended as spontaneous abortion for the 14 families where one of the parents is heterozygous for a variant, as compared to 7.41% in controls and 17.11% for the total free trisomy 21 families analysed in the present study (details are given in Chapter V). The observed high frequency of spontaneous abortions in the couples where one of the parents is heterozygous for a variant is in agreement with the hypothesis that it may have a harmful effect on reproduction. It is also suggested that the chromosomal rearrangements might probably have some interchromosomal effects predisposing non-disjunction, resulting in aneuploid offspring which are aborted during the previous pregnancies and are followed by the subsequent birth of a Down Syndrome child.

After the first suggestion by Lejeune (1963) that interchromosomal effect may be present in man, a number of investigations have led to conflicting results. Several individuals have been identified with trisomy 21 and sex chromosome aneuploidy who had a parent with structural rearrangements unrelated to chromosome 21 or sex chromosomes. Mikkelsen (1971) estimated that women carrying Robertsonian translocations between D group chromosomes had a significantly higher risk (of about 2%) of having a child with Down Syndrome. Aurias et al. (1978) have suggested that the meiotic segregation of chromosomes such as 21 may be influenced by balanced
structural rearrangements following the hypothesis of Lejeune (1963). Stoll et al. (1978) have also suggested the existence of an interchromosomal effect between non-disjunction and balanced translocations.

A possible relationship between inversion and non-disjunction has been postulated (Hernandez et al., 1979; Kaiser, 1984). There are also reports that autosomal inversions (1,9,16) are found to be relatively more frequent among parents of Down Syndrome as compared to the general population (Borgaonkar, 1985). Lindenbaum et al. (1985) reported that there was a considerable rise in the frequency of reciprocal translocations in the parents of children with regular trisomy 21 over the new born population and that this could reflect a real interchromosomal effect in man. Couzen et al. (1987) observed 10 times the frequency of variants in parents of trisomy 21 than new born population and supported the existence of interchromosomal effect. A possible association between inv9qh in the parents and trisomy 21 in the offspring has also been suggested by Serra et al. (1980; 1990) and Neri et al. (1981). In contrast there have been other reports which did not show any association of inv9qh with pathological conditions (Carothers et al., 1982; Hsu et al., 1987).

By means of human sperm-hamster oocyte fusion, many authors have analyzed sperm chromosomes from individuals with rearrangements, including reciprocal translocations. Some have reported no increase in hyperploid sperm in these carriers as compared with karyotypically normal males (Martin et al., 1990) suggesting no indication of an interchromosomal effect as regards segregation. Burns et al. (1986), on the other hand, reported remarkable interchromosomal effects in a male heterozygous for two reciprocal translocations suggesting that when the proportion of chromosomes related to the rearrangements become larger, they may affect meiotic
disjunctions of other chromosome pairs more efficiently and induce more remarkable interchromosomal effects.

An interchromosomal effect has been suggested but not proven yet, as one mechanism able to explain the observed increase of reciprocal translocations or other structural rearrangement among the parents of DS individuals (Lindenbaum et al., 1985; Couzen et al., 1987). The presence of the aberrant chromosome could at meiosis, upset the disjunction or distribution of other chromosomes not directly involved in the rearrangement because of asynapsis or precocious desynapsis (Hulten et al. 1987).

A number of investigators have reported (Natarajan and Ahnstrom, 1969; Natarajan and Gropp, 1971; Gagne et al., 1974; Mattei et al., 1974; Schmid et al., 1976; Stahl et al., 1975,1976,1983; Miklos and Nankivell, 1976; Driscoll et al., 1979; Miklos and John, 1979; Mirre et al., 1980; Stahl and Hartung, 1981; Hulten et al., 1987; Julian et al., 1987) that:

1. The centromeric regions of acrocentric chromosomes are frequently associated with the heterochromatic regions of chromosome 1,16 and especially 9, where the satellite DNA III is highly represented as in the centromeric regions of D and G chromosomes;

2. The nucleolus associated chromatin contains heterochromatin from both the small arms of D and G chromosomes and the secondary constrictions of chromosome 1 and 9;

3. Heterosynapsis between non-homologous heterochromatin regions can occur at meiosis in man, as it occurs in mice and Drosophila, followed by a general disturbance in pairing and distribution;
1qh, 9qh and 16qh regions and the p arms of chromosomes, 13, 14, 15 and 21, 22 in normal as well as in structurally abnormal conditions, are often delayed in synapsis, followed sometimes by complete asynapsis of the homologues and possibly heterosynapsis with disturbing effects upon disjunction and chromosome distribution;

the pericentric inversion of chromosome 9 increases the likelihood of mitotic non-disjunction (Ford and Lester, 1978).

