Missegregation of Chromosome 21 In Oocyte: What Genetic Causes Imperil a Healthy Egg to Have Bad Fate?

Review Article

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Abstract

Down syndrome represents the most frequent live born aneuploidy and genetic form of intellectual disability. The overwhelming majority of live born Down syndrome is caused by trisomy 21 condition, although a small fraction is due to the inheritance of de novo translocated chromosome 21. The extra copy of chromosome 21 originates owing to non-separation or nondisjunction of chromosome at anaphase in meiotic cell division of gametogenesis. Sincere research attempt have recognized that the higher incidence of Down syndrome birth is associated with maternal advanced age of conception and reduced recombination on chromosome 21. Using panel of short tandem repeat (STR) markers scientists have proved for overwhelming majority of cases, the error originates in maternal first meiotic division when the oocyte grows in fetal ovary and the maternal age effect is restricted only to these maternally originated cases. Several hypotheses have been proposed to explain this maternal age associated increase in the incidence of Down syndrome birth. With large population sample and refined analytical approaches scientists have determined that the effect of recombination error on the nondisjunction is bimodal. On one hand, overall reduction in recombination frequency imparts a risk of nondisjunction irrespective of maternal age. On other hand, some susceptible chiasma configurations increase the chance of chromosome malsegregation with advancing maternal age. Thus the risk factors for the chromosome 21 nondisjunction are of two categories namely, maternal age independent and maternal age dependent. Beside these, some genetic polymorphisms show high degree of susceptibility for Down syndrome conception among women. The gene MTHFR is such candidate which is actually involved in folic acid metabolism pathway and its specific polymorphisms exhibits predisposition to missegregation of Ch21 irrespective of ethnicity of population across the globe. The present review is focused to address the latest development in understanding the role of genetic and molecular risk factors for Down syndrome birth.

Keywords: Down syndrome; Nondisjunction; Maternal Age; Recombination; Genetic polymorphism

Introduction

Down syndrome is the most frequent among all known recognizable live born human aneuploidies, estimating approximately 1 in 700 live-births [1] and for overwhelming cases it is caused by trisomy 21 chromosome complement. The trisomy 21 condition arises due to non-separation or nondisjunction (NDJ) of chromosome 21(Ch21) during gametogenesis and as a result disomic gametes form with two copies of a particular chromosome and upon fertilization by haploid gamete from opposite sex the trisomic fetus is formed. 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 occur in maternal oogenesis particularly at meiosis I (MI) stage. A little fraction of NDJ errors arise at paternal spermatogenesis.

In search of etiology of Ch21 NDJ, researchers have identified two risk factors unambiguously namely, advancing maternal age and altered pattern of meiotic recombination. Beside these two risk factors, other environmental and behavioural factors have also been identified associated with 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

Advanced maternal age is the primary risk

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 [2]. For all the populations studied so far, estimated mean maternal age of conception of DS baby is higher than that of controls having euploid baby and women with MII NDJ is older than women affected with MI NDJ.

