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Etiology of Down Syndrome: Risk of Advanced Maternal Age and Altered Meiotic Recombination for Chromosome 21 Nondisjunction

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1. Introduction

Down Syndrome (DS) is the most frequent live born aneuploidy and recognizable form of mental retardation among all the ethnic groups of human population across the globe. The overwhelming majority of this birth defect is caused by trisomy 21 due to nondisjunction (NDJ), i.e., failure of chromosomes to separate properly during meiosis at parental gametogenesis and the fact was initially reported by Lejeune et al. (1959). Since that time attempts were made to explore the etiologic factors that are associated with the underlying mechanism of NDJ of chromosome 21(Ch21). Like that of other autosomal aneuploidy, the errors during maternal oogenesis accounts for about 90% of DS births (Antonarakis, 1991; Freeman et al. 2007), of which majority occurs at first meiotic division(MI) (Antonarakis et al. 1992; Yoon et al. 1996). In searching the maternal risk factors for DS birth, researchers have identified advanced maternal age (Hassold and Chiu, 1985) and altered meiotic recombination (Warren et al. 1987; Sherman et al. 1991) as two strong correlates associated with underlying mechanism of Ch21 NDJ in oocyte and the risk factors are preferentially present in oocyte due to its mode of development in the lifetime of women.

The meiosis in fetal ovary initiates at about 11-12 weeks of gestation (Gondos et al. 1986) and becomes arrested at late prophase I following pairing, synopsis and recombination. The process resumes at the onset of puberty after the follicle receives proper hormonal signal and immediately completes the MI and progress through metaphase of meiosis II (MII) where it pauses until it is fertilized and the meiosis is then completed. Thus the individual oocyte remains arrested in prophase I for 10 to 50 years, depending on the time of ovulation in reproductive life. This protracted event of oocyte growth includes three distinct error prone phases (Hassold et al., 2007). First, the prophase event in fetal ovary, at which change in usual pattern of recombination might lead to subsequent aneuploid oocyte formation. The second risk prone phase is the follicular growth during which the meiosis remains arrested and the genetic and environmental challenges get chance to accumulate in ovarian milieu. The third and the final risk phase is the maturation of oocyte which is associated with the adverse effect of advancing maternal age on protein components involved in
chromosome separation system and rapidly deteriorating endocrine environments. In contrast, spermatogenesis begins at puberty and spermatogonial cells complete both MI and MII without any delay (Sherman et al. 2007).

As mentioned earlier, the overwhelming majority of Ch21 NDJ is maternal in origin among all the ethnic varieties of human population studied to date. Based on results from the US (Allen et al. 2009) and other population-based studies (Mikkelsen et al., 1995; Gomez et al., 2000), it has now been estimated that over 90% of NDJ errors leading to trisomy 21 arise in the oocyte and the majority of those occur at MI.

We carried out similar study on Indian trisomy 21 samples, particularly from eastern part of the country and obtained strong replication of those observations (Table 1). This study was started from the year 2001 and till date we included about 400 families having free trisomy 21 child. Our STR (short tandem repeat)-PCR analyses estimated over 88% maternal errors with majority of cases (~77%) having NDJ events at MI. The paternal errors account for about 10% with almost equal distribution of MI and MII NDJ events. The post zygotic mitotic error was estimated about 2%. Very concordant results were also reported for Ukraine and Russian cohorts (Machatkova et al. 2005), and Spanish cohort (Gomez et al. 2000). Little difference among these datasets that does exist is probably due to sampling variation. In this article we discuss the maternal stress factors responsible for the origin of nondisjunction.

2. Effect of advanced maternal age

The effect of ‘maternal age’ remains as ‘black-box’ for DS birth. Initially Penrose identified that advanced maternal age as risk for DS birth (Penrose 1933, 1934) and postulated that the maternal age dependent increase in birth rate of DS is in some way associated with the NDJ mechanism. But this effect is restricted only to NDJ that occur in the oocyte (Antonarakis et al., 1992; Ballesta et al., 1999; Muller et al., 2000; Sherman et al., 2005). That is, adverse effect of advanced maternal age is not evident among mothers whose offspring received an extra copy of chromosome 21 as a result of: (1) a NDJ error in spermatogenesis i.e., paternal errors (Yoon et al., 1996; Sherman et al., 2005), (2) a post zygotic mitotic error (Antonarakis et al., 1993; Sherman et al., 2005), or (3) a translocation (inherited or de novo) (Hook, 1983).

