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2. REVIEW OF LITERATURE

Down syndrome (DS) or trisomy 21 has always been of special interest to the human geneticists and is one of the most intensively studied human aneuploidies. It is the most common single genetic cause for intellectual disabilities in childhood, the most prevalent syndrome with an unknown etiological factor and a postnatally viable chromosomal abnormality with profound effects on development resulting in a constellation of phenotypes. An association of its increased incidence with advanced maternal age provided an interesting paradigm for the investigation of chromosomal anomalies and was an early milestone in the history of screening for Down syndrome. With the introduction of cytogenetic methodologies, the chromosomal basis for this condition was identified to be an extra chromosome 21, resulting mainly from chromosomal nondisjunction during meiosis (Sherman et al., 2005).

The first distinctive description of this condition was provided by Sir John Langdon Down in 1866 (Appendix 1). Edouard Onesimus Seguin had also in 1866 described persons with Down syndrome, but as having "furfuraceous cretinism". By 1877 Ireland clearly delineated the "Mongolian idiot" from the "cretinoid idiot". The first illustrations in the medical literature of the face, foot, and skull of an individual with Down syndrome appeared in 1876 in a classic paper by Fraser and Mitchell. These authors also published a pathologic description of the brain at autopsy. The term "Mongolian idiocy" or "Mongolism" was widely used throughout the latter part of the 19th and
first half of the 20th century. Waardenburg in 1932 and Bleyer in 1934 suggested chromosome anomaly and genetic mutation respectively resulting in trisomy to be the basis of this condition. However, only in 1959 could Lejeune, Gautier and Turpin cytogenetically demonstrate an additional chromosome 21 to be the cause for Down syndrome (Lejeune et al., 1959). Sparked by the discovery of trisomy 21 in persons with Down syndrome as well as by complaints from Chinese and Japanese scientific investigators, the term "Down syndrome" or "trisomy 21" was proposed to replace the term "Mongolism". Since then enormous amount of literature has accumulated describing various aspects of this common disorder. The knowledge of the complete DNA sequence of human chromosome 21 (long arm) in 2000 (Hattori, 2000) has further revolutionized our genetic understanding of this condition. There are over 18,305 articles available as on date (Pubmed) and therefore, an attempt has been made to briefly review the relevant information in this chapter.

2.1 PREVALENCE

Trisomies of chromosomes 21, 18 or 13 are consistently detected with live birth incidence of 1.23, 0.15 and 0.046 per 1000 respectively (Fitzpatrick et al., 2002). Trisomy 21 is 13 times as frequent as trisomy 18, 26 times as frequent as trisomy 13 and 3 times as frequent as any of the sex chromosomal trisomies (Millington-Ward and Pearson, 1988). Autosomal trisomy has been reported in approximately 4% of clinically recognized pregnancies. In particular, trisomy 21 accounts for about 0.1% of live births and 0.5% of all conceptions (Hassold and Jacobs, 1984; Rajangam, 2000). Viability of
trisomy 21 in embryonic and in early fetal life is 25% and in mid trimester 34% (Rajangam, 2000).

There is a wide variation in the incidence of DS reported from various parts of the world. The global incidence of DS is stated to be 1 to 1.67 in 1000 live borns (Epstein, 1995; Christianson, 1996; Antonarakis et al., 2001; Reeves et al., 2001; Yamaki et al., 2001; Chango et al., 2005) and 1 in 150 conceptions (Hernandez and Fisher, 1996). Recently the population-based large data set of the Hungarian Congenital Abnormality Registry showed an increase in the recorded total (birth + fetal) prevalence rate of informative offspring with Down syndrome. The previous birth prevalence of 1.17 per 1000 in the 1970s increased to 1.50 per 1000 between 1989 and 1999 with a maximum 1.77 in 1992 (Méteneki and Czeizel, 2005). Two recent studies from County Galway (O'Nualláin et al., 2007) and Dubai (Murthy et al., 2007) showed an increased frequency of 2.68/1000 and 2.2/1000 live births respectively.

In India, the prevalence of DS is 0.88 per 1000 (1 out of 1139) to 1.09 per 1000 (1 out of 916) and three DS children are reported to be born every hour (Rajangam and Thomas, 1992; Verma, 2000; Malini and Ramachandra, 2006).

2.2 SEX RATIO

In general, sex ratio among individuals with Down syndrome was skewed toward an excess of males in the majority of studied populations, either in large epidemiological studies or in selected groups. The ratio showed
a tendency to increase since 1940s, reaching a mean value of 1.35 in 1980s varying from 1.3 to 1.62 in different populations, which might be a consequence of the growing use of karyotyping to confirm diagnosis and of a real increase in the proportion of males. In the 1990s, the ratio fell to 1.22 varying from 1.03 to 1.27 (Kovaleva, 2002).

Mutton et al. (1996) in a large National Down Syndrome Cytogenetic Register from England and Wales showed sex ratio to be raised (1.23:1). Kovaleva (2002) compiled data from 55 publications providing the sex ratio in cases of Down syndrome and found predominance of males in the overall sex ratio. In another study from Lebanon the male to female sex ratio of 1.66 was significantly elevated (Zahed and Megarbane, 1998). Jyothy et al. (2000) in a large study from south India reported the sex ratio among Down syndrome patients to be 1.41:1. In contrast to these findings, Carothers et al. (2001) reported an unusual finding of a markedly lower sex ratio (98 males per 100 females) than has been reported in other DS samples.

Meta-analysis of data from epidemiological studies suggests that the phenomenon is not restricted to free trisomy 21 alone but also appears in translocation cases, both in de novo (1.31:1) and inherited (1.36:1) translocation carriers (Kovaleva, 2002). A male excess was observed for Robertsonian translocation D/21 (1.70:1) and G/21 (1.38:1) DS individuals (Hook, 1981). On the other hand, an excess of females (0.83:1) (Kovaleva, 2002) and (0.67:1) (Mokhtar et al., 2003) among DS mosaics was noticed. Contradicting reports from Indian studies showed the male to female ratio in case of mosaics to range from 2:1 (Thomas et al., 1992) to 2.34:1 (Jyothy
et al., 2000). In a sample of 75 children with trisomy for chromosome 21, Verma and Huq (1987) noticed that more males with Down syndrome were born to young couples, while elderly couples had an excess of girls.

2.3 CLINICAL FEATURES

Trisomy for chromosome 21 is characterized by a complex and specific phenotype being the consequence of many developmental abnormalities. Virtually all the major organ systems of the human body are affected in some degree in a very consistent fashion. Further, there is a temporal dimension to the evolution of the phenotype so that the issues of concern to affected individuals, their families and society change with age (Antonarakis and Epstein, 2006). A chronology of the major components of the DS phenotype is presented in Table 1.

DS infants tend to be born prematurely on an average of seven to ten days earlier than normal babies. This difference, although not great, has been shown to be statistically significant (Berg et al., 1969). Trisomy 21 is associated with a rich variety of phenotypes, about 80 clinical traits, including typical facial features, anomalies of intestinal tract, muscular hypotonia, and increased risk of leukemia, congenital heart diseases and mental retardation (MR). Existence of MR and neonatal hypotonia is universal being noticed in almost 100% of individuals with DS (Reeves et al., 2001; Sanchez-Font et al., 2003; Wolvetang et al., 2003a).

Jackson et al. (1976) conducted a prospective study correlating clinical features with cytogenetic analysis with the aim of improving the accuracy of
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<th></th>
<th>At birth</th>
<th>Infancy and childhood</th>
<th>Adulthood</th>
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<tbody>
<tr>
<td>Structural</td>
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<tr>
<td>Dysmorphic features(^a)</td>
<td>Growth retardation and obesity</td>
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<tr>
<td>Congenital heart disease</td>
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<tr>
<td>Duodenal stenosis or atresia</td>
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<tr>
<td>Imperforate anus</td>
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<tr>
<td>Hirschprung disease</td>
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<td>Central nervous system</td>
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<tr>
<td>Hypotonia</td>
<td>Developmental and mental</td>
<td>Decrease in cognitive</td>
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<td></td>
<td>retardation</td>
<td>function</td>
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<td></td>
<td>Decreased sensitivity to pain</td>
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<td>Alzheimer disease</td>
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<td>Immune and hematopoietic</td>
<td></td>
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<tr>
<td>systems</td>
<td>Transient myeloproliferative</td>
<td>Leukemia</td>
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<tr>
<td></td>
<td>disorder</td>
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<tr>
<td></td>
<td>Other</td>
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<tr>
<td>Thyroid dysfunction</td>
<td>Male sterility</td>
<td></td>
<td>Reduced longevity</td>
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\(^a\) The dysmorphic features include (in order of frequency with which they are noted): upslanting palpebral fissures (82%), loose skin on the nape of the neck, narrow palate, brachycephaly, flat nasal bridge, wide gap between the first and second toes, short broad hands, short neck, abnormal teeth, epicanthic folds, short and incurved fifth fingers, open mouth with down turned corners and protruding tongue, transverse palmar creases, and folded or dysplastic ears (50%).

**Ref.:** Antonarakis and Epstein (2006), p. 474
clinical diagnosis. In individuals with 13 or more signs of the 25 physical signs of DS (Table 2), the clinical diagnosis could be confidently confirmed while those exhibiting less than five could be diagnosed as normal. Cardinal signs of DS in newborns are: flat facial profile, hypotonia, slanting palpebral fissures, redundant skin of neck, brachycephaly, clinodactyly, small folded ear, simian crease and sandal gap (Jackson et al., 1976; Rex and Preus, 1982). Autism is not extremely rare in DS (Satge et al., 1998).

Four features found to be common in DS are MR (92%), clinodactyly of the fifth finger (89%), open mouth (88%) and oblique eye fissures (85%), and these characteristics were present in more than 85% of those individuals with a duplication of 21q (Forster-Gibson et al., 2001). Hypotonia was less often observed in persons with rec dup(21q) (Lazzaro et al., 2001). The dysmorphic features and malformations are described below:

**Head**

Individuals with trisomy 21 show an overall reduction in the size of the skull, flattened occiput, brachycephaly, small midface, reduced maxilla and mandible and reduced inter-orbital distance (Epstein, 2002). The fontanels may be large and close later than usual. The weight of the brain is in the low normal range. The frontal lobes are shortened and cerebellum and brain stem are reduced in volume. Abnormalities of dendritic spines and defects in neuronal architecture and brain histogenesis are found in the literature (Villar and Epstein, 2003). There is a progressive and virtually linear decline in development quotient, intelligence quotient and social quotient, with a wide
Table 2. Jackson's checklist of 25 physical signs

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Clinical features</th>
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<tbody>
<tr>
<td>1</td>
<td>Brachycephaly a</td>
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<tr>
<td>2</td>
<td>Oblique eye fissure a</td>
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<tr>
<td>3</td>
<td>Epicanthic eye-fold</td>
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<tr>
<td>4</td>
<td>Blepharitis, conjunctivitis</td>
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<tr>
<td>5</td>
<td>Brushfield spots</td>
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<tr>
<td>6</td>
<td>Nystagmus a</td>
</tr>
<tr>
<td>7</td>
<td>Flat nasal bridge a</td>
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<tr>
<td>8</td>
<td>Mouth permanently open</td>
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<tr>
<td>9</td>
<td>Abnormal teeth</td>
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<td>10</td>
<td>Protruding tongue</td>
</tr>
<tr>
<td>11</td>
<td>Furrowed tongue</td>
</tr>
<tr>
<td>12</td>
<td>High-arched palate</td>
</tr>
<tr>
<td>13</td>
<td>Narrow palate a</td>
</tr>
<tr>
<td>14</td>
<td>Folded ear a</td>
</tr>
<tr>
<td>15</td>
<td>Short neck a</td>
</tr>
<tr>
<td>16</td>
<td>Loose skin of neck</td>
</tr>
<tr>
<td>17</td>
<td>Short and broad hands</td>
</tr>
<tr>
<td>18</td>
<td>Short 5th finger</td>
</tr>
<tr>
<td>19</td>
<td>Incurved 5th finger a</td>
</tr>
<tr>
<td>20</td>
<td>Transverse palmar crease</td>
</tr>
<tr>
<td>21</td>
<td>Gap between 1st and 2nd toes a</td>
</tr>
<tr>
<td>22</td>
<td>Congenital heart defect</td>
</tr>
<tr>
<td>23</td>
<td>Murmur</td>
</tr>
<tr>
<td>24</td>
<td>Joint hyperflexibility</td>
</tr>
<tr>
<td>25</td>
<td>Muscular hypotonia a</td>
</tr>
</tbody>
</table>

* - 10 most discriminating signs of DS in infants

Ref.: Jackson et al. (1976), p. 484
range for the latter two. DS individuals have greater difficulty in recalling sequences of verbal information presented orally. Anomalous dominance of language has been observed in young adults with DS (Villar and Epstein, 2003).

Intellectual disability is generally defined by an IQ score of <70; with a score of 50–70 being categorised as mild, 20–50 severe and <20 profound. It is estimated that 10% of DS children have profound intellectual disability, 70% severe and 20% mild or none (Noble, 1998). There is usually a steady and fairly rapid decline in intellectual performance. It is about 50 at the age of 2-3 years and drops to 40 after adolescence (Wishart, 1993; Kumar et al., 2000).

Nose

The nose tends to be small, and the bridge of the nose is somewhat low so that the face appears relatively flat. Half of children with DS suffer from obstructive sleep apnea, caused by the flattened mid face, narrowed nasopharynx, hypotonic upper air way muscles and enlarged adenoids and tonsils (Grech, 2001).

Eyes

The eyes tend to slant upward (slanting palpebral fissures). There may be small folds of skin at the inside corners of the eyes (inner canthal folds). The outer portion of the iris of the eye may be speckled with lightly colored spots (Brushfield spots) especially noticeable in blue-eyed babies. Myopia is
found in 30% of school aged children. Strabismus is seen in 27% and cataract in 15% (Leonard et al., 1999).

Ears

The ears are low set and usually small. The top rim of the ear (helix) is frequently folded over slightly. The ear lobes may be small. Ninety percent of all DS individuals have significant hearing loss, which is usually conductive (Grech, 2001).

Mouth

The tongue is of normal size but the mouth may be relatively small and the roof of the mouth a little short. For this reason and because of generally poor muscle tone, the tongue of a baby with DS may intermittently protrude. In older children a furrowed tongue sometimes develops. The teeth may be small and sometimes are abnormally shaped. They may be formed late and occasionally are placed in an unusual position. Sometimes one or more teeth are missing. The voice may have a slightly deep quality in early to late childhood. Onset of speech is late and learning to talk articulately is generally difficult (Mary et al., 2007).

Neck

The neck is short. Babies may have loose folds of skin across the back of the neck, which becomes less prominent with time. This forms a sonographic sign for fetal diagnosis in second trimester (Benacerraf et al., 1985).
Hands

The hands are often small, with relatively short fingers. Clinodactyly of the fifth finger is frequent. There may also be a single flexion on the 5th finger. Simian crease across the upper palm forms one of the classical features. The axial triradius is in t’ or t” in more than 70% of the patients. There are frequent hypothenar patterns, an excess of ulnar loops and an increased index of transversality (Dar and Schmidt, 1976).

Feet

The foot appears short, broad and thick. There may be a small gap between the first and second toes, with a short crease running up between them on the sole of the foot. The development of early onset of pedal arthritis is associated with severe flat feet (Roizen and Patterson, 2003).

Genitalia

The genitalia are usually normal. Sometimes a small pelvis and cryptorchidism may be encountered. In adolescent girls with DS, the age of onset of puberty is similar to that of other adolescents. In boys the primary and secondary sexual characteristics and, pituitary and testicular hormone concentrations are similar to typical adolescents (Pueschel et al., 1985). Women are able to bear children (Kivivuori et al., 1996) but men have diminished capacity to reproduce (Bovicelli et al., 1982). Infertility in males has been attributed to defective spermatogenesis. Exceptional cases of fertile
non-mosaic trisomy 21 males who have fathered a child are reported (Bobrow et al., 1989; Pradhan et al., 2006).

DS newborns have hypotonia but the basis for the decrease in muscle tone is unknown. In infancy, there are, in addition, delayed dissolution of early reflexes and automatisms such as grasp reflexes, the Moro response and automatic stepping (Villar and Epstein, 2003).

Congenital heart disease (CHD) is the most common clinical defect with about 40-50% risk among live born children with DS, as compared to less than 1% of the total population (Park et al., 1977; Villar and Epstein, 2003). Approximately 40% of individuals with DS have a clinically apparent congenital heart defect, with the bulk attributable to entities resulting from deficient outgrowth and fusion of endocardial cushion. A wide spectrum of heart anomalies has been reported in DS. The most common defects are atrioventricular septal defect (45%) and ventricular septal defect (35%). Besides these, isolated secundum atrial septal defect (8%), isolated persistent patent ductus arteriosus (7%), isolated tetralogy of Fallot (4%) and rarely transposition of the great arteries are encountered (Roizen and Patterson, 2003). Arrhythmias in DS are uncommon and are related to coexisting CHD or its treatment. DS individuals have a reduced risk of coronary artery disease and this was attributed to favorable blood lipid profile.

Children with DS have a 10- to 20-fold higher risk of developing an acute lymphocytic or myeloid leukemia (Delabar, 2003). At least 10% of children with DS are born with megakaryocytosis commonly called transient
myeloproliferative disorder, transient abnormal myelopoiesis, or transient leukemia. The risk of acute megakaryoblastic leukemia is about 400 times (Rainis et al., 2003). On the other hand, trisomy 21 confers protection against the development of solid tumors like neuroblastomas, cancers of the digestive system and breast cancers (Delabar, 2003).

The most common non-cardiac congenital anomalies in DS are duodenal atresia, Hirschsprung disease and annulæ pancreas which cause chronic vomiting, pancreatitis and tracheoesophageal fistula. Uncommon anomalies include spina bifida, anencephaly, conjoined twins, intestinal duplication and other mid-line body defects (Torf and Christianson, 1998). Thyroid disease is found in 15% of these individuals and 1% have congenital hypothyroidism. The prevalence of insulin-dependent diabetes mellitus in DS patients is 1.4 to 10.6% compared to 0.1% in the general population (VanGoor et al., 1997; Grech, 2001).

Epilepsy occurs in 5-10% of DS patients and exhibits a bimodal peak, infantile spasms in the first two years of life and tonic–clonic variety at 15–25 years. Coeliac disease could be as high as 7 to 16 percent and the symptoms range from diarrhea, vomiting, weight loss, malnutrition, irritability and decreased appetite (Grech, 2001).

Many disorders such as arthritis, atlantoaxial subluxation, obstructive sleep apnea and seizures are seen. Eighty seven percent of children develop skin disorders such as palmoplantar hyperkeratosis (40.8%), seborrhoeic dermatitis (30.9%), fissured tongue (20%), geographic tongue (11.2%), cutis
marmorata (12.6%) and xerosis (9.8%) (Roizen and Patterson, 2003). There is an increased susceptibility to infection, which appears to be the result of abnormalities in the immune system, particularly in the maturation and function of T lymphocytes.

Attempts have been made to explain the occurrence of phenotypic similarities in many aneuploid states and the observation of phenotypic traits of Down syndrome in euploid people, but at much lower frequencies (Patterson and Costa, 2005). However, other aneuploid conditions do not result in the appearance of the neuropathological signs of Alzheimer disease or an increased risk of Alzheimer disease. This apparent uniqueness of Alzheimer disease-like pathology in DS adults may be argued to be simply a consequence of the increased longevity of these individuals compared with those with other autosomal aneuploidies. DS individuals also show an increased risk to develop myelodysplastic syndrome and acute myeloid leukaemia (Hasle, 2001). Further, the most frequent form of cardiac malformations seen in people with Down syndrome are atrioventricular canal defects, whereas ventricular septal defects are more common in trisomies of 13, 18 and 22 (Bacino et al., 1995; Cohen. 1999; Tolmie, 2002). Additionally, duodenal atresia, which is not characteristic of other aneuploidies, affects 2–5% of infants with Down syndrome. Twenty to thirty percent of all children with duodenal atresia have Down syndrome (Cohen, 1999; Tolmie, 2002).

The principal causes of death in DS are infection, CHD and malignancy (Yang et al. 2002). The mean life expectancy for persons with DS has improved dramatically in the last 50 years. The major determinant of
survival during the first decade of life is the presence of CHD. The life expectancy of an individual with DS at any point in time is 10-20 years or less with this difference being greater for females than males (Baird and Sadovnick, 1988).