Hypotheses have been proposed to explain the relationship between advancing maternal age and higher incidence of aneuploid oocyte formation but no one has proved to be completely satisfactory. The most popular hypothesis [3] holds that the protracted tenure of oogenesis interrupted with meiotic halts (Figure 1), probably makes the eggs more vulnerable to the age effect than sperms. 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 [3], 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 fertilization. Thus, the oocyte, whose ovulation marks the menarche, remains in pause for shortest period and the one 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. The deterioration in egg includes diminishing amount of a meiotic proteins, like those maintaining sister chromatid adhesion [4,5] or meiotic checkpoints components [6] or weakening of centromere cohesion due to age-related reduction in centromere associated proteins MCAK [7] . This list also includes accumulation of environmentally induced damage to the meiotic machinery over time or genetic changes such as mitochondrial deletions [8]. Among all these variables, the spindle assembly check point (SAC) components and sister chromatid cohesion (SCC) were investigated thoroughly [9], as they are prospective genetic candidate that may explain the maternal age 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 attached to the spindle. The SAC includes MAD2L1, BUB1B, and
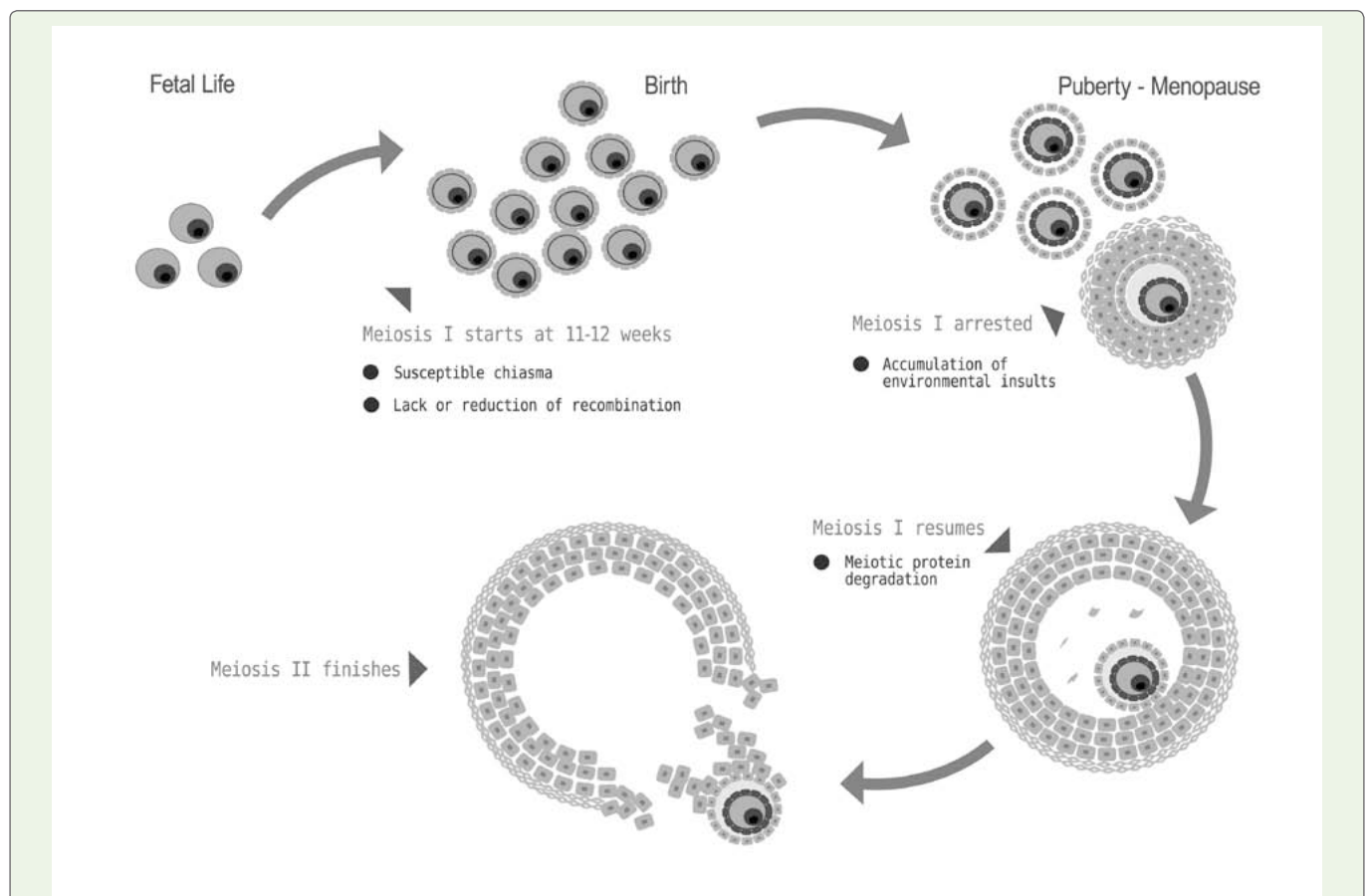

Figure 1: Phases of oocyte development and its various challenges that increases the risk of chromosome nondisjunction.

TTK [10,11] which show decline in concentration with age in mouse leading to misaligned chromosomes [12] and suggests that errors in SAC function contribute to age-related aneuploidy. Disrupted spindles, misaligned chromosomes and decreased expression of SAC components Mad2L1 and Bub1 have evident in aged human oocytes [13,14] and the findings are consistent with hypothesis related to maternal aging. On the other hand, the SCC mediates physical pairing of duplicated chromosome which is essential for appropriate distribution of chromosomes. The cohesion along chromosome arms keeps 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 of 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 [14]. The loss of cohesion with maternal age for distally placed chiasma [15] is consistent with the idea that cohesion defects may contribute to age related aneuploidy [9].

Another component that supposed to decline with age and contributes significantly to maternal age effect on DS birth is the surveillance system of ovary that ensures achiasmate chromosome segregation [16]. Chiasma formation and subsequent recombination is prerequisite of faithful separation of homologues at meiotic anaphase. Absence of chiasma, faulty configurations of chiasma and reduction in chiasma frequency have been attributed; risk of NDJ of Ch21 and subsequent DS birth [17-19]. A high proportion of achiasmate Ch21 tetrad was reported among the mothers of DS having age >35 year [16]. As the decision regarding chiasma formation is taken in foetal ovary, high frequency of achisamate 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 [14,20].