Although the knowledge of function and correlated mechanisms of heterochromatin, especially during meiosis, remains a challenge, the reported facts point to the existence of interchromosomal effect bound to the presence of heterochromatic blocks both in normal and abnormal conditions, a greater effect being reasonably expected in the abnormal ones. Yet like many other associations, the underlying mechanism remains obscure. The causes of aneuploidy in general, are also still unknown particularly in humans, eventhough a large body of experimental data has been collected in various animal species (Bond and Chandley, 1983).

Moorhead (1976) stressed that the "understanding of the significance of specialized regions such as 1qh, 9qh and 16qh would have important implications for clinical cytogenetics. One is somewhat uncomfortable in assigning no significance for fetal development to the presence of a 'variant chromosome'".

However, the figures for prevalence of inversions and balanced rearrangements in the general population are also crucial. It may yet prove that the currently accepted figures considerably underestimate this incidence.

The higher frequency of inversions and variants observed in the parents of the DS families of the present study, support an association between chromosome

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rearrangements and trisomy 21. Further, taking into account the data of parental origin of extra chromosome 21 and spontaneous abortion analysis in these families, the interchromosomal effect is suggested. However, further studies are needed on the origin of non-disjunction of chromosome 21 in the families where one parent is carrier for inversion or chromosomal rearrangement in a large population.

(C) Analysis of Acrocentric Chromosome Associations in Down Syndrome

The presence of nucleolar organizing regions on the short arms of all human acrocentric chromosomes and their intimate association in nucleoli has led to frequent speculation that NORs may be important in non-disjunction involving acrocentric chromosomes. Polani et al. (1960) suggested that nucleolar persistence might interfere with the normal pairing process, which ultimately leads to random assortment of univalents at meiosis I. They further suggested that this could be the basis for the maternal age effect in trisomy 21, as nucleoli of older oocytes might be more resistant to the normal breakdown process than nucleoli of younger oocytes.

Subsequently, large number of investigations were carried out by enlarging upon the initial hypothesis by Polani et al. (1960). Satellite association between the human acrocentric chromosomes have received much attention as a possible factor in non-disjunction and some of the first few reports was by Ohno et al. (1961). It was said that satellite associations and fusions of nucleoli are different manifestations of the same phenomenon and if "sticky" material from the large fused nucleoli persists through cell division, non-disjunction may result.

Ferguson-Smith (1964) has shown in meiotic preparations that the five acrocentric bivalents can be associated with nucleoli, and he suggested that this
phenomenon corresponds to the mitotic satellite associations, so that the satellite association reflects the participation of chromosomes in the organization of nucleolus. Also, the connectives between the satellite regions, which can sometimes be observed in stained mitotic preparations (Zang and Back, 1968) represents the remnants of nucleolus. Furthermore, Henderson et al. (1973), by means of hybridization of $^{3}$H-labelled rRNA to the human chromosomes, demonstrated the presence of rDNA connectives between the satellite regions of chromosomes involved in satellite associations. These connectives are occasionally visible in light microscopy as thin fibres. Moreover, Henderson also found grains arranged between satellite regions even when the connectives were not visible, which indicated the presence of invisible labelled strands.

A number of reports suggested that satellite associations involving acrocentric chromosomes, and specifically associations involving chromosome 21, are more frequently observed in parents of DS patients than in controls (Hansson and Mikkelsen, 1974; Mattei et al., 1974). These reports lend support to the idea that satellite associations play a significant role in non-disjunctional events leading to DS.

Hansson and Mikkelsen (1978) analysed both the parental origin of the additional chromosome 21 and satellite association in 72 families with DS. They observed a highly significant increase in satellite association involving chromosome 21 in the parent in which the non-disjunction was known to have occurred. They also found a significantly increased association frequency for certain chromosomes including chromosome 21, in the parent in which the non-disjunction had not occurred. The above authors suggested that satellite association may play a role in the etiology of non-disjunction. However, there are also a number of contradictory reports in which
increase in satellite associations has not been found in parents of DS children (Cooke and Curtis, 1974).

