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Report

Maternal Folate Polymorphisms and the Etiology of Human Nondisjunction

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Attempts to identify genetic contributors to human meiotic nondisjunction have met with little, if any, success. Thus, recent reports linking Down syndrome to maternal polymorphisms at either of two folate metabolism enzymes, methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR), have generated considerable interest. In the present report, we asked whether variation at MTHFR (677C→T) or MTRR (66A→G) might be associated with human trisomies other than trisomy 21. We analyzed maternal polymorphisms at MTHFR and MTRR in 93 cases of sex-chromosome trisomy, 44 cases of trisomy 18, and 158 cases of autosomal trisomies 2, 7, 10, 13, 14, 15, 16, 18, or 22, and compared the distributions of genotypes to those of control populations. We observed a significant increase in the MTHFR polymorphism in mothers of trisomy 18 conceptuses but were unable to identify any other significant associations. Overall, our observations suggest that, at least for the sex chromosomes and for a combined set of autosomal trisomies, polymorphisms in the folate pathway are not a significant contributor to human meiotic nondisjunction.

Since the identification of the first human trisomy, >40 years ago, many attempts have been made to elucidate factors that influence the rate of meiotic nondisjunction in our species. These efforts have been remarkably unsuccessful; aside from the well-established association between increasing maternal age and trisomy, no etiological factor has been convincingly linked to human nondisjunction (Abruzzo and Hassold 1995).

Given this background, recent observations of a link between trisomy 21 and maternal polymorphisms in the folate pathway were greeted with considerable excitement (Rubin 1999). In an initial report, James et al. (1999) compared the frequency of C→T mutations at nucleotide 677 in the enzyme methylenetetrahydrofolate reductase (MTHFR [MIM 236250]) between mothers of individuals with Down syndrome (DS) and age-matched control females. The 677C→T mutation affects both folate metabolism and cellular methylation reactions and is a known risk factor for neural tube defects (van der Put et al. 1998). James et al. (1999) hypothesized that aberrant DNA methylation as a result of abnormal folate metabolism due to the mutation might increase the likelihood of meiotic nondisjunction, thus making the mutation a risk factor for trisomy 21, as well as for neural tube defects. In their analyses of 57 case and 50 control women, they observed significant increases in plasma homocysteine concentrations among mothers of DS individuals, consistent with a functional folate deficiency among these individuals. Further, they found a highly significant increase in the proportion of CT heterozygotes and TT mutant homozygotes among the case mothers, consistent with a gene-nutrient effect on chromosome 21 nondisjunction.

In a follow-up study (Hobbs et al. 2000), James and colleagues expanded their study population to 157 case and 144 control mothers, reexamined the association between MTHFR polymorphisms and trisomy 21, and analyzed maternal polymorphisms at a second gene in the folate pathway, methionine synthase reductase (MTRR [MIM 602568]); an A→G mutation at position 66 of MTRR has been linked to an increase in spina

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bifida (Wilson et al. 1999). Hobbs et al. (2000) confirmed the initial observations of a link between MTHFR and trisomy 21 and found a highly significant increase in GG mutant homozygotes at MTRR among DS mothers. Furthermore, the combined presence of both mutations appeared to increase the risk, since the highest odds ratios were observed among women with either CT or TT genotypes at MTHFR and a GG genotype at MTRR—that is, among women having a "susceptible" genotype at each locus.

Subsequently, another group has also linked MTRR polymorphisms to trisomy 21 (O'Leary et al., in press). Specifically, in an analysis of 48 mothers of individuals with DS and 192 control mothers, they identified a highly significant increase in the G allele among the case mothers.

Taken together, these reports provide preliminary evidence of a genetic component to human nondisjunction which, if confirmed, would represent the first known genetic contributor to meiotic chromosome malsegregation in our species. Further, these results suggest the possibility of relatively simple preventative strategies, since dietary folate and vitamin B_{12} supplementation might overcome the risk of nondisjunction associated with the susceptible genotypes. The potential importance of this association makes it essential that the original observations on trisomy 21 be confirmed or refuted, and that the possibility of an effect on nondisjunction of other chromosomes be investigated. In the present report, we summarize studies that address the latter issue, specifically analyzing maternal folate-pathway polymorphisms in sex-chromosome trisomies and autosomal trisomies 2, 7, 10, 13, 14, 15, 16, 18, and 22.