A second hypothesis relates the "biological aging" or "ovarian aging" with the increasing rate of meiotic errors [21,22]. 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 depend upon the biological age of the woman rather than the chronological age [21]. 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 [23,24]. 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 [21]. This change in hormone balance is related to increased rate of aneuploidy at advanced maternal age. Support to this prediction is available from the experiments in mouse model [25]. Alternative to this prediction was provided in the 'limited oocyte pool hypothesis' [21] 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 [21] and women having trisomic pregnancy have average earlier (~1 year) age of menopause [26]. If this were the

case, 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. In support to this prediction, recent data from prenatal diagnosis after a previous trisomic conception shows that the risk of a subsequent trisomy is about 1.7 times the maternal age-related risk [21]. Mathematical model proposed by Kline and Levin (1992) [27] 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 [26]. Elevated level of FSH is reported among the women with DS pregnancy [28,29] which suggests precocious aging among them. Very recently, Kline et al. (2011) [30] have 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 is 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 related to 'genetic age' of women and stated that it is the genetic aging of women that underlies all kind of degenerative changes in ovary and oocyte. The hypothesis was proposed by Ghosh et al., (2010) [18]. The authors estimated the telomere length of peripheral lymphocyte of women having DS child and compared the values with that of age matched controls. They found that beyond of age 29 years the DS bearing mothers exhibit rapid telomere attrition than do 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 do 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 affects the chromosome separation system leading to NDJ. The notion suggested some intuitive link between telomere maintenance system (i.e., system of molecular aging) and chromosome segregating apparatus at molecular level.

Altered pattern of recombination and its interaction with maternal age

Aside from maternal age, there is only factor that has been shown to associate increased susceptibility of maternal NDJ, namely altered recombination patterns. Warren et al. (1987) [31] 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 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 absent or reduced levels of recombination, along with sub-optimally placed recombinant events, increases the likelihood of NDJ [32-38]. 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 [39] 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 indicated by estimated length (cM) of age-specific linkage map of Ch21. In exploring the interaction between maternal age and recombination to gain further insight into the potential mechanisms of abnormal chromosome segregation, comparison had been performed [17] among women of various maternal ages who had an infant with DS due to a maternal MI error for frequency and location of meiotic exchanges along 21q. 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 [17,40] 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) [16] and on the Indian population by Ghosh et al., (2009) [19] 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 error 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 [16,19], 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) [16] and supported by Ghosh et al. (2009, 2010) [18,19] 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 achisamate chromosome containing disomic oocyte may ovulate at any time in reproductive life and hence it is maternal age independent risk factor of Ch21 NDJ.

In both the studies on US and Indian populations [16,19] 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 NDJ error at meiosis I stage of oogenesis and there were 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 formation represent 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 of the mechanism how single telomeric chiasma causes chromosomal mis-segregation has been obtained from the observation in model organisms like Drosophila [36], Saccharomyces [37] and Caenorhabditis elegans [35]. 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 [41,42,36]. Most likely, this susceptibility is related to the minimal amount of sister chromatid cohesion complex remaining distal to the exchange event [43]. 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 [44,45].

Another chiasma configuration that poses susceptibility for NDJ of Ch21 is the pericentromeric exchange. In both the studies on US and Indian DS populations [16,19], 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. 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 [40]. Upon MII division, the bivalent divides reductionally, resulting in disomic gamete with identical centromeres. 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 [36], proximal chiasmata lead to a premature sister chromatid separation just prior to anaphase I. Resolution of chiasmata requires the release of sister chromatid cohesion distal to the site of exchange [42]. 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 product of meiosis at MII, resulting in an apparent MII NDJ. Similar

observation is reported from the study in Yeast in which centromere-proximal crossover promotes local loss of sister-chromatid cohesion [46]. Studies of NDJ in both human [45] and Drosophila [47] 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 indicated by greater proportion of DS births among older women who have experienced the particular pattern of chiasma 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 chromatid 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 [16]. The former explanation is likely to the 'two hit model' proposed previously by Lamb et al., (1996) [40]. 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 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) [19] found that two or more chiasmata formation is prevalent particularly in older age group (≥ 34 years). This signifies that even when Ch21 experiences formation of two or more chiasmata which are believed to be protective of NDJ, particularly when aging effect imparts various degenerative changes in ovary. Analyzing the effect of multiple chiasmata of the 21q, Oliver et al. (2011) [48] found a decrease in the interval between two simultaneous chisamata 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 correctly placed proximal chiasma disjoined erroneously at MI. Moreover, the authors found more intimate positioning of proximal chiasma with the centromere of the chromosomes with two exchange 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 [16,19]. 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) [16] and Ghosh et al. (2009) [19].