Mirre et al., (1980) proposed that NOR-related non-disjunction derives from an error in chromosome pairing or separation due to physical proximity of acrocentric chromosomes in nucleoli. Schmickel (1985) suggested that recombination errors involving the short arms of non-homologous acrocentric chromosomes could give rise to non-disjunction. Although Green et al. (1989) demonstrated significant difference in number of chromosome association per cell, mean NOR per cell and average NOR size for parents of DS than controls, they still indicated that the role of NORs in the mechanism of non-disjunction remains unclear.

In the present study, the acrocentric chromosome association was observed to be significantly higher in DS patients as compared to controls. The results are in accordance with the previous reports suggesting relationship between satellite association and non-disjunctional event (Hansson and Mikkelsen, 1974; Mattei et al., 1974).

Jackson-Cook et al. (1985) presented data suggesting a strong association between a specific variant of the nucleolar organizing region and trisomy 21. They observed a significantly increased frequency of double nucleolar organizing regions (dNORs) on any of the acrocentric chromosomes among parents of trisomy 21 individuals. They further reported that the families where parental origin of the extra chromosome could be determined, the non-disjunction had occurred in the parent carrying dNOR. Thus, they concluded that dNORs were important in the etiology of trisomy 21, and suggested that the presence of this variant might increase the risk of having a DS child by as much as 20 fold. Further, Underwood and Giri (1988)
indicated that the NOR might be a diagnostic discriminator of malignancy. Jones et al. (1988) have implicated the dNOR in non-disjunction of sex chromosomes as well.

However, these initial reports on dNOR were not supported by several subsequent studies. Hassold et al. (1987) studied parents of spontaneously aborted trisomic fetuses and chromosomally normal fetuses. Among the 150 individuals in their study, they did not observe a single dNOR in both the cases and the controls.

The relationship of the dNOR variant to non-disjunction was again questioned by Spinner et al. (1989). The dNOR frequencies observed were 12% for parents of DS and 14% in the controls and so concluded that dNOR do not correlate with a tendency for non-disjunction events of the acrocentric chromosomes. Similar conclusion was also reached by Schwartz et al. (1989), and Serra and Bova (1990) that a dNOR carrier has no increased risk of having a DS offspring.

In the present study also, not a single dNOR was observed in 25 DS children and 25 age matched controls. Thus, the observations made are contrary to the observations made by Jackson-Cook et al. (1985). The results are similar to those of Spinner et al. (1989) and Serra and Bova (1990) and it is concluded that no association appears to exist between the presence of dNOR and non-disjunction.

The acrocentric short arms have been considered to be redundant, since they contain highly reiterated DNA sequences and because persons with balanced Robertsonian translocations have no phenotypic abnormalities (Schmickel et al., 1985). Further, the above authors also suggested that the dNORs most likely arise from unequal homologous recombination within the ribosomal genes. Thus, there is no proven relationship between dNORs and any disease or phenotype.

From the observations made in the present study, it could be found that an
increased acrocentric satellite association (and not dNORs) can be related with a non-disjunctional event.

(D) Telomeric Associations in Down Syndrome

It has been established that certain chromosomal defects are consistently associated with some types of human cancer (Yunis, 1983). In 1930, the association between DS and Leukaemia was first described (Brewster and Cannon, 1930). Later the association between trisomy 21 and leukaemic states has been confirmed and expanded (Robinson et al., 1984; Fong and Brodeur, 1987). Further, not only do children and adults with DS have a 20-50 fold increased risk of leukaemia (both lymphoblastic and myeloblastic), but 19% of phenotypically normal children with leukaemia have an extra chromosome 21 in their blast cells (in association with aneuploidy and hyperdiploidy) (Rowley, 1981). Mittelman et al. (1990) reported that trisomy 21 is one of the five most frequent numerical abnormalities seen as the sole anomaly in acute myeloid leukaemia (AML), myeloproliferative disorders (MPD), myelodysplastic syndromes (MDS) and acute lymphocytic leukaemia (ALL). In contrast, they did not find +21 as the only change in any solid tumour.

The presence of a trisomy 21 line has been associated with a transient leukaemoid reaction occurring during the new born period (Weinstein, 1978). Acute lymphoblastic leukaemia is the type of leukaemia most commonly seen in DS patients (Peeters and Poon, 1987). Children with DS can manifest extensive defects of bone marrow function including aplastic anaemia, myelofibrosis with myeloid metaplasia, congenital leukaemoid reactions, preleukaemia and transient leukaemias (Peeters and Poon, 1987).
The ends of chromosomes were called telomeres by Müller nearly 60 years ago (Müller, 1938). He coined the word from the Greek telos for "end" and meros for "part". Analyses of telomere length as a function of age, either in cells from people of different ages, or as a function of cell division number in primary cultures of human fibroblasts, and in certain cancerous cells, show that mean telomere length gradually decreases with increased age or number of cell divisions (Hastie et al., 1990).