The results of earlier studies (Antonarakis et al. 1992; Ballesta et al. 1999; Muller et al. 2000), revealed that the average age of mother at the time of conception of a fetus with DS is significantly higher than that of mothers with normal euploid baby. This observation was confirmed further in the population based study in the Atlanta Down syndrome project (Allen et al. 2009) for US population and recently by us (Ghosh et al. 2010a) for Indian population. All these reports suggest that the advanced maternal age is risk factor for both the MI and MII errors and both types of error are potentially related in respect to their association with risk factors. Further, Atlanta Down syndrome project suggests (Allen et al. 2009) that maternal age specific incidence rate for live birth with free trisomy 21 may differ between MI and MII errors: the increasing risk for MII errors is shifted to the older maternal ages compared with MI errors. Interestingly, the women with MII errors are in average older than mothers with MI errors, as evident in both US (Allen et al. 2009) and Indian cohorts (Table 1). All these observations led the workers to propose several hypotheses to explain the intriguing association between advanced maternal age and an increasing chance of Ch21 nondisjunction.
### Parental Origin

<table>
<thead>
<tr>
<th>Meiotic Stage of Nondisjunction</th>
<th>Sample size</th>
<th>Proportion</th>
<th>Frequency</th>
<th>Maternal Age at Conception (Years±SD)</th>
<th>Paternal Age at Conception (Years±SD)</th>
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</thead>
<tbody>
<tr>
<td>Maternal Meiosis I (MI)</td>
<td>242</td>
<td>MI/(MI+MII)=242/314</td>
<td>77.07%</td>
<td>29.91±6.12</td>
<td>33.95±2.04</td>
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<tr>
<td>Meiosis II (MII)</td>
<td>72</td>
<td>MII/(MI+MII)=72/314</td>
<td>22.9%</td>
<td>31.01±3.44</td>
<td>34.01±4.66</td>
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<tr>
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<td></td>
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<tr>
<td>Subtotal</td>
<td>331</td>
<td>Maternal/All=331/373</td>
<td>88.73%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paternal Meiosis I (PI)</td>
<td>11</td>
<td>PI/(PI+PII)=11/27</td>
<td>40.74%</td>
<td>24.55±3.02</td>
<td>31.85±5.6</td>
</tr>
<tr>
<td>Meiosis II (PII)</td>
<td>16</td>
<td>PII/(PI+PII)=16/27</td>
<td>59.25%</td>
<td>26.92±4.91</td>
<td>33.98±4.4</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Subtotal</td>
<td>34</td>
<td>Paternal/All=34/314</td>
<td>10.82%</td>
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<tr>
<td>Post Zygotic Mitotic Error</td>
<td>8</td>
<td>8/373</td>
<td>2.14%</td>
<td>25.66±3.26</td>
<td>31.76±5.21</td>
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<tr>
<td>Origin Unknown</td>
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<td>19/392</td>
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<td>Total Informative Cases</td>
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<td></td>
<td></td>
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<tr>
<td>Total Cases</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>206</td>
<td></td>
<td></td>
<td>24.82±3.9</td>
<td>32.01±4.04</td>
</tr>
</tbody>
</table>

Table 1. Origin of Trisomy 21 in Indian Cohort and Parental Age at conception of Trisomy Foetus