Very recently, a denser recombination map along the long arm of nondisjoined Ch21 has been made using SNP (single nucleotide polymorphism) markers by Oliver et al. (2014) [49]. The authors enquire whether the altered patterns of recombination associated with maternal nondisjunction of Ch 21 could be explained by differences in the relationship between recombination placement and recombination-related genomic features (i.e., GC content, Chg. fraction, Poly(A)/Poly(T) fraction or gene density) on 21q or differential hot-spot usage along the no disjoined chromosome 21 (Oliver et al. 2014). They included a total of 297 maternal MI and 277 maternal MII cases of trisomy 21 and genotyped them at 1536 SNP loci on 21q using the Illumine Golden Gate Assay platform. Authors have identified several recombination hot-spots along the length of 21q and examined proportion of single and double recombination events within these hotspots and compared them with the pattern of hot-spot use among controls. For MI cases, the use of recombination hotspot did not differ significantly from the control, which suggests altered pattern of recombination among MI cases was not due to differential use of hot-spots. For MII single recombinant events compared with those for MI-single recombinants events and controls, authors found proportion of single recombinant within the hot spot is significantly positively correlated. The authors infer that potentially factors characteristic of pericentromeric DNA such as chromatin structure or epigenetic modifications may affect the accessibility of a specific chromosome region to recombination in at least a proportion of oocytes with meiotic errors. In analysis of double recombinants events, the authors found similar results with respect defined hotspots among MI and MII errors. They detected a significant relationship between defined hotspots for the distal recombinant events among doubles, but not the proximal events. Furthermore, the lack of evidence for an association in the proximal region differed from that in controls where an association was detected (i.e., significant interaction). The authors did not provide consistent evidence that genomic features present at the site of recombination or differential hotspot usage are implicated in the nondisjunction of Ch 21.

Genetic polymorphism and increasing susceptibility of Down syndrome birth

Maternal genetic factors such as polymorphisms of certain genes 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 homolog pairing, assembly of the synaptonemal complex, chiasmata formation and chiasma positioning, sister chromatid cohesion, spindle formation. Genetic variations of these genes are predisposing factors for chromosome NDJ. Study on Yeast revealed that synaptonemal complex protein Zip1 is essential for nonexchange chromosome segregation and mutation of it causes NDJ [50]. Mutation in synaptonemal complex protein SYP3 is known to associate the recurrent pregnancy loss due to abnormal chromosome behavior [51]. Yeast med1 mutant revealed both meiotic homologous NDJ and precocious separation of sister chromatids [52]. In Drosophila the SAC component BubR1 has been proved to be essential for sister chromatid cohesion and synaptonemal complex and mutation of this gene causes NDJ [53]. Unfortunately, mutation or polymorphisms of these genes have not been examined among the women bearing DS child.

The gene that has been identified first in this category is MTHFR (methylene tetrahydrofolate reductase), which is not directly related to the meiotic process. The case-control study by James et al., (1999) [54] provided preliminary evidence that the 677C→T polymorphism in the 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 [55]. Folate is essential for the production of S-adenosylmethionine, which is the primary methyl donor (Figure 2) for epigenetic DNA methylation and essential for gene expression regulation and maintenance of chromosomal integrity at centromere [54,56,57]. Folate deficiency reduces S-adenosylmethionine synthesis, leading to DNA hypomethylation [58-60]. The pericentromeric hypomethylation could impair the heterochromatin formation and kinetochore establishment (Figure 2) resulting in chromosomal NDJ [54]. This can happen 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 [61].

