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Risk Factors for Down Syndrome Birth: Understanding the Causes from Genetics and Epidemiology

Sujay Ghosh and Subrata Kumar Dey

1. Introduction

Aneuploidy can be defined as presence of erroneous number of chromosome in organisms and in human aneuploidy is the major cause of birth wastage. Among all known recognizable human aneuploidies, trisomy 21 shows the highest frequency of occurrence, estimating approximately 1 in 700 live-births (Kanamori et al., 2000). The trisomy 21 condition originates due to non-separation or nondisjunction (NDJ) of chromosome 21(Ch21) during gametogenesis and as a result disomic gametes with two copies of a particular chromosome are formed and upon fertilization by haploid gamete from opposite sex lead to the formation and implantation of trisomic fetus. The trisomy 21 condition is popularly known as Down syndrome (DS) after the name of John Langdon Down who described the syndrome for the first time in 1866 (Down, 1866). Beside chromosomal NDJ, a small proportion of DS occurs due to post zygotic mitotic error or translocation of chromosome 21 to other autosomes.

Within the category of free trisomy 21 due to NDJ, overwhelming majority of errors occurs in maternal oogenesis particularly at meiosis I (MI) stage (Table 1). A little fraction of NDJ errors arise at paternal spermatogenesis. This preferential occurrence of maternal meiotic error is probably due to the mechanism of oocyte maturation in the ovary. Meiosis is initiated in the human foetal ovary at 11–12 weeks of gestation (Gondos et al., 1986), but becomes arrested after completion of homologous chromosome pairing and recombination. This meiotic-halt lasts for several years until the elevated level of LH and FSH resume the process at the onset of puberty. Then the oocyte completes meiosis I (MI) and enters meiosis II (MII) and again undergoes a phase of pause. It completes the meiosis II after the sperm enter its cytoplasm following fertilization. Thus, the oocyte, whose ovulation marks the menarche, remains in pause for shortest period and that ovulates just preceding menopause experiences longest period of arrest. This long tenure of oocyte development makes it vulnerable to
acquire environmental hazards within its microenvironment which inevitably increases the risk of chromosomal NDJ.

| Parental Origin | Meiotic Origin of Nondisjunction | Frequency | Maternal Age at Conception (Years±SD) | Paternal Age at Conception (Years±SD) |
|----------------|---------------------------------|-----------|--------------------------------------|--------------------------------------|
| Maternal       | Meiosis I                        | 79.03%    | 29.07±6.11                          | 34.98±3.88                          |
| Maternal       | Meiosis II                       | 29.97%    | 32.54±2.45                          | 35.02±4.66                          |
| Paternal       | Meiosis I                        | 39.23%    | 24.07±6.22                          | 33.02±5.9                           |
| Paternal       | Meiosis II                       | 59.26%    | 28.03±4.6                           | 34.09±3.9                           |
| Post Zygotic   |                                 | 2.2%      | 29.66±7.3                           | 32.08±5.32                          |

Table 1. Distribution of mean parental age for Down syndrome birth and nondisjunctional errors of chromosome 21 stratified by parent and meiotic stage of origin.

In search of etiology of Ch21 NDJ, researchers have unambiguously identified two risk factors namely advancing maternal age and altered pattern of meiotic recombination. Beside these two risk factors, other environmental and behavioural factors have also been identified as risk of Ch21 NDJ and they exhibit several degrees of interactions with advancing maternal age and recombination pattern of Ch21. These make the etiology of DS birth a puzzle in the field of medical genetics.

2. Genetic risk factors

2.1. Advanced maternal age and related hypotheses

The age of the mother at the time of the conception of a fetus with DS is, by far, the most significant risk factor for meiotic NDJ of Ch21. As a woman ages, her risk for having a fetus with trisomy 21 significantly increases. This association was noted initially by Penrose in 1933 (Penrose, 1933). For all the populations studied so far, estimated mean maternal age of conception of DS baby is higher than that of controls i.e., having euploid baby and women with MII NDJ is older than women affected with MI NDJ.