### 3. Biological aging hypothesis

The hypothesis was originally proposed by Brook et al (1984). The central idea of this hypothesis is that the increasing rate of meiotic errors and subsequent aneuploid birth is related to ‘biological aging’ of ovary not to the chronological age of women. Two different views do exist about how the biological aging is implicated for increased incidence of trisomic birth. The first view relates the suboptimal level of hormonal signal with higher rate of meiotic errors in aging ovary. The number of antral follicle at various stages of development also declines with increasing maternal age as the fact has been confirmed in...
several studies (Reuss et al. 1996; Gougeon 1998; Scheffer et al. 1999; Kline et al. 2004). This decline in antral follicle count, together with the accompanying decrease in total oocyte pool generates an imbalance in the hormonal environment in ovary (Warburton, 2005) which predisposes the women for aneuploid conception. Support to this postulate came from the studies on human and mouse (Freeman et al., 2000; Roberts et al. 2005). Alternate to this concept has been proposed by Warburton (1989) in her “limited oocyte pool” hypothesis which suggests a more direct effect of antral oocyte pool size on the risk of aneuploidy. Among older women available antral follicles are limited and ovary has to compromise in selecting a suboptimal or erroneous oocyte for ovulation. The ‘biological aging’ can also be interpreted in term of senescence associated degradation of ovarian protein components that are implicated in chromosome separation system in oocyte (Sherman 2005). Interestingly, level of hundred of transcripts, including cell cycle genes have been reported to decrease with increased maternal age in mice and women (Hamatani et al., 2004; Steuerwald et al., 2007).

4. Genetic aging hypothesis

We proposed ‘genetic aging’ hypothesis (Ghosh et al. 2010b), which states that some of the mothers who have DS baby are genetically older than the mothers of same chronological age who have euploid baby (Ghosh et al. 2010b) and this genetic aging is the underlying cause of biological aging in ovary. In this analyses we estimated the telomere length (TL) of age matched controls and cases to get insight into the state of molecular aging, stratifying the mothers by stage of NDJ and their age of conception (young, <29 years; middle, 29-35 years; and old, >35 years). Our results showed that all three groups(M1,MII & control) have similar TL on average for younger mothers. As age increases, all groups show telomere loss, but that loss is largest in the meiosis II mother group and smallest in the euploid mother group with the meiosis I mother group in the middle(Figure 1). Our results do not support the theory that younger women who have babies with Down syndrome do so because they are ‘genetically older’ than their chronological age, but we proposed that older mothers who have DS baby are “genetically older” than controls, who have euploid babies at the same age. This finding, however, is consistent with the previous result (Dorland et al. 1998), showing no difference in genetic age among young DS mothers and young controls. The fact of telomere shortening among women with DS child can be explained in several ways. Apparently, the result suggests a possible functional link between telomere maintenance system and chromosome segregating apparatus at molecular level. Degradation of this possible ‘molecular link’ with age may affect the both system simultaneously. In this regard BubR1 is most promising candidate as mutation in this gene causes rapid senescence and high rate of aneuploidy in mouse (Baker et al., 2004) and the protein shows rapid fall with age. Alternatively, the environmental factor that induces rapid telomere loss at advanced reproductive age might simultaneously affect the chromosome separation system in oocyte. (Chen et al.,2007; Sebastián et al., 2009; Eichenlaub-Ritter et al., 2007; Susiarjo et al., 2007).

5. Reduced meiotic recombination and its interaction with maternal age

Aside from maternal age, only single factor that has been identified unambiguously to be associated with maternal NDJ is altered pattern of meiotic recombination. The first evidence
for association of reduced recombination with the events of NDJ of Ch21 was provided by Warren et al. (1987). Chiasmata are physical connections between homologous chromosomes at the site of recombination and they function to stabilize the paired homologues or tetrad at MI along with sister chromatids and centromere cohesion. It aids in proper chromosome orientation on the meiotic spindle (Carpenter 1994) and ensure their proper segregation to opposite poles. Absence of chiasma formation left the homologous pair free to drift randomly to the poles and if they move together to same pole aneuploidy results. As far as chromosome 21 NDJ is concerned, achiasmate meiosis is the major cause of reduction in recombination frequency (Lamb et al., 2005a, 2005b), although fall in double exchange frequency was reported too (Hawley et al. 1994).

