Introduction
1. INTRODUCTION

Aneuploidy is the most frequently observed chromosomal abnormality in human liveborns, abortuses, blastomeres and oocytes. Down syndrome (DS) or trisomy 21 is the most common genetic cause of moderate mental retardation that is accompanied by many other specific and complex phenotypes. Down syndrome was first distinctively and comprehensively described in clinical terms by John Langdon Down in 1866, but its cause remained a deep mystery for almost a century. Although it was recognized as early as the 1930s that a chromosome abnormality could explain this condition, it was only in 1959 that Jerome Lejeune and colleagues could demonstrate that Down syndrome was the result of an extra chromosome 21 with the help of improved cytogenetic methods.

Trisomy 21 is presently one of the most intensively studied human aneuploid conditions. Down syndrome has always been of special interest to the human geneticists, perhaps because of its historical perspective but, more important, owing to the strong correlation between the birth of a DS child, with advanced maternal age and increased meiotic nondisjunction, thereby providing an interesting paradigm for the investigation of chromosomal anomalies (Hassold, 1985).

Down syndrome, the most common autosomal aneuploidy in humans, has an incidence of 1 in 700 neonates. It is one of the few autosomal trisomies that survive to term, although 80% of conceptuses with trisomy 21 are spontaneously aborted (Hook, 1995). In general, sex ratio is skewed toward
an excess of males both in populations with a high level of case ascertainment (epidemiological studies) and in selected groups (Kovaleva, 2002). The overall sex ratio was reported to be 1.23:1 and there was also an excess of associated male sex chromosomal aneuploidy (Mutton et al., 1996).

Trisomy for chromosome 21 has profound effects on development resulting in a constellation of phenotypes. Virtually, all the major organ systems of the human body are affected in some degree in a very consistent fashion (Grech, 2001). DS appears to be associated with more than 80 clinical manifestations, including typical facial features, anomalies of the intestinal tract, muscular hypotonia, and increased risk for leukemia, congenital heart defect and mental retardation (Hassold and Jacobs, 1984; Epstein, 1995). Despite a vast amount of knowledge gained on the pathology, biochemistry and physiology of DS, it is still not known how the individual genes on chromosome 21, either singly or in combination, produce the abnormal phenotypic features characteristic of the syndrome.

A variety of abnormal karyotypes such as free trisomy 21, Robertsonian translocations, isochromosome 21, mosaicism, partial trisomy, double trisomies, and tandem translocations have been reported in individuals with Down syndrome. In general, Down syndrome is basically categorized into three groups based on cytogenetic analysis, namely free trisomy 21, Robertsonian translocation and mosaicism. Trisomy 21 accounts for over 95% of the cases while about 2-4% result from translocations and 1-2% due to mosaicism (Nussbaum et al., 2001).
Molecular analysis of individuals who have partial trisomy 21 with or without the characteristic Down syndrome phenotype, has facilitated mapping of the chromosomal region, which if triplicated, results in the phenotypic features of Down syndrome (McCormick et al., 1989a; Rahmani et al., 1989; Korenberg et al., 1990; Delabar et al., 1993; Korenberg, 1993). Delabar et al. (1993) identified an area of approximately 5 Mb between loci D21S58 and D21S42 to be associated with mental retardation and most of the facial features of the syndrome. In particular, a sub-region that includes D21S55 and MX1, located in band 21q22.3, has been associated with mental retardation and several specific morphologic features including oblique eye fissure, epicanthal fold, flat nasal bridge, protruding tongue, short broad hands, clinodactyly of the fifth finger, gap between first and second toes, hypotonia, short stature, brushfield spots, and characteristic dermatoglyphic pattern. Additional phenotypic traits may map outside the minimum critical region.

The landmark publication of the (almost) entire sequence of the long arm of chromosome 21 published on May 18, 2000 (Hattori et al., 2000) concluded the research efforts of many investigators and laboratories that studied the infrastructure of human chromosome 21. A total of 225 genes, of which 127 are known genes, 98 predicted genes and 59 pseudogenes were reported to comprise within 33.5 Mb of 21q (Gardiner and Davisson, 2000). Cloning of genes on chromosome 21 within the critical region for DS has greatly contributed to an understanding of the basis of these clinical features. In fact, a few candidate genes have been proposed to play a pathogenetic role in Down syndrome (Ferrando-Miguel et al., 2004). Several mouse models are
available which have served as important tools for understanding the cellular, biochemical and molecular bases for the various features of DS (reviewed by Davisson and Costa, 1999; Dierssen et al., 2001; Antonarakis and Epstein, 2006).