Several hypotheses have been put forward to explain the link between advancing maternal age and higher incidence of aneuploid oocyte formation but no one has proved to be completely satisfactory. The most popular hypothesis (Gondos *et al.*, 1986) holds that the protracted tenure of oogenesis interrupted with meiotic halts (Figure 1), probably makes the
eggs more vulnerable to the aging effect than sperms. This long period of oocyte maturation results in the aging associated deteriorative changes to accumulate over time either in the oocyte or its milieu. Examples of such factors would be a diminishing amount of a meiotic proteins, like those maintaining sister chromatid adhesion (Hodges et al., 2005; Hunt & Has‐sold, 2008) or meiotic checkpoints components (Garcia-Cruz et al., 2010) or weakening of centromere cohesion due to age-related reduction in centromere associated proteins MCAK (Eichenlaub-Ritter et al. 2010). This list of age related risks may also include the accumulation of environmentally induced damage to the meiotic machinery over time or genetic changes such as mitochondrial deletions (Van Blerkom, 2011). Among all these variables, the spindle assembly check point (SAC) components and sister chromatid cohesion (SCC) were investigated thoroughly (Chiang et al.; 2010), as they are prospective genetic candidates that may explain the aging effect on aneuploid oocyte formation. The SAC is a molecular machine that ensures proper chromosome separation in both mitosis and meiosis. In meiosis SAC prevents anaphase until all chromosomes properly attach to the spindle. The SAC includes MAD2L1, BUB1B, and TTK (Hached et al., 2011; Niault et al., 2007) which show decline in concentration with age in mouse leading to misaligned chromosomes (Pan et al., 2008) and errors in SAC function contribute in age-related aneuploidy. Disrupted spindles, misaligned chromosomes and decreased expression of SAC components Mad2L1 and Bub1 have evident in aged human oocytes (Mc Guinness et al., 2009; Steuerwald et al., 2001) and these findings are consistent with aging hypothesis. On the other hand, the SSC mediates physical pairing of duplicated chromosomes which is essential for appropriate distribution of chromosomes. The cohesion along chromosome arms keeps the bivalents intact in MI and centromere cohesion holds sister chromatids together in MII. A defect in cohesion distal to crossover sites may result in a shift in chiasmata placement (alternatively known as ‘chiasma slippage’) or even premature bivalent separation in MI, whereas reduced centromere cohesion may result in premature separation of sister chromatids in MII (Steuerwald et al., 2001).

The loss of cohesion with maternal age for distally placed chiasma (Subramanian and Bickel, 2008) is consistent with the idea that cohesion defects may contribute to age related aneuploidy (Chiang et al., 2012). Another component that supposed to decline with age and contributes significantly to aging effect on DS birth is the meiosis surveillance system of ovary that ensures achiasmate chromosome segregation (Oliver et al., 2008). Chiasma formation and subsequent recombination are prerequisite of faithful separation of homologues at meiotic anaphase. Absences of chiasma, faulty configurations of chiasma and reduction in chiasma frequency have been attributed to NDJ of Ch21 and subsequent DS birth (Lamb et al., 2005; Ghosh et al., 2010). A high proportion of achiasmate Ch21 tetrad was reported among the mothers of DS having age >35 year (Oliver et al., 2008). As the decision regarding chiasma formation is taken in foetal ovary, high frequency of achiasmate nondisjoined Ch21 in older oocyte can only be explained by down regulation of surveillance system. Human proteins involved in segregation of nonexchange chromosome show down regulation with increasing ovarian age (Steuerwald et al., 2001; Baker et al., 2004).
A second hypothesis relates the “biological aging” or “ovarian aging” with the increasing rate of meiotic errors (Warburton, 1989; 2005). The central theme of this hypothesis is the prediction that biological aging is different among women of the same chronological age and that the frequency of trisomic conceptions depends upon the biological age of the woman rather than the chronological age (Warburton, 2005). The biological age of women can usually be assessed by counting the falling number of antral follicles with chronological age together with decrease in total oocyte pool size (Scheffer et al. 1999; Kline et al. 2004). These altogether alter the optimum hormonal balance in ovary, which is marked by falling concentration of serum inhibin A and B, decline in estrogens surge and elevated level of FSH (Warburton, 2005). This change in hormone balance is related to increased rate of aneuploidy at advanced maternal age. Support to this prediction is available from the experiment on mouse model (Robert et al. 2005). Alternative to this prediction was provided in the ‘limited oocyte pool hypothesis’ (Warburton, 2005), which stated that with biological age there is a decrease in the number of antral follicles, leaving only the premature or post mature oocyte to ovulate. The “biological aging” hypothesis predicts that women with a trisomic conception should on the average have an older “ovarian age” than other women of the same chronological age with a normal conception (Warburton, 2005) and women having trisomic pregnancy have average earlier (~1 year) age of menopause (Kline et al., 2000). If these were the facts, one would expect that after a trisomic conception, the risk of a subsequent trisomy for any chromosome should be higher than the maternal age-related risk. Support to this

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**Figure 1.** Time line for oocyte development in human and probable time of occurrence of risk factors for chromosome 21 nondisjunction.
prediction comes from the recent data from prenatal diagnosis after a previous trisomic conception which shows that the risk of a subsequent trisomy birth is about 1.7 times the maternal age-related risk (Warburton et al., 2005). Mathematical model proposed by Kline and Levin (1992) estimated that women with trisomy pregnancy experience 0.9 years early menopause which suggests that such women suffer from advanced ovarian aging than the women with chromosomally normal pregnancies. Population sample survey for calculating the median age of menopause among the women with trisomic pregnancy loss also suggested an early cessation of menstrual cycle among them than the mothers with chromosomally normal foetus (Kline et al., 2000). Elevated level of FSH is reported among the women with DS pregnancy (Nasseri et al., 1991; van Montfrans et al., 2002) which suggests precocious aging among them. Very recently, Kline et al. (2011) conducted the survey on the hormonal level of women with trisomic pregnancy and supported the ‘reduced oocyte pool hypothesis’, suggesting that some women have smaller follicle content than the others of same chronological age. The former group are susceptible for rapid ovarian aging and associated trisomic conceptions. All these findings suggest intuitive existence of some predisposing factors among some women for their earlier aging that relates their trisomic conception too.