2.4 CYTOGENETIC ASPECTS

Trisomy 21 accounts for over 95% of Down syndrome cases while about 2-4% result from translocations and 1-2% due to mosaicism (Nussbaum et al. 2001). Few reports of partial trisomy 21 (El-Ruby et al., 2007) are also available. In the study by Mokhtar et al. (2003) regular trisomy 21 constituted 95.4%. Robertsonian translocation 2.7% and mosaicism 0.7% of 673 Down syndrome patients in Alexandria. Further, free trisomy 21 was found to be associated with structural or numerical chromosome anomalies in 8 cases (1.2%).

Compilation of results from India exhibited the frequency of free trisomy 21 to range from 83.6-97.8% with a mean of 91.6%. Mosaic karyotypes were observed only in some studies, varying from none to 11.76% with a mean of 4.1%. The frequency of Robertsonian translocations varied from 2.2% to 13.7% with a mean of 4.1%. Rare karyotypes were noted in 0.3% of the cases and chromosomal variants in 0.074% of the cases (Verma et al., 1991). However, Jyothy et al. (2000) reported the frequencies of full trisomy, mosaicism and translocation to be 87.92%, 7.69% and 4.39% respectively.
2.4.1 Free trisomy

Free trisomy for chromosome 21 is the most frequent genetic cause for Down syndrome. It may result from either nondisjunction which is the failure of chromosomes/chromatids to separate during meiosis in one of the parents or anaphase lag. Approximately 90-95% of Down individuals have free trisomy. Study of DNA polymorphisms showed the origin of extra chromosome 21 to be maternal in about 95% of the cases. Further, among maternal errors, about 75% arise in the first meiotic division and 25% in the second meiotic division (Antonarakis, 1998).

2.4.2 Robertsonian translocation

Robertson for the first time in 1916 described Robertsonian translocations in Grasshoppers (Locustidae). Robertsonian translocations are the most common chromosomal rearrangements occurring in humans D and G group chromosomes (13–15, 21 and 22) and other organisms. They are formed when long arms of acrocentric chromosomes are translocated at or near the centromere (Hamerton et al., 1975; Jacobs, 1981). The prevalence is about 1 in 1000 newborn infants and has a relatively high rate of de novo formation with a mutation rate approximately $3.92 \times 10^{-4}$ per gamete per generation (Jacobs, 1981). However, subsequent studies have estimated the frequency of reciprocal translocations to be greater than Robertsonian translocations (de Brackeleer and Dao, 1991. Kékesi et al., 2007).

The short arms of acrocentric chromosomes are known to frequently undergo a nose-to-nose association or fusion within the nucleoli during
meiosis I. This has led to the speculation of a causal relationship between such an association and predisposition to nondisjunction and translocation (Cheung *et al*., 1990). Cytogenetically, all the acrocentric chromosomes have a similar morphology in that each chromosome contains a centromere, and a short arm that can be subdivided into three identifiable bands p11, p12 and p13. The p11 has satellites I, II, III and IV in the pericentromeric regions, βsatellite and the 724 DNA. The p12 region is known as nucleolus organizing region (NOR) and contains the genes encoding the 18S and 28S rRNA. Three DNA sequences have been mapped to p13 region - (i) *ACR1*, a well conserved single copy sequence (ii) beta-satellite, a tandemly repeated DNA with a monomeric length of 68bp and (iii) a sequence representing the “724” family of very low-copy interspersed repeats (Fig. 1). All these sequences are found on the short arms of all acrocentric chromosomes. Despite the apparent similarity the sequences within the centromere-short arm regions are not uniform amongst the five chromosomes (Choo, 1990).

Both non-homologous and homologous Robertsonian translocations involving chromosome 21 can lead to DS (Berend *et al*., 1998). Approximately 5% of cases of DS are caused by Robertsonian translocations (Shaffer *et al*., 1992). Molecular studies using highly polymorphic microsatellite markers indicate that the majority of t(21q21q) are in fact isochochromosomes and only a small percentage are true Robertsonian translocations (Shaffer *et al*., 1991). About one-half of rob(14q21q) are inherited. However, over 95% of der(21q21q) arise *de novo* (Berend *et al*., 1998). Translocations were parentally inherited in 33.3% of the cases with
The short arm p11-p13 region contains "similar" sequences on all 5 chromosomes, except for chromosome 15, which carries an identifiably separate satellite III family in p11. The different alphoid subfamilies (designated I to VII) within the centromeric (cen) regions are represented by different symbols but their relative positions on this map presently remain hypothetical. Note the presence of a common alphoid subfamily on 13, 14 and 21, and the likeness of the cen-pter region between 13 and 21.

Ref.: Choo 1990. (441)
maternal transmission being twice as common as paternal. Mean maternal age was not raised in translocation group (25.3 years) in comparison with regular trisomy 21 (38.2 years) (Mokhtar et al., 2003)

Robertsonian translocations offer a unique opportunity for studying different aspects of the mechanisms involved in the etiology of nondisjunction. It is proposed that the sequence found on chromosomes 13, 14 and 21 mediates homologous recombination resulting in these translocations. This model predicts that the sequence on chromosome 14 are in opposite orientation to the homologous sequence on chromosomes 13 and 21 to facilitate the formation of t(13q14q) and t(14q21q), but they do not favour the formation of t(13q21q) (Page et al., 1996). This is supported by the relatively rare occurrence of t(13q21q) in the population (Shaffer, 2002).

Shaffer (2002) suggested that cloning the breakpoints of t(13q14q) and t(14q21q) would help in understanding the mechanisms involved in the formation of Robertsonian translocations, in determining whether or not some individuals have a predisposition to form these rearrangements and in studying the relationship between Robertsonian translocation formation and nondisjunction resulting in DS. The possible reasons for the increased level of aneuploidy in gametes of heterozygous Robertsonian translocation carriers include (i) difficulties during pairing of homologous chromosome arms in prophase I that have been translocated to a different chromosome, and (ii) disturbance in the proper orientation of centromeres during prometaphase-metaphase I that causes chromosomal mal-segregation (Eichenlaub-Ritter and Winking, 1990).
Balanced Robertsonian translocations between homologous chromosomes are rare (Pangalos et al., 1992). The causes of de novo occurrence in the Robertsonian translocation between homologous chromosomes have been postulated to be (i) a meiotic translocation or an isochromosome formation in one parent producing a disomic gamete, ultimately fertilized by a nullisomic gamete, and (ii) a zygotic translocation followed by loss of the nullisomic cell line (Creau-Goldberg et al., 1987). Carriers of balanced Robertsonian translocations are phenotypically normal, even though most of the short arm is lost (Berend et al., 1998). However, they are at increased risk of having phenotypically abnormal and chromosomally unbalanced offspring. The risk of an abnormal outcome depends on the chromosomes involved in the translocation and the gender of the carrier parent. When a woman carries a t(14q21q) she is at 10–14% risk of having a child with DS. In contrast, when a man is the carrier of the same translocation, his risk of having a child with DS is less than 2% (Shaffer, 2002).

In theory, alternate segregation should produce equal number of normal and balanced gametes (Nussbaum et al. 2001). Segregation ratios observed for the t(21:22) translocation were in concordance with all the other studied D:D and G:G translocations where there was an equivalent number of normal and balanced karyotypes in spermatozoa. The frequency of imbalance from Robertsonian translocations studied by sperm chromosome analysis is higher than the frequency of imbalance studied in fetuses and newborns.
(Syme and Martin, 1992). The meiotic segregation results in higher rates of unbalanced gametes in females than in males.

Robertsonian translocations are a common contributing factor in the genesis of trisomy, because of their prevalence (Cheung et al., 1990). In carriers with t(21q21q) balanced translocation, it is accepted that all viable offspring will be DS (Sudha and Gopinath, 1990a) unless both partners have t(21q21q) (Ercis and Balci, 1999). Further, familial incidence of DS appeared to be the result of the inheritance of a translocation (21q21q) (Sudha and Gopinath, 1990a).

2.4.3 Mosaicism

Mosaicism is defined as the presence of two or more cell lines derived from a single stem cell line in the same individual but with different chromosome complements (Lorda-Sanchez et al., 1992). Two different mechanisms have been proposed for chromosome mosaicism: (i) mitotic nondisjunction, a disturbance of early mitosis in a normal zygote resulting in a mosaic embryo, and (ii) somatic reduction, an early loss of the supernumerary chromosome in some cells of a trisomic embryo (Chuang, 1994). Approximately 1-2% of DS individuals have cells with both 46 and 47 chromosomes (Blachford, 2002).

The phenotype in mosaics may be milder than that seen in typical trisomy 21 patients. However, there is a wide variability in phenotype among mosaic patients, possibly reflecting the variable proportions of trisomy 21 cells in the embryo during early development. The ascertained patients with
mosaic DS probably represent the more clinically severe cases, because very mildly affected persons are less likely to be karyotyped (Nussbaum et al., 2001). An excess of males seen in all cytogenetic groups of DS was not reported in the mosaic group where the male: female ratio was 0.67 (Mokhtar et al., 2003).

2.4.4 Partial trisomy

About 4% of DS individuals are the result of an unbalanced translocation of which a small proportion of cases are the result of partial trisomy of chromosome 21 arising either from reciprocal translocations or from intrachromosomal duplications (Bartsch et al., 1993; Forster-Gibson et al., 2001; Nadal et al., 2001). Down syndrome patients with partial trisomy are extremely rare (Horn et al., 2003). Patients with no cytogenetically visible chromosomal abnormalities are even more rarely identified. Genotype-phenotype correlation in such patients help to determine which region(s) of chromosome 21 plays a crucial role in the pathogenesis of specific anomalies of DS. The DS phenotype mostly results from the trisomy of the band 21q22 (Nussbaum et al., 2001; Horn et al., 2003). Very rarely partial trisomies for the distal segment of chromosome 21 including the bands 21q22 → qter, resulted in DS (Delabar et al., 1993).

Huret et al., (1987) reported a case of an 18-month-old boy with many typical Down syndrome features but a normal cytogenetic analysis. High-resolution banding techniques on lymphocytes and fibroblasts of the propositus and his parents did not show any detectable abnormality including
that of mosaicism for trisomy 21. However, CuZn superoxide dismutase (CuZn SOD) in the patient's red cells was increased as in trisomy 21. DNA analysis (Southern blots) using a human CuZn SOD probe showed that the genotype of the propositus contained three CuZn SOD genes. These results indicate that the Down syndrome phenotype of this patient was due to microduplication of a chromosome 21 fragment containing the CuZn SOD gene.

Forster-Gibson et al. (2001) described an adult male who was diagnosed with Down syndrome at 9 months of age and had exhibited normal karyotype upon repeated investigations. However, studies using FISH probes revealed a possible duplication of 21q22.13 to 21q22.3. The presence of these features thus, did not appear to depend on the specific portion of chromosome 21 that was duplicated. A review of 18 additional clinical features also showed no consistent phenotype-genotype correlation.

An evaluation of the patient with phenotypic features of DS but with an apparently normal karyotype would require to exclude undetected mosaicism or an extremely subtle chromosome 21 rearrangement. Undetected chromosome 21 material located elsewhere in the karyotype may be found, using "chromosome painting" for relatively large regions. Smaller regions may be detected with the use of chromosome 21 region-specific DNA sequences that generate a more intense hybridization signal. Even finer distinction of duplicated regions may be assessed by using either in situ hybridization with well-defined cosmids or gene dosage with unique probes (Epstein et al., 1991).
Melkild (1994) reported a ring chromosome 21 [r(21)] with mental retardation. Ring 21 is a rare chromosomal abnormality known to cause a great variety of clinical abnormalities. Familial DS cases of r(21) are usually due to maternal transmission. Adult males with r(21) are usually infertile. Phenotypically normal women who are heterozygous for ring 21 chromosomes, with or without the presence of a normal cell line, have a high risk for reproductive mishaps including offspring with r(21) and trisomy 21. Duplication of segments within the ring may cause functional trisomy 21 and features of DS.

McGinniss et al. (1992) studied the mechanism of r(21) formation in 13 patients (7 familial and 6 de novo). Of these, 11 r(21)s were unique with r(21) not showing any evidence for dup(21q) in nine of them. However, they did show molecular evidence of partial deletion of 21q. The phenotype of r(21) patients varies with the extent of chromosome 21 monosomy or trisomy. Palmer et al. (1995) described a patient with an asymmetric double ring 21 in mosaic form. 45,XX,-21 /46,XX,-21,+r(21)pat, who had limited manifestations of Down syndrome and who developed acute myelofibrosis and megakaryocytic leukemia. In situ hybridization studies, and gene dosage and DNA polymorphism analyses showed that the ring chromosome carried a duplicated region extending from D21S406 to D21S3 that included the candidate genes for leukemia, AML-1, ETS, and ERG.

Delabar et al. (1987) reported a patient with the phenotype of Down syndrome with a normal karyotype in blood lymphocytes and fibroblasts. Assessment of chromosome 21 markers SOD1, CBS, ETS2, D21S11 and
BCE1 showed partial trisomy by duplication of a chromosome segment carrying the SOD1, CBS and ETS2 loci and flanked by the BCE1 and D21S11 loci, which are not duplicated. This submicroscopic duplication at the interface of 21q21 and 21q22.1 reduces to about 2000-3000kb the critical segment, the trisomy of which is responsible for the phenotype of trisomy 21.

Pangalos et al. (1992) studied three Down syndrome patients for whom karyotypic analysis showed a reverse tandem ("mirror") duplication of chromosome 21. High-resolution R-banding analysis suggested a partial deletion of distal 21q in two cases. The evaluation of eight chromosome 21 single-copy sequences of the 21q22 region namely, SOD1, D21S15, D21S42, CRYA1, PFKL, CD18, COL6A1, and S100B by a slot blot method showed a partial deletion of 21q22.3 and partial monosomy in all three cases. DNA polymorphism analysis indicated a homozygosity of the duplicated material in all cases suggesting that the reverse tandem chromosomes did not result from a telomeric fusion between chromosomes 21 but from a translocation between sister chromatids. The phenotypes of these patients did not differ significantly from that of individuals with full trisomy 21. The fact that monosomy of distal 21q22.3 in these patients resulted in a phenotype very similar to Down syndrome suggests that the duplication of the genes located in this part of chromosome 21 is not necessary for the pathogenesis of the Down syndrome features observed in these patients.

Majority of the partial trisomy cases are due to unbalanced translocation involving chromosome 21. Sebastio et al. (1996) reported an aneuploidy syndrome due to the unbalanced segregation of a familial
translocation t(4;21)(p16.3;q22.1) causing a partial 4p monosomy and a partial 21q trisomy, with most of the Down syndrome critical region triplicated, and giving the overall appearance of the Down syndrome. Braddock et al. (2000) presented two individuals with an unreported tertiary trisomy for chromosomes 5p and 21q in an eight-generation pedigree. The propositus was described as atypical for Down syndrome and the karyotype showed 47,XY,+der(21)t(5;21)(p15.1;q22.1)mat. incorporating partial trisomies of both chromosomes 5 and 21. The translocation originated from the maternal great-grandmother, but only the propositus and his mentally retarded aunt had a similar phenotype and the derivative chromosome. FISH showed absence of band 21q22.2 in the derivative chromosome of the propositus and his aunt, indicating that neither had trisomy for the Down syndrome critical region.

Parmar and Sira (2003) reported partial trisomy 21 resulting from balanced translocation t(21q;22q) in a 36-year-old woman with pericentric inversion of chromosome 9. The lady had history of recurrent spontaneous first trimester abortions and only one live child. Lee et al. (2005) reported a case with partial trisomy 21 due to a paternal cryptic insertion (4;21) which was ascertained by a rapid overnight FISH on uncultured amniotic fluid cells. The fetus was delivered at term and had classical features of Down syndrome.

Two recent reports described cases with lack of distinct clinical features of Down syndrome. The first was an 8-year-old female with a supernumerary chromosome der(21)t(4;21)(q25;q22)mat resulting in partial trisomies for 4q25-qter and 21(pter-q22) (El-Ruby et al., 2007). The second
described two patients with moderate mental retardation and short stature, but the typical facial appearance of DS was not observed. FISH analysis showed that each of their extra chromosomes 21 contained a distal part of chromosome 3p or 14q at the telomeric region of chromosome 21q lacking all of DSCR on 21q22 (Kondo et al., 2006).

2.4.5 Non-Classical Down karyotype

Non-classical chromosomal anomalies have been reported in the last few years in cytogenetic surveys on DS individuals with a frequency ranging up to 2.4% (Mutton et al., 1996; Mokhtar et al., 2003; Sheth et al., 2007). Regular trisomy 21 was associated with structural or numerical chromosome anomalies in 8 out of 673 Down syndrome patients referred for chromosomal investigation (Mokhtar et al. 2003).

Double aneuploidy leading to trisomy and/or monosomy of two different chromosomes arises because of two meiotic non-disjunctional events. Both these aneuploidies could have the same or different parental origin (Lorda-Sanchez et al., 1991). The co-occurrence of two numerical chromosomal abnormalities in the same individual is a relatively rare phenomenon and the clinical presentations are variable depending on the predominating aneuploidy or a combination effect of both. Double aneuploidy involving Down and Turner syndromes is a rare occurrence. Townes et al. (1975) reported the first example of a Turner-Down patient in whom there was no mosaicism for the X chromosome.
The coincidence rate of both Down and Klinefelter syndromes in the same individual is estimated to lie in the range $0.27$ to $0.7 \times 10^{-5}$ (Yamaguchi et al., 1989). However, neonatal survey data has revealed that the incidence of XXY and trisomy 21 double trisomy at birth is higher than expected from the incidence of either alone (Taylor and Moores, 1967). Several cases of double aneuploidy of XXY and trisomy 21 have been published since the first report by Ford et al. (1959). Iliopoulos et al (2004) described a rare case of possible coincidence of double aneuploidy in newborn twins. The newborns exhibited typical clinical features of Down syndrome, of which one revealed 48.XXY,+21 karyotype. The second newborn died two days after its birth. Due to identical phenotypic features of the twins, the common placenta and amniotic sac, it was speculated that they were monozygotic twins and therefore, the second newborn should also have possessed the same karyotype. Recently, a 5-year-old Turkish child, the second-born of non-consanguineous parents, was described to possess an extra X chromosome in addition to trisomy 21. The proband's parents and his brother showed normal karyotype (Karaman and Kabalar, 2008).

Double aneuploidy involving XYY and trisomy 21 is seldom described. Furthermore, there was only one case of XYY Down syndrome male showing mosaicism for the Y chromosome (Schwanitz and Hagner, 1978). The clinical features of DS were apparent in these individuals. XYY males are generally fertile and bear normal children in contrast to DS males who are sterile. Thus, it would be reasonable to propose that DS males with XYY chromosome pattern may also be infertile (Parmar et al., 2002).
**Tandem translocations** have been observed very rarely in Down syndrome. Karukaya and Yokoyama (1980) reported a girl with atypical Down syndrome characterized by a mosaic karyotype 46,XX,-21,+t(21q21q)/46,XX. The tandem translocation chromosome with dicentric bisatellited structure was confirmed by G-, Q- and C-banding methods. Buchanan (1975) described a rare occurrence of double aneuploidy and a *de novo* reciprocal translocation in a DS infant with atypical features.

### 2.4.6 Normal karyotype in Down syndrome

Evaluation of patients exhibiting phenotypic features of DS but with apparently normal karyotype requires additional consideration. Two possible explanations for this could be presence of (i) undetected mosaicism and (ii) extremely subtle translocation/rearrangement involving chromosome 21 (Epstein *et al.*, 1991).

Nadal *et al.* (1997) found a patient with a typical DS phenotype and a normal karyotype. FISH using painting probes, revealed that the patient had partial trisomy of chromosome 21 owing to an unbalanced translocation t(15:21)(q26; q22.1) of paternal origin. The father, a brother and a sister of the patient carried the balanced translocation and had a normal phenotype.

