Technology Update

Indian Pediatrics 2003; 40:115-123

Gene Polymorphism and Folate Metabolism: A Maternal Risk Factor for Down Syndrome

Jayesh J. Sheth and Frenny J. Sheth

From the Foundation for Research in Genetics and Endocrinology (FRIGE), Genetics Center, Ahmedabad 380 015, India.

Correspondence to: Jayesh J. Sheth, 20/1, Bimanagar, Satellite, Ahmedabad 380 015, India. E-mail: jshethad1@sancharnet.in

Manuscript received: July 16, 2001; Initial review completed: August 23, 2001;

Revision accepted: November 9, 2002.

The high birth frequency of Down syndrome (DS), trisomy 21 (T21), has been a subject of interest to the clinicians and researchers due to its complexity in phenotypic expression. In addition to the maternal age, identification of the mechanistic basis for T21 requires an understanding of the cellular-molecular events and other biochemical pathways that could promote maternal meiotic nondisjunction. Recent studies have linked the increased frequency of polymorphism of methylenetetrahydrofolate reductase (MTHFR, C677T) and methionine synthase gene (MTRR, A66G) in mothers with DS child. Based on evidence that abnormal folate and methyl metabolism can lead to DNA hypomethylation and abnormal chromosomal segregation, researchers have observed that mothers with mutation in MTHFR (C677T) and MTRR (A66G) gene have elevated levels of plasma homocysteine. This was found to be associated with a 2.6 to 2.9 fold increased risk of having child with DS compared to mothers without the mutation. Subsequent studies evaluating Italian, Irish, French, and Indian-Gujarati women could not demonstrate an association of MTHFR gene polymorphism in mothers with DS child. However, the Irish study did find an increased risk of DS associated with the MTRR polymorphism and an interactive effect of MTRR and MTHFR polymorphisms with increased risk. Interestingly, an increase in plasma homocysteine was found to be a risk factor for DS in several of the studies. Despite the differences, the published studies suggest a common theme of abnormal folate metabolism associated with increased risk of having a child with DS.

These observations suggest that there seems to be a geographic variation in gene polymorphism and it could not be attributable to meiotic nondysjunction in all mothers with DS child but increased homocysteine in all different study group does suggest that there may be a gene-nutritional or gene-gene or gene-nutritional-environmental factors involved in increased frequency of meiotic nondisjunction which needs transnational and multinational study design.

Key words: *Down syndrome, folate, homocysteine, meiotic nondisjunction.*

DOWN syndrome (DS) is the first clinically defined genetic disorder shown to be chromosomal in origin -the result of trisomy 21 (T21)(1). Since then, human chromosome 21 and its genes have an important place in human genetics because T21 is the most common aneuploidy at birth and the most commonly recognized genetic cause of mental retardation. The premature pregnancy failure is higher in conceptus with T21 and about 1/150 conception has T21. Eighty per cent of these are lost during pregnancy(2) with the birth frequency of 1 : 700 to 1 : 1000(3,4).

It has been recognized that the risk of having a child with DS increases with maternal age(5) and that the distribution of maternal age in the population of women having children is the primary determinant of the overall incidence of DS(6). For example, the risk of having alive born infant with DS at maternal age 30 is 1 in 1,000 and at maternal age 40 is 9 in 1,000(4) .

Origin of Trisomy 21

Almost 95% of individuals with T21 have 3 free copies of chromosome 21; in about 5% of patients, 1 copy is translocated to another acrocentric chromosome, most often chromo-some 14 or 21(4). It has been shown(7) that all

de novo translocations involving #14 and #21 originated from maternal germ cells but the *de novo* translocation involving two #21 is an isochromosome (dup 21q) rather than the result of Robertsonian translocation caused by a fusion between 2 heterologous chromatids(8). About half of translocations are of paternal and half are of maternal origin. In 2 to 4% of cases with free trisomy 21 there is recognizable mosaicism for a trisomic and a normal cell line(9). Antonarakis(10) has shown that 95% of T21 cases are maternal in origin due to meiotic nondisjunction and most errors in maternal meiosis occur in meiosis I and the mean maternal age in this population is 32 years. Subsequently, a population based analysis using DNA markers have shown that maternal nondisjunction accounts for 86 per cent of all cases of T21, with 75 percent of cases occurring at meiosis-I (M-I) and 25 percent at meiosis-II (M-II)(11). This study has further shown that 9 percent of cases were paternally derived (50 percent M-I, 50 percent M-II) and 5 percent were somatic in origin.