The third hypothesis is concerned with ‘genetic age’ of women and stated that it is the genetic aging that underlies the all kind of degenerative changes in ovary and oocyte. The hypothesis was proposed by Ghosh et al., (2010). The authors estimated the telomere length of peripheral lymphocyte of women with DS child and compared with age matched controls. They found that beyond of age 29 years the DS bearing mothers exhibit rapid telomere shortening and hence rapid genetic aging than the controls. The authors inferred that DS bearing younger mothers do not experience any accelerated genetic aging; it is only the chronological older age when DS bearing mothers suffer from rapid genetic and molecular aging than the age matched mothers of euploid child. The authors proposed ‘Genetic aging hypothesis’ which stated that some women are predisposed to rapid genetic and molecular aging and its effect is exacerbated at advance age when age-related deteriorative changes also affect the chromosome separation system leading to NDJ. The notion has suggested some intuitive link between telomere maintenance system (i.e., system of molecular aging) and chromosome segregating apparatus at molecular level.

2.2. Altered pattern of recombination and its interaction with maternal age

Aside from maternal age, there is only one other factor that has been shown to associate increased susceptibility of maternal NDJ, namely altered recombination patterns. Warren et al. (1987) provided the first evidence to suggest that a proportion of maternal NDJ errors were associated with reduced recombination along Ch 21. Further examination has shown that, in addition to the absence of an exchange along the nondisjoined Ch 21, the placement of an exchange is an important susceptibility factor for NDJ. Examination of recombination along the maternal nondisjoined Ch 21 has suggested three susceptible exchange patterns: 1) no exchange leads to an increased risk of MI errors, 2) a single telomeric exchange leads to an increased risk of MI errors, and 3) a pericentromeric exchange leads to an increased risk of so-called MII errors. These patterns are similar to those observed in model organisms where
absence or reduced recombination, along with sub-optimally placed recombinant events, increases the likelihood of NDJ (Rasooly et al., 1991; Moore et al., 1994; Sears et al., 1995; Zetka and Rose, 1995; Koehler et al., 1996; Ross et al., 1996; Krawchuk and Wahls, 1999). Exchanges too close to the centromere or single exchange too close to the telomere seem to confer chromosomal instability.

Subsequently, researchers have identified a potential interaction between maternal age and pattern of recombination. The study on US population (Sherman et al., 1994) provided the first evidence in this regard and proved an age related reduction in recombination frequency among the MI cases, with older women (35 yrs. and more) having less recombination along 21q than younger women (< 35 yrs.), as suggested by estimated length (cM) of age-specific linkage map of Ch21. In exploring the interaction between maternal age and recombination and to gain further insight into the potential mechanisms of abnormal chromosome segregation, comparison had been made for frequency and location of meiotic exchanges along 21q (Lamb et al. 2005) among women of various ages who had an infant with DS due to a maternal MI error. While there was no significant association between maternal age and overall frequency of exchange, the placement of meiotic exchange differed significantly by age of conception. In particular, single telomeric recombination event was present in highest proportion among the youngest age group (80%), while the proportion in the oldest group of women and in control group were almost equal (14% and 10% respectively). Moreover, studies (Lamb et al., 1996, 2005) suggested that in maternal MI error cases, majority of single exchanges were located in the telomeric end of Ch21, whereas the single exchange within the peri-centromeric region was associated with maternal MII errors. In the independent age-stratified analysis on the US population by Oliver et al., (2008) and on the Indian population by Ghosh et al., (2008) a universal pattern of interactions among maternal age groups, chiasma placement and amount of meiotic recombination has been discovered. In these studies a major fraction of MI errors was recorded due to absence of any detectable exchange between non-sister chromatids of nondisjoined homologues. A trend of decreasing frequency of achiasmate meiosis (meiosis without recombination) with increasing maternal age is also observed in both the studies (Oliver et al., 2008; Ghosh et al., 2009), which suggests achiasmate meiosis without any recombination is maternal age-independent risk. According to the model of maternal risk factors for DS birth proposed by Oliver et al., (2008) and supported by (Ghosh et al. 2009, Ghosh et al., 2010) that any risk factor which is maternal age independent should present in highest frequency in the younger mother, the age group in which other risk factors are usually absent. In contrast, any risk factors whose frequency increases with increasing maternal age is regarded as maternal age dependent risk factor as its effect gets exacerbated in interaction with increasing maternal age. The chiasma stabilizes the tetrad and counter balances the pull from opposite poles which ensure the faithful segregation of homologues. In absence of chiasma, the chromosomes move randomly at MI, resulting in formation of disomic gametes. As the chiasma formation takes place in foetal ovary, the achiasmate chromosome containing disomic oocyte may ovulate at any time in reproductive life and hence it is maternal age independent risk factor of Ch21 NDJ.
Figure 2. Model for mechanism of nondisjunction of chromosome 21: a) Normal segregation of chromosomes; b) First meiotic nondisjunction; c) Second meiotic nondisjunction. The first meiotic nondisjunction involves telomeric chiasma with premature sister chromatid separation followed by mono-orientation of homologous chromosome at MI. The second meiotic nondisjunction involves peri-centromeric chiasma formation with chromosome entanglement. Noted that the error actually arises at MI but its effect appeared at MII.
In both the studies on US and Indian populations (Oliver et al., 2008; Ghosh et al., 2009), the single telomeric chiasma and subsequent recombination were found in highest frequency among the women of younger age group i.e., age group below 29 years, who had a NDJ error at meiosis I stage of oogenesis and there was a gradual decrease in telomeric chiasma frequency with advancing maternal age. This observation suggests that the single telomeric chiasma formation is the risk of NDJ of Ch 21 even in younger women who otherwise do not suffer from deterioration related to the aging. Thus within the total risk probability of Ch21 NDJ, the single telomeric chiasma representation is the highest proportion among the younger women of MI NDJ category. Two important inferences have been drawn from this finding. The first one is that the single telomeric chiasma formation is maternal age independent risk of Ch21 NDJ. The second is that the single telomeric chiasma probably induces some structural instability of Ch21 that segregates randomly at meiosis I which takes place in fetal ovary.