Two other reports of DS phenotype with a normal karyotype by standard chromosomal analysis involved a translocation between chromosomes 18 and 21. A case of an infant with clinical features of Down syndrome and a normal karyotype with her two uncles who had unusual appearance but not apparent Down syndrome, and a severely retarded girl
with dysmorphv and epilepsy from the same family was reported. FISH using whole chromosome painting and unique-copy probes, and high-resolution banding revealed a familial subtelomeric translocation of chromosomes 18 and 21 with breakpoints located in bands 18q23 and 21q22.1, resulting in partial trisomy 21 in the infant and her two uncles, and partial monosomy 21 in the girl (Bartsch et al., 1997). Horn et al. (2003) reported a three-generation family that included two patients with severe mental retardation and additional anomalies. A clinical diagnosis could not be made in the propositus, but facial anomalies of Down syndrome were recognized in the maternal uncle of the propositus. Standard cytogenetic analysis did not reveal any abnormalities. FISH using subtelomeric DNA probes identified a familial cryptic translocation of chromosomes 18 and 21, resulting in partial trisomy 21 and partial monosomy 18q in both patients.

Ahlbom et al. (1996) described a woman with clinically typical DS but apparently normal chromosomes. Southern blot analysis using radioactive probes for the sequences D21S59, D21S1, D21S11, D21S8, D21S17, D21S55, ERG, D21S15, D21S112, and COL6A1 mapped on chromosome 21 showed the presence of these selected markers in two copies. FISH with chromosome 21-specific genomic library showed only two copies of chromosome 21. Nineteen markers from the critical region studied with PCR amplification of di- and tetra-nucleotide repeats did not indicate any partial trisomy 21. It was concluded that she suffered from an autosomal recessive disorder which was phenotypically indistinguishable from DS and that she did not have any partial submicroscopic trisomy for chromosome 21.
Contradicting the above report, an infant who presented with a *de novo* t(21:21) translocation trisomy 21 was found to have an atypical phenotype for Down syndrome (Keppler-Noreuil *et al.* 2002). Clinical evaluation using both the diagnostic criteria for DS and the Jackson checklist of 25 signs was inconsistent with the diagnosis for DS. Findings included microcephaly, small stature, downslanting palpebral fissures, absence of Brushfield spots, moderate micrognathia, left ptosis, left torticollis, severe developmental delay, seizures, and hypertonia. Blood karyotype revealed 46,XX,+21,del(21:21)(p11.2;p11.2). FISH analysis confirmed the trisomy 21 translocation. On the other hand, chromosomal and FISH analyses performed on skin fibroblasts revealed a mosaic pattern with the second cell line consisting of a ring chromosome 21 derived from the translocation t(21:21).

### 2.4.7 Chromosomal variants

Heteromorphisms of chromosomal regions also referred to as polymorphisms or normal variants, have been observed since the earliest studies of chromatin visualization. Heteromorphisms are defined as microscopically visible regions that vary in size, morphology and staining properties in different individuals and have no impact on the phenotype. They are typically stable and are inherited in a Mendelian fashion. These regions include the proximal long arm of chromosomes 1, 9 and 16, the distal two-thirds of the long arm of the Y chromosome, and the short arms and satellites of the acrocentric chromosomes. They have been invaluable aids in studies in various areas such as forensics, paternity testing, marrow engraftment, linkage
analysis and gene mapping (Gardner and Sutherland, 1989; Rao et al., 2006; Brothman et al., 2006).

Population studies, using Q-, G- and/or C-band techniques have revealed significant differences in the frequencies of these polymorphisms in various ethnic groups. Examination of parental chromosomes and study of the variant chromosome, using a combination of different banding techniques, usually allow the distinction between a polymorphism and a structural abnormality and help to identify the origin of the polymorphism (Borgaonkar, 1998; Wyandt and Tonk, 2004). Heterochromatic regions on chromosomes 1, 9, 16, and Y were considered heteromorphisms by 97% of the 226 participants in a clinical cytogenetics proficiency survey and 24% of participants indicated they would include these findings in their reports. Pericentric inversions including inv(1)(p13q21), inv(2)(p11.2q13), inv(3)(p11.2q12), inv(3)(p13q12), inv(3)(p13q13), inv(5)(p13q13), inv(9)(p12q13), inv(10)(p11.2q11.2), inv(16)(p11.2q12.1) and inv(Y) were considered heteromorphisms with more than 75% of respondents indicating they would report these findings (Brothman et al. 2006).

Pericentromeric polymorphisms of chromosome 9 include variations in the size of heterochromatin, pericentric inversions, and, more rarely, additional C-band-negative, G-band-positive material in either the proximal short arm or long arm or within the heterochromatin. Rearrangements involving the different classes of satellite DNA present in this relatively unstable region of the human genome have been postulated to constitute a mechanism for the origin of these variants (Park et al., 1998). Pericentric
inversions of chromosome 9 are among the most frequent chromosomal rearrangements. Although it been considered as a normal variant, many recent reports implicate pericentric inversions of chromosome 9 with infertility, recurrent abortions, and a number of other abnormal conditions (Rao et al. 2006; Sheth et al. 2007). Pericentric inversion of Y chromosome has also been reported with trisomy 21 (Sparkes et al. 1970; Krishnamurthy et al. 1989; Sheth et al., 2007).

2.5 GENOTYPE – PHENOTYPE CORRELATION

The term “phenotype” refers to a measurable parameter and includes clinical, physical, cellular and physiological components. Phenotypic mapping means the molecular definition of a physical region that is likely to contain the gene(s) whose over-expression is ultimately responsible in part for the phenotype (Korenberg et al., 1994). It is an extremely difficult task to define how an extra copy of all or part of HSA21 results in the phenotype of DS. While some loci may have a greater phenotypic effect, it is the cumulative effect of imbalance of many genes that determines the overall phenotype. There are two principal hypotheses for how trisomy 21 causes Down syndrome. Both are based on the assumption that a gene present in three copies rather than two would lead to an elevation in the level of its expression in the simplest case, by 50 percent.

The gene dosage effect hypothesis states that an elevated expression of specific trisomic genes leads to specific features of Down syndrome directly from the cumulative effect of overexpression of specific chromosome 21 gene
products or indirectly through the interaction of these HSA21 genes products with the whole genome, transcriptome, or proteome (Pritchard and Kola, 1999; Dierssen et al., 2001). On the other hand, the amplified developmental instability hypothesis states that an elevated activity of sets of genes, regardless of their identity, will lead to a decrease in genetic stability or homeostasis (Shapiro, 1983). The larger the number of trisomic genes, the more susceptible the fetus will be to developmental abnormalities. These two hypotheses are not mutually exclusive. The chief proponents of the latter hypothesis admit that there might be some DS phenotypes that are influenced to a large extent by specific trisomic genes.

Evidence from different murine models points to specific genes affecting phenotypes rather than the unspecific effect of the amount of extra genetic material. However, we still cannot answer the question of how three copies of normal genes contribute to the abnormal phenotype of DS (Dierssen et al., 2001). The specific genes present in the region of imbalance mainly determine the aneuploid phenotypes. Although stochastic, environmental and other genetic factors may influence the phenotype in any individual case, they are not determinants of the phenotype (Epstein et al., 1991). Furthermore, comparisons of syndromes resulting from overlapping duplications or deletions or from double aneuploidy have shown that it is frequently possible to attribute individual components of a phenotype to imbalance of a particular chromosomal region.

The majority of features that occur in DS are not present in every individual with trisomy 21, and those features that are present can vary
considerably in severity (Epstein et al., 1991). Several genetic factors are likely to contribute to individual variability in DS. First, different allele combination of chromosome 21 genes might have different effects when present in 3 copies. Second, the genetic background of the individual in whom trisomy 21 occurs is an obvious source of phenotypic variation. Third, many traits are sensitive to environmental influences (Reeves et al., 2001). Trisomic individuals have in addition, unique genetic influence in the hundreds of genes expressed at inappropriate levels in diverse genetic pathways (Shapiro, 1997).

Very little is known about the genes involved in the different phenotypes of Down syndrome and the molecular pathophysiology of these phenotypes. The working hypotheses for the phenotypes of DS are: (i) few genes contribute to the different phenotypes of DS (ii) single genes may be involved in specific DS phenotypes (iii) the effect of expression of three copies of these genes to the DS phenotypes could be either direct or indirect through the global dysregulation of gene expression (iv) some phenotypes may be related to trisomy for specific alleles of HC21 genes or to the interaction of HC21 products with specific alleles of genes on other chromosomes and (v) some DS phenotypes may be related to disturbances of gene regulation due to the presence of the extra chromosomal material and not specifically to particular genes (Antonarakis et al., 2002).

Delabar et al. (1993) pooled data from 10 patients with overlapping partial trisomies of chromosome 21. Their results were consistent with previous data and significantly increased the number of DS phenotypes
mapped on chromosome 21. Further, the physical map of chromosome 21 has eliminated the uncertainty of cytogenetic analyses and has made possible the molecular definition of regions responsible for specific phenotypic features of DS (Fig. 2).

The notion of a minimal critical region (Delabar et al., 1993) that harbors majority of the genes involved in the DS phenotypes, needs to be re-evaluated by the study of additional patients with partial trisomy 21, systematic analysis of the phenotypes, and diagnosis of the triplicated genomic regions using current methodology (Antonarakis et al., 2002). The completed sequence of genes encoded on chromosome 21 provides excellent basic information. However, the molecular mechanisms leading to the phenotype of DS remain to be elucidated (Ferrando-Miguel et al., 2004).

2.6 ETIOLOGY

The actual etiology for nondisjunction still remains an enigma even after a vast amount of epidemiological, cytogenetic and molecular research (Arbuzova et al., 2001). It is therefore necessary to understand the cellular-molecular events and other biochemical pathways and epigenetic factors that could promote meiotic nondisjunction. Numerous hypotheses have been suggested to explain the underlying mechanism, in particular the maternal factors in the etiology of DS.
Fig. 2. DS Phenotypic map of 25 features based on cytogenetic analyses.

P - profound MR; M - moderate MR

Ref.: Korenberg et al. (1994), p. 1256
2.6.1 Maternal age

Maternal age has been long recognized as the primary risk factor for nondisjunction. In fact, it is the only well established risk factor for DS (Lamb et al., 2005a). The increased risk of older mothers to have a child with DS was noted long before chromosomal basis of the disorder was discovered (Fraser and Mitchell, 1876). In 1933, Penrose and Smith in their review and analysis concluded that there are two maternal age distribution “one in which the maternal age is not significant, and the other in which maternal age is a fundamental influence”. The frequency of DS has been proved to increase with maternal age (Table 3). The risk is 1 in 385 at the age of 35 years and by the age of 45 years the frequency is 1 in 25 births (Epstein, 2007). The strength of the maternal age effect involves a near 100-fold increase in risk between maternal ages of 15 and 45 years (Carothers et al., 1999). The associated risk increases exponentially at age more than 30 years (Hassold and Jacobs, 1984). The age effect results from the maternal age-dependent loss of affected fetuses in utero irrespective of parental origin (Carothers et al., 1987).

The association of advanced maternal age and nondisjunction of chromosome 21 has been explained in several ways as below: (i) Aging changes in the ova itself could predispose to a meiotic defect of chromosome 21 (ii) The “older egg” model – the increased production of abnormal eggs or decreased destruction of abnormal embryos - most errors involving the numerical assortment of chromosome 21 occur in oocytes with the error rate increasing as women age (iii) ‘Production line’ theory - Oogonia that are
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Ref.: Nussbaum et al. (2001), p.158
committed to meiosis later in intrauterine life have less chiasmata than those that start meiosis early and, in addition, are ovulated later in menarche (Henderson and Edwards, 1968). Thus, age-dependent trisomy 21 results primarily from a mechanism that favors maturation and utilization of euploid oocytes in preference to the pre-existing aneuploid products of mitotic (premeiotic) nondisjunction. The increased utilization of aneuploid oocytes at later stages of maternal life would result from their increased proportion following many progressive cycles of selection against their maturation in earlier stages (Zheng and Byers, 1992) and (iv) 'In utero selection' - with increase in maternal age there is reduced rate of spontaneous abortion of the aneuploid embryos (Warburton et al., 1983).

Gaulden (1992) proposed that aneuploid oocytes arise from a concenation of events. Hormonal imbalance causes a less-than-optimal microvasculature to develop around the maturing and mature follicles. The resulting decrease in the size of the perifollicular capillary bed reduces the volume of blood flow through the area, leading to an oxygen deficit and a concomitant excess of carbon dioxide and anaerobic products, such as lactic acid increase inside the follicle. This in turn causes a decrease in the intracellular pH of the oocyte that diminishes the size of the spindle, with consequent displacement and nondisjunction of a chromosome. The author stated that this compromised microcirculation hypothesis explains the occurrence of aneuploidy in primary and secondary oocytes, sperm precursor cells, tumor and embryonic cells. It also explains why women of all reproductive ages may have a Down syndrome child.
The "relaxed-selection" suggests that age-related increase in frequency of DS might ensue from an inability of older mothers to reject trisomy 21 conceptuses (Ayme and Lippman-Hand, 1982). But DS resulting from an unbalanced translocation does not show significant maternal age dependence (Hook, 1980).

Lamb et al. (2005b) hypothesized that environmental and age-related insults accumulate in the ovary as a woman ages, leading to malsegregation of oocytes with stable exchange patterns. It is this risk due to recombination-independent factors that would be most influenced by increasing age leading to the observed maternal age effect. For young women, the meiotic machinery such as the spindle, sister chromatid adhesive proteins, microtubules, motor proteins, etc. function optimally and correctly segregate configurations. Then the greatest risk factor for nondisjunction is the presence of susceptible exchange pattern in the oocyte. As a woman ages, her meiotic machinery accumulates the effects of years of environmental and age-related in results, becoming less efficient and more error prone. Suboptimal exchange bivalents are still susceptible to nondisjunction, but even correctly placed bivalents are now at risk. Maternal age would not necessarily be increased considering that younger woman, depending upon her social circumstances may be as susceptible as the older (Juberg and Mowrey, 1983).

Erickson (1979) suggested that the relationship between maternal age and the incidence of trisomy 21 resulted from biased ascertainment, which did not take into account spontaneous abortions of trisomy 21. Secondly there was a frequent and significant paternal age effect. Morris et al., (2005b)
conducted a study of 13,745 live births born to women ≥45 years of age to determine the risk of a Down syndrome live birth for these women. The risk was considerably lower than has often been previously assumed. The authors explained that women of this age were more likely to miscarry DS pregnancies than younger mothers.

2.6.2 Paternal age

There is no clear evidence of paternal age effect (Petersen et al., 1993). Various studies on aneuploid fetuses and children have been performed to estimate aneuploidy in relation to paternal age (Stene et al., 1981; Hook et al., 1995; Lorda-Sanchez et al., 1992; Zaragoza et al., 1994). However, the weight of evidence indicated no clear association between advanced paternal age and the incidence of autosomal trisomies commonly found in human fetuses and newborns (Jung et al., 2003). An increased risk for trisomy 21 offspring for paternal age of 41 years and above has been reported (Matsunaga et al., 1978; Stene et al. 1981) although Hook and Cross (1982) found no paternal effect. Dagna-Bricarelli et al. (1989) inferred that aging enhanced nondisjunction at both first and second divisions in the female while, aging in the male was presumably associated mostly (or only) with first division errors. The existence of a paternal age effect on Down syndrome is controversial.

Fisch et al. (2003) evaluated 3,419 cases of Down syndrome in a 15-year period and found that the incidence of Down syndrome was influenced by paternal age. Paternal age has an effect on Down syndrome but
only in mothers 35 years and older. In younger women, in whom maternal age was not a risk factor for Down syndrome, there was no paternal effect. The paternal effect was greatest in couples older than 40 years, where the risk of Down syndrome was 6 times the rate in couples younger than 35 years. With increasing paternal age an increase in the rate of Down syndrome is seen in women ≥35 years of age, most likely due to a combined effect of maternal and paternal factors (Fisch et al., 2003).

In contrast to female fertility which begins to decline in the early 30s, spermatogenesis continues well into male senescence (Kidd et al., 2001). The data of several autosomal and X chromosome dominant disorders support a positive relationship between paternal age and de novo gene mutations (Jung et al., 2003). However, data from de novo cytogenetic abnormalities could not provide evidence for a relationship between paternal age and cytogenetically conspicuous offspring.

Studies of the paternally derived cases led to conflicting results about an association of paternal age with trisomy 21. For other autosomal trisomies found in spontaneous abortions and live births, parental contribution is estimated to be more substantial ranging from 11% for trisomy 22, to 12% for trisomy 13 and 15 and 17% for trisomy 14 (Antonarakis et al., 1991; Sherman et al., 1991; Jacobs and Hassold, 1995).

2.6.3 Advanced grand maternal age

Penrose (1964) suggested that some mosaic mothers could have started as trisomic zygotes due to advanced grandmaternal age. Papp et al. (1977)
investigated on the age distribution of grandparents of free trisomy and showed the mean maternal grandmaternal age of mothers less than 30 years old when giving birth to children with DS was slightly higher than that of control. Baker (1963) suggested that in females the first meiotic division of the oocytes is initiated during the second to the seventh month of fetal life. This implies that an ovum giving rise to nondisjunction at the first meiotic division had entered meiosis during the fetal life of the mother of the patient. Advanced maternal age as well as advanced grand maternal age on the mother's side independently of each other, are factors that increase the risk of first meiotic nondisjunction (Aagesen *et al.*, 1984).

Advanced age of grandmother is responsible to bring disturbance in the meiosis of her daughter. At the advanced age the grandmother's reproductive system may fail to make the essential proteins like spindle associated proteins, factors responsible for resting of oocyte, chiasma-binding proteins, DNA repair enzymes, etc. which are needed for proper meiotic segregation in the germ cells of her daughter. The non-availability or non-functioning of proteins leads to impairment in the meiotic process, which in turn results in nondisjunction of chromosome 21 in the oocytes of the daughter. It is also possible that recombination is reduced in the oocytes, which brings about the nondisjunction of chromosome 21 (Malini and Ramachandra, 2006).

2.6.4 Genetic control

Hecht *et al.* (1964) proposed two types of effects that such genes might have - one leading to recurrence of the same chromosomal abnormality
(homo-aneuploidy) and the other resulting in aneuploidy of either the same or a different chromosome (hetero-aneuploidy). There was only suggestive evidence for genetic control of recombination or disjunction in mammals. Relatives of a proband with trisomy 21 appeared to have slightly increased risk of having a child with Down syndrome (Tamaren et al., 1983). The finding of increased DS incidence in highly inbred population has led to the hypothesis that autosomal recessive genes are involved in the genetic control (Alfi et al., 1980). They speculated the possible existence of a gene that induces mitotic nondisjunction in the homozygous fertilized ovum or an autosomal recessive gene resulting in meiotic nondisjunction in homozygous parents.

In model organisms, genetic mutations that reduce or abolish recombination are associated with meiotic arrest, abnormalities of chromosome segregation, and increased levels of nondisjunction (Roeder, 1997). These findings indicate that genetic mechanisms are likely to be involved in at least some cases of aneuploidy. It has been demonstrated that earlier menopause is associated with prior trisomic spontaneous abortion (Kline et al., 2000), again indicating a possible genetic mechanism (Bianco et al., 2006).

2.6.4.1 Consanguinity

Parental consanguinity, as a recognized risk factor for congenital anomalies, has mainly been studied with a focus on the types of parental relationships and their effects on genetic syndromes or birth defects in
general. Alfi et al. (1980) observed an increased occurrence of DS in offspring of consanguineous marriages. The authors suggested that trisomic DS is etiologically heterogeneous, and that in a subgroup of the trisomic Down patients, nondisjunction may be genetically determined. The influence of genetic factors and consanguinity, for the nondisjunction phenomenon has also been supported by other investigators (Penrose, 1961; Roberts et al., 1991) and disputed by yet others (Hamamy et al., 1990; Basaran et al., 1993).

Rajangam and Thomas (1998) examined the effect of parental consanguinity in 417 cytogenetically confirmed DS patients. Statistical comparison of parental consanguinity between the parents of trisomy and mosaic DS patients and that of the general population did not reveal any significant difference as in an earlier study too (Gahlib and Isaac, 1991). Based on the inbreeding coefficient the influence of consanguinity could not be established in trisomy and translocation DS. First cousin marriages were seen to occur more frequently than uncle-niece and second cousin marriages. Among Palestinian Arabs the rate of consanguinity was very high and 44.3% of the marriages were between relatives (22.6% of them between first cousins) (Zlotogora, 1997). The author did not find any significant difference in the rate of consanguineous marriages between the parents and grandparents of children with trisomy 21 and the general population.
2.6.4.2 Genes involved in nondisjunction

a) Presenilin-1

A statistically significant association between AD and a family history of DS in first degree relatives has been reported (van Duijn et al., 1991), and a study demonstrated an increased risk of AD in younger mothers of DS individuals (Schupf et al., 1994). The involvement of chromosome 21 both in Down syndrome and Alzheimer’s disease (AD) suggested a shared genetic susceptibility to these disorders. Rare forms of autosomal-dominant AD are caused by mutations in the amyloid precursor protein (APP) and Presenilin genes (PS-1 and PS-2). The Presenilin proteins have been localized to the nuclear membrane, kinetochores, and centrosomes, suggesting a function in chromosome segregation (Petersen et al., 2000).