Natural chromosome ends do not exhibit any tendency to fuse together spontaneously, and differ in this way from artificial chromosome ends caused by chromosome breakage. Such artificial ends show a strong capacity for re-fusion and behave as if they were sticky (Fitzgerald and Morris, 1984). The chromosomes of man can be found to be joined by the association of the normal ends of two chromosomes with minimal or no loss of material from either chromosome end. Such anomalous rearrangements of telomeres has been referred to as: telomere fusion (Dutrilaux et al., 1978), or terminal chromosome attachment (Novitski et al., 1981), or telomeric association (Fitzgerald and Morris, 1984).

Telomeric association has been reported in metaphase cells of abnormal lymphocytes of patients with the rare inherited disorder ataxia telangiectasia (Oxford et al., 1975; Taylor et al., 1981), in cultured epidermal fibroblasts and kidney cells of normal origin but subsequently infected with SV40 virus (Wolman et al., 1980), and in heteroploid or senescent fibroblasts (Benn, 1976).

Fitzgerald and Morris (1984) reported two cases of B-Cell leukaemia, each of which had different types of telomeric involvement. They observed apparent translocations in about 20% of leukaemic bone marrow cells from each of the two
patients which they reported to be the result of telomeric association. The above authors suggested that telomere association may be considered as a potential origin of new stable cytogenetic combinations that have a role in oncogene transposition and tumour biology.

Morgan et al. (1986) studied telomeric association in a patient with rapidly progressive pre-T-cell acute lymphoblastic leukaemia and from their findings suggested that telomeric association may function as a mechanism for the development of chromosome rearrangements that may play a role in human neoplasia. Pathak et al. (1988) analysed telomeric associations in two morphologically distinct squamous-cell carcinoma lines and proposed that telomere associations are characteristic of cancer cells. Telomere associations have also been reported in leukaemic cells undergoing apoptosis (Pathak et al., 1994a; 1994b). The tendency to form telomeric associations has been described by other authors as well, in a variety of leukaemias (Raimondi et al., 1987; Saltman et al., 1989), in solid tumours and meningomas (Aledo et al., 1988; Vagner-Capodeno et al., 1992).

Multani et al. (1997) reported an increased telomeric associations in a female with primary amenorrhea and suggested that this may increase her risk for predisposition to certain types of cancer. Dhaliwal et al. (1994) reported a high frequency of telomeric associations in a family with multiple congenital neoplasia. The above authors found 23.3% of metaphases showing telomere-telomere associations involving single and double chromatids in the father's blood, while such associations were not observed in the metaphases of the mother. They speculated that the genotype of father may be responsible for the congenital malignancies in their twin daughters.

It has also been demonstrated that telomeric association is one mechanism that
can initiate chromosome instability by generating subclones with unstable chromosome intermediates and result in ring chromosomes and subsequent chromosome loss (Sawyer et al., 1996). Mondello et al. (1995) showed that a telomeric association leads to a variety of balanced and unbalanced chromosome rearrangements, which may result from asymmetric interchanges between sister chromatids, ‘bridge-breakage-fusion’ events during cell cycle division, breakage and reunion of isochromatids, and breakage followed by healing of ends.

Several lines of evidence suggest that telomere shortening plays a causal role in cellular aging. It has been shown that, in human fibroblasts, telomeres shorten as a function of cell doublings *in vitro* and *in vivo* and that initial telomere length predicts the replicative capacity of these cells (Harley et al., 1990). Loss of telomeric DNA during ageing *in vivo* has also been observed in peripheral blood cells and colon mucosa epithelia (Hastie et al., 1990). Telomere shortening in normal (non immortalized) human cell strains is associated with the inability to detect telomerase in extracts from these cells (Counter et al., 1992). In contrast, immortal cells express telomerase, and their telomeres do not progressively shorten (Counter et al., 1992).

The sudden increase in the number of dicentric chromosomes in senescent fibroblasts (Saksela and Moorhead, 1963; Benn, 1976) and the significant age related increase of these abnormalities in human peripheral blood lymphocytes (Bender et al., 1989) suggest that the integrity of chromosome ends is compromised during cellular ageing. These observations were the basis of the telomere hypothesis of cellular ageing and immortalization (Harley, 1991).