Understanding the exact mechanism how does single telomeric chiasma cause chromosomal mis-segregation has been obtained from the observations in model organisms like Drosophila (Koehler et al., 1996), Saccharomyces (Ross et al. 1996) and Caenorhabditis elegans (Zetka and Rose, 1995). As the telomeric chiasma located far from the kinetochore, the point of spindle-attachment links the homologues less efficiently and orients each kinetochore to the same spindle pole and prevents bi-orientation of homologues (Nicklas, 1974; Hawley et al., 1994; Koehler et al., 1996). Most likely, this susceptibility is related to the minimal amount of sister chromatid cohesion complex (Figure 2b) remaining distal to the exchange event (Orr-Weaver, 1996). 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 univalent during MI, as has been evident in human oocyte (Angell, 1994; 1995).

Another chiasma configuration that poses susceptibility for NDJ of Ch21 is the pericentromeric exchange. In both the studies on US and Indian DS populations (Oliver et al., 2008; Ghosh et al., 2009), highest frequency of pericentromeric exchange was scored in older women having age >34 years. A trend of gradual increase in centromeric chiasma frequency with increasing age was recorded in both the studies with gradual shifting of chiasma from middle of the chromosome in younger age group to more proximal to centromere in older age group. In explaining the effect on chromosome segregation that single centromeric chiasma imparts two hypotheses have been put forward by the authors. The chiasma that is positioned 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 (Figure 2c). In this manner, proximal pericentromeric exchange, which occurs at MI, is resolved and visualized as MII error. According to an alternate model, studied in Drosophila (Koehler et al., 1996), proximal chiasma leads to a 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 chiasma that is very close to centromere could result in premature separation of chromatids (Figure 2c). If the sister chromatids migrate to a common pole at MI, they have 50% proba-
bility to move randomly into the same product of meiosis at MII, resulting in an apparent MII NDJ. Similar observation is reported from the study in Yeast in which centromere-proxi- 
mal crossover promotes local loss of sister-chromatid cohesion (Rockmill et al., 2006). Studies of NDJ in both humans (Angell, 1995) and Drosophila (Miyazaki & Orr-Weaver, 1992) 
have provided preliminary supports for this model.

The effect of pericentromeric exchange on meiotic chromosome separation gets exacerbated 
with maternal age related insults in ovarian environment, as suggested by greater propor-
tion of DS births among older women who have experienced the particular pattern of chias-
ma formation. This relationship 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, perhaps leading to an increase in premature sister chroma-
tid segregation 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). The former explanation is likely to the ‘two hit model’ proposed previously 
by Lamb et al., (1996). Alternatively, a pericentromeric exchange may protect the bivalent 
from maternal age related risk factors. The effect of degradation of centromere or sister chro-
matid 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 which is a maternal age dependent risk for NDJ of Ch21.