Although the normal and pathological functions of the presenilins are still largely unknown, remarkable mutations in PSEN1 and PSEN2 are associated with an early and excessive formation and deposition of amyloid Aβ, particularly for the longer form of Aβ_{42}, which is suspected to initiate the formation of amyloid plaques in AD and DS (Iwatsubo et al., 1994; Hutton and Hardy, 1997). In addition to the dominant mutations, a genetic association between PSEN1 intron 8 polymorphism and AD was suggested, but not consistently found, in different studies (Wragg et al., 1996; Wu et al., 1999). Controversial findings from association studies with PSEN1 intron 8 polymorphism might reflect the occurrence of linkage disequilibrium with sequence variations of higher functional relevance elsewhere in the PSEN1 gene (Theuns et al., 2000).
A polymorphism in intron 8 of the PS-1 gene was analyzed in 168 probands with free trisomy 21 of known parental and meiotic origin and their parents from a population-based material. An increased frequency of allele 1 in mothers with a meiosis II error (70.8%) was found compared with mothers with a meiosis I error (Petersen et al., 2000). The authors concluded that the PS-1 intronic polymorphism might be involved in chromosomal nondisjunction through an influence on the expression level of PS-1 or due to linkage disequilibrium with biologically relevant polymorphisms in or outside the PS-1 gene.

b) *Apolipoprotein E*

Apolipoprotein E is a plasma protein involved in cholesterol transport and metabolism. ApoE is produced in most organs, including the ovary, in which it might be involved in chromosomal segregation (Mahley, 1988). The ApoE gene is located on chromosome 19, and the three common alleles are ε2, ε3 and ε4. The ApoE allele ε4 has been identified as a risk factor for early onset and late onset Alzheimer’s disease in both familial and sporadic cases (Schupf et al., 1994).

An increase in the apoE allele ε4 in younger mothers with meiosis II (MII) error suggested this allele to be a risk factor for MII nondisjunction of chromosome 21. Meiosis II spindle dysfunction could be explained by both the isoform-specific binding of ApoE to microtubule-associated proteins and by the possible interference of ApoE with microtubule stability and function (Strittmatter et al., 1993). The frequency of ε4 varies from 7% in Chinese to
37% in New Guineans but the incidence of DS is fairly constant among different populations (Avramopoulos et al., 1996). Genetic variations at the ApoE and TNF-α loci appear to modulate the risk of AD and DS related dementia, interacting with brain deposition of amyloid β protein (Lucarelli et al., 2003).

c) **Genes involved in folate metabolism**

Folic acid or pteroylglutamic acid is a well-known water-soluble vitamin of the B-complex group. Folic acid is essential for the de novo synthesis of nucleotide precursors for normal DNA synthesis and for normal cellular methylation reactions. Studies have shown that dietary folate/methyl deficiency in vivo and in vitro has been associated with abnormal DNA methylation, DNA strand breaks, abnormal gene expression and altered chromosome segregation. A low folate status is observed in elderly people, smokers, alcoholics and oral contraceptive users. Folate requirements are increased during pregnancy apparently due to increased metabolic breakdown of folate in addition to fetal transfer. Chromosomal instability and aneuploidy exhibited in human tumors is related to genome-wide DNA hypomethylation (Lengauer et al., 1997).

Recent studies from western countries have linked increased frequency of polymorphism of many other genes involved in folate metabolism including MTHFR gene in mothers with Down syndrome (James, 2004). Associations between specific alleles of genes encoding enzymes in the methionine/homocysteine pathway and plasma homocysteine levels have
been examined in different populations. The most common polymorphisms analyzed are *MTHFR A222V* (677C>T), *MTHFR E429A* (1298A>C), *MTRR 122M* (66A>G), *MTR D919G* (2756A>G) and *CBS 844ins68* and *RFC 1* (80G>A). These gene variants implied as risk factors for nondisjunction are briefly described below:

**i) Methylene tetrahydrofolate reductase gene (*MTHFR*)**

*MTHFR* gene comprises of 11 exons, each of which is 102–432 bp in size. The *MTHFR* gene, ranging from base pair 11,769,246 to base pair 11,788,568 is localized on chromosome 1p36.3 and is 20kb in size. It codes for a cytosolic flavoprotein that catalyses the reduction of 5,10-MTHFR to 5-methyl tetrahydrofolate, the predominant circulating form of folic acid (Weisberg *et al.*1998). The MTHFR enzyme serves as a key enzyme in folate and homocysteine (Hcy) metabolism and also plays a role in processing amino acids. In the absence of sufficient folic acid, intracellular Hcy accumulates, methionine resynthesis is reduced and essential methylation reactions are compromised. An increase in homocysteine and a decrease in methionine result in a decreased ratio of S-adenosyl methionine (SAM) to S-adenosyl homocysteine (SAH) (Fig. 3), which has been associated with DNA hypomethylation, a cause of abnormal gene expression and chromosome segregation (Hobbs *et al.*, 2000).

Research of the metabolic events related to DNA hypomethylation led to the observation that 5,10-methylenetetrahydrofolate (*MTHFR*) acts at a critical metabolic juncture in the regulation of cellular methylation reactions.
Overview of the interactive and interdependent reactions involved in cellular one-carbon metabolism.

Ref.: Hobbs et al. (2000), p. 624
There are two well described, commonly occurring polymorphisms in the MTHFR gene; C677T in exon 4 and A1298C in exon 7 (Frosst et al., 1995; Weisberg et al., 1998). Other polymorphisms have been reported at positions 1059, 1289, 1317 and 1793 (Weisberg et al., 1998; Trembath et al., 1999; Rady et al., 2002). These polymorphisms are less common than C677T and A1298C polymorphisms and their functional relevance has not yet been investigated (Lucock, 2000).

Studies have linked increased frequency of MTHFR C677T polymorphism in mothers with Down syndrome, based on the evidence that abnormal folate and methyl metabolism can lead to DNA hypomethylation and abnormal chromosomal segregation (James et al., 1999). Several studies with conflicting results have also been published (Stuppia et al., 2002; Yanamandra et al., 2003; Coppede et al., 2006).

**MTHFR C677T**

The C→T transition in exon 4 results in an alanine to valine (A222V) substitution in the MTHFR protein, causing higher thermolability and reduced enzyme activity due to dissociation of dimer into monomers and loss of flavine adenine dinucleotide (FAD)-binding capacity. Relative to the normal CC genotype, the specific activity of MTHFR is reduced by ~35% with the heterozygous CT genotype and ~70% with the homozygous TT genotype (Carothers et al., 1999). The preliminary study showed a mildly elevated plasma homocysteine levels and a 2.6-fold increase in frequency of the 677 C→T polymorphisms in the MTHFR gene in mothers of DS patients (James
et al., 1999). The hypothetical effect of 677 C→T in maternal nondisjunction was linked to an altered DNA methylation pattern in the oocyte, secondary to reduced MTHFR activity.

The expanding spectrum of common conditions linked with the 677 C→T allele includes certain adverse birth outcomes, pregnancy complications, cancers, adult cardiovascular diseases and psychiatric disorders, with several of these associations remaining still unconfirmed or controversial (Ueland et al., 2001). In addition to ethnicity, age may also negatively influence folate status. Non-Hispanic women of reproductive age with the TT genotype, who consumed low folate diets, are at greater risk for impaired folate status than women with CC genotype (Shelnutt et al., 2003). It is well known that the effect of the TT genotype on plasma homocysteine concentration is related to folate intake, the TT genotype only inducing hyper-homocysteine if associated with low intake of folate. Hyper-homocysteine may be one of these factors, and it is probably of value to determine homocysteine in the plasma of pregnant women early during the pregnancy, along with hormonal markers and to correlate plasma homocysteine concentration with the birth of DS fetuses (Vekemans et al., 2002).

Wilcken et al., (2003), in their study of comparing geographical and ethnic variation of the 677 C→T allele of MTHFR showed that homozygous TT genotype was particularly common in northern China (20%), southern Italy (26%) and Mexico (32%). The TT genotype frequency was low among the newborns of African ancestry and intermediate among newborns of American Hispanic ancestry. In southern Italy the TT genotype is common
but the rate of neural tube defects was not particularly high (Stuppia et al., 2002).

In Indian scenario, homozygosity for the C677T MTHFR SNP was detected in 1.38% (1/72) and the frequency of the C677T heterozygotes was 18.1% (13/72). The T allele frequency for C677T mutation was 0.104 (Angeline et al., 2004). Rama Devi et al. (2004) reported the 677T allele frequency to be 10.12%. Kumar et al. (2005) studied 19 Indian populations selected on the basis of their linguistics lineage and geographical location, and recorded homozygous mutant frequency as 2.16% for C677T which is much lesser when compared to Caucasian (8.9%) and Chinese (16.9%) populations.

**MTHFR A1298C**

The A→T transversion in exon 7 of MTHFR gene results in a glutamine to alanine (E429A) and is not associated with thermolability of the enzyme with no impact on plasma total homocysteine. However, it lowered the enzyme activity to 60% of the control values in individuals showing combined heterozygosity with the C677T polymorphism (Sheth and Sheth, 2003).

The prevalence of 1298 A→T polymorphism among different ethnic populations was found to vary as 9% in Canada and Netherlands and 13.8%, 17% and 41.1% in populations from Germany, China and Brazil, respectively. The frequency of compound heterozygosity was 15% in Canada, 20% in Netherlands and 17% in United States (Abu-Amero et al., 2003).
On the basis of their linguistics lineage and geographical location, 19 Indian populations selected for the study of A1298C polymorphism frequency. The homozygous mutant frequency was found to be 19.46% which is higher than the Caucasian (9.4%) and Chinese (3.3%) populations. The frequency of A1298C genotypes was 37.5% normal, 47.2% heterozygous and 15.3% homozygous mutant in another study from south India (Angeline et al., 2004). Rama Devi et al. (2004) reported the 1298C allele frequency to be 40.69%. These results show a high frequency of 1298 A→T in Indian population than 677 C→T (Kumar et al., 2005).

In addition to ethnicity, age may also negatively influence folate status. Limited data are available from Asian populations, especially Indians (Mukherjee et al., 2002; Nair et al., 2002; Angeline et al., 2004; Rama Devi et al., 2004).

ii) Methionine synthase reductase gene (MTRR)

Methionine synthase reductase (MTRR) is a related flavoprotein that maintains the methionine synthase enzyme in an active state for the remethylation of homocysteine to methionine. MTRR gene is localized at 5p15.3-5p15.2. The A66G polymorphism of the MTRR gene results in the substitution of isoleucine with methionine at codon 22. Homozygosity for 66 A→G mutation was associated with a 2.57-fold increase in the risk of DS. Because of the importance of methionine synthase reaction in maintaining normal folate metabolism and DNA methylation, MTRR gene polymorphism
was considered to be a second maternal genetic risk factor for DS (Hobbs et al., 2000).

**iii) Methionine synthase gene (MTR)**

The *MTR* gene, localized on 1q43, is maintained in its active form by methionine synthase reductase (*MTRR*). Protein binding region of the enzyme contains aspartic acid. The A2756G polymorphism of the *MTR* gene replaces aspartic acid with glycine. This polymorphism reduces the activity of the MTR enzyme and in turn, it increases the plasma folate level. Bosco et al. (2003) provide evidence that homocysteine level and *MTR* polymorphism are two potent risk factors for mothers to have a DS child in Sicily.

**iv) Cystathionine beta synthase gene (CBS)**

*CBS* gene is mapped to 21q22.3. Several polymorphisms in this gene have been reported among which the 68bp insertion at the position 844 in the coding region of exon 8 is the most common polymorphism. This insertion reduces the plasma homocysteine level due to its increased activity (Tsai et al., 1998). T2199C polymorphism in the *CBS* gene also reduces the activity of the enzyme. Fetuses and infants with DS have functional folate deficiency due to overexpression of CBS (Martinez-Frias et al., 2006).

**v) Reduced folate carrier gene (RFC 1)**

*RFC 1* gene is localized on chromosome 21q22.3. This is responsible for the internalization of 5-methylene tetrahydrofolate within the cells. A single nucleotide polymorphism 80G>A (Arg27his) has been discovered in
the RFC 1 gene by using the restriction enzyme CfoI (Chango et al. 2000). This polymorphism has an impact on folate status separately or in combination with the MTHFR 677C>T genotype. A significant increase in plasma Hcy levels was detected in doubly homozygous 80GG/677TT individuals (Chango et al. 2000). Coppède et al (2006) observed an association between reduced folate carrier gene polymorphism (80G>A) and the risk of a Down syndrome pregnancy in young Italian women.

d) α1-antitrypsin

Alpha1-protease inhibitor (PI) has a role in the regulation of chromosome behaviour during cell division. Proteolytic enzymes and their inhibitors are involved in the phenomena of nondisjunction and anaphase lag by influencing the microtubule network and spindles. Assessment of the alpha 1-antitrypsin/alpha 1-protease inhibitor (PI) types in the mothers of DS children revealed a significantly higher value of non-M PI variants particularly when only the MS and MZ types were recognized as deficiency variants (Jongbloet et al., 1981). The value was 3- to 4-fold higher than for MM homozygous women. On the other hand, a variant PI phenotype was inherited from the parent contributing the extra chromosome in four families and from the parent not contributing the extra chromosome in three families, indicating that there is no simple correlation between the PI variant and nondisjunction. Further, the increase in PI variant in Down syndrome families was independent of maternal age (Bufton et al., 1982).
2.6.4.3 Mitochondrial mutations

A wide variation in the onset and severity of dementia in DS individuals was suggested to imply the involvement of factors besides the genes on chromosome 21 in the development of Down syndrome. Mitochondrial (mt) mutations were shown to increase with age in different cells, particularly in oocytes (Keefe et al., 1995). Further, mtDNA is of maternal origin, as is the origin of the extra chromosome 21 in a vast majority of DS cases. Mitochondrial mutations are associated with Alzheimer’s disease, diabetes and hypothyroidism which have been reported to be more common in mothers of DS individuals (Schupf et al., 1994; Wallace, 1994; Narchi and Kulaylat, 1997). An etiological role of mitochondrial mutations in DS was suggested to explain the association of DS with Alzheimer’s disease, diabetes, hypothyroidism and premature aging and occurrence of free radical damage (Arbuzova et al., 2001). Mutations in mitochondrial DNA bring about an increase in the generation of free radicals and reduce ATP levels and there by could affect the synaptonemal complex, chromosome segregation and mitotic spindle, alter recombination (since the enzymes participating in recombination and DNA repair are ATP-dependent) and so could lead to aneuploidy (Arbuzova et al., 2001).

Spontaneous deletions of mtDNA (deltamtDNAs) were observed at low levels in normal heart muscle and brain from adult humans but not in the respective fetal tissues. The specific mitochondrial DNA deletion was previously found only in patients affected with certain types of neuromuscular disease (Cortopassi and Arnheim, 1990). Further, the deletion occurred at
much higher levels in nervous and muscle tissues than in all other tissues studied. The proportion of such deltamtDNAs in normal muscles increases exponentially as a function of age, with the accelerating part of the curve beginning at approximately 30-40 years (Cortopassi et al., 1992; Schon et al., 2000). It was postulated that, like muscle and brain, a similar time-dependent accumulation of deltamtDNAs may occur in normal oocytes. An accumulation of such aberrant genomes which are functionally inactive would compromise ATP-dependent energy-utilization in the somatic follicular cells that surround, and secrete important paracrine factors to the oocyte and would eventually culminate in errors in meiosis (an ATP-dependent process).

2.6.5 Nucleolus organizing regions (NORs) and acrocentric associations

One of the most interesting phenomena of human acrocentric chromosomes is their tendency to associate because of the nucleolus organizing regions (NORs) localized on their short arms. Nucleoli, which disappear during mitosis, are formed at telophase at specific chromosome sites called NORs. NORs can be strongly stained with a silver nitrate solution because a specific nucleolar protein quickly reduces the silver ions to native silver (Goodpasture and Bloom, 1975). An increased frequency of associations among these acrocentric chromosomes might predispose them to nondisjunction during cell division (Ohno et al., 1961).

The region of the nucleolus where rRNA synthesis occurs has a characteristic appearance, with a core of fibrillar nature surrounded by glandular cortex. The fibrillar core is where the rRNA is transcribed from the
DNA template, and the granular cortex is formed by the ribonucleoprotein particles into which the rRNA is assembled. The whole area is called the nucleolus (Lewin, 1997). The nucleoli are conspicuous solitary or multiple bodies visible in most interphase nuclei. The nucleolus is an unique nuclear structure because it includes the actual genes (rDNA), the transcription machinery, the transcription product (precursor rRNA) and the processing machinery producing the final 18S, 28S rRNA and 5S rRNA, synthesized outside the nucleolus, ribosomal proteins and finally the newly formed preribosomes (Schwarzacher and Wachtler, 1991).

The chromosome regions involved in the formation and maintenance of nucleoli in the interphase nuclei are called nucleolus organizing regions (NORs). As interphase progresses, the number of nucleoli decreases because nucleoli tend to fuse together into larger nucleoli, by an unknown mechanism. Nucleolar fusion attributed to increased rDNA clusters in DS, might also predispose to an increased tendency of acrocentric chromosomes to associate (Murthy, 1987). Even at metaphase there is a residual trace of this fusion called satellite association.

The presence of NORs on the short arms of all human acrocentric chromosomes and their intimate association in nucleoli has led to frequent speculation that NORs may play an important role in nondisjunction involving the acrocentric chromosomes (Hassold et al., 1987). The frequency with which a chromosome is involved in satellite associations is strongly correlated with the size of its silver-stained NOR and less strongly with its
number of rRNA genes, reflecting the importance of rRNA gene activity for nucleolar fusion (Miller et al., 1977).

The entanglement of ribosomal DNA fibers has been claimed to be a major cause of meiotic nondisjunction of the acrocentric chromosomes resulting in trisomy (Ferguson-Smith and Handmaker, 1961). It is possible that such associations arise because of the large amount of homology between the NORs and may be responsible for both somatic crossing over and meiotic nondisjunction. This also explains as to why not all of the chromosomes involved in acrocentric associations are stained with the ammonical silver method (Varley, 1971).

Polani et al. (1960) suggested that nucleolar persistence may interfere with the normal pairing process, ultimately leading to random assortment of univalent at MI. Additionally they suggested that this could be the basis for maternal age effect in trisomy 21, as nucleoli of older oocytes may be more resistant to normal breakdown process than nucleoli of younger oocytes. Persistence of nucleolus may play a role in nondisjunction leading to trisomy 21 and centric fusion resulting in Robertsonian translocations (Murthy, 1987).

The preferential involvement of active NORs in nondisjunction led to the following speculations: (i) after desynapsis of chiasmata between long arms of chromosome 21, chiasma terminalization on the short arm is delayed because of tight association of NORs, which share common ribosomal cistrons. (ii) chromosome 21 with functional NORs may have a rigid orientation and common polarity due to a common nucleoprotein matrix
which might block the separation of bivalent at anaphase, (iii) synaptonemal complexes formed in active NORs may be more rigid than those with active/inactive types of association, and (iv) the regulatory mechanism of transcriptional activity of rDNA is affected by parental age that could in turn influence nondisjunction (Verma et al., 1986). They demonstrated that the acrocentric associations of homologous and nonhomologous nature involving chromosome 21 were the most frequent in the contributing parent. It was speculated that if chromosome 21 bearing NORs have intimate physical connections with one another, the parents in whom both chromosome 21s are active (Ag-stainable) should have a higher risk of producing Down children.