Search for Origin of Nondisjunction

Despite decades of investigation we still do not understand the basis of the very strong effect of maternal age on nondisjunction. It appears that both age dependant and age independent factors may be operating simul-taneously. It could be due to an age dependant decay in spindle fibers or their components, a failure in nucleolar breakdown or an accumulation of the effects of radiation, hormonal imbalances and/or infection. Hypothesis of this type would be compatible with the predominantly meiotic origin of nondisjunction(12).

Clinical and experimental studies have shown that age independent DNA hypo-methylation is associated with chromosomal instability and abnormal segregation. Based on these observations, a link between dietary folate and methyl deficiency *in vivo* and DNA hypomethylation was suggested(13). Further research of the metabolic events related to DNA hypomethylation lead to the observation that 5-10 methylenetetrahydrofolate reductase (MTHFR) acts at a critical metabolic juncture in the regulation of cellular methylation reactions by catalyzing the conversion of 5,10-methylenetetrahydrofolate to 5-methyl-tetrahydrofolate (5-MTHF), the cofactor form required for remethylation of homocysteine (Hcy) to methionin(4). Mutation in the MTHFR gene (MIM-236250) was subse-quently found to be associated with DNA hypomethylation(15). These cellular observa-tions led the scientists(16,17) to postulate a link between abnormal folate metabolism and mutation of MTHFR gene as a maternal risk factor for DS.

These reports provided preliminary evidence of a genetic component to human nondisjunction responsible for T21, which if confirmed, would represent the first known genetic contribution to meiotic chromosome segregation in humans.

Polymorphism of MTHFR and MTRR Gene

The enzymatic properties of MTHFR indicate stimulation of MTHFR by high levels of S-adenosylmethionine (SAM)(18). In subsequent years an extensive scientific literature accumulated demonstrating the association of hereditary enzyme deficiency with disturbances of the metabolism of homocysteine leading to hyperhomo-cysteinemia (*Fig. 1*). The cloning of the MTHFR gene provided the basis for identifying the mutations associated with different degrees of MTHFR deficiency(19). The most common polymorphism in this gene, C®T substitution at nucleotide 677 [C677T] was the cause of the thermolabile enzyme activity(20). It has been shown that this polymorphism is a genetic risk factor for cardiovascular and cerebrovascular disease. Two common genetic polymorphisms associated with a reduced MTHFR activity have been identified. One is located in exon 4 at the folate-binding site (C677T) converting an alanine into a valine residue (14). In homozygous TT subjects this loss of functional mutation results in approximately 50% of enzyme activity as compared to those without mutation and leads to decrease syn-thesis of 5-methyltetrahydrofolates. Among healthy subjects, the C677T genotype is associated with a significantly higher homoc-ysteine and low red cell folate levels than in heterozygotes or individuals with wild type C alleles. The other polymorphism (A1298C) is in exon 7 within the presumptive regulatory domain(21,22). This transversion changes glutamine into alanine residue and lowers the enzyme activity to 60% of the control values with combined heterozygosity with the C677T polymorphism.

Another enzyme methionine synthase reductase (MTRR) catalyzes the remethyla-tion of homocysteine to methionine via reaction in which methylcobalmin serves as an intermediate methyl donor. Therefore patients with disorders of folate/cobalmin metabolism who are defective in methionine synthase activity exhibit megaloblastic anemia, developmental delay, hyperhomo-cysteinemia and hypomethioninemia(23).

The cloning of MTRR gene led to the identification of a polymorphism (A66G), that was shown to be associated with increased risk of spina bifida(24). Later on Hobbs *et al.*(17) reported this polymorphism to be associated with maternal risk for DS.

Down Syndrome, MTHFR (C677T), MTRR (A66G) Polymorphism and Folate Metabolism

Although the perception of DS as a metabolic disease is not yet prevalent, the over expression of genes coding for specific enzymes translates directly into biochemical aberrations and contributes to the unique pathogenesis of DS(25). This will require better understanding of the biochemical and molecular events taking place in mother with DS child and the child as well.