As far as effect of multiple chiasmata formation on the nondisjoined Ch 21 is concerned, two 
important reports have been published very recently. In their study Ghosh et al. (2010) 
found that two or more chiasmata formation is prevalent particularly in older age group (≥ 
34 years). This infers that the older oocyte suffers from nondisjunctional errors even when 
Ch21 experiences formation of two or more chiasmata which are believed to be protective of 
NDJ; this is due to aging effects that imparts various degenerative changes in ovary. Analyz-
ing the effect of multiple chiasma of the 21q, Oliver et al. (2011) found a decrease in the 
interval between two simultaneous chiasmatas on the chromosome that disjoined at MI and 
this closeness is due to shifting of distal chiasma towards centromere. The author argued 
that as the proximal chiasma remains at its usual position, similar to that on the normally 
disjoined chromosome, it is the distal chiasma whose dislocation towards the proximal 
chiasma nullifies the ‘good-effect’ of the latter that is needed for faithful segregation of the 
chromosome. The Ch21 experiences such distal chiasma dislocation in association with cor-
rectly placed proximal chiasma disjines erroneously at MI. Moreover, the authors found 
more intimate positioning of proximal chiama with the centromere of the chromosomes 
with two exchanges and this tendency increases with advancing age. This pattern is very 
similar to the single chiasma shifting related to MII errors reported in earlier studies (Oliver 
et al., 2008; Ghosh et al., 2009). Moreover, the authors further extend their realization that 
the centromeric chiasma may not be protective of NDJ, the notion previously assumed both 
by Oliver et al. (2008) and Ghosh et al. (2009).
2.3. Genetic polymorphism and increasing susceptibility of Down syndrome birth

Maternal genetic factors such as polymorphism of certain gene probably make them susceptible for NDJ error. Experimental organisms have been used to identify genes that are important in the proper segregation of chromosomes. The potential candidates are those genes involved in the meiotic process such as homologue pairing, assembly of the synaptonemal complex, chiasma formation and chiasma positioning, sister chromatid cohesion, spindle formation. Genetic variations of these genes are predisposing factors for chromosome NDJ.

The gene that has been identified first in this category is \textit{MTHFR} (methylene tetrahydrofolate reductase), which is not directly related to the meiotic process. The case-control study by James et al., (1999) provided primary evidence that the 677C→T polymorphism in the \textit{MTHFR} gene increases the risk of having a child with DS (Odds Ratio = 2.6) in North American population. This polymorphism is associated with elevated plasma homocysteine and/or low folate status (Sherman et al., 2005). Folate is essential for the production of S-adenosylmethionine, which is the primary methyl donor (Figure 3a) for epigenetic DNA methylation essential for gene expression regulation and maintenance of chromosomal integrity at centromere (James et al., 1999; Dworkin et al., 2009; Scandrello et al., 2004). Folate deficiency reduces S-adenosylmethionine synthesis, leading to DNA hypomethylation (Pogrribny et al., 1997; Beetstra et al., 2005; Wang et al., 2004). The pericentromeric hypomethylation could impair the heterochromatin formation and kinetochore establishment (Figure 3b) resulting in chromosomal NDJ (James et al., 1999). This happens because the stable centromeric chromatin depends on the epigenetic inheritance of specific centromeric methylation patterns and it binds with specific methyl-sensitive proteins in order to maintain the higher-order DNA architecture necessary for kinetochore assembly (Migliore et al., 2009).

This initial report had inspired several follow-up studies on the \textit{MTHFR} 677C→T polymorphism, as well as several other allelic variants in the folate pathway genes to identify genetic risk factors for having a child with DS. But the results are inconsistent (James et al., 2004a, 2004b), especially those that have evaluated genotype alone without biomarkers of metabolic phenotype. Those who have examined blood homocysteine levels, a broad-spectrum indicator of nutritional and/or genetic impairment in folate/B12 metabolism have documented a significantly higher level among the mothers of children with DS compared with control mothers from the same country. One possible explanation for the inconsistent results among the numerous studies may reflect the complex interaction between effects of genetic variants and nutritional intake (James et al., 2004b). Nevertheless, support to the notion regarding the association between \textit{MTHFR} 677C-T polymorphism and risk of DS birth was provided by other studies in different populations. Wang et al., (2004) reported significant increase in the risk of DS conception among Chinese women bearing two polymorphisms namely, polymorphisms of \textit{MTHFR} 677C→T and the polymorphism \textit{MTRR} (Methionine synthase reductase) 66A→G. The estimated risks were more than three folds and five folds for \textit{MTHFR} (Odd Ratio=3.7; 95% CI, 1.78–8.47) and \textit{MTRR} (Odd Ratio= 5.2; 95% CI, 1.90–14.22) respectively. The combined presence of both polymorphisms was associated with a greater risk of DS than the presence of either alone, with an odds ratio of 6.0 (95% CI, 2.058–17.496). The study on Italian population also agreed the link between DS birth and \textit{MTHFR} and \textit{MTRR}.
polymorphisms (Coppedè et al., 2010). Cyril et al., (2009) conducted such association study on Indian women and confirmed the association of MTHFR 677C→T polymorphism with DS birth risk.