Several studies have shown an increased frequency of acrocentric association among parents of DS individuals (Mattei et al., 1974; Taysi, 1975; Hansson, 1979; Jackson-Cook et al., 1985; Vishwanath et al., 1996). Contradictory reports have observed no significant difference (KrishnaMurthy and Ambani, 1981; Hassold et al., 1987). Further, one cannot extrapolate the results obtained through evaluation of acrocentric associations in blood cultures with results in germ cells in the origin of trisomy 21 (KrishnaMurthy and Ambani, 1981). Two possible mechanisms for the role of the dNOR variant (double NORs) in nondisjunction include the promotion of nucleolar persistence and the facilitation of non-homologous pairing and/or crossing over (Jackson-Cook et al., 1985; Schwartz et al., 1989).
2.6.6 Reduced genetic recombination

Warren et al. (1987) provided the first direct evidence of an association between reduced recombination and human trisomy. They showed a significant reduction in pairing and/or recombination contributed to a substantial proportion of cases of trisomy 21 in a study of cytogenetic and DNA markers in 104 DS individuals and their parents (Sherman et al., 1991).

Since bivalents are held together during MI by chiasmata a reduction in chiasma number may predispose to univalent formation and ultimately to nondisjunction (Stewart et al., 1988). The factor responsible for nondisjunction leading to trisomy 21 is (i) asynapsis (failure of normal pairing of homologous chromosomes at meiosis I) or (ii) desynapsis (premature unpairing of the homologous chromosomes after normal pairing) (Hamers et al., 1987). Studies suggested that asynapsis of the parental homologous chromosomes, rather than premature or delayed desynapsis of chiasmata between the long arm predisposed to nondisjunction (Warren et al., 1987).

More recombination events occur during meiosis in females than in males and that fewer recombination events occur near to the telomere than near to the centromere in both sexes. Recombination is eight times more likely to occur near to the centromere in females than in males. Conversely, recombination is 1.8 times more likely to occur in the most telomeric region in males than in females (Delaber, 2003).

Molecular studies have identified alterations in genetic recombination as an important predisposing factor in both age-independent and
age-dependent trisomy 21. Significant reduction in recombination has been reported in paternally and maternally derived sex chromosome trisomies and maternal trisomies of chromosomes 15, 16, 18 and 21 (Hassold and Sherman, 2000). Genetic linkage analysis of trisomy 21 families revealed alterations in levels and positioning of meiotic recombinational events. Specifically, increases in zero exchange events or in distal-only or pericentromeric exchanges were significantly increased in trisomy 21-generating meioses. These observations led to the idea that chromosome 21 nondisjunction requires 'two hits' - first, the establishment in prophase I of a 'vulnerable' bivalent and second, abnormal processing of the bivalent at metaphase I or II (Hassold and Sherman, 2000). Alterations in genetic recombination are an important correlate of nondisjunction in species with chiasmate meioses and can be of two types – i) either homologous chromosomes fail to pair and/or recombine at meiosis I, or ii) they are united by chiasmata that are suboptimally positioned. Studies on 23 cases revealed that recombination in proximal 21q was infrequent in trisomy-generating meioses and that, in a proportion recombination did not occur anywhere on 21q, thus, implying that failure to recombine is responsible for a proportion of trisomy 21 cases (Shen et al., 1998).

2.6.7 Interchromosomal effect (ICE)

Pericentric inversion could lead to increased nondisjunction in children of the parent with the inversion because of reduced chiasmata between the inverted chromosome and its homologue (Sparkes et al., 1970). The pericentric inversion of chromosome 9 is a common chromosomal variant and
is estimated to have a frequency of 1 to 3% in general population (Teo et al., 1995). This inversion has been reported to be associated with repeated spontaneous abortions, infertility and congenital malformations (Rao et al., 2006).

Thomas et al. (1992) stressed the role of interchromosomal effect (ICE) in nondisjunction by showing that parents of DS children with abnormal chromosome(s) transmit them to their offspring and those with variants are prone to nondisjunction. Stoll et al. (1978), Lindenbaum (1981) and Murthy and Prabhakaran (1990) had also proposed ICE effects due to chromosome variants and double aneuploidy in the etiology of nondisjunction in DS.

2.6.8 Disturbances in spindle structure

Ford (1984) described a mechanism of chromosome error in mothers of Down syndrome patients and concluded that they may have a condition of microtubular dysfunction which contributed to an increased rate of hyperdiploidy in all their dividing cells. It was suggested that sporadic microtubular dysfunction may occasionally be induced in otherwise “non-susceptible” individuals.

The mitotic assembly is very dynamic since the mitotic chromosomes are mechanically driven towards spindle poles by microtubules. Any disturbance with the dynamic structure can result in aneuploidy. Other factors include: (i) failure to form a bipolar spindle, formation of a monopolar spindle or spindles with more than two poles, (ii) loss of chromosomes lacking
functional kinetochores which cannot attach to the spindle. (iii) chromatid breakage or aneuploidy owing to forces acting on both the kinetochores from the two poles of the spindle resulting from chromosomes with 2 widely separated kinetochores, and (iv) instability of the spindle (Joshi, 1987).

Oocytes from mice which were heterozygous for multiple Robertsonian translocations implied that disturbances in chromosome orientation and spindle structure, rather than a failure in pairing and crossing-over between homologous chromosome arms, might be the predominant cause of nondisjunction in these cells (Eichenlaub-Ritter and Winking, 1990).

2.6.9 Maternal abortion

Buck et al. (1966) were the first to point out that “if a group at risk for non-disjunction existed then one might find an increased incidence of fetal loss in their obstetric history”. They also observed highest fetal loss in pregnancies occurring closest to Down syndrome. The relative risk to produce Down syndrome was associated with an increase in number of abortions in younger mothers and women with translocation carrier status (Hook and Cross, 1983). A positive correlation between maternal history of abortion and the birth of a DS offspring was also seen in a retrospective study of 417 DS families (Rajangam et al., 1997).

A significant proportion of aneuploid embryos and fetuses are spontaneously aborted, and many spontaneous abortions are aneuploid (Sullivan et al., 2004). Therefore, many women with prior miscarriages will have had prior aneuploid pregnancies and would appear to be at increased risk
for recurrence. Bianco et al., (2006) conducted a study to examine the association between history of spontaneous abortions and aneuploidy in a subsequent pregnancy. Women with no prior spontaneous abortions had a 1.39% risk for any aneuploidy. In women with one prior spontaneous abortion, this risk increased to 1.67%; for women with 2 previous spontaneous abortions, the risk increased to 1.84%; and for those women who had had 3 or more prior spontaneous abortions, the risk increased further to 2.18%. These findings support the hypothesis that an increased risk of aneuploidy is present in women with an increasing number of spontaneous abortions.

2.6.10 Parity

Major criticisms concerning studies on parity and Down syndrome have been related to inadequate control for maternal age, differential use of prenatal diagnosis, and inclusion of terminations of pregnancy in the analysis (Doria-Rose et al., 2003; Clementi et al., 2004). Early investigators have shown that DS tended to affect the last born in a large family (Fraser and Mitchell, 1876; Shuttleworth, 1909; Penrose, 1934; Rundle et al., 1974; Erickson, 1978). In India most Down individuals were first- or second-born (Rajangam and Thomas, 1992; Jyothy et al., 2001).

Trisomy risk was shown to increase with increase in parity (Eidelman et al., 1988; Kallen and Masback, 1988; Castilla and Paz, 1994; Kallen, 1997; Schimmel et al., 1997; Doria-Rose et al., 2003). Higher parity was associated with an increased risk of giving birth to a DS infant, both for women under
35 years of age and for those aged 35 years or more, after results were controlled for maternal age. This association was particularly strong among older women (Doria-Rose et al., 2003). However, a positive association between parity and DS was not observed in two other studies (Haddow and Palomaki, 1994; Chan et al., 1998). The positive correlation between grand multiparity and the incidence of DS was suggested to be due to a combination of two different phenomena - a decreased risk for primiparas to give birth to an infant with DS which could have a biological explanation, and an increased risk for DS in the offspring of grand multiparas which could have a social explanation (Kallen, 1997).

2.6.11 Seasonality

The month-of-birth effect of infants with Down syndrome appears to be very consistent and independent of maternal age and education, social level and year of birth (Bennet and Abroms, 1979). This was recognized by taking into account the inherent biological fluctuation of total births as a reflection of ancestral reproductive biology which is an alteration of ovulatory seasons (spring and fall) and anovulatory seasons (winter and summer) (Jongbloet and Vrieze, 1985). The DS conceptions appeared to be disproportionately more frequent during the seasonal transitions of increasing and decreasing ovulation rate, and disproportionately less during the seasons in which the ovulation rate is stabilized. This was in accordance with the seasonal Preovulatory Over-ripeness Ovopathy (SPOO) hypothesis which presumes two tightly linked phenomena in humans: (i) Ovulation is delayed during the transitions from anovulatory seasons to ovulatory seasons and vice versa, and
(ii) Pre-ovulatory over-ripeness ovopathy elicited by delayed ovulation leads to premature loss of tetrads and dyads from the maturation spindle in the oocyte, causing nondisjunction (Jongbloet et al., 1982).

2.6.12 Parental occupation

In 1977, Uchida presented correlation between radiation and nondisjunction in experimental animals. Various studies have indicated that radiation certainly increases frequency of DS. Studies of radiation-induced mitotic nondisjunction of human lymphocytes showed significantly increased susceptibility of X chromosome and chromosome 21 to abnormal segregation. These studies supported the relatively high frequency of mosaicism found in subjects with trisomy 21 and X chromosome aneuploidy (Uchida, 1981). Verger (1997) examined the epidemiologic and experimental studies into the possible role ionizing radiation might play in Down syndrome. It was prompted by a report of a temporal cluster of DS cases observed in West Berlin exactly 9 months after the radioactive cloud from Chernobyl passed. Most epidemiologic studies into trisomies and exposure to ionizing radiation examine only the first period (before the completion of the first meiosis) while the Chernobyl cluster is related to the second (around the time of ovulation). The experimental results, although sometimes contradictory, demonstrate that irradiation may induce nondisjunction in oogenesis and spermatogenesis. However, the results cannot be easily extrapolated to humans (Verger, 1997).
Chia et al. (2003) evaluated the association between the prevalence of birth defects among maternal and paternal occupation groups in Singapore for live births between January 1, 1994 and December 31, 1998. Mothers and fathers who worked as 'cleaners, labourers and related workers' appeared to have a higher risk of giving birth to children with chromosomal single birth defect, non-chromosomal single birth defects and multiple birth defects, with 'legislators, senior officers and managers' as the reference. However, there was no significant risk for overall birth defects between working and non-working parents.

2.6.13 Other predisposing factors

Maratou et al., (2000) used pulsed-field gel electrophoresis to examine the chromosome 21 alphoid DNA array lengths in 23 families with a child with trisomy 21 resulting from maternal meiosis I error, and in 38 controls. The results indicated an association between small combined alphoid (alpha21-I) size and maternal meiosis I nondisjunction. On the other hand, Vorsanova et al. (2005) reported lack of a correlation between alphoid DNA variation and non-disjunction of chromosome 21.

Amiel et al. (2000) demonstrated a relation between loss of replication control, centromere dysfunction, and predisposition to nondisjunction. Couples with a Down syndrome offspring were the high-risk probands. Replication pattern of two pairs of alleles, RB-1 and 21q22, were studied, and the rate of aneuploidy was estimated using two alpha-satellite probes of chromosomes 8 and 18. The results suggested the existence of an association
between replication timing and the rate of non-disjunction. A higher rate of allele asynchrony and aneuploidy was found in older women and in mothers of a Down syndrome offspring. These findings may reflect a predisposition for meiotic nondisjunction in these women.

Christianson et al. (2004) evaluated whether the association of socioeconomic risk factors for trisomy 21 differed by type of maternal meiotic error. They found maternal lifetime exposure to poor socioeconomic environment is a risk factor for trisomy 21, particularly if nondisjunction occurred in a maternal meiosis II.

Freeman et al. (2000) in a population-based, case-control study of Down syndrome demonstrated that women who reported surgical removal of all or part of an ovary or congenital absence of one ovary were significantly more likely to have delivered a child with DS than were women who did not report a reduced ovarian complement. It has also been observed that women who have had an ovary removed exhibit elevated levels of FSH and similar hallmarks of advanced maternal age. The findings suggested that the physiological status of the ovary is the key to the maternal-age effect. In addition, it also implied that women with a reduced ovarian complement should be offered prenatal diagnosis (Freeman et al. 2000).

A higher incidence of DS (2.75 times higher) in infants of diabetic mothers was found in a study of 1870 infants of diabetic mothers out of a total of 22,300 neonates born between January 1987 and April 1994 at an institution in Saudi Arabia, when compared with infants of non-diabetic
mothers of similar age (Narchi and Kulaylat 1997). Another study noted that 75% of mothers of infants with DS had an altered carbohydrate metabolism (Navaratte et al., 1967).

Major histocompatibility antigens are thought to play a role in maintenance of pregnancy. Increased HLA sharing between spouses has been observed in repetitive abortions (Gill, 1983; Redman, 1983). An excess of HLA sharing had been reported in parents of DS children also (Mottironi et al., 1981). These investigators found a very high number of DS parents sharing two antigens A or B (45% versus 9%). In contrast, Ayme and Lippman-Hand (1982) reported more HLA sharing at locus A or B in controls, while DS parents with the mothers under 35 years of age shared more HLA-B antigens than control parents. The tolerance in normal pregnancy is thought to be dependent on immune response to TLX antigen coded by genes linked to HLA. Although differences in the frequency of B antigens between DS families and controls were recorded, they were not significant in the study by Soubiran et al. (1985). They suggested that the excessive tolerance of older mothers towards a DS fetus might be due to previous immunization of the mother during anterior pregnancies or through early pregnancy wastage.

Vig (1984) proposed out-of-phase separation of a rare chromosome, like premature separation in mitosis of the X chromosome in elderly humans or of chromosome 18 in parents of trisomy 18 children. The suggestion is made that such out-of-phase separation results in aneuploid cell lines by total failure of the centromere to separate or by it separating too early, before
the spindle is formed. The prematurely separating centromeres, it appears, do not attach to spindle fibers and hence cause nondisjunction. Such nondisjunction in embryonic stages will produce apparently normal individuals with mosaicism in somatic and/or gametic tissue. An individual carrying mosaicism in gonadal tissue will produce a large number of abnormal gametes, one of which may have a reasonable chance of entering fertilization.

In meiosis I, exchanges provide a connection between homologous chromosome pairs that facilitates their proper attachment to the meiotic spindle. In many eukaryotes, homologous chromosomes that fail to become linked by exchanges exhibit elevated levels of meiotic errors, but they do not segregate randomly, demonstrating that mechanisms beyond exchange can promote proper meiosis I segregation. The experiments described by Kemp et al. (2004) demonstrate the existence of a meiotic centromere pairing mechanism in budding *S. cerevisiae*. This centromere pairing is DNA-sequence-independent and mediates the meiosis I bipolar spindle attachment of nonexchange chromosome pairs. It also likely plays the same role for all homologous chromosome pairs. Meiotic centromere pairing and paired centromeres separate precociously at metaphase I. The experiment also suggests that pairing orients the kinetochores of the non-exchange partners such that they are likely to encounter microtubules that radiate from opposite spindle poles. Thus the centromere pairing in meiotic chromosomes plays a role in segregation.
Keefe *et al.* (2006) proposed a unifying theory of reproductive aging, based on **telomere shortening**. Telomeres are repetitive sequences of DNA that cap chromosome ends and dissipate during divisions. Oocytes do not divide, but oogonia do, and telomerase, the enzyme responsible for maintaining telomere length, is inefficient, and remains inactive in oocytes and embryos until blastocyst stage. Telomere shortening may mediate both 'hits' involved in reproductive aging, which is late exit from the fetal production line and long interval to ovulation in the adult. As women age egg dysfunction increases, with meiotic nondisjunction, embryonic arrest, apoptosis and miscarriage. Reactive oxygen also shortens telomeres, so the prolonged interval between birth and ovulation would further shorten telomeres from chronic exposure to reactive oxygen. In support of this theory, experimental shortening of telomeres in mice produced a phenotype similar to reproductive aging in women, with abnormal chiasmata, spindles, cell cycles, apoptosis, and genomic instability (Keefe *et al.*, 2006).

A checkpoint mechanism operates at the metaphase/anaphase transition to ensure that a bipolar spindle is formed and that all the chromosomes are aligned at the spindle equator before anaphase is initiated. Since mistakes in the segregation of chromosomes during meiosis have particularly disastrous consequences, it seems likely that the meiotic cell division would be characterized by a stringent **metaphase/anaphase checkpoint**. LeMaire-Adkins *et al.* (1997) investigated if the presence of an unaligned chromosome activates the checkpoint and delays anaphase onset during mammalian female meiosis, and concluded that mammalian female meiosis lacks chromosome-
mediated checkpoint control. The lack of this control mechanism provides a biological explanation for the high incidence of meiotic nondisjunction in the human female. Furthermore, since available evidence suggests that a stringent checkpoint mechanism operates during male meiosis, the lack of a comparable checkpoint in females provides a reason for the difference in the error rate between oogenesis and spermatogenesis.

In a population-based case-control study, maternal smoking and oral contraceptive use were determined as possible risk factors in cases of trisomy 21 categorized by parent of origin and timing of the meiotic error. The odds ratio for maternal smoking was significantly increased among younger mothers while oral contraceptive use alone was not a significant risk factor. The risk was further increased with the combined use of cigarettes and oral contraceptives (Yang et al., 1999). On the other hand, Chen et al. (1999) found no clear relation between maternal smoking and the risk of Down syndrome after adequately controlling for maternal age.

2.7 RECURRENTRCE OF TRISOMY 21

The risk of recurrence of trisomy 21 in young women is between 1-2% (Hamers et al., 1987; Carothers et al., 1999). This has been attributed to parental mosaicism, structural chromosomal rearrangements and factors eliciting increased nondisjunction later such as mendelian genes or exogenous factors (Stene, 1984; Al Awadi et al., 1999). In translocation cases, when parents are neither balanced carriers, the risk is equivalent to that of trisomy 21. The risk is 1-3% for male carriers but 10-15% for female carriers. For the
21/21 translocation carriers, irrespective of the sex, the recurrence is 100% (Carothers et al., 1999).

Gair et al. (2005) described an unusual pedigree with four cases of DS with free trisomy 21 born to four separate women related through three generations of one family. The authors suggested that recurrence of trisomy in the same couple could occur due to several reasons: i) gonadal mosaicism in one or the other parent (Pangalos et al., 1992; Bruyère et al., 2000; Warburton et al., 2004), ii) chance particularly when the mother is >35 years of age, iii) mutations affecting chromosome segregation at meiosis (Hunt and Hassold, 2002), iv) variation in recombination rates (Lynn et al., 2000), v) rate of ovarian aging (Kline et al., 2000), and vi) cryptic translocations involving the pericentromeric regions of acrocentric chromosomes (Cockwell et al., 2003).

Pangalos et al., (1992) suggested that gonadal mosaicism in one or the other parent was an important etiologic factor in recurrent free trisomy 21 and that chance alone could explain the recurrent trisomy 21 in many of the remaining cases. Bruyère et al., (2000) reported a case of recurrent trisomy 21 in a couple with a child presenting trisomy 21 mosaicism and maternal uniparental disomy for chromosome 21 in the euploid cell line.

Penrose (1964) suggested that some mothers of children with DS might be mosaic in their gonads. Contribution of cryptic mosaicism to the total incidence of aneuploidy may be small, but it seems to be a strong risk factor (Joshi, 1987). In contrast, a high percentage of trisomic cells in mother’s
ovaries cannot be a complete explanation for the multiple DS recurrence (Neilsen et al., 1988). Gómez et al., (2000) carried out a population-based study on the origin of the extra chromosome 21 in 38 families with Down syndrome. The percentage of parental mosaicism was 2.7%.

**Gonadal mosaicism** for a trisomy may explain an increased recurrence risk for trisomies of the same chromosome (homotrisomy), producing a very high rate of recurrence in a small number of families (Sachs et al. 1990). Mosaicism will be underestimated by studies of parental lymphocytes (Uchida and Freeman 1985) or genetic markers (Pangalos et al. 1992; Bruyere et al. 2000), which have suggested rates of ~2% in cases of Down syndrome. In contrast, gonadal mosaicism cannot explain an increased recurrence risk for a different trisomy (heterotrisomy). On the other hand, such an increase would imply that some couples have a higher risk for meiotic nondisjunction than do others of the same age. Since most trisomy is of maternal origin, maternal factors probably would be implicated (Hassold and Hunt 2001).