Study material was obtained from two centers that have been actively engaged in analyses of the origin of human trisomies, namely the Wessex Regional Genetics Laboratory in Salisbury, England ("Wessex") and the Department of Genetics at Case Western Reserve University in Cleveland, OH ("Cleveland"). We analyzed maternal DNA samples from trisomies that were known to be maternal in origin (Fisher et al. 1993, 1995; MacDonald et al. 1994; Zaragoza et al. 1994, 1998; Thomas et al. 2001); a total of 311 such samples were available for study. At Wessex, maternal DNA samples were available for 104 cases of sex-chromosome trisomy (70 cases of 47,XXY and 34 cases of 47,XXX) and 45 cases of trisomy 18. Virtually all cases of sex-chromosome trisomy involved live-born individuals, whereas cases of trisomy 18 were ascertained from spontaneous abortions, stillbirths, and therapeutic abortions; details regarding the ascertainment of the cases have been provided elsewhere (Fisher et al. 1993, 1995; MacDonald et al. 1994). At Cleveland, maternal DNA samples were available for 10 cases of trisomy 2, 10 cases of trisomy 7, 5 cases of trisomy 10, 27 cases of trisomy 13, 8 cases of trisomy 14, 20 cases of trisomy 15, 66 cases of trisomy 16, and 16 cases of trisomy 22. With the exception of four cases of trisomy 13, all trisomies were ascertained from cytogenetic studies of spontaneous abortions (Zaragoza et al. 1994).

For controls, we utilized DNA samples being analyzed at the Wessex or Cleveland centers for reasons unrelated to those of the present study; whenever possible, these involved mothers of chromosomally abnormal conceptuses who were known to have contributed the "right" number of chromosomes. For example, at Wessex, controls consisted of DNA samples from 57 mothers of paternally derived cases of 47,XXY. Similarly, at Cleveland we used DNA samples from 25 mothers of androgenetic triploids, although we augmented this group with an additional 24 samples from volunteer donors being analyzed for other purposes. The racial composition of the Wessex and Cleveland study groups was markedly different: at Wessex, all individuals were white, whereas, at Cleveland, both case and control groups consisted of ~85% white and ~15% black individuals. Since the mutant-allele frequencies at MTHFR 677 vary with race and ethnicity (Botto and Yang 2000) and because the two centers analyzed different types of trisomies, we made no attempt to pool cases and controls between centers. Instead, we made separate case-control comparisons at each of the two study centers.

All case and control individuals were genotyped at MTHFR and MTRR, using standard PCR-based methodology (Hobbs et al. 2000). We obtained genotypes at both loci for 280 of the 311 case individuals and for 100 of the 106 control individuals; for the remaining individuals, we were able to score only one of the two loci. We compared case and control allele frequencies at MTHFR and MTRR separately for the two study centers, using standard goodness-of-fit tests. For each of the two loci, we performed three different types of statistical analyses: first, a comparison of allele frequencies between case and control individuals; second, a comparison of the frequencies of the three genotypes among case and control individuals; and, third, a comparison of the frequencies of "susceptible" genotypes among case and control individuals (i.e., at MTHFR, CT heterozygotes and TT homozygotes, and, at MTRR, GG homozygotes). The distributions of genotypes for MTHFR are provided in tables 1 and 2, and those for MTRR are provided in tables 3 and 4. Additionally, the tables provide the results of statistical tests comparing the proportion of "susceptible" genotypes between controls and each type of trisomy; other statistically significant results (i.e., case vs. control differences in allele frequencies or in the proportions of the three genotypes) are described below.