The other way to find out the genes involved in human NDJ is to analyze the association of consanguinity and trisomy 21(Sherman et al., 2005). If such an association really does exist, it would provide evidence for a genetic effect for NDJ. The study of Alfi et al., (1980) provided one of the earlier reports suggesting an association between increased consanguinity among parents of individuals with DS in a study population in Kuwait. Authors postulated the existence of a gene that increases the risk for mitotic NDJ. Alternatively, they suggested that increased rates of consanguinity among parents would be correlated with those in grandparents and therefore, an autosomal recessive gene may be postulated to be involved in mitotic NDJ in the homozygous parents. But the reports from subsequent studies in other populations are contradictory and did not find any evidence for an association between consanguinity and human NDJ (Devoto et al., 1985; Hamamy et al., 1990; Roberts et al., 1991; Basaran et al., 1992; Zlotogora, 1997; Sayee & Thomas, 1998; Rittler et al., 2001).

Lastly, differences in the prevalence of DS among different racial groups may provide indirect evidence for genetic factors involved in human NDJ. However, such studies are difficult to conduct and to interpret. Differences (or similarities) may reflect the maternal age distribution of the population, accuracy of diagnosis, cultural preference and/or access to selec-
tive prenatal termination of pregnancies with trisomic fetuses, and as yet unidentified environmental factors (Sherman et al., 2005). Only one such study by Allen et al., (2009) reported demographic differences in mean maternal age of DS conception recorded in two different sample sets from USA. This study included DS samples from Atlanta Down syndrome project and National Down syndrome project and found that mothers enrolled in National Down syndrome project were on an average older than those of Atlanta. Moreover, the authors have also reported some ethnic differences in maternal age distribution. The Atlanta Down syndrome project had a higher proportion of cases and controls that were black and a significantly smaller proportion of Hispanics than did the National Down syndrome project. Comparison of mean maternal ages indicated variation by ethnic groups. In both the Atlanta Down syndrome project and National Down syndrome project, white mothers tended to be older than their black or Hispanic counterparts. Specifically, for both cases and controls, white mothers were found to be significantly older than black mothers (P< 0.01) and Hispanic mothers (P< 0.01); blacks and Hispanics were not significantly different from each other (P>0.05). To confirm such effect of demographic and ethnic differences on the etiology of DS birth, further large scale population based studies are needed to be conducted.

2.4. Paternal risk factor for chromosome 21 nondisjunction

The paternal error constitutes nearly 5 to 10% of total occurrence of live born DS cases, depending upon the populations studied. Unlike maternal cases the studies on the etiology of paternal NDJ are limited by insufficient sample size. The first significant report was provided by Savage et al., (1998) who found reduction in recombination in MI nondisjoined cases, but not in MII errors. Moreover, the authors inferred that altered chiasma positioning may not associate with NDJ in spermatogenesis, as the authors recorded very concordant pattern of chiasma distribution among DS cases and control. In their extension study with more paternally derived samples, Oliver et al., (2009) determined that majority of Ch21 NDJ errors in spermatogenesis occurs at MII (32%MI:68%MII), and the authors did not found significant reduction in recombination either in MI or in MII errors. Moreover, their sample did not exhibit any advanced age effect for either of meiotic outcome groups. The authors argued that the time scale of spermatogenesis is much shorter starting at puberty runs continuously without meiotic halt and this explains why advancing paternal age does not exacerbate and associate Ch21 NDJ in spermatogenesis. This study is significant in the realization that etiology of Ch21 NDJ differs in two sexes and case of paternal errors remains an enigma. In general the frequency of recombination for normally segregating chromosome is less in male than in female. But further reduction in recombination frequency may not cause NDJ in male. Moreover, epidemiological study on the risk factors for paternal NDJ of Ch21 is yet to be conducted.

3. Habitual risk factor for chromosome 21 nondisjunction

Beside maternal age and altered pattern of recombination, set of prospective environmental or habitual risk factors have been identified in several epidemiological studies. These factors
show various degrees of associations with DS birth. The list includes maternal cigarette smoking, use of oral contraceptive, peri-conceptional alcohol consumption by mother, exposure to radiation and low socio-economic status. Number of studies reported a negative association between maternal smoking around the time of conception and the risk for DS birth (Kline et al., 1983, 1993; Hook & Cross, 1985, 1988; Shiono et al., 1986; Chen et al., 1999). One explanation for the negative association was that trisomic conceptuses were selectively lost prenatally among women who smoke (Hook and Cross, 1985; Kline et al., 1993). But evidence against this speculation is also available (Cuckle et al., 1990; Kallen, 1997; Torf & Christianson, 2000). Study conducted by Yang et al., (1999) suggested that maternal-smoking was significantly associated with MII error and probably due to compromise in blood and oxygen supply surrounding the developing follicles. Besides smoking, the other maternal risk factor for which epidemiological studies have been conducted most is oral contraceptive. The use of oral contraceptive by women at the time of conception is subject of speculation as risk for DS births (Yang et al., 1999). The study by Martinez-Frias et al., (2001) showed that the risk for DS in infants born to mothers with less than 35 years of age (as a group) who became pregnant while taking oral-contraceptive is near the risk for mothers of DS with more than 35 years of age. In their epidemiological study, Yang et al., (1999) found that women having simultaneous habits of smoking and using oral contraceptive have seven folds increased risk of having DS pregnancy and they argued that this is due to anoxic condition in ovarian microenvironment related to toxicant induced reduction in blood flow surrounding ovary. This speculation is similar to that proposed by Gaulden (1992) to explain the cause of maternal-age related NDJ. She suggested that the follicular microcirculation may be compromised in an aging ovary because of abnormal hormone signaling. Although sufficient evidence is lacking (Henderson et al., 2007), alcohol consumption by women increases the chance of having DS pregnancy as suggested by Kaufman (1983).