Couples with a previous trisomy 21 pregnancy have a significantly recurrence risk above that expected for the maternal age (Warburton et al., 2004). It was concluded that: i) the risk of a subsequent trisomy 21, following a previous pregnancy with trisomy 21, is greater than the age-related risk for women whose first trisomy occurred at age <30 years and ii) there is a 1.6- to 1.8-fold increase in risk for a different viable trisomy, both after a previous trisomy 13, 18, or 21 and after a previous nonviable trisomy detected in a spontaneous abortion.
2.8 PARENTAL ORIGIN AND STAGE OF NONDISJUNCTION OF THE EXTRA CHROMOSOME 21

Studies of parental origin of the additional chromosome 21 and meiotic stage of nondisjunction in families with Down syndrome children and case-control surveillance of these factors are important for an understanding of the pathogenesis of chromosome 21 nondisjunction (Macek et al., 2003).

2.8.1 Chromosomal heteromorphisms

Juberg and Jones, and DeGrouchy independently in 1970 were the first to identify the maternal origin of the supernumerary chromosome 21 in the pre-banding period through examination of a Gp- chromosomal variant. Analysis of quinacrine fluorescence heteromorphisms was then employed for the first time by Lieznerski and Lindsten in 1972 to identify a first meiotic division failure in the mother. Subsequently it became apparent that nondisjunction during paternal meiosis could also result in trisomy 21 (Uchida 1973; Sasaki and Hara, 1973). A number of studies evaluating maternal and paternal meiotic failures were published (Robinson 1973; Magenis et al., 1977; Hansson and Mikkelsen 1978; Mattei et al., 1979; Mikkelsen et al., 1980; Roberts and Callow, 1980; Juberg and Mowrey, 1983).

Chromosome 21 fluorescent heteromorphisms were used to establish the parental and meiotic stage of origin in 31 cases of 42 patients as maternal in 77% and paternal in 23% cases. The error had occurred during meiosis I in 3 of 24 maternally derived cases and in 5 out of 7 cases of paternal origin
(Magenis et al., 1977). Mikkelsen et al., (1980) evaluated a larger number of
110 families and estimated the parental origin of the extra chromosome 21 to
be maternal in 80% and paternal in 20% of DS cases. Juberg and Mowrey
(1983) compiled all the available cases since 1970 and elucidated that 20% of
the cases indeed arose from spermatogenic nondisjunction. Further, the ratio
of first: second meiotic errors among the maternal cases were 80:20 and 60:40
among the paternal cases.

In a pioneering study on parental origin in DS individuals from India,
Rajangam (1994) reported 53.33% maternal and 46.67% paternal origin of
extra 21 in DS individuals. On the other hand, analysis of chromosomal
heteromorphisms revealed the meiotic error to be of maternal origin in
79.24% and paternal in 20.76% cases of Down syndrome subjects in another
study from south India, performed over a 20-year period (Jyothy et al., 2001).

2.8.2 Combined analysis

Molecular analysis using DNA markers associated with restriction
length fragment polymorphisms in the early 1980’s, further expanded the
knowledge of parental origin (Davies et al., 1984; Rudd et al., 1988; Stewart
et al., 1988). Later, short sequence repeats typed using polymerase chain
reaction amplification were employed as highly informative markers for the
study of nondisjunction in Down syndrome (Petersen et al., 1991b).

Stewart et al., (1988) using cytogenetic heteromorphisms and DNA
polymorphisms analyzed the parental origin and stage of meiotic errors
resulting in trisomy 21 in each of five nuclear families. The 16 DNA
fragments used to detect polymorphisms spanned the length of the long arm and detected recombinational events on nondisjoined chromosomes in both maternal meiosis I and II errors. This study illustrated the necessity of combining cytogenetic polymorphisms on 21p with DNA polymorphisms spanning 21q to determine (i) the parent of origin and stage of meiotic error that lead to trisomy 21 and (ii) whether an association exists between nondisjunction and meiotic recombination.

Using a conservative scoring system for Q-banding to distinguish chromosome 21 homologues and FISH heteromorphisms for repetitive sequences on 21p. Lorber et al., (1992) were able to identify the parental origin of the extra chromosome 21 in only 10% of cases. However, DNA marker studies were found to be informative for parental origin in almost all cases. In 4 of 13 cases in which the molecular studies contributed to the interpretation of the cytogenetic findings, the two results did not agree with respect to the meiotic stage of nondisjunction which was explained due to a relatively high frequency of crossing-over on either the short arm or proximal long arm of chromosome 21. A comparative study of cytogenetic heteromorphisms and seven PCR-based DNA polymorphisms at D21S215, D21S120, D21S192, IFNAR, D21S156, HMG14, and D21S171 loci was carried out by Petersen et al. (1992) in 68 cases of Down syndrome. The parental and meiotic division origin could be determined in 51% of the cases by using the cytogenetic markers and in 88% of the cases by using the DNA markers. Although there were no discrepancies between the two scoring
systems regarding parental origin, there were eight discrepancies regarding meiotic stage of nondisjunction.

Sherman et al., (1991) analyzed cytogenetic and DNA markers in 104 trisomy 21 individuals and their parents to assess the association between recombination and nondisjunction of chromosome 21. DNA marker studies were informative in 100 cases, with the majority (94) being maternal in origin. This value was significantly higher than the 75%-80% maternal nondisjunction rate typically observed in cytogenetic studies of trisomy 21 and illustrated the increased accuracy of the molecular approach. The study combining cytogenetic and molecular tools to examine for the first time the contribution of paternal nondisjunction to trisomy 21, found twice as many meiosis II paternal cases as meiosis I (Petersen et al., 1993). However, in an analysis of recombination and nondisjunction for a large paternally derived population of free trisomy 21 conceptuses (n = 67), Savage et al., (1998) observed a near 1:1 ratio of meiosis I (MI) to meiosis II (MII) errors. This contrasted with the maternal cases where an MI error was nearly three times as likely as that during MII. The authors attributed this variation to the differences between the meiotic process in males and females and/or to the different mechanisms of surveillance and segregation of susceptible tetrads.

2.8.3 DNA polymorphisms

Antonarakis et al., (1992) studied DNA polymorphisms at loci in the pericentromeric region on the long arm of chromosome 21 in 200 families with trisomy 21, in order to determine the meiotic origin of nondisjunction.
Among the 188 maternal cases, nondisjunction occurred in meiosis I in 128 cases (77.1%) and in meiosis II in 38 cases. Among the 9 paternal nondisjunction cases the error occurred in meiosis I in 2 cases and in meiosis II in 7 (77.8%) cases.

PCR amplification of short sequence repeats were used to study the parental origin of the additional chromosome 21 in 87 DS cases. The parental origin was determined in 68 cases by studying the segregation of polymorphic alleles in the nuclear families (either by scoring three different alleles in the proband or by dosage comparison of two different alleles in the proband) (Petersen et al., 1991b). Using DNA polymorphic markers on 21q, it was observed that parental mosaicism was an important etiologic factor in recurrent free trisomy 21 (5 of 22 families) and that chance could explain the recurrence in many of the remaining families (14 of 22 families). However, in a small number of families (3 of 22), a familial predisposing factor or undetected mosaicism could not be excluded (Pangalos et al., 1992).

Zaragoza et al., (1994) studied the parental and meiotic stage of origin of the additional chromosome in 432 fetuses with trisomy 13, 14, 15, 21, or 22. The proportion of cases of paternal origin was similar among the five trisomies: 12% for trisomy 13, 17% for trisomy 14, 12% for trisomy 15, 9% for trisomy 21, and 11% for trisomy 22. The stage of nondisjunction was also similar among the five trisomies, with the majority of cases of maternal origin being due to nondisjunction at meiosis I, whereas for paternally derived cases, nondisjunction occurred primarily at meiosis II.
Using highly polymorphic microsatellite DNA markers located in the pericentromeric region and along the long arm of chromosome 21, it was confirmed that in cases with maternal origin, meiosis I error was nearly three times as likely as a meiosis II error. In cases of paternal origin, a 1:1 ratio between the stages was found with a slight increase of meiosis II errors (Sherman et al., 1994; Lamb et al., 1996; Savage et al., 1998). In a study of 43 Down syndrome individuals with atrioventricular septal defects and 51 DS cases without cardiac defects Zittergruen et al., (1995), using 20 polymorphic chromosome 21-specific microsatellite markers, found no significant differences between the groups. The parental origin of the nondisjoined chromosome was maternal in 86.2% of the families. Further, a meiotic I nondisjunctional error was seen in 76.5% of maternally derived trisomies while it was a meiosis II error in 76.9% of paternally derived trisomies.

Ko et al., (1998) used fluorescence microsatellite analysis of the markers D21S1435, D21S1436, D21S1437, D21S1446, D21S156, D21S258, D21S263, D21S265 and D21S270 to study the parental origin of the supernumerary chromosome in Down syndrome. One of each pair of DNA primers was labeled with a fluorescence dye. The amplified products were subjected to electrophoresis in a semi-automated DNA sequencer and then analyzed with Genescan software. The extra chromosome 21 originated from the mother in 93% of the patients and from the father in 7% of the cases. In the largest meta-analysis study of 807 Down syndrome patients, the parental origin was maternal in 90.7%, paternal in 5.5% and mitotic in the remaining 3.8% of cases (Petersen and Mikkelsen, 2000).
Ballesta et al., (1999) reported the parental origin and the meiotic stage of non-disjunction determined from analysis of DNA polymorphisms in 139 Down syndrome patients with regular trisomy 21 and their parents. The meiotic error was maternal in 91.6% cases and paternal in 8.39% of cases. Of the maternal cases, 72.41% were due to meiosis I errors and 27.58% were due to meiosis II errors. Of the paternal cases, 45.45% were due to meiosis I and 54.54% due to meiosis II. Using nine distinct DNA polymorphisms, Muller et al. (2000) investigated the parental origin of the extra chromosome in 110 families with a prenatally diagnosed trisomy 21 fetus. Parental origin was maternal in 89.2% cases and paternal in 10.8%, when compared with studies using DNA polymorphisms conducted in liveborn trisomy 21-affected infants showing paternal origin of trisomy 21 in only 6.7% of cases (Antonarakis 1998). The increased frequency of paternal origin of nondisjunction in trisomy 21-affected fetuses could not obviously be explained by factors leading to selective loss of paternal origin fetuses (Muller et al., 2000).

A population-based study on the origin of the extra chromosome 21 in 38 families with Down syndrome offspring in El Vallès (Spain) from 1991 to 1994 showed that the origin was 88% maternal (90.6% meiosis I, 6.2% meiosis II, 3.1% maternal mosaicism), 5.6% paternal (50% meiosis I, 50% meiosis II) and 5.6% mitotic (Gómez et al., 2000). The results support the hypothesis that 'achiasmate' chromosomes may be subject to aberrant segregation regardless of maternal age. Valle et al., (2006) analyzed 20 nuclear families (parents and child with Down syndrome) using the markers D21S11, D21S1260, and D21S265 to identify the meiotic and parental origin
in children with regular trisomy 21. The error was found to have occurred during the first meiotic division in 13 cases and at the second meiotic division in the other seven. Twelve out of the 13 errors from the first group were maternal and one paternal. As in other reports, the origin of trisomy 21 in this population was mainly due to a maternal nondisjunction in M1. Twenty-two Colombian families were studied, each with one affected Down syndrome (free trisomy 21) child. A maternal origin of the trisomy was seen in approximately 90% of cases; paternal and mitotic origin shared the remaining 10% (Ramírez et al., 2007). However, differences were found in the frequency of maternal meiotic stage errors reported in this study (46.1% meiosis I and 53.9% meiosis II) and those published previously (70% meiosis I and 30% meiosis II).

DNA polymorphism analysis in 17 families with mosaic trisomy 21 probands showed that the majority of cases resulted from a trisomic zygote with mitotic loss of one chromosome (Pangalos et al., 1994). Of these cases, nine were due to errors during maternal meiosis (six meiosis I and three meiosis II) and one paternal meiosis I. The postzygotic loss of chromosome 21 seemed random.

Determining the parental origin and the stage of translocation formation (meiotic or mitotic) is of importance in understanding the mechanisms underlying the common rob(13q14q) and rob(14q21q), and rare (other) Robertsonian translocations (ROBs) (Bandyopadhyay et al., 2002). In ~50% of cases of ROBs, the rearrangements have been reported to occur de novo (Shaffer et al., 1992). Further, ~95% of the de novo cases of
rob(13q14q) and rob(14q21q) originate during maternal meiosis (Page and Shaffer, 1997). On the other hand, Shaffer et al. (1991) found a two-fold increase of paternally derived cases in a study of 12 proband with de novo rea(21q21q) using QFQ/Ag-NOR/RFLP analyses. Brahe et al. (1990) investigated parental origin in eight DS cases, inclusive of two Robertsonian translocation cases in their pilot study. The extra long arm of chromosome 21 was of maternal origin in both and the de novo 21-21 rearrangement was suggested to have originated as an isochromosome 21q. Petersen et al., (1991a) investigated eight families with DS due to de novo Robertsonian translocation rob(14q21q) and demonstrated maternal origin of extra chromosome 21 in all cases. Rajangam et al. (1997b) reported a case of DS with biparental inheritance of two der(14q21q) and a maternal origin of the extra chromosome 21. Further, the two derivative chromosomes were found to be non-identical by descent using a combination of FISH and microsatellite analyses.

Shaffer et al. (1992) used cytogenetic heteromorphisms and molecular polymorphisms to determine the parental origin in thirty de novo non-homologous Robertsonian translocations. The parent-of-origin was maternal in 86.7% based on chromosomal heteromorphisms and 92.3% from molecular analysis. Further, most de novo Robertsonian translocations ascertained through unbalanced probands with DS were of maternal origin. Published reports have provided evidence that most de novo ROBs are of maternal origin, probably forming prior to or during meiosis 1 in oogenesis (Petersen et al., 1991a; Shaffer et al., 1992; Page and Shaffer, 1997; Bandyopadhyay
et al., 2002). Haplotype analysis of 23 patients with DS and de novo rob(14q21q) showed that all translocations and all nondisjoined chromosomes 21 were maternally derived and that meiosis II nondisjunction occurred in 21 of 23 families. Berend et al. (2003) stated that these Robertsonian translocations would form when the mother was a fetus, by 7–9 months of gestation, but that meiosis II nondisjunctional event would not occur until fertilization of that particular ovum, several decades later. Thus, environmental or intrinsic factors that may influence either of these events occur at very different times during gametogenesis in human females.

2.9 MOUSE MODELS FOR DOWN SYNDROME

Individuals with trisomy 21 show several abnormalities to various extents. The variability of clinical features may be due to combined influence of heterogeneous genetic background, environmental factors and altered dosage of individual genes. Mouse genetic models have played an important role in the elucidation of the contribution of specific genes to the DS phenotype (Davisson and Costa, 1999). The common model for research on DS for over 20 years beginning in the mid-1970s was the Ts16 mouse based on similarities in pathology. Gropp and colleagues (1975) developed a breeding scheme that would produce a high frequency of mouse embryos with whole chromosome trisomies. Robertsonian translocation chromosomes were used from the wild population of Mus domesticus (Polani and Adinolfi 1980). Later, comparative mapping revealed that HSA21 shared a large region of genetic homology with MMU16. The trisomy models are advantageous in that they overexpress the orthologues of multiple genes located on HSA21,
which permits the study of how arrays of genes interact to control each other at the level of transcription (Dierssen et al. 2001). Although still used for developmental studies, this model has the disadvantages that Ts16 mice die in utero and hence, did not enable postnatal studies including aging, learning, memory, behavior, cancer development and immunological deficits. Further, Ts16 mice are not trisomic for some HSA21 genes and are at a dosage imbalance for other genes that are not implicated in the pathogenesis of DS (Davisson 2005).

Regions on human chromosome 21 were also found to be conserved within the segments of mouse chromosomes MMU 17 and MMU 10 besides MMU 16 (Fig. 4). Mice triplicated for a chromosomal segment however, survived to adulthood. Production of adult mice trisomic for specific segments was based on two facts: (a) mice heterozygous for reciprocal translocation chromosomes, where the translocation chromosomes are small, can produce progeny trisomic for the small translocation product and (b) mice trisomic for small chromosomal segments usually survive to adulthood (Dierssen et al. 2001).

In 1990, Davisson et al. created a segmental trisomic mouse called Ts65Dn [Ts(17^6)65Dn], which was trisomic for the regions β-amyloid precursor protein (App) to the myxo-virus susceptibility gene (Mx1) of MMU16 (predicted to contain at least 86 homologous genes) but lacked the remaining approximately 40% of HSA21 genes (Dierssen et al., 2001). FISH studies revealed that the Ts65Dn chromosome contains >80% of the human chromosome 21 sequences (Akeson et al., 2001). Reciprocal translocations
Fig. 4. Comparative map of the segments conserved between human chromosome 21 and mouse chromosomes

Comparative map of the segments conserved between human chromosome 21 and mouse chromosomes 10, 16 and 17 showing selected orthologous genes and the genetic extent of the trisomic segments present in Ts65Dn, Ts1Cje and Ts1Rhr mice. Arrows on the chromosomes 10 and 17 segments indicate the direction of the centromere.

Ref.: Davisson (2005), p. 106
were produced in germ cells of male mice by cesium irradiation of testes and progeny from the animals were screened for the small translocation chromosome containing the entire distal end of MMU16. Cytological analysis indicated that the extra chromosome was no more than 5% of MMU17. The mice possessed many physical, behavioural and neurological features characteristic of Down syndrome individuals (Table 4) (Patterson and Costa, 2005).

Sago et al. (1998) proposed another model of segmental trisomy for HSA21, Ts1Cje [Ts(16C-ter)1Cje]. This segment of MMU16 contained the region from disrupted Sodl to Znf295 (72 known genes). Ts1Cje mouse is a translocation between mouse chromosomes 12 and 16. Ts1Cje mice have fewer similarities to DS than do Ts65Dn mice but are important to study the particular effects of trisomy for a subset of genes triplicated in Ts65Dn mice (Antonarakis et al., 2002). The Ts1Cje mice exhibit learning deficits in the Morris water maze, perform as well as the controls in the cued or non-spatial (visible platform) part of the test, only show moderate to severe impairment in the hidden platform, probe and reverse learning parts of the test and absence of age-dependent degeneration of basal forebrain cholinergic neurons (Table 5). Subsequently, Ms1Ts65 (Ms1Cje/Ts65Dn) mice were produced that were trisomic for the genes present in Ts65Dn and missing from Ts1Cje (the segment from APP to SOD1) (Dierssen et al. 2001).

The use of transgenic techniques to model Down syndrome has led to major advances in the understanding of underlying pathogenic mechanisms of the various clinical features of this syndrome. In other words, a transgenic
Table 4. Features in people with Down syndrome and Ts65Dn mice

<table>
<thead>
<tr>
<th>Feature</th>
<th>Down syndrome</th>
<th>Ts65Dn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Learning and memory deficits that potentially implicate abnormal hippocampal function</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Learning and memory decline with age</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Altered synaptic endocytosis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Degeneration of basal forebrain cholinergic neurons</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Decreased TrkA receptors with increasing age</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Decreased hippocampal volume</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Decreased dendritic spines on cortical pyramidal cells</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>Some</td>
<td>Yes</td>
</tr>
<tr>
<td>Seizures</td>
<td>Some</td>
<td>Some</td>
</tr>
<tr>
<td>Stereotypical (repetitive) behaviours</td>
<td>Some</td>
<td>Yes</td>
</tr>
<tr>
<td>Difficulty in suppressing inappropriate behaviour</td>
<td>Some</td>
<td>Yes</td>
</tr>
<tr>
<td>Pain response</td>
<td>Altered</td>
<td>Altered</td>
</tr>
<tr>
<td>Decreased cerebellar volume</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Decreased cerebellar granule cells</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Gait abnormalities</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Growth and development</td>
<td>Slow growth, short stature and obesity</td>
<td>Lag in growth and body weight</td>
</tr>
<tr>
<td>Craniofacial dysmorphogenesis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Haematological and/or immunological abnormalities</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Male sterility</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Ovulation difficulties, shorter reproductive life</td>
<td>Small litters, shorter reproductive life</td>
</tr>
<tr>
<td>Ageing</td>
<td>Shorter life expectancy</td>
<td>Shorter life expectancy</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Likely</td>
<td>Likely</td>
</tr>
<tr>
<td>Upregulation of trisomic gene transcription</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Global transcription dysregulation</td>
<td>Yes?</td>
<td>Yes?</td>
</tr>
<tr>
<td>Brain myo-inositol levels</td>
<td>Elevated</td>
<td>Elevated</td>
</tr>
<tr>
<td>Vasooactive intestinal peptide anomalies</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Altered plasma amino acids</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Ref.: Patterson and Costa, 2005
mouse enables identification of genes related to specific pathophysiological features. The creation of multigene duplications using 'Cre-loxP' and embryonic stem cell technologies provides a bridge between aneuploids and single gene transgenic mouse model (Zheng et al., 2000). Several transgenic mice that express from one or a few genes to entire segments of HSA21 (YAC transgenic mice) have been produced (Table 5) (Antonarakis et al., 2002). Transgenic mice containing human SOD1 were the first mice to be produced (Epstein et al., 1987). Although transgenic mice can be used to understand the function of individual genes, only trisomic mouse models are suitable for modeling and characterizing a syndrome (or a part of it) and in testing therapeutic strategies (Dierssen et al., 2001).