This initial report had inspired several follow-up studies on the 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 [62,63]. 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 [63]. Nevertheless, support to the notion regarding the association between MTHFR 677C-T polymorphism and risk of DS birth was provided by other studies in different populations. Wang et al., (2004) [60] reported significant increase in the risk of DS conception among Chinese women bearing two polymorphisms namely, polymorphisms of MTHFR 677C→T and the polymorphism MTRR (Methionine synthase reductase) 66A→G. The estimated risks were more than three folds and five folds for MTHFR (Odd Ratio=3.7; 95% CI, 1.78~8.47) and 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 MTHFR and MTRR polymorphisms [64,65]. 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 [55]. If such an association really does exist, it would provide evidence for a genetic effect for NDJ. The study of Alfi et al., (1980) [66] 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
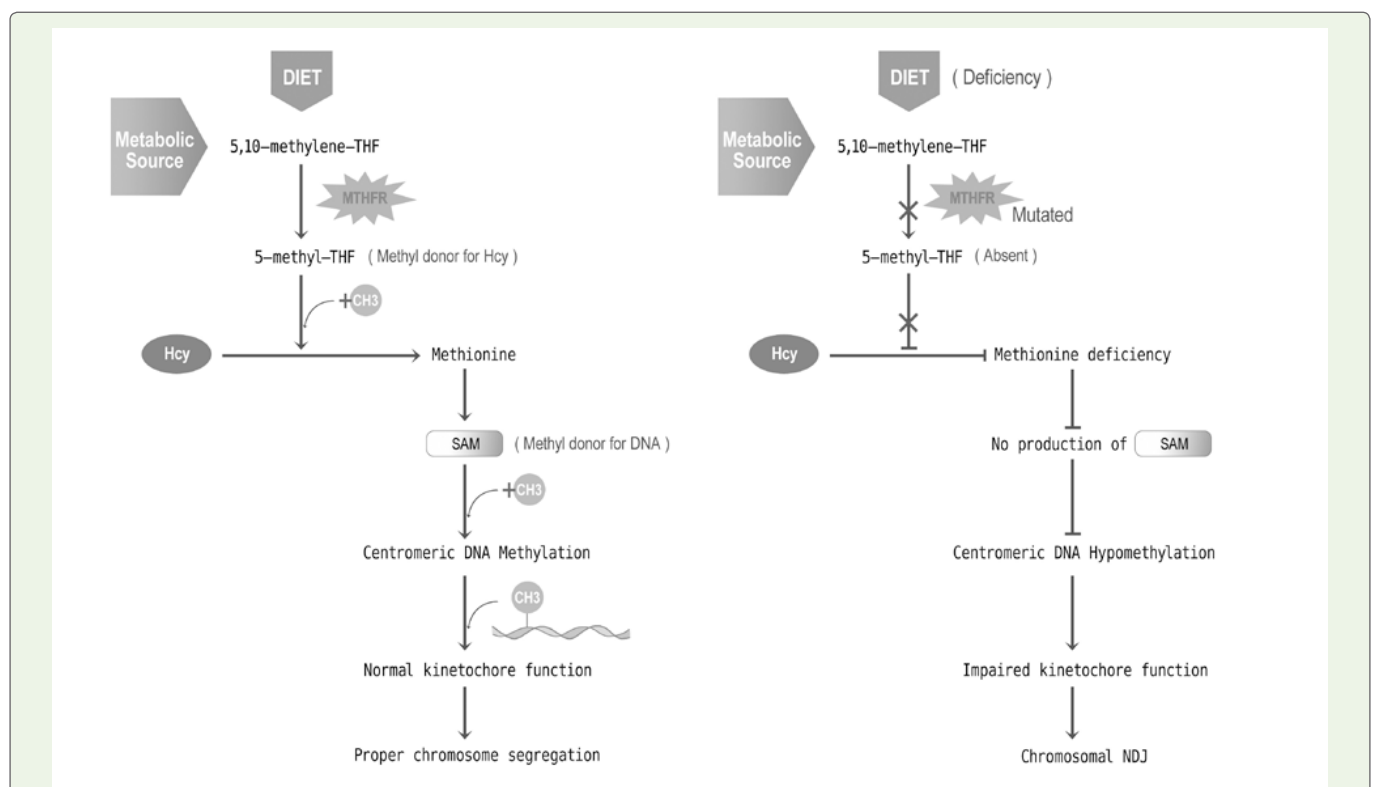

Figure 2: Pathway for folic acid metabolism and mutation in MTHFR gene that increases the risk of chromosomal nondisjunction.

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 meiotic 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 [67-72].

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 selective prenatal termination of pregnancies with trisomic fetuses, and as yet unidentified environmental factors [55]. Only one such study by Allen et al., (2009) [73] 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 are 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 large scale population based studies are needed to be conducted.

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, 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. For searching the cause of recombination anomaly, PRDM9 would be the good target of investigation, as it is a documented regulator of mammalian recombination [74,75]. Telomere maintenance system and their genetic components such as TERT and TERC may be the other targets of research and would help us to realize the cause of molecular aging and related genetic susceptibility of NDJ [76,77]. 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 be associated with elevated level of chromosomal missegregation and aneuploidy [78-80]. But their role and allelic variation 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 is 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 indepth 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.

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