Very recently, population based epidemiological study by Ghosh et al., (2011) analyzed the effect of chewing tobacco and contraceptive pill use on the Ch21 NDJ in interaction with known risk variables like maternal age, meiotic stage of NDJ and pattern of recombination i.e., amount of exchange and positioning of chiasma on the recombining homologues. Various logistic regression models have been designed to examine every possible interaction among all above mentioned risk factors. Smokeless chewing tobacco was associated with significant risk for MII NDJ and achiasmate (nonexchange) MI error among the younger mothers. For both of these groups, the highest frequency of tobacco user was recorded in young age group (≤28 yrs) with successive gradual decrease in middle (29-34 years) and old (≥35 years) age group. According to risk prediction model (mentioned above) of DS birth, the chewing tobacco may impart some maternal age-independent risk of DS birth. In explaining the possible adverse influence of chewing tobacco on subcellular components of oocyte, the authors speculated that, regardless of oocyte age and the amount and location of recombination, tobacco probably affects some molecular system common both to meiosis I and meiosis II stages, for example the spindle apparatus. Conversely, the prevalence of oral contraceptive pill exhibited a trend of increasing frequency of occurrence with advancing...
maternal age, suggesting maternal age dependent risk of contraceptive pill in both the meiotic I and meiotic II error groups. Moreover, both risk factors, when present together, exhibited a strong age-dependent effect.

4. Epidemiology of environmental pollutants associated with Down syndrome birth

The epidemiological evidences in favour of the association between DS birth and environmental pollution are also surprisingly high, although controversial. Several pollution events are known to be followed by higher incidence of DS birth in an affected geographical locality. Early reports in the 1950s from USA suggested that fluoridation of water supplies might result in an increase in the frequency of DS birth (Dolk & Vrijheid, 2003). Subsequent comparison of overall DS birth rates in fluoridated and non-fluoridated areas in Massachusetts found no evidence for a difference (Needleman et al., 1974). In this study prevalence rates of DS at birth were compared for Massachusetts residents ingesting fluoridated and non-fluoridated water. The observations included nearly all children born alive with DS in Massachusetts during the 17-year period 1950–1966. A rate of 1.5 cases per 1000 births was found both for fluoride-related births and appropriate comparison groups. Analysis of data from 51 American cities also found no difference in maternal age-specific DS rates between fluoridated and non-fluoridated areas (Erickson, 1980).

Similarly, water contamination with pesticide trichlorfon has been reported to cause an outbreak of DS birth incidence. It was reported in the village of Hungary in 1990s (Czeizel et al., 1993) to increase in teratogenic births, including that of DS. In Woburn, Massachusetts, toxic chemicals (industrial solvents, mainly trichloroethylene) from a waste disposal site were detected in municipal drinking water wells (Dolk & Vrijheid, 2003) and people of this area reported increased incidence of several congenital anomalies. Lagakos et al., (1986) followed up this finding by compiling an exposure score for residential zones in Woburn, using information on what fraction of the water supply in each zone had come from the contaminated wells annually since the start of the wells. The authors found a positive correlation between contaminated water use and higher birthrate of DS in this locality.

The increase in DS birth incidence due to accidental exposure to radioactive materials or radiation remains as a subject of research interest for long time. The disaster at nuclear power plant of Chernobyl, located in former Soviet Union, now at Ukraine, is the worst nuclear accident of the century. The immediate fallout of the incidence was the exposure of a large number of people to the various degree of ionizing radiation, which created a new situation for epidemiological investigation. The accidental event prompted numerous studies on the genetic effects of low dose ionizing radiation in man and almost all studies reported a significant increase in Down syndrome birth along with other birth defects in the parts of Germany, Scandinavia and the Lothian region of central Scotland, nine months after the disaster (Burkart et al., 1997; Sperling et al., 1994; Verger, 1997). This incidence was suggestive for the
deleterious effect of ionizing radiation on the chromosome segregation system in oocyte of the women who are exposed to the radiation. After conducting month wise birth prevalence study on DS birth in West Germany from January 1980 to December 1989, Sperling et al., (1994) suggested that low dose of ionizing radiation might cause birth of cluster of trisomy21 children in that area. Further they hypothesized that the effect of radiation got worse owing to error susceptible process of oogenesis and rapid accumulation of radioactive iodine (I$^{131}$) in body, as the people of that area suffered from iodine deficiency. Although the notion is intuitive, it is very compelling and needs further scientific investigation. Similarly, the effect of irradiation to which the women remained exposed for medical purpose has also been evaluated as DS birth risk in few studies (Uchida et al., 1979; Strigini et al., 1990; Padmanabhan et al., 2004), which suggest radiation may affect the younger women more severely and may increase the chance of having DS conception.