Recently, a new strategy has been adopted by producing "transchromosomal" (trans-species aneuploid) mouse strains which carry an extra human chromosome 21 and thus proving that modelling of whole human chromosome aneuploidy syndrome is feasible in the TcI mouse. A selectable marker was placed into HSA21 and the chromosome was transferred from a human somatic cell line into mouse embryonic stem (ES) cells using irradiation microcell-mediated chromosome transfer and these 'Transchromosomal' ES cells containing different Hsa21 regions were used to create chimeric mice (Hernandez and Fisher 1999; Hernandez et al. 1999). This approach potentially reflected more closely the 3:2 dosage difference present between trisomic and disomic individuals through the introduction of an extra copy of each HSA21 gene in contrast to transgenic methods single-gene transgenics, which cannot model the complexity of Down syndrome
Table 5. Mouse models of Down syndrome

<table>
<thead>
<tr>
<th>Model</th>
<th>Genotype</th>
<th>Neurological phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Segmental trisomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ts16</td>
<td>Trisomy 16</td>
<td>Reduced brain size and some structural changes</td>
</tr>
<tr>
<td>Ts65Dn</td>
<td>Trisomic for App-Znf295 (~86 known genes)</td>
<td>Learning and behavioural deficits, degeneration of basal forebrain cholinergic neurons Reduction of the cerebellar volume and granule cell density Reduced cell number and volume in the hippocampal dentate gyrus Reduction in excitatory (asymmetric) synapses in the temporal cortex at advanced ages Age-related degeneration of basal forebrain cholinergic neurons Astrocytic hypertrophy and increased astrocyte numbers</td>
</tr>
<tr>
<td>Ts1Cje</td>
<td>Trisomic for Znf295-Sod1 (72 known genes)</td>
<td>Learning and behavioural deficits (less severe than in Ts65Dn)</td>
</tr>
<tr>
<td>Ms1Ts65</td>
<td>Trisomic for App-Sod1 (~14 known genes)</td>
<td>Learning deficits (less severe than in Ts1Cje)</td>
</tr>
<tr>
<td>Single Genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TgSod1</td>
<td>Transgenic for human SOD1</td>
<td>Learning defects</td>
</tr>
<tr>
<td>TgPfk1</td>
<td>cDNA, highly over-expressed</td>
<td>-</td>
</tr>
<tr>
<td>TgS100B</td>
<td>2-12 copies</td>
<td>Astrocytosis, neurite degeneration</td>
</tr>
<tr>
<td>TgApp</td>
<td>YAC, low copy</td>
<td>Cognitive/behavioural defects</td>
</tr>
<tr>
<td>TgEts2</td>
<td>cDNA, highly over-expressed</td>
<td>-</td>
</tr>
<tr>
<td>TgHmg14</td>
<td>2-6 copies</td>
<td>-</td>
</tr>
<tr>
<td>TgMnb</td>
<td>YAC, 1-3 copies, and cDNA, highly over-expressed</td>
<td>Learning/memory defects</td>
</tr>
<tr>
<td>TgSim2</td>
<td>BAC, 1-2 copies, and cDNA, highly over-expressed</td>
<td>Behavioural defects</td>
</tr>
</tbody>
</table>

Ref.: Antonarakis et al. (2002), p. 727
(O’Doherty et al., 2005). Additionally, the complete genomic sequence can be included, including upstream and downstream regulatory elements of unusually large genes or those with complex regulatory elements and multiple transcripts. Furthermore, as human chromosome 21 has orthologs on more than one mouse chromosome, the mice with partial trisomies cannot recreate the complexity of Down syndrome although only partially can represent it.

2.10 MOLECULAR ASPECT OF CHROMOSOME 21

Chromosome 21 is the smallest of the human autosomes constituting approximately 1.7% of the length of the haploid genome and having a genetic length of 46 cM. In physical terms, chromosome 21 is acrocentric, with two arms and its centromere close to one end. The short arm (21p) consists of the nucleolar organizer region that contains multiple copies of genes coding for ribosomal RNA and a more proximal region composed of highly repetitive DNA sequences (Epstein, 2007). Historical landmarks of research on molecular aspects of chromosome 21 include the first cloning of superoxide dismutase (SOD1) gene from chromosome 21 in 1982 (Lieman-Hurwitz et al., 1982), first disease-related gene mutation identified in 1989 (Kishimoto et al., 1989), first linkage map of 17 markers on the long arm of human chromosome 21, including six genes and two anonymous loci with a variable number of tandem repeats, in 1989 (Warren et al., 1989) and completion of the physical map in 1992 (Chumakov et al., 1992). The landmark publication of the (almost) entire sequence of 21q published on May 18, 2000 concluded the research efforts of many investigators and laboratories that studied the infrastructure of Human Chromosome 21. A total of 225 genes comprising of
127 known genes, 98 predicted genes (13 similar to known proteins, 17 anonymous ORFs featuring modular domains, and 68 anonymous transcription units with no similarity to known proteins), and 59 pseudogenes were reported to constitute 33.5 Mb of the long arm of chromosome 21. The pseudogenes were classified on the basis of lack of introns, presence of multiple in-frame stop codons, and inability of transcripts to produce a complete protein (Hattori et al., 2000).

The sequence is of high quality (error rate less than 1 in 10000 nucleotides) with only 3 cloning gaps on 21q, each of which is no more than 30-40 kb. One of these gaps was partially filled by the sequence generated by Celera Genomics (Venter et al., 2001). There are four contigs for 21q (from 21cen to 21qter) of lengths 28515, 219, 1378, and 3429 kb, accounting for a total of 33,546,361 nucleotides (Antonarakis et al., 2001). There are also 7 sequencing gaps in regions with long stretches of repeats in which it was impossible to know if the entire repeat was sequenced. A region of 281kb from 21p was also sequenced and shown to contain just one gene TPTE (transmembrane phosphatase with tensin homology) achieving 99.7% coverage of the chromosome (Antonarakis, 2001). This gene, encoding a putative tyrosine phosphatase, consists of 24 exons and spans approximately 87kb (Guipponi, 2000). This is the first description of a protein-coding gene mapping to the p arm of an acrocentric chromosome (Hattori et al., 2000).

Only approximately 3% of the entire sequence encodes for proteins and 1.3% encodes for short sequence repeats that may be polymorphic in human populations. About 10.8% of the sequence are SINE repeats, 15.5%
LINE repeats and an additional 11.7% are other human repeats (total interspersed repeats account for 38%). Gene flanking regions, introns and other DNA of unknown function constitute the remainder (Antonarakis, 2001).

Over-expression of one specific gene would be responsible for each pathological feature of DS. However, functional interactions would result in additive or even synergistic effects. In others, the positive effect of one gene might nullify the detrimental effects of other dosage-sensitive genes acting on the same pathway. Thus, the phenotypic outcome cannot be foreseen on the basis of analyzing the over-expression of single genes (de la Luna and Estivill, 2006). The study of patients with partial trisomy 21 and exhibiting several phenotypes of Down syndrome suggested that there is a region of about 2.5 Mb between DNA marker D21S17 and ERG that if triplicated, is associated with numerous features of Down syndrome. This segment of 21q22.2 is referred to as the Down Syndrome Critical Region (DSCR).

2.10.1 Down syndrome critical region

It has been presumed that several dosage-sensitive genes in a section of human chromosome 21 called the Down syndrome critical region are responsible for many of the features of this disease. The distal half of the long arm of 21 (21q22) possesses most of the gene transcribing sites of the chromosome. It was this region that was thought to contain loci essential for production of the clinical syndrome. Subsequent studies identified subregions of this band as “minimal” or “critical” sites necessary and sufficient to
produce the clinical condition (Shapiro, 1999). Since, the early 1970s numerous attempts have been made to determine whether specific segments of chromosome 21 when triplicated, are responsible for the clinical condition of DS.

The study of patients with partial trisomy and several phenotypes of DS led to the suggestion that a region of 4 Mb between DNA markers D21S17 and ETS2 that if triplicated, was associated with numerous features of DS (Antonarakis, 1998). Later, investigations indicated that a region as small as 1.6 to 2.5 Mb could contain all of the genes with a dosage imbalance that produces most of the features of DS. Current research does not exclude the possibility of the involvement of other genes mapped outside DSCR is a number of DS phenotypes (Korenberg et al., 1994), nor is there evidence currently to link dosage imbalance of a single gene with a specific feature (Reeves et al., 2001). The minimal DSCR contains several known genes including SIM2, HLCS, DSCR5, TTC3, DSCR3, DYRK1, KCNJ6, DSCR4, KCNJ15 and ERG, identified in the initial gene catalog of the 33.5 Mb HC21 sequence (Hattori et al., 2000).

A major problem with these assignments was that different investigators defined different critical regions. Molecular analysis to define the extent of triplication of regions of chromosome 21 assigned the full DS phenotype to bands distal q22.1 to proximal q22.3. The immediate consequences of an aneuploid state are a gene dosage effect of each of the loci present on the unbalanced chromosome segment. Such gene dosage effects,
amounting to the expected 1.5 times diploid levels, were reported for nine chromosome 21 loci (Villar and Epstein, 2003).

The facial features of DS may be determined by genes in the region of DNA marker D21S55→21qter (Korenberg et al., 1988, 1990; Rahmani et al., 1989). Rahmani et al. (1990) suggested that the region around D21S55, between D21S17 and ETS2 probably contain genes contributing significantly to the pathogenesis of some of the facial features. MR results from imbalance of genes mapping throughout the chromosome. Congenital heart decrease and duodenal stenosis have also defined as D21S55→21qter and D21S8→D21S15 respectively (Korenberg et al., 1989, 1990).

**Down Syndrome Critical Region Protein 1 (DSCR1),** mapped to 21q22.1-q3, encodes a protein that binds to the catalytic subunit of calcineurin, inhibiting its phosphatase activity. Over-expression of DSCR1 inhibited calcineurin-dependent gene transcription. Consequently, it is also called modulatory calcineurin-interacting protein 1 or calcipressin 1 to reflect this function. Elevated levels of DSCR1 protein have been implicated in the pathogenesis of neurodegenerative diseases such as Alzheimer’s disease and Down syndrome (Kim et al., 2006). The DSCR1 gene consists of seven exons: exons 1–4 can be alternatively spliced, resulting in four different transcripts (denoted DSCR1.1 through DSCR1.4). DSCR1.1 and DSCR1.4 are widely expressed, particularly in the central nervous system, skeletal muscle, and heart. However, DSCR1.2 has only been detected in fetal liver and brain, and DSCR1.3 has not been detected in any tissue. The DSCR1 gene has received a lot of attention, and many names have been given to its protein
products: ADAP78, MCIP1, RCN1, CBP1 and calcipressin 1. The Human Genome Organization (HUGO) initiative to rename DSCR1 as RCAN1 (regulator of calcineurin 1) is underway (de la Luna and Estivill, 2006).

**DSCR2** is located in the plasma membrane as an integral membrane protein. Possik *et al.* (2004) observed this protein in the endoplasmic reticulum of cells. DSCR2 is synthesized as a 43 kDa precursor protein, from which the N-terminus is cleaved resulting in a polypeptide of 41 kDa. The polypeptide is modified by still uncharacterized co- or post-translational modifications increasing the predicted molecular weight of 32.8 kDa by about 10 kDa. Analyses of the only putative N-glycosylation site by *in vitro* mutagenesis excluded the possibility of the contribution of N-glycosylation to this increase in molecular weight (Vesa *et al.*, 2005).

**DSCR4** gene, located on 21q22.2, consists of three exons, and the size of cDNA is 1083bp. The ORF encodes a predicted protein of 118 amino acids. Although the functional sites such as TNF ligand family like domain are expected from the predicted amino acid sequence, the function of DSCR4 is unknown. Northern hybridization and RT-PCR analysis of various human tissues showed that DSCR4 was expressed only in placenta. DSCR4 protein was found mainly in the cytoplasm. DSCR4 may contribute to the development of embryo in the placenta tissue of early pregnancy (Satoko *et al.*, 2005).

Shibuya *et al.*, (2000) isolated two novel genes, designated **DSCR5** and **DSCR6**, from the Down syndrome critical region on chromosome
21q22.2 which has been defined as minimal overlapping region of partial trisomy 21 patients of approximately 1.6 Mb. DSCR5 and DSCR6 genes consist of 6 and 5 exons, respectively. Alternative use of transcription start sites and alternative splicing events produce different RNA species and proteins from both genes. Three different transcripts of DSCR5 gene encode three putative transmembrane proteins of 158, 134, and 108 amino acids, while 4 different transcripts of DSCR6 gene encode two forms of proteins with 190 and 106 amino acids. The DSCR5 gene is expressed in various human tissues examined, whereas the DSCR6 gene is expressed only in limited tissues at low level. Both DSCR5 and DSCR6 genes are candidates for the pathogenesis of Down syndrome, although the function of these genes remains to be elucidated.

Expression levels of seven proteins whose genes are encoded on chromosome 21 – DSCR4, DSCR5, DSCR6, KIR4.2 GIRK2, KCNE1 and KCNE2 – were evaluated in fetal cortex brain of DS and controls at the early second trimester of pregnancy. DSCR5 (phosphatidyl-inositol-glycan class P protein, PIG-P), a component of glycosylphosphatidylinositol-N-acetylglucosaminytransferase (GPI-GnT), was over-expressed about two-fold, even when levels were normalized with neuron-specific enolase (NSE). DSCR6 was over-expressed in addition, but when normalised versus NSE, the levels were comparable to controls (Fernando-Miguel et al., 2004). Potassium channels KIR4.2 and GIRK2 were comparable between DS and controls, whereas KCNE1 and KCNE2 were not detectable. Quantification of these proteins revealed that not all gene products of the DS critical region are
overexpressed in DS brain early in life, indicating that the DS phenotype cannot be simply explained by the gene dosage effect hypothesis. Over-expression of PIG-P (DSCR5) may lead to or represent impaired glycosylphosphatidylinositol-N-acetylglucosaminyltransferase-mediated posttranslational modifications and subsequent anchoring of proteins to the plasma membrane (Ferrando-Miguel et al., 2004).

NFAT (nuclear factor of activated T cells) transcription factors are well-known regulators of development and normal physiology in mammals (Graef et al., 2001). Their activity is controlled by cycles of dephosphorylation and phosphorylation that determine the amount of the transcription factor in the nucleus and, therefore, the final transcriptional response. The phosphatase calcineurin is responsible, following increases in intracellular Ca2+, for the NFAT dephosphorylation step and subsequent nuclear entry. Several kinases, including glycogen synthase 3 and casein kinase 1, participate in the phosphorylation step that acts as a switch-off mechanism (Hogan et al., 2003).

The regulation of various developmental pathways and of the immune response relies on processes that are activated by the entry of calcium into the cell, and the NFAT signaling pathway mediates many of these processes. Following the influx of calcium, phosphate groups are removed from NFATc factors in the cytoplasm by the enzyme calcineurin. This allows NFATc to enter the nucleus and activate its target genes. However, once in the nucleus, NFATc can have phosphate groups added back to it by a kinase enzyme
(phosphorylation), forcing it to return to the cytoplasm and halting its effects on the genes (Fig. 5) (Epstein, 2006).

Arron et al. (2006) looked for genes in the DSCR that could regulate NFATc. They identified **DYRK1A**, which encodes a kinase, and **DSCR1**, which encodes a known inhibitor of calcineurin. The DYRK1A and DSCR1 proteins acted synergistically to block NFATc-regulated gene expression. **DSCR1** acts as a negative regulator of this pathway by virtue of its inhibitory activity on calcineurin. Dscr1 knockout mice are viable and present phenotypes that link the gene to the regulation of calcineurin–NFAT-dependent transcription programs (Ryeom et al., 2003).

The authors found that DYRK1A acts as a priming kinase that enables additional phosphorylation of NFATc by another kinase, glycogen synthase kinase 3, and leading to NFATc inactivation. Furthermore, mice genetically engineered to have increased levels of DYRK1A alone, or of both DYRK1A and DSCR1, had abnormal heart-valve development. As expected, in these mice NFATc was mostly phosphorylated and found in the cytoplasm. An increased activity of **DYRK1A** and **DSCR1** was suggested to contribute not only to mental retardation, but also to many other features of Down syndrome (Arron et al., 2006; Gwack et al., 2006).

### 2.10.2 Over-expression of some known genes on chromosome 21

The identification of all genes on chromosome 21 provides the opportunity to study the level of their over-expression (or under-expression) in different cells, tissues and at different stages of development. Furthermore,
Fig. 5. NFAT signalling and Down syndrome

Calcium signalling through the NFATc pathway mediates many developmental processes and the immune response. a. The entry of calcium ions into the cell activates the enzyme calcineurin to remove phosphate groups (P) from NFATc factors in the cytoplasm, allowing NFATc to enter the nucleus and activate its target genes. However, once in the nucleus, the NFATc can be phosphorylated, and so returns to the cytoplasm. The genes encoding DSCR1 and DYRK1A are found in the ‘Down’s syndrome critical region’ of human chromosome 21, which has an extra copy in people with Down’s syndrome. The increased expression of DSCR1 and DYRK1A disturbs the balance of NFATc phosphorylation, so that most of the protein is found in the cytoplasm. Thus, NFATc-dependent genes will not be properly regulated, which could markedly affect development.

Ref.: Epstein CJ (2006). p. 582
by using either microarray technologies or SAGE (serial analysis of gene expression) the global dysregulation of gene expression in trisomy 21 (or in model organisms) could be determined. The effects of over-expression of one gene using transfected cell lines or transgenic animals could also be evaluated at the transcription level. All of the above studies could be also performed and validated at the protein level by qualitative and quantitative analysis of proteins. These studies will provide a better understanding of the pathophysiology of DS and may reveal target molecules for therapeutic interventions of certain phenotypes (Antonarakis et al., 2002).

The precise gene catalogue of 21q is still not entirely known. The total number of protein-coding genes is estimated to be between 271 and 364 (Gardiner et al. 2003; Antonarakis et al., 2004). These estimates, which are based on computational methods, expressed sequence tags (EST) sequencing, comparative genomic analysis and laboratory verification are likely to be an underestimation because single-exon protein-coding genes and non-coding RNA molecules are difficult to identify and characterize. Recent studies of the transcriptional activity of the entire 21q suggest that the potentially transcribed genome is about ten-fold larger than the current genic annotation (Kampa et al., 2004). These findings raise the possibility that important discoveries concerning non-protein-coding RNA molecules will happen in the years to come (Antonarakis and Epstein, 2006).

The unique increased risk of Alzheimer disease-like pathology and dementia in people with Down syndrome led to the hypothesis that a gene on HSA21 must be involved in Alzheimer disease and the demonstration that
mutations in a gene that encodes the amyloid precursor protein, APP cause early-onset Alzheimer disease (Goate et al., 1991; St George-Hyslop et al., 1987). This apparent uniqueness of Alzheimer disease-like pathology in DS adults may be argued to be simply a consequence of the increased longevity of these individuals compared with those with other autosomal aneuploidies. 

**β-Amyloid precursor protein (β-APP)** is encoded by a gene mapped to 21q21.3-q22.05 and is thus triplicated and overexpressed in Down syndrome. Although the function of APP protein is not precisely known, various fragments have been shown to be associated with the promotion of cell survival, stimulation of neurite outgrowth and synaptogenesis, modulation of synaptic plasticity, regulation of cell adhesion, and neuroprotection against excitotoxic and oxidative insults (Mattson, 1997). APP gene expression is regulated during CNS development and has been found to be overexpressed in the brain of at least one fetus with DS (Capone, 2001).