MTHFR: At Wessex, we found no obvious difference in allele frequencies between mothers of 47,XXX/XXY

Table 1

Maternal MTHFR Polymorphisms in Maternally Derived Cases of Sex Chromosome Trisomy or Trisomy 18 and in Control Individuals

Study Population	No. (%) of Individuals with MTHFR Genotype			Case vs. Control "Susceptible" Genotypes (CT or TT)[a]
	CC	CT	TT	
47,XXX or XXY ($n = 93$)	48 (51.6)	40 (43.0)	5 (5.4)	$\chi^2 = .01$; NS
Trisomy 18 ($n = 44$)	13 (29.5)	22 (50.0)	9 (20.5)	$\chi^2 = 5.00$; $P < .05$
Controls ($n = 56$)	29 (51.8)	22 (39.3)	5 (8.9)	…

[a] NS = not significant.

individuals and control mothers, nor was there any increase in susceptible genotypes among the 47,XXX/XXY mothers (table 1). However, among mothers of trisomy 18 conceptuses, there was a significant increase in the T allele ($\chi^2 = 6.10$; $P < .05$), and a significant increase in the proportion of susceptible (i.e., CT or TT) genotypes by comparison with controls ($\chi^2 = 5.00$; $P < .05$).

At Cleveland, the maternal allele frequencies and genotypic distributions were virtually identical for the combined trisomy data set ($n = 158$) as for the controls (table 2). Further, there was no evidence for significant deviations from the controls for any of the individual trisomies.

MTRR: At Wessex, the maternal allele frequencies and genotypic distributions were very nearly the same for the 47,XXX/XXY individuals as for the controls (table 3). Similarly, there were no obvious differences between mothers of trisomy 18 conceptuses and controls.

At Cleveland, values for the combined trisomy data set were almost the same as those for controls (table 4). Among individual trisomies, there was a nonsignificant increase in mutant GG homozygotes among mothers of trisomy 13 conceptuses, but there were no obvious differences from control individuals for any of the other trisomies.

Taken together, these data provide little support for a major effect of maternal polymorphisms in the folate pathway on human meiotic nondisjunction. At MTHFR, values for 47,XXX/XXY individuals and for spontaneously aborted trisomies were little different from those of controls. Similarly, at MTRR none of the categories of trisomies showed significant differences from controls; indeed in most instances the case and control values were remarkably alike. However, one significant association was identified, involving an increase in MTHFR susceptible genotypes among mothers of trisomy 18 conceptuses. This may indicate an effect of MTHFR variants on chromosome 18 nondisjunction, or it may be a fortuitous observation reflecting the relatively small number of cases yet studied and/or the number of statistical comparisons that we conducted. Further analyses will be required to confirm or refute this association.

With the exception of MTHFR and trisomy 18, we observed no link between folate-pathway polymorphisms and any of the trisomies analyzed in the present report. These results contrast with the previously reported results for trisomy 21 (James et al. 1999; Hobbs et al. 2000), which implied a substantial contribution of both maternal MTHFR and MTRR polymorphisms to chromosome 21 nondisjunction. Although the reasons for this discrepancy are not yet clear, we can think of several possible explanations:

First, it could be that there are significant differences between the study populations (e.g., with the maternal age distribution of our cases being different from that in the previous studies) or in the meiotic origins of the trisomies (e.g., with one data set involving meiosis I [MI] errors and the other involving meiosis II [MII] errors). However, in our data set, we found no evidence for maternal age-related alterations in genotype frequencies at either MTHFR or MTRR; thus, it seems unlikely that

Table 2

Maternal MTHFR Polymorphisms in Maternally Derived Cases of Trisomies 2, 7, 10, 13, 14, 15, 16, or 22 and in Control Individuals

Study Population	No. (%) of Individuals with MTHFR Polymorphisms			Case vs. Control "Susceptible" Genotypes (CT or TT)[a]
	CC	CT	TT	
Trisomy:				
2	2	7	1	$\chi^2 = 2.20$; NS
7	6	2	2	$\chi^2 = .66$; NS
10	2	2	1	$\chi^2 = .06$; NS
13	14	11	2	$\chi^2 = .25$; NS
14	2	5	1	$\chi^2 = 1.23$; NS
15	10	10	0	$\chi^2 = .99$; NS
16	30	27	5	$\chi^2 = .07$; NS
22	7	9	0	$\chi^2 = .02$; NS
Total ($n = 158$)	73 (46.2)	73 (46.2)	12 (7.6)	$\chi^2 = .01$; NS
Controls ($n = 48$)	22 (47.8)	22 (47.8)	4 (8.3)	

[a] NS = not significant.