Aging of the immune system could account for some of the morbidity of elderly individuals and DS patients (Vaziri et al., 1993). The above authors analysed
the loss of telomeric DNA during ageing of normal and trisomy 21 human lymphocytes and reported terminal repeat loss during ageing of human lymphocytes in vivo and in vitro. They further showed a significantly higher rate of loss in age-matched DS patients. They proposed that since DS is characterized by immune dysfunction, including thymus abnormalities, derangements of both lymphoid and myeloid cell compartments, premature T-cell ageing. The accelerated loss of telomeres in peripheral blood lymphocytes (PBLs) could reflect a generalized early senescence of immune cells in these individuals. They also indicated that ageing of the lymphoid and myeloid lineages is characterized by similar rates of telomeric DNA loss.

Vaziri et al. (1993) speculated that the increased rate of telomere loss in PBLs from DS patients could reflect a higher turnover rate of these cells in vivo. The possible explanation for the above is that either the immune cells in DS individuals could have reduced viability or abnormalities in maturation due to trisomy 21, which will lead directly to increased cell turnover, or the amount of telomere loss in PBLs from DS patients is greater per cell doubling than that in normal individuals. It has been demonstrated in yeast, that an altered expression of some genes results in abnormal regulation of telomere length (Lundblad and Szostak, 1989). Similarly, if the expression of genes involved in telomere length regulation are altered due to trisomy 21, then the rate of telomere loss could increase in DS (Vaziri et al., 1993).

The previous reports show that some of the manifestations of DS include predisposition to leukaemia and signs of precocious ageing and that DS ranks first among human "segmental progeroid syndromes", defined as those genetic disorders in which multiple major aspects of the senescent phenotype appear prematurely (Franceschi et al., 1986). Thus, DS can be considered a model of accelerated ageing.
Further, a reduction of life span of cultured cells from DS patients has also been reported (Segal and McCoy, 1974).

From the above description it is clear, that DS patients are predisposed to certain malignancies and precocious ageing and that a variety of cancer cells have a tendency to form telomeric associations. As mentioned earlier, Vaziri et al. (1993) have reported a significantly higher telomeric loss in age matched DS patients. Lymphocytes of young DS patients and old normal individuals have been reported to share several characteristics, including diminished response of T cells to activate and proliferate in response to antigen, low replicative capacity, and reduced B- and T-cell counts (Franceschi et al., 1991). Thus, it would be interesting to analyse telomeric associations in DS patients and compare them with age matched controls. In the present study, both the single and double strand telomeric associations were observed. The proportional involvement of the single and double chromatid in the associations was similar in both the subject groups and mostly involved single chromatid. The total frequency of telomere-to-telomere association was observed to be increased for DS patients as compared to the controls and the values were highly significant.

Moreover, the participation of chromosomes in the telomeric associations was of random manner and practically all the chromosomes were involved. This random involvement of chromosomes in telomere associations is similar to the previous reports in B-cell lymphoid leukaemia (Fitzgerald and Morris, 1984) and in pre-T-cell acute lymphoblastic leukaemia (Morgan et al., 1986). In two morphologically distinct squamous-cell carcinoma cell lines, Pathak et al. (1988) observed semirandom involvement of chromosomes in telomeric association where they observed chromosome 2,3,5 and 16 to be more frequently associated than the other
chromosomes.

The random involvement of chromosomes in association in the present study suggests the ability of each chromosome to fuse with any other. It has also been postulated that this randomness of fusion may serve as a potential origin of new cytogenetic combinations that may have a role in tumour etiology (Fitzgerald and Morris, 1984). Previous studies show that under certain circumstances the telomere-telomere association may not produce untoward phenotypic effects, whereas, telomeric associations with ends resulting from chromosome breakage, may have consequences on the biology of the affected cells (Morgan et al., 1986).

Telomeric associations can result into dicentric chromosome (Morgan et al., 1986). The inactivation of one of the two centromeres may occur, which will preserve both centromeres, one as functional and the other latent in the resultant mitotically stable chromosome (Hsu et al., 1975). Evolution of the human chromosome 2 has been believed to have occurred by this manner from two acrocentric chromosomes present in the karyotype of other primates (Lejeune et al., 1973).