Recent observations of a possible link between T21 and maternal polymorphisms in the folate pathway have opened up the new era of investigation of pathogenesis of DS related to abnormal folate metabolism(16,17). A study by Pogribna *et al.*(25) have shown that the increased activity of cystathione beta synthase (CBS) in children with DS alters Hcy metabolism such that the folate-dependant resynthesis of methionine is compromised (16.1 ± 3.3 µmol/L vs 30.6 ± 6.5 µmol/L) in DS and in control children respectively. The decreased availability of Hcy (5.1 ± 1.1 µmol/L in DS vs 6.7 ± l.6 µmol/L in control children) promotes the well-established folate trap, creating a functional folate deficiency that may contribute to the metabolic pathology of this complex genetic disorder. James *et al.*(16) hypothesized the possibility that gene-nutrient interactions associated with abnormal folate metabolism and DNA hypomethylation might increase the risk of maternal chromosome nondisjunction and T21. They have found a higher frequency of both the MTHFR C/T and T/T genotype in the mothers of children with DS as compared to control mothers (*Table I*). Although the absolute number of mothers with the T/T genotype was twice as high in case mothers than in control mothers, the sample size was small and the difference was not significant. When the frequencies of mothers with at least one T allele were compared, the odd ratio for case mothers was 2.6 ($p < 0.03$). In their analyses of 57 cases and 50 control women, they observed significant increases in plasma Hcy concentrations among mothers of DS child compared to control mothers (10.9 µmol/L versus 7.9 µmol/L), although the increase was not dependant on MTHFR genotype(16) (*Table II*). In addition, they carried out *in vitro* lymphocyte sensitivity to methotrexate in mothers of children with DS as an indicator of functional folate metabolism. A significant increase in lymphocyte cytotoxicity to methotrexate was observed in the mothers of children with DS as compared to control mothers. Again this was independent of MTHFR genotype suggesting interaction of other genetic/environmental factors. Finally, mothers of children with DS, regardless of MTHFR genotype had low dietary intakes of folate from food and interestingly 26% of DS mothers with T allele were reported taking vitamin supplement containing 400 µg folic acid at the time of conception. A subsequent study by Hobbs, *et al.*(17) in a larger sample size have shown data consistent with the preliminary observation of MTHFR C677T polymorphism in mothers with DS child than among controls with an odd ratio of 1.91 (95% CI 1.19-3.05). In addition, they observed(17) (*Table III*) that polymorphism in MTRR gene (A66G) was independently associated with a 2.57 fold increase in DS childbirth with an estimated risk at (95% CI 1.33-4.99). It was observed that combined presence of both polymorphism (MTHFR C677T and MTRR A66G) was associated with a greater risk of DS than was the presence of either alone with an odd ratio of 4.08 at (95% CI 1.94-8.56). Because of the importance of methionine synthase reaction in maintaining normal folate metabolism and DNA methylation, the authors have hypothesized that this poly-morphism could be a second maternal genetic risk factor for Down syndrome. It was also shown in a case report study that altered folate status and homozygous TT mutation in the MTHFR gene in both mother and child would be expected to increase the risk of neural tube defect in addition to meiotic nondisjunction resulting in T21(26). Bailey and Gregory(27) have shown the reduction in enzyme activity associated with MTHFR polymorphism (C67.7T) that raises the dietary requirement for folic acid to maintain normal remethylation of Hcy to methionine. Consequently, low folate level in individual with MTHFR polymorphism results in an increase of Hcy and decrease of methionine levels. This chronic elevation in intracellular Hcy can lead to a decrease in the ratio of S-adenosylmethionine (SAM) to S-adeno-sylhomocysteine (SAH) which results in inhibition of the DNA methyltransferase and DNA hypomethylation(28). Thus, the association between folate deficiency and DNA hypomethylation provides evidence that gene/nutritional deficiencies that affect folate metabolism may be responsible for increased risk of nondysjunction and DS. However, further study in different population of Italian and French women did not support the presence of an increased risk of DS birth in mothers with T allele of MTHFR gene(29-31); however, the high intake of food folate in France and Italy may neutralize the metabolic impact of the MTHFR polymorphism. As shown in *Table 1*, the primary study of MTHFR polymorphism carried out in young Indian-Gujarati women having DS child do not support the link between increased fre-quency of T allele and DS birth. (Sheth *et al*. unpublished data). This result is consistent with genetic studies that have shown an unusually low prevalence of the T