In our analysis of etiology of DS birth in Indian cohort, we recorded only ~22% detectable crossover on MI nondisjoined chromosome in maternal meiosis (Ghosh et al., 2009). This observation was very consistent with the previous observation by Sherman et al. (2007), who reported 45% achiasmate meiosis associated with MI NDJ of Ch 21 in US population. Sherman and her co-workers constructed the linkage map of nondisjoined Ch21 (1994) and estimated 55% reduction in map length than the control CEPH map (39.4cM in contrast to 72.1cM). With similar approach for Indian DS population (Ghosh et al. 2010a), we scored 30.8cM map length of maternal MI nondisjoin ed Ch 21, which further confirmed the fact that reduced recombination due to absence of chiasma or less recombination frequency in some way increases the risk of NDJ.

In elucidation of the relationship between reduced recombination and maternal age, Sherman et al. (1994) hypothesized that the trisomy 21 conception at advanced maternal age is strongly associated with reduction in recombination frequency. The authors estimated shorter map length of Ch21 for mothers of >35 years with their linkage analysis approach. Very recently, Oliver et al. (2008) also reported a highest occurrence of non-exchange Ch21 pair among the old age (>34 years) women in compare to young (<29 yrs) and middle (29-35 yrs.), although the frequency of non-exchange tetrads remain most frequent among all the risk factors when only young mothers (<29 years) were considered. The authors proposed a model for explaining the risk of Ch21 NDJ in relation to maternal age categories. Among the young mothers risks related to aging is minimum and therefore absence of recombination becomes the predominant cause of NDJ in total risk scenario. If this remains true, then lack of recombination is an age-independent risk factor for Ch21 NDJ. This hypothesis was supported by our previous studies (Ghosh et al. 2009; 2010a) in which we estimated about 80% of younger mothers with achiasmate Ch21 who had NDJ at MI.

The highest frequency of non-exchange Ch 21 among older mothers is difficult to explain as the events of chiasma formation and recombination take place in foetal ovary. The fact led workers (Oliver et al. 2008; Ghosh et al. 2009) to speculate presence of maternal age dependent NDJ mechanism which gains support from the studies on model organisms. Mutation in the gene nod (no distributive disjunction) in Drosophila causes high frequency of NDJ of non-exchange chromosome (Knowles and Hawley, 1991) and it suggests existence of the genetic component that acts as surveillance system to ensure proper segregation of non-exchange meiotic chromosomes. Presence of such ‘back-up system’ is also evident in yeast in which, the gene Mad3 performs the same function (Gillett et al. 2004). Interestingly, proteins with similar function in human have been shown to be down regulated with increasing ovarian age (Baker et al. 2004; Steuerwald et al. 2001). Thus, age-dependent down-regulation of these essential proteins may lead to the decreased ability to segregate properly the non-
exchange chromosomes in aging oocyte. However, more direct evidence is needed to establish this speculation as fact.

6. Susceptible chiasma formation and its interaction with maternal age

Aside reduced recombination, unusual chiasma placement is another risk for Ch21 NDJ. Chiasma formation usually takes place at the middle of normally disjoining chromosomes (Lynn et al. 2000). This medially placed chiasma probably maintains the proper balance by counteracting the pull from opposite poles which is needed for proper segregation of chromosomes. But a chiasma close to centromere or close to telomere seems to confer instability and makes the Ch21 susceptible for random segregation and subsequent NDJ (Lamb et al. 1996; 2005a, 2005b). The increased risk of NDJ due to sub-optimally placed chiasma on the chromosome is also evident in model organisms such as Drosophila (Rasooly et al. 1991; Moore et al. 1994; Koehler et al. 1996a), yeast (Sears et al. 1995; Krawchuk and Wahls, 1999) and Caenorhabditis elegans (Zetka and Rose, 1995). The study of Lamb et al. (1996), suggested for the first time that a single telomeric chiasma is a risk for malsegregation of Ch21 at MI in oocyte in contrast to single pericentromeric chiasma which increases risk of MII NDJ.