The occurrence of the telomeric associations of chromosomes with minimal or no loss of DNA material have been explained in different ways by a number of features characteristic of telomere structure and function. It was previously known that telomeres of eukaryotic chromosomes contain repeated base sequences particularly (CA)$_n$ sequences (Szostak and Blackburn, 1982) and that such base homology could be the basis of telomere pairing. Dancis and Holmquist (1977) have shown that telomere-telomere fusions are normal part of chromosome replication and they suggested that during normal chromosome division, transient telomere-telomere fusion occurs before replication, followed by a subsequent dissociation which is required for
the free telomeric ends to maintain chromosome structure. Telomeric associations observed in metaphase chromosome possibly reflect the persistence of these replication intermediates because of faults in the processes of fission although renewed fusion can also take place (Holmquist and Dancis, 1979). Such associations may occur in certain human syndromes, virus infected cells, ageing cultures and cancer cells (Fitzgerald and Morris, 1984; Morgan et al., 1986; Pathak et al., 1988). The possibility of telomeric "stickiness" caused by a substance or substances produced in these abnormal cells that have lost normal cell properties has also been suggested (Pathak et al., 1988). However, since then, the mechanism of telomere replication has been explained by the discovery of telomerase (Harley, 1991). Hastie et al. (1990) reported that telomeric association might be a result of the loss of long stretches of G-rich repeats found at the ends of chromosomes or of a telomerase defect. Broccoli and Cooke (1993) have suggested that telomeric loss may result in fusion of non telomeric ends which will lead to chromosome instability. Further, Harley (1991) in his theory proposed that telomere loss reflects the replicative history of normal somatic cells and contributes to chromosomal abnormalities in cell aging and transformation. The report of Vaziri et al. (1993) that the cells from DS patients have a significantly higher rate of telomere loss compared with age matched controls support the observation of increased telomeric associations in the present study. Thus it could be assumed that an increased telomeric loss in the cells of DS patients is the underlying mechanism responsible for these chromosomal rearrangements.

According to Kovacs et al. (1987) telomeric associations only reflect a genetic instability, while others (Pathak et al, 1988) reported that the presence of telomeric associations in a wide variety of tumors suggests that this phenomenon is a
characteristic of malignant cells. Fitzgerald and Morris (1984) and Morgan et al. 
(1986) suggested that telomeric fusion may function as a mechanism for the 
development of chromosome rearrangements that may play a role in human neoplasia.

The effects of telomeric association on cells of DS patients are not yet known. 
Increased telomeric associations have been reported in leukaemia (Fitzgerald and 
Morris, 1984; Morgan et al., 1986; Raimondi et al., 1987; Saltman et al., 1989) and 
in other human malignancies (Pathak et al., 1988; Aledo et al., 1988). The presence 
of telomeric associations in cells from patients with ataxia telangiectasia should be 
emphasized because this lymphoid disorder has a high incidence of transformation to 
malignant diseases (Harnden, 1974). Thus, it is suggested that the significant increase 
of telomeric associations present in the DS children compared to age matched controls 
seem to increase their risk for predisposition to leukaemia and precocious ageing. It 
is further added that the lack of reports of telomeric associations in DS patients may 
lie in the non-recognition rather than absence of this phenomenon.

The increased telomere to telomere associations observed in the present study 
is in accordance with the previous reports of a possible relationship between these 
chromosomal rearrangements and malignant transformation. The follow-up of these 
cases with telomeric associations will help to determine whether these arrangements 
might play a causal role in predisposition to leukaemia and precocious ageing as 
suggested in the present study. If such a relation is confirmed to exist by further 
studies and subsequent follow-ups, it could provide an excellent tool for genetic 
counselling and for the better management of DS children. Furthermore, DS cells may 
provide a sensitive model to study the sequential changes in the carcinogenic processes 
and ageing.
The detection of telomere loss has only become possible with the molecular characterization of human telomeres. As the understanding of chromosome structure advances, it is likely that many more such abnormalities will be discovered in the future. With the increased understanding of telomere structure and function, it is becoming clear that this sequence plays a larger role in contributing to genetic load than was previously suspected (Broccoli and Cooke, 1993).

To summarize, it is suggested that the data on the telomere association in the DS children indicates that these individuals have a high risk for predisposition to cancer and premature ageing. However, further studies are needed to confirm the magnitude to which an increased telomeric association could be used to assess the risk of predisposition to cancer.
It is not the fruits of scientific research that elevate a man & his nature, but the urge to understand.

- Albert Einstein