allele in Indian populations(32). A study by O' Leary *et al.*(30) observed that Irish women who have polymorphism of MTHFR and MTRR gene are at increased risk of producing offspring with DS. However, MTHFR C677T genotype was not an independent risk factor (p = 0.74) in mothers of DS child while the frequency of MTRR polymorphism (AG, GG) was significantly higher in mothers of DS child (p = 0.0028). Hassold *et al.*(33) could not demonstrate the polymorphism variability in the folate pathway as a significant contributor to human meiotic nondisjunction in chromosomes other than #21. They analyzed maternal polymorphisms of MTHFR and MTRR gene in 93 cases of sex chromosome trisomy, 44 cases of trisomy 18, 158 combined cases of autosomal trisomies 2, 7, 10, 13, 14, 15, 16, 18 or 22 and compared the distribution of genotypes to those of control populations. MTHFR polymorphism was observed in mothers of trisomy 18 conceptuses but they were unable to identify any other significant associations. This study suggests that factors involving, both genotype and nutrition may underlie susceptibility to nondisjunction involving chromosome 21 but the same may not be true for other autosomal trisomies. Therefore genetic-environmental interactions will continue to be unmasked as an increasing number of genes involved in Hcy and folate metabolism is cloned. Prime candidate genes for further study in this regard are both methionine synthase and methionine synthase reductase for which polymorphism have been found.

Table I

Prevalence of Maternal MTHFR Genotype in Down syndrome affected Pregnancies and Control Mothers

	DS Mothers				Control mothers			
Genotype	**C/C (%)**	**C/T (%)**	**T/T (%)**	**CT/TT**	**C/C (%)**	**C/T (%)**	**T/T (%)**	**CT/TT**
James et al.(16)	26.3	59.6	14.0	73.6	48.0	44.0	8.0	52.0
Hobbs et al.(17)	32.0	54.0	14.0	68.0	48.0	42.0	10.0	52.0
Stupia et al.(31)	31.0	50.7	18.3	69.0	24.1	55.4	20.5	75.9
O'Leary et al.(30)	44.0	51.0	5.0	57.0	47.0	44.0	9.0	53.0
Sheth et al. (unpublished)	71.4	25.0	3.5	28.5	46.15	46.15	7.7	53.84

DS mothers = mother with Down syndrome child. Control mother = mother with normal children and no history of abortion, C/C or C/T = Type of MTHFR genotype observed in mothers at nucleotide position 677.

Table II

Plasma Homocysteine and Methionine Concentration in Age Matched Control Mothers and in Mothers of Children with DS

	Homocysteine µmol/L		Methionine µmol/L		
Author	**Control mothers**	**DS Mothers**	**Control mothers**	**DS mothers**	**P Value**
James et al.(16)	8.30± 0.4	12.0± 0.3	33.6± 3.8	28.3± 1.4	0.001
O'Leary et al.(30)	8.44± 2.6	8.40± 2.6	–	–	0.91
Sheth et al.(unpublished)	6.41± 1.8	8.81± 2.4	25.08± 5.1	21.5± 6.0	0.025

Values are mean ± SD, DS = Down syndrome.

Table III

Association between maternal MTRR genotype in DS and Control Pregnancies

	DS mother				Control mothers			
Genotype	**AA**	**AG**	**GG**	**AG/GG**	**AA**	**AG**	**GG**	**AG/GG**
Hobbs et al.(17)	18	44	38	82	28	49	23	72
O'Leary et al.(30)	2	48	50	98	18	53	29	82

Values expressed are in percentage, AA/AG=Genotype of mothers observed in MTRR gene at nucleotide position 66.

Acknowledgements

Our sincere acknowledgement to Dr. S. J. James for providing help in mutation study, to WHO for the financial support which has made this review possible, Prof. A. Campana and other scientific committee members for giving an opportunity to participate in the 11th WHO course held at Geneva and Dr. Stylianos E. Antonarakis, for his suggestions in writing this review. Dr. James Jill, Centre for Toxicological Research, US-FDA, has carried out MTHFR mutation study and thiol estimation for our study group.