5. Future research

Attempt to resolve the etiology of DS birth is a continuous process and we hope this will bring new insight in the understanding the hidden truth in near future. But the problem lies in its multi factorial nature (Table 2) which inevitably suggests necessity of multi-faceted research efforts from the several directions. For example, it is needed to analyze the polymorphisms of certain genes that regulate meiotic recombination or genes that control maternal molecular aging or those who are involved in faithful chromosome segregation system in meiosis. In searching the cause of recombination anomaly, PRDM9 would be the good target of investigation, as it is a documented regulator of mammalian recombination (Borel et al., 2012). Telomere maintenance system and their genetic components such as TERT and TERC may be the other targets of research and exploration of these genes would help us to realize the cause of molecular aging and related genetic susceptibility of NDJ. The component of sister chromatid cohesion complex and their role in chromosome segregation have been evident in mammals and non-mammalian model organisms. Their functional impairment is known to associate with increased rate of chromosomal missegregation and aneuploidy. But their role and allelic variations have not been explored in the context of Ch21 NDJ and subsequent DS birth. Apart from genetic components, several environmental influences are known to associate with DS birth as risk factors. But proper molecular study on how their adverse effect interacts and imperils faithful chromosome separation apparatus is tantalizingly low. At this level it is almost certain that environmental hazards or aneugen in various forms are associated with accidental increase in DS birth rate at different parts of world. But scientific evidence in favor of their interaction with genetic component is lacking and needs in depth study. If these could be resolved properly in future great advances will be made in the field of medical science and potential couple would enjoy their parenthood with physically and mentally healthy babies.
| Risk Factors | Relation with maternal age | Interaction with other risk factors | Meiotic stage of errors | Reference |
|-------------|----------------------------|------------------------------------|-------------------------|-----------|
| Reduced meiotic recombination | Maternal age independent | Not clear, possibly affected by genetic polymorphisms | MI | Lamb et al. (2005), Oliver et al. (2008), Ghosh et al. (2009), Ghosh et al. (2011). |
| Telomeric single chiasma | Maternal age independent | Not evident | MI | Oliver et al. (2008), Ghosh et al. (2009). |
| Pericentromeric single chiasma | Maternal age dependent | The risk exacerbates with increasing maternal age | MII | Oliver et al. (2008), Ghosh et al. (2009). |
| Shifting of distal chiasma towards proximal one when two simultaneous recombination occur | Maternal age independent | Not evident | MI | Oliver et al. (2008), Ghosh et al. (2009). |
| Shifting of proximal chiasma towards centromere when two simultaneous recombination occur | Maternal age dependent | The risk exacerbates with increasing maternal age | MII | Oliver et al. (2008), Ghosh et al. (2009). |
| Genetic polymorphisms: MTHFR 677C→T, MTRR 66A→G | Possibly maternal age independent | Not evident | Not analyzed | James et al. (2004), Wang et al. (2004). |
| Maternal cigarette smoking | Maternal age independent | Not evident | Not analyzed | Kline et al. (1983), Hook & Cross (1985); Yang et al. (1999). |
| Maternal chewing tobacco use | Maternal age independent | Possibly affects system that ensure non recombinant chromosome segregation and some components common to both MI and MII phases | Both MI and MII | Ghosh et al. (2011) |
| Maternal oral contraceptive use | Debatable | Supposed to affect ovarian hormone level | MI | Martínez-Frías et al. (2001), Ghosh et al. (2011) |
| Combined exposure to tobacco and oral contraceptive | Maternal age dependent | The risk exacerbates with increasing maternal age | Both MI and MII | Yang et al. (1999), Ghosh et al. (2011) |
| Maternal low socioeconomic exposure | Maternal age independent | Not evident | MII | Christianson et al. (2004) |

Table 2. Summary of maternal risk factors for Ch21 nondisjunction and their probable mode of action.
Acknowledgements

We are extremely grateful to Prof. Eleanor Feingold, Pittsburgh University, USA and Prof. Stephanie Sherman, Emory University, Atlanta, USA for their cooperation in Down syndrome research.

Author details

Sujay Ghosh$^{1,2}$ and Subrata Kumar Dey$^1$

*Address all correspondence to: g.sujoy.g@gmail.com

1 Centre for Genetic Studies, Department of Biotechnology, School of Biotechnology and Biological Sciences, West Bengal University of Technology, Salt Lake City, Kolkata, West Bengal, India

2 Genetics Research Unit, Department of Zoology, Sundarban Hazi Desarat College (Affiliated to University of Calcutta), Pathankhali, West Bengal, India

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