Trisomy 21 is a consequence of nondisjunction which is the failure of a pair of homologous chromosomes to disjoin during meiosis I or two chromatids of a chromosome to disjoin during meiosis II or mitosis. Despite years of intensive study, an advanced maternal age remains the only factor indisputably linked to nondisjunction resulting in human aneuploidy. A large number of other genetic or environmental risk factors have been suggested, including allelic combinations at specific loci, the presence of certain types of chromosome heteromorphisms, acrocentric associations, consanguinity, reduced recombination, parental irradiation, oral contraceptives and fertility drugs, thyroid antibodies, alcohol consumption, seasonality, parity and maternal diabetes. Altered genetic recombination has been identified as the first molecular correlate of chromosome nondisjunction in both humans and model organisms. However, none of these or any other associations have been proven. Thus, analyses of putative aneuploidy-inducing agents would profit from knowledge of the parent and meiotic/mitotic stage of the origin of trisomy (Hassold and Hunt, 2001). On the other hand, recurrent aneuploidy was suggested to be due to gonadal mosaicism, parental translocation and/or structural rearrangements (Al-Awadi et al., 1999).

An increased frequency of associations among acrocentric chromosomes bearing nucleolus organizing regions (NORs) was proposed to
predispose them to nondisjunction during cell division (Ohno et al., 1961). NOR-related nondisjunction results from an error in chromosome pairing or separation due to physical proximity of acrocentric chromosomes in nucleoli (Mirre et al., 1980) or recombination involving the short arm of non-homologous acrocentric chromosomes (Schmickel et al., 1985). The causal relationship between NOR heteromorphism and nondisjunction has not been established unequivocally (Mattei et al., 1974; Hansson, 1979; Krishnamurthy and Ambani, 1981; Jackson-Cook et al., 1985; Hassold et al., 1987).

Chronic folate/methyl deficiency in vivo and in vitro has been associated with abnormal DNA methylation, DNA strand breaks and altered chromosome segregation. Recent studies have linked the increased frequency of polymorphism of methylenetetrahydrofolate reductase (MTHFR, C677T) and methionine synthase gene (MTRR, A66G) in mothers with DS child. Based on evidence that abnormal folate and methyl metabolism can lead to DNA hypomethylation and abnormal chromosomal segregation, researchers have observed that mothers with polymorphisms in MTHFR and MTRR genes have elevated levels of plasma homocysteine (James et al., 1999; Hobbs et al., 2000). Several studies with conflicting results have also been published (Stuppia et al., 2002; Yanamandra et al., 2003; Coppede et al., 2006).

Nondisjunction in trisomy 21 has traditionally been studied by cytogenetic heteromorphisms. Over the past several years an increased accuracy of detection of the parent and meiotic stage of origin of nondisjunction has been demonstrated by DNA polymorphism analysis. These studies have revealed that the vast majority of cases arise due to errors
in the egg, as nearly as 90% of cases possess an additional maternal chromosome. The rest are due to paternal and post-zygotic nondisjunctional errors (Hassold and Sherman. 2000). Further, the ratio of maternal errors scored as meiosis I compared with meiosis II is 3:1 and 1:1 in paternal cases.

It is only recently that it has become possible to understand the underlying pathogenetic mechanisms and to apply this knowledge to device approaches for the prevention and treatment of the numerous features of this syndrome. The earlier pessimism was largely due to: (i) the nature of the genetic defect that leads to the syndrome. (ii) the multiplicity of systems involved, and (iii) the high degree of variability of the phenotype (Antonarakis and Epstein. 2006).

In the light of the above reports, the present study was therefore designed to ascertain the role of potential epidemiologic (questionnaire-based), cytogenetic and molecular risk factors in the etiology of nondisjunction in Down syndrome.

The objectives of the study are the following:

1. To determine the constitutional karyotype in referred cases of Down syndrome to confirm the diagnosis to aid in further management of the condition.

2. To document the clinical features recorded in the different chromosomal types of Down syndrome patients and to correlate the phenotype with the abnormal karyotype,
3. To ascertain the origin of Robertsonian translocations/chromosomal rearrangements by investigating the parents and thus, to determine the recurrence risk for genetic counseling.

4. To examine the possible association of a few epidemiologic factors in the etiology of nondisjunction.

5. To determine the frequency of acrocentric associations in the parents as a risk factor for nondisjunction employing GTG- and AgNOR-GTG banding methods.

6. To elucidate the role of C677T and A1298C polymorphisms in methylenetetra-hydrofolate reductase (MTHFR) gene in the etiology of nondisjunction. and

7. To determine the parental origin and meiotic stage of nondisjunction of the extra chromosome 21 using cytogenetic and molecular polymorphisms.