Contributors: Principal authors have carried out karyotyping of children with Down syndrome, biochemical study of mothers for homocysteine and DNA seperation. Both authors wrote the manuscript. JJS shall act as guarantor.

Funding: None for practical work except WHO fellowship to carry out review work on the subject as a part of 11th PG course in Reproductive Biology and Reproductive Medicine held at Geneva, Oct 2001.

Competing interest: None stated.

Key Messages

• Polymorphism in the MTHFR (C677T) and MTRR gene (A66G) negatively affects folate metabolism and its role in meiotic nondisjunction.

• Hyperhomocystenemia and low methionine in mothers with DS child suggest involvement of other genes in folate metabolic pathway as well as nutritional deficiencies including folate and B12.

• Folate is the key factor in DNA methylation and synthesis. However, whether folate deficiency is causally associated with trisomy 21 will require larger studies comparing different ethnic and geographical populations with different dietary habits.

References

1. *Lejeune J, Gauthier M, Turpin R. Les chromosomes humans enculture de tissues. C R Acad Sci 1958; 248; 602-603.*

2. *Boue J, Bone A, Lazar P. Retrospective and prospective epidemiological studies of 1506 karyotyped spontaneous abortions. Teratology 1975; 12: 11-26.*

3. *Krivchenia E, Hether CA, Edmond LD, May DS. Comparative epidemiology of Down syndrome in two United States populations. Am J Epidemiol. 1993; 137: 815-825.*

4. Hook C G. Epidemiology of Down syndrome. In: Pueschel, SM Rynders, JE. Down syndrome. Advances in Biomedicine and the Behavioral Sciences. Cambridge, Ware Press, 1982: pp 11-18.

5. Penrose LS. The relative effects of paternal and maternal age in mongolism. J Genet 1933; 27: 219-224.

6. Adams MM, Erickson JD, Layde PM, Oakley GP. Down's syndrome. Recent trends in the United States. JAMA 1981; 246: 758-760.

7. Petersen MB, Adlsberger PA, Schinzel AA, Binkert F, Hindkel GK, Antonarakis SE. Down syndrome due to de novo Robertsonian translocation t(14; 21): DNA polymorphism analysis suggests that the origin of the extra 21q is maternal. Am J Hum Genet 1991; 49: 529-536.

8. Antonarakis SE, Adel Berger PA, Peterson MB, Binkert F, Schinzel AA. Analysis of DNA polymorphism suggests that most de novo dup (21q) chromosomes in patients with DS are isochromosome and not translocations. Am J Hum Genet 1990; 47: 968-972.

9. Mikkelsen M. Down Syndrome. Cytogenetical epidemiology. Hereditas 1977; 6(1): 45-50.

10. Antonarakis ES and the Down Syndrome Collaborative Group. Prenatal origin of the extra chromosome in trisomy 21 as indicated by analysis of DNA polymorphism. N Engl J Med 1991; 324: 871-876.

11. Yoon PW, Freeman SB, Sherman SL, Tarf LF, Gu Y, Pettay D, et al. Advanced maternal age and the risk of Down syndrome characterized by the meiotic stage of the chromosome error. A population based study. Am J Hum Genet 1996; 58: 628-633.

12. Chandley AC. Maternal aging as the important factor in human aneuploidy. In: Dellaco VL, Voytek PE, Hollaender A (Eds): Aneuploidy. Etiology and Mechanisms. New York, Plenum, 1985; p 409-415.

13. Christman JK, Sheikhnejad G, Dizik M, Abileah S, Wainzan F. Reversibility of changes in nucleic acid methylation and gene expression induced in rat liver by severe dietary methyl deficiency. Carcinogenesis 1993; 14: 551-557.

14. Frost P, Blom J, Milos R, Goyette P, Sheppard CA, Mathews TG, et al. A candidate genetic risk factor for vascular disease, a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995; 10: 111-113.

15. Stern LL, Mason JB, Selhub J, Choi SW. Genomic DNA hypomethylation, a character-istics of most cancers, is present in peripheral leucocytes of individuals who are homozygous for the C677T polymorphism in the MTHFR gene. Cancer Epidemiol Biomarkers Prev. 2000; 9: 849-853.