Very recently, Oliver et al. (2008) and we (Ghosh et al. 2009) independently conducted population based studies on US and Indian DS populations respectively to get an insight into the interaction between susceptible chiasma configuration on Ch21 in oocyte and maternal age. In doing so we used family linkage approach to detect exchange pattern on nondisjoined Ch21, using set of microsatellite markers and all the analyses were done by stratifying the participating mothers into three age groups: young (>29 yrs.), middle (29-34 yrs) and old (>34 yrs). Surprisingly, the two sets (US set and Indian set) of results were very concordant and revealed that single telomeric exchange is prevalent among younger mothers whose Ch21 nondisjoined at MI. In contrary, single centromeric chiasma is risk for MII NDJ, particularly at older age. For Indian DS sample, we recorded susceptible single chiasma within the 3.1 Mb peri-telomeric and 4 Mb peri-centromeric segment of 21q for MI younger and MII older categories, respectively (unpublished data). These observations led us (Oliver et al. 2008; Ghosh et al. 2009) to propose a hypothesis which states that maternal age independent risk factor is one which affects all the age groups equally and be detected in highest frequency among younger mothers for whom aging related risk factors are minimum. Alternately, age-dependent risk factors usually intensify with advancing age and so one would expect highest frequency of such factors among older age group (Figure 2). If our prediction is true, the telomeric single chiasma is maternal age independent risk, whereas, the single peri-centromeric chiasma is maternal age dependent factor.

The relationship between centromeric exchange and advancing maternal age can be interpreted in two different ways: 1) pericentromeric exchange set up a sub-optimal configuration that initiates or exacerbates the susceptibility to maternal age-related risk factors, or 2) a pericentromeric exchange protect the bivalent against age related risk factor allowing proper segregation of homologues, but not the sister chromatids at MII (Oliver et al., 2008). A chiasma very close to centromere may cause ‘chromosomal entanglement’ at MI, with the bivalent being unable to separate, passing intact to MII metaphase plate (Lamb et al.1996). Upon MII division, the bivalent divides reductionally, resulting in disomic gamete with identical centromeres. In this manner, proximal pericentromeric exchange, which occurred during MI, is resolved and visualized as MII error. According to an
alternate model, studied in Drosophila (Koehler et al. 1996b), proximal chiasma lead to premature sister chromatid separation just prior to anaphase I. Resolution of chiasma requires the release of sister chromatid cohesion distal to the site of exchange (Hawley et al., 1994). Attempt to resolve chiasmata that are very near to centromere could result in premature separation of chromatids. If the sister chromatids migrate to a common pole at MI, they have 50% probability to move randomly into the same pole at MII, resulting in an apparent MII NDJ. Similar observation is evident in yeast in which centromere-proximal crossover promotes local loss of sister-chromatid cohesion (Rockmill et al., 2006). One of the members of centromeric cohesion complex shugoshin, when down regulated due to aging shows high frequency of MII NDJ of bivalent with peri-centromeric exchange (Marston et al. 2004). Alternatively, a pericentromeric exchange may protect the bivalent from maternal age related risk factors. The effect of degradation of centromere or sister chromatid cohesion complexes or of spindle proteins with age of oocyte may lead to premature sister chromatid separation. Perhaps the pericentromeric exchanges help to stabilize the compromised tetrad through MI. This would lead to an enrichment of MII errors among the older oocytes. Although there is no specific model system in favor of this mechanism, but some findings in model organisms can be interpreted in this way.

Fig. 1. Telomere length (Kbp) among control and meiotic outcome groups stratified by age categories.

A telomeric chiasma imparts its susceptibility for MI NDJ probably due to recruitment of minimal amount of sister chromatid cohesion complex remaining distal to the exchange event (Orr-Weaver, 1996). Specifically, when the exchange is too far from kinetochore, this could prevent the bi-orientation of the homologues on the meiotic spindle (Nicklas 1974; Hawley et al.1994; Koehler et al.1996b). Alternatively, the integrity of chiasma may be
compromised when a minimum amount of cohesin remains to hold homologue together. Thus bivalent may act as pair of functional univalents during MI, as has been evident in human oocyte (Angell 1994, 1995).