**Human Erythroblastosis virus oncogene homolog-2 (ETS-2)** is mapped to 21q22.3. A four- to seven-fold higher expression of β-APP mRNA in DS as compared to controls suggested the existence of another gene(s) on HSA 21 which, directly or indirectly, further up-regulate(s) β-APP mRNA levels. Wolvetang et al. (2003a) showed that the chromosome 21 transcription factor ETS2 transactivates the β-APP gene via specific Ets binding sites in the β-APP promoter and cooperates with the transcription factor complex AP1. The authors concluded that ETS2 is a transcriptional regulator of β-APP and that overexpression of ETS2 in DS may play a role in the pathogenesis of the brain abnormalities in DS and possibly AD. Increased expression of ETS2
was found to predispose to p53-dependent apoptosis in fibroblasts, neurons and thymi of subjects with DS (Wolvetang et al., 2003b). Over expression also causes skeletal abnormalities.

Mutation of the gene for superoxide dismutase (SOD-1), mapped to 21q22.1, is associated with a familial form of amyotrophic lateral sclerosis (ALS) (Deng et al., 1993; Rosen et al., 1993). ALS is a progressive degenerative disorder of large motor neurons in the brain and spinal cord. Gene dosage effects for SOD-1 have been documented in DS. Elevations in SOD-1 activity and increased lipoperoxidation were observed in the brains of those with DS as early as 15 to 25 weeks gestation (Brooksbank and Balazs, 1984).

The gene for S100 protein (β subunit) maps to 21q22.2-22.3. The S100 protein is a 10.5kD, dimeric, zinc- and calcium-binding protein implicated in signal transduction pathways, which regulate the cell-cycle and neuronal differentiation (Kligman and Hilt, 1988). S100β mRNA and protein expression increase dramatically during postnatal maturation of the cerebellum and gene dosage effects have been documented in the brains of those with DS (Marks et al., 1996).

The SIM2 gene, one of the two human homologs of the Drosophila single-minded SIM gene, maps to 21q22.2. SIM2 coded helix-loop-helix nuclear protein has a putative transcriptional repressor activity (Ema et al., 1996). The human SIM2 and mouse Sim2 proteins are very similar and have a basic helix-loop-helix motif, two Per-Arnt-Sim (PAS) domains and HIF1α-
SIM-TRH (HST) domain. Their intron3 sequences reveal a clustering of interferon response elements, suggesting an unexpected role for this gene in interferon action. In human, SIM2 is expressed in fetal kidney and fetal neuroepithelium region in the ventricle. In murine, it is expressed in brain and has been postulated to play an important role in the development of central nervous system. The Sim2 over-expressing transgenic mice have mild impairment of learning and memory. Three copies of SIM2 may contribute to the pathogenesis of DS, particularly mental retardation (Yamaki et al., 2001).

The human homolog (MNBH/DYRK1) of the Drosophila minibrain gene maps to 21q22.1 within the Down syndrome (DS) critical region (Guimera et al., 1999). MNB codes for a novel type of serine-threonine protein kinase. Transgenic mouse models suggest that over-expression of MNB results in impaired learning and memory function (Smith et al., 1997). Its putative role in the regulation of neuronal proliferation and cell division in humans awaits further study (Capone, 2001). Both in trisomic mice and in DS subjects, the brain levels of DYRK1A protein were increased approximately 1.5-fold, indicating that this protein is overexpressed in gene dosage-dependent manner. MNBH was over-expressed 1.5-fold in DS brains and Dyrk1 about 2.1-fold in the brains of the Ts65Dn mice (Guimera et al., 1999). Over-expression of DYRK1A encoding dual-specificity Tyrosine-Y-phosphorylation-regulated kinase can potentially contribute to AD-like pathogenesis and cognitive deficits in DS (Dowjat et al., 2007; Park et al., 2007).
The human gene coding for the trifunctional protein **glycinamide ribonucleotide synthetase (GARS)**, **aminoimidazole ribonucleotide synthetase (AIRS)** and **glycinamide ribonucleotide formyltransferase (GART)** maps to 21q22.1. The GARS-AIRS-GART protein complex catalyzes the second, third and fifth steps respectively in *de novo* purine synthesis (Kan *et al.*, 1993). The GARS-AIRS-GART complex is normally expressed during prenatal development of the human cerebellum, but in the brain of those with DS, over-expression continues for several months postnatally (Brodsky *et al.*, 1997).

The **DS cell-adhesion molecule (DSCAM)** gene maps to 21q22.2-q22.3. DSCAM is expressed in all regions of the brain and in cells of neural crest origin (Yamakawa *et al.*, 1998). Its role in the neuropathogenesis of DS is unknown (Capone, 2001). Of the potential candidate genes in the narrowed DS-CHD region, **DSCAM** is notable in that it encodes a cell adhesion molecule and is expressed in the heart during cardiac development. Barlow *et al.* (2001) proposed DSCAM as a candidate for DS-CHD.

**Cystathionine β-synthase (CBS)** gene (localized to 21q22.3) is one of the 3 enzymes able to produce hydrogen sulfide. The human CBS gene product is a 63KDa polypeptide. CBS gene spans over 30kb and consists of 23 exons ranging in size from 42–209bp. Elevated CBS expression in DS results in low plasma homocysteine and has been suggested to contribute to decreased atherosclerosis (Ge *et al.*, 2001). CBS is over expressed in DS with levels 166% of normal values in fibroblast and 1200% in myeloblast from DS children with AML (Taub *et al.*, 2000). This endogenous over production of
hydrogen sulfide is potentially able to induce some of the clinical signs of DS such as hypotonia and mental retardation (Kamoun et al., 2003).

Expression of COL6A1 mRNA from the transgene was copy number-dependent, and the increased gene dosage correlated with increased production of collagen VI alpha 1 (COL6A1) in skin and heart of transgenic mice with BAC containing the human COL6A1 gene. COL6A1 maps to 21q22.3 and this gene is a candidate for contributing to cardiac defects and skin abnormalities in Down syndrome (Xing et al., 2007).

α-crystallin A chain (CRYA-1) is localized at 21q22.3. Overexpression of CRYA-1 causes cataract. Hawkins et al. (1987) suggested that juvenile cataract of Down syndrome may be related to trisomy of the CRYA1 gene.

Sanchez-Font et al. (2003) demonstrated for the first time a direct link between dosage imbalance of a chromosome 21 gene and altered expression of a downstream gene mapped to another chromosome. The human PREP1 (Pbx regulating protein 1) ortholog, named PKNOXI (Pbx/knotted homeobox 1), is mapped to 21q22.3. PKNOXI was shown to bind to Pbx/POU site at the FABP7 promoter and thus, to regulate FABP7 transcription. The gene encoding fatty acid-binding protein 7 (FABP7) is mapped to 6q22-23 and most family members show dual cytoplasmic and nuclear localization. FABP7 is expressed in radial glial cells and immature astrocytes of the developing central nervous system and later becomes restricted to the glia limitans, radial glial cells of the hippocampus and Bergmann glial cells in the adult mouse
brain. DS brain abnormalities include functional disturbances in glial cells, delayed myelination and diminished neuronal density in the brain cortex. This over-expression (1.63-fold increase) of *FABP7* in DS brain may contribute to the DS-associated neurological disorders (Sanchez-Font *et al.* 2003).

Bahn *et al.* (2002) investigated abnormalities in gene expression in human neuronal stem cells and progenitor cells from DS and control post-mortem fetal tissue. Differential display PCR analysis showed that *SCG10* - a neuron-specific growth-associated protein regulated by the neuron-restrictive silencer factor REST - was almost undetectable in DS sample. Other REST-regulated genes such as *L1*, synapsin and β4 tubulin were also downregulated in cells from DS fetuses. These findings suggested a link between dysregulation of the REST transcription factor and some of the neurological deficits seen in Down syndrome (Bahn *et al.* 2002).

**GATA1**, localized on X chromosome, encodes a zinc-finger transcription factor that has been shown to be critical for normal erythroid and megakaryocytic development. GATA1 is mutated in the majority of DS patients with transient myeloproliferative disorders (Groet *et al.*, 2003). The presence of GATA1 mutations in a congenital disorder imply that they are acquired *in utero*, and suggest that cooperation between increased dosage of a gene(s) on chromosome 21 and mutations in exon 2 of GATA1 is associated with the prenatal initiation of the clonal proliferation of megakaryocytic precursors (Rainis *et al.*, 2003). The authors suggest that the cooperation between these GATA1 mutations and trisomy 21 are insufficient for
progression to acute megakaryoblastic leukemia as patients with transient myeloid disorder usually achieve remission.

Gulessrian et al. (2002) studied aberrant expression of **centractin** and **capping proteins** in fetal DS brain. Centractin α, F-actin capping protein α-1, α-2 and β subunits were significantly reduced in fetal DS cortex. Deranged centractins and F-actin capping proteins may represent or induce deficient axonal transport and contribute to deterioration of the cytoskeleton's mitotic functions in trisomy 21. The authors concluded that the chromosomal imbalance by trisomy 21 may be the underlying mechanism for the decrease of centractins and F-actin capping proteins rather than neuronal or glial loss in the fetal brain with DS (Gulessrian et al., 2002).

**Interferon (α, β and ω) receptor 1 (IFNAR1)**, located at 21q22.1, is involved in binding the >15 interferon (IFN) ligands whose genes are located on human chromosome 9. The type I interferon system has been thought to function as antiviral, antiproliferative, differentiation-modulating and natural killer cell-stimulating agents. The type I IFN system has also been proposed to function in embryonic development on the basis of its growth regulatory activities and regulated temporal expression of its components during embryonic development (Kola and Hertzog, 1997).

DS may be understood best as a syndrome complex of genetic and epigenetic origin with protean neurobiologic consequences and several characteristic neurodevelopmental manifestations. To advance our understanding of this biologically complex condition, and toward the
development of novel therapeutic approaches, clinician-scientists must be able to integrate information from many disparate disciplines, including molecular genetics, developmental biology, and the neurosciences. Throughout this century, distinct genetic, neurobiologic, metabolic, developmental, and medical models of DS have evolved, each having its own set of principles, pedagogy, and practices that has produced a "separate definition of reality" among basic scientists, health care practitioners, and parents, as well as an array of treatment options both real and imagined (Capone, 2001).

2.11 PRENATAL DIAGNOSIS

Techniques used in making definitive prenatal diagnoses include amniocentesis, cordocentesis and chorionic villus sampling. If all pregnant women, 35 years or older chose to have amniocentesis, about 30 percent of trisomy 21 pregnancies would be detected (Wald et al., 1988). Second-trimester amniocentesis has been used the most extensively, and the safety of this technique continues to improve as technical advances have occurred. Chorionic villi sampling offers the opportunity for first-trimester diagnosis, when elective pregnancy termination carries the lowest risk of maternal morbidity, as compared with the risk in the second and third trimesters. Early amniocentesis offers a similar advantage, but the fetal loss rate associated with this technique is higher than that of chorionic villus sampling (Khoshnood et al., 2006).
Prenatal testing designed to detect congenital malformations, in particular Down syndrome has progressed considerably over the past three decades. Prenatal screening techniques for Down syndrome include assessment of ultrasonographic markers, especially measurement of nuchal translucency in the first trimester of pregnancy and maternal serum screening during both the first and second trimesters (Khoshnood et al., 2006). Table 6 lists a few of the frequently encountered ultrasonographic findings associated with fetal Down syndrome.

Maternal serum screening (multiple-marker screening) allows the detection of trisomy 21 pregnancies in women. Alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG) and unconjugated Estriol are the serum markers most widely used to screen for Down syndrome (Palomaki et al., 1997). The "triple test" or "triple screen" which is a combination of these assays was found earlier to detect 60 percent of trisomy 21 pregnancies (Wald et al., 1988). An estimate of gestational age by ultrasound examination further improves the performance of the triple test. The use of ultrasound raised the sensitivity of the triple test from 60 percent to 74 percent and decreased the initial false-positive rate from 9 percent to 5 percent (Benn et al., 1997).

*Lens culinaris* agglutinin reactive AFP (AFP-L3) was significantly higher in women who were pregnant with a fetus with Down syndrome (Yamamoto et al., 2001; Azuma et al., 2002). Further, Yamamoto et al. (2004) suggested that AFP-L3 MoM (multiples of the median) should be considered as an effective replacement for AFP-L3% in prenatal trisomy 21 screening. The level of inhibin A, a protein secreted by the ovary, was also
Table 6. Ultrasonographic Findings Associated with Fetal Down Syndrome

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<tr>
<th>Condition</th>
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<tr>
<td>Intrauterine growth restriction</td>
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<td>Mild cerebral ventriculomegaly</td>
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<td>Choroid plexus cysts</td>
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<td>Increased nuchal fold thickness</td>
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<td>Cystic hygromas</td>
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<td>Echogenic intracardiac foci</td>
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<td>Congenital heart defects</td>
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<td>Increased intestinal echogenicity</td>
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<tr>
<td>Duodenal atresia (&quot;double-bubble sign&quot;)</td>
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<td>Renal pelvis dilation</td>
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<td>Shortened humerus and femur</td>
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<td>Increased iliac wing angle</td>
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<tr>
<td>Incurving (clinodactyly) and hypoplasia of the fifth finger</td>
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<tr>
<td>Increased space between first and second toes</td>
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<td>Two-vessel umbilical cord</td>
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Ref.: Newberger. 2000
found to be increased in the blood of mothers of fetuses with Down syndrome (Canick et al., 2003). This protein inhibits the production of the hormone FSH by the pituitary gland.

The measurement of subcutaneous oedema in the fetal neck, visualised by ultrasonography as nuchal translucency, has been studied in combination with new biochemical markers as a potentially useful first-trimester screening test for trisomy 21. The estimated trisomy 21 risk, from maternal age and fetal nuchal-translucency thickness, was 1 in 300 or higher in 7907 (8.3%) of 95476 normal and 268 (82.2%) of 326 trisomy 21 pregnancies (Snijders et al. 1998). Combining this procedure with measurement of maternal serum free beta-hCG subunit and pregnancy-associated plasma protein A (PAPP A) could increase the detection rate to 80 percent, with a 5 percent false-positive rate (Chitty, 1998).

Definitive prenatal diagnosis of trisomy 21 requires cytogenetic analysis of cells obtained by one of three invasive procedures. Fluorescent in situ hybridization technique allows rapid diagnosis of trisomy 21 after amniocentesis (Newberger. 2000). Recently, a noninvasive method of prenatal diagnosis involving molecular detection of fetal DNA in maternal blood was introduced (Lee et al., 2002). Dennis Lo et al. (2007) outlined nonpolymorphism-based method using digital PCR for the noninvasive prenatal detection of fetal trisomy 21. Analysis of fetal nucleic acids in maternal plasma by digital relative chromosome dosage method involves the direct assessment of whether the total copy number of chromosome 21 in a fetal DNA sample is overrepresented with respect to a reference chromosome.
2.12 MANAGEMENT

In the management of patients with Down syndrome, all primary care teams are faced with the challenges of the condition. Nothing can be done about the underlying impairment but the associated disabilities require active management if serious handicaps are to be minimized (Howells, 1989).

A well organized clinical trial was carried out in Chicago to determine whether nutritional supplements improve the level of intellectual functioning. The results demonstrated that the use of a combination of nutritional supplements in school-aged children with Down syndrome did not lead to improvements in intellectual test performance (Smith et al., 1983; 1984). Although there is no specific evidence that megavitamin therapy has a place in management of DS patients, there is a great deal of anecdotal evidence that their general health is often dependent on vitamin and mineral supplementation. It would seem reasonable to provide vitamins A, B, C and D together with a zinc supplement (Howells, 1989).

Mental handicap is the most critical of all the disabilities in Down syndrome. There is growing evidence to support the use of early central cholinergic enhancement to improve cognitive functioning in these individuals (Spiridiglioizzi et al., 2007). Presenile dementia is more common for DS patients surviving to the age of 40 years and the incidence of Alzheimer's disease is high (Burger and Vogel, 1973; Wisniewski et al., 1985). Acetyl cholinesterase inhibitors may help in reversing the symptoms of dementia during early and middle stages of cognitive decline (Lott et al.,

Many infants with DS have co-occurring congenital malformations requiring intensive surgical and medical management (Cleves et al., 2007). A reduction in the recurrence rate of isolated cleft lip with or without cleft palate after periconceptional supplementation with a multivitamin including a very high dose (10mg) of folic acid was noticed (Czeizel et al., 1999).

The ideal timing and optimal management of surgical repair for isolated complete atrioventricular septal defect still remains controversial. Pulmonary vascular obstructive disease (PVOD) is also observed among Down syndrome children. In order to avoid progression to irreversible PVOD, surgical intervention within four months of birth was suggested to be appropriate in such patients (Kobayashi et al., 2007).

Among children, the most commonly encountered problem is middle ear effusion associated with a flat tympanogram and conductive hearing loss. Owing to the chronic nature of the middle ear effusion, antibiotic therapy is not usually helpful (Howells, 1989). Post aural hearing aids can be worn by such patients. Virtually all adults and children with Down syndrome suffer from problems relating to the eyes like myopia, hypermetropia and astigmatism but fortunately many of these are amenable to treatment.

Type 1 insulin-dependent, diabetes mellitus (Type 1 DM) is thought to be more prevalent in individuals with Down syndrome. Glycaemic control was therefore necessary with the frequent use of simple insulin regimens,
which may relate to the more stable lifestyle of these patients (Anwar et al., 1998).

Individuals with DS develop obesity as they have reduced resting metabolic rates. Hence, diet should be planned to favour nutrient-rich foods that are high in fibre, and low in calories and fat. Special attention should be paid for the intake of calcium and vitamin D, since DS adults have lower bone density. Regular dental care should be given to prevent periodontal disease (Table 7) (Roizen and Patterson, 2003).

Complications such as hypothyroidism, celiac disease, and obesity occur more frequently in adults with Down syndrome. These individuals are predisposed to a variety of medical conditions which can impose an additional, but preventable, burden of secondary disability. Although there are guidelines for health checks and medical management of children with DS, the needs of adults are relatively neglected (Henderson et al., 2007).

Cognitive and language disabilities may predispose this population to unwanted pregnancy, sexually transmitted disease, and sexual exploitation. Sex education tailored to cognitive level, learning style, and living arrangements is essential to the education of children and young adults with DS (Van Dyke et al., 1995).

Menstruation for girls with Down syndrome is no different than for their peers in the general population. While it is recommended that all females with Down syndrome have a baseline pelvic examination and pap smear between 17 and 20 years of age, this recommendation is infrequently
## Table 7. Management of Down syndrome

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<th>Evaluation</th>
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<td>Ophthalmological assessment</td>
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<td>Hearing assessment</td>
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<td>Atlantoaxial subluxation</td>
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<td>Diabetes mellitus</td>
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<td>Obstructive sleep apnea</td>
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<td>Seizures</td>
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<th>Other</th>
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<td>Dermatological problems</td>
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<td>Behaviour problems</td>
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**Ref.**: Roizen and Patterson (2003), p. 1287
followed. Men with Down syndrome need to learn testicular self-examination as their cognitive level permits; likewise, women need to learn breast self-examination and the necessity for regular gynecological care (Elkins et al., 1987; Doty, 1995). Health care providers and professionals need to initiate structured, nonjudgmental discussion of contraception and to provide clear information tailored to patient developmental levels (Grant, 1995).

Individuals with Down syndrome need individualized instruction and education to develop appropriate socio-sexual behaviors. Education and counseling to prevent unplanned pregnancy, abuse, and sexually transmitted disease should be part of the routine medical care and education for these individuals (Van Dyke et al., 1995). They must be taught the boundaries of normal physical interactions in the social sphere, as well as the self-assertion skills to enlist help if necessary.

Genetic counseling involves a complex interaction of social, medical, and psychological factors. It is not enough to present information about the genetic aspects and risks of the situation. A competent and effective genetic counselor must recognize and deal with the psychological defense mechanisms which affected persons and parents of affected children use to cope with the strain of genetic disease in the family. Parents raising a child with significant developmental challenges are profoundly aware of the often sustained impact of that child's special needs upon their other children. Supported by recent research on siblings of developmentally challenged children, clinicians are advocating family-based interventions that take into account the needs of siblings (Schuntermann, 2007).