16. James SJ, Pogribna M, Pogribny IP, Melynk S, Jean Hine R, Gibson JB, et al. Abnormal folate metabolism and mutation in the MTHFR gene may be maternal risk factors for Down syndrome. Am J Clin Nutr 1999; 70: 495-550.

17. Hobbs CA, Sherman SL, Yi P, Hopkins SE, Torfs CP, Hine RJ, et al. Polymorphisms in genes involved in folate metabolism as maternal risk factors for Down syndrome. Am J Hum Genet 2000; 67: 623-630.

18. Kutzback C, Stokstad EL. Mammalian MTHFR. Partial purification, properties and inhibition by 5-adenosylmethinone. Biochim Biophys Acta. 1971; 250: 459-477.

19. Goyette P, Summer J, Milos R, Duncan A, Rosenblatt D, Matthews R, et al. Isolation of cDNA for Human MTHFR and identification of mutations in MTHFR deficient patients. Am J Hum Genet 1993; 53(suppl): A 153.

20. Goyettee P, Summer JS, Milos R, Duncan AMV, Rosenblatt DS, Mathews RG, et al. Human MTHFR gene and genotype/phenotype correlations in severe MTHFR deficiency. Am J Human Genet 1994; 56: 1052-1059.

21. Goyette P, Aditya P, Renate M, Frosst P, Tran P, Chen Z, et al. Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). Mammalian Genome. 1998; 9: 652-656.

22. van der Put NM, Gabreels F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, et al. A second common mutation in the MTHFR gene: an additional risk factor for neural tube defects. Am J Hum Genet 1998; 62: 1044-1055.

23. Leclerc D, Wilson A, Dumas R, Gafuik C, Song D, Watkins D, et al. Cloning and mapping of cDNA for methionine synthase reductase, a flavoprotein defective in patients with homocysteinuria. Proc Natl Sci Acad USA. 1998; 95: 3059-3064.

24. Leclerc D, Odievre MH, Wu Q, Wilson A, Hizenga JJ, Rozen R, et al. Molecular cloning, expression and physical mapping of the human methionine synthase reductase gene. Gene 1999; 240: 75-78.

25. Pogribna M, Stepan M, Pogribny I, Chango A, Yi P, James SJ. Homocysteine metabolism in children with Down syndrome: In vitro modulation. Am J Hum Genet 2001; 69: 88-95.

26. Al-Gazali LI, Padmanabhan R, Melynk P, Yi P, Pogribny LP, Pogriban M, et al. Abnormal folate metabolism and genetic polymorphism of the folate pathway in a child with DS and neural tube defect. Am J Med Genet 2001; 103: 128-132.

27. Bailey LB, Gregory J. Polymorphisms of MTHFR and other enzymes; metabolic significance, risks and impact on folate requirement. J Nutr 1999; 129: 919-922.

28. Melynk S, Pogribna M, Pogribny IP, Yi P, James SJ. Measurement of plasma and intra-cellular S-adenosylmethionine and S-adenosyl-homocysteie utilizing colorimetric electro-chemical detection; alterations with plasma homocysteine and pyriodoxal S-phosphate concentrations. Clin Chem 2000; 46: 265-272.

29. Chadefaux VB, Conde M, Muller F, Oury JF, Chabli A, Jais J, et al. Methylene-tetrahydrofolate reductase polymorphism in the etiology of Down syndrome. Pediatr Res 2002, 51(6); 766-776.

30. O'Leary VB, Parlie-McDermott A, Molloy AM, Kirke PN, Johnson Z, Colney M, et al. MTRR and MTHFR polymorphism; Link to Down syndrome. Am J Med Genet 2002; 107: 151-155.

31. Stupia L, Gatta V, Gaspar AR, Antonucci I, Morizio E, Calabrese G, et al. C677T mutation in the 5,10-MTHFR gene and risk of Down syndrome in Italy. Eur J Hum Genet 2002; 10: 388-390.

32. Mukherjee M, Joshi S, Bagadi S, Dalvi M, Rao A, Shetty KR. A low prevalence of the C677T mutation in the methylenetetrahydrofolate reductase gene in Asian Indians. Clin Genet 2002; 61: 155-159.

33. Hasold T, Burrage L, Chan E, Judis L, Schwartz S, James SJ, et al. Maternal folate poly-morphisms and the etiology of human nondysjunction. Am J Hum Genet 2001; 69: 434-439.