Further, we evaluated the interaction between maternal age and multiple chiasmata on MI nondisjoined Ch21 and found that there is a linear increase in multiple chiasma frequency with advancing age (Ghosh et al.2010a). Interestingly, similar trend is also evident for chromosome 15, 18 and X chromosome (Robinson et al. 1998; Thomas et al. 2001; Bugge et al.1998). This finding suggests two important possibilities. The first one is that the multiple chiasmata might be protective and chromosomes with multiple recombinants probably more resistant to NDJ at least at MI because of an increase in bivalent stability. Secondly, instead of enjoying multiple recombinations some bivalents segregate improperly, particularly in older oocyte, which suggests presence of some aging associated factors that impart risk to these otherwise recombination perfect chromosomes.

7. Summary and conclusion

We have paved half of a century after the initial discovery of cause of DS, but we are still in dark regarding etiology of DS. Although advanced maternal age has been identified unambiguously as risk, its molecular relation with chromosome separation system is enigmatic. It is still elusive whether or not some women are genetically predisposed to altered meiotic recombination and subsequent chromosomal NDJ. Very recently, the gene
PRDM9 has drawn the interest. The gene controls the recombination hotspot of meiotic homologues (Parvanov et al. 2010). The variant of this gene has been reported to make the women susceptible for recurrent miscarriages, infertility and aneuploid pregnancy (Cheung et al. 2010). So PRDM9 is prospective candidate gene whose altered functional state might increase susceptibility of Ch21 NDJ. Similarly, genetic variant of any component of meiotic chromosome separation system could increase the risk for chromosome missegregation in oocyte. Intuitively, the gene BubR1 is of special interest as it is a member of centromere cohesion complex and also known for its role in cellular aging (Baker et al. 2004). In Drosophila hypomorphic bubR1 causes high rate of NDJ at MII due to premature sister chromatid separation and these nondisjoined chromosome exhibited centromeric exchange. All these findings suggest the possibility of BubR1 to be a ‘missing link’ between the molecular mechanism of cellular aging and higher incidence of chromosomal NDJ at advanced age.

The effect of environmental agents on chromosome segregation, particularly in connection with maternal age and recombination remains unexplored. As environmental aneugen have great opportunity to become accumulated within the ovarian microenvironment during protracted oocyte growth phase, their probable effects cannot be underestimated. Although epidemiologic association of some environmental agents with DS birth have been identified, their influence on meiotic recombination and aging is intriguing. The periconceptional smoking and contraceptive use have been identified as potential risk for Ch21 NDJ (Yang et al. 1999), but this observation needs further confirmation. We conducted an epidemiological study on the risk of chewing tobacco and contraceptive use among mothers having DS baby and found some association of chewing tobacco with MI NDJ and contraceptive for both MII and MI (unpublished data). Moreover, the fetal incidence of chiasma formation and recombination make us curious to the probable ‘grand maternal’ influence on DS birth. Presently we are in position to realize at least that the risk factors associated with DS birth is multidimensional and several mechanisms are involved for chromosome 21 NDJ in women. At this point, it is worth mentioning that the etiology of maternal Ch21 NDJ and subsequent DS birth may be similar across the human population divides irrespective of ethnic and socio-cultural differences. The future investigations should be focused to resolve all these pending issues so that we could move towards complete understanding of risk factors associated with DS birth.

8. Acknowledgements

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9. References


This book provides a concise yet comprehensive source of current information on Down syndrome. Research workers, scientists, medical graduates and paediatricians will find it an excellent source for reference and review. This book has been divided into four sections, beginning with the Genetics and Etiology and ending with Prenatal Diagnosis and Screening. Inside, you will find state-of-the-art information on: 1. Genetics and Etiology 2. Down syndrome Model 3. Neurologic, Urologic, Dental & Allergic disorders 4. Prenatal Diagnosis and Screening Whilst aimed primarily at research workers on Down syndrome, we hope that the appeal of this book will extend beyond the narrow confines of academic interest and be of interest to a wider audience, especially parents and relatives of Down syndrome patients.

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