Table 3

Maternal MTRR Polymorphisms in Maternally Derived Cases of Sex Chromosome Trisomy or Trisomy 18 and in Control Individuals

Study Population	No. (%) of Individuals with MTRR Genotype			Case vs. Control "Susceptible" Genotype (GG)[a]
	AA	*AG*	*GG*	
47,XXX or XXY (n = 98)	31 (31.6)	46 (46.9)	21 (21.4)	χ^2 = .26; NS
Trisomy 18 (n = 44)	13 (29.5)	25 (56.8)	6 (13.6)	χ^2 = 1.99; NS
Controls (n = 56)	17 (30.4)	25 (44.6)	14 (25.0)	…

[a] NS = not significant.

factors such as maternal age are the reason for the discrepancy. Further, we found no evidence of an association between genotype and the stage of origin of trisomy, since the genotypic distributions were similar for MI- and MII-derived cases. Specifically, for MTHFR the distribution of CC, CT, and TT genotypes was .48, .45 and .07 for MI-derived cases (n = 148) and .41, .45, and .14 for MII-derived cases (n = 66); for MTRR the distribution of AA, AG, and GG genotypes was .28, .54, and .18 for MI trisomies (n = 152) and .25, .49, and .26 for MII trisomies (n = 68). Thus, we think it improbable that this is the source of the difference.

Second, the discrepancy could be attributable to differences in gene-nutrition interactions between the present and previous study populations. Cellular methylation reactions involving MTHFR and MTRR require folate and B_{12}, respectively (Hobbs et al. 2000); thus, the adverse consequences of the MTHFR and MTRR variants—including the risk of neural tube defects and, possibly, the risk of meiotic nondisjunction—are highly dependent on nutritional status and will be maximized by inadequate nutritional levels of folate or B_{12}. Consequently, any systematic differences in nutritional status between study populations will complicate interstudy comparisons. Because the nutritional status of mothers in our study was not ascertained, we cannot eliminate this as a explanation for the differences.

Third, it could be that the effect of polymorphisms at MTHFR or MTRR is restricted to certain chromosomes, including chromosome 21, and that we either studied the "wrong" chromosomes or had too few data on those chromosomes that are affected by these polymorphisms. Consistent with this possibility, we identified an effect for chromosome 18, with a sample size of 44. Further, for several of the other autosomes (e.g., chromosomes 2, 7, 10, 14, and 22) <20 cases were available for analysis; thus, for these trisomies, even large effects would have been difficult to detect. Nevertheless, we might expect that the pooled data from acrocentric trisomies 13, 14, 15, and 22 would display an effect similar to that reported for trisomy 21—after all, available data indicate little difference in maternal age patterns or in the

Table 4

Maternal MTRR Polymorphisms in Maternally Derived Cases of Trisomies 2, 7, 10, 13, 14, 15, 16, or 22 and in Control Individuals

Study Population	No. (%) of Individuals with MTRR Genotype			Case vs. Control "Susceptible Genotype" (GG)[a]
	AA	*AG*	*GG*	
Trisomy:				
2	1	6	3	χ^2 = 0.54; NS
7	1	5	1	χ^2 = 0.12; NS
10	0	3	2	χ^2 = 1.11; NS
13	8	9	9	χ^2 = 2.00; NS
14	1	7	0	χ^2 = 1.88; NS
15	2	9	5	χ^2 = 0.93; NS
16	16	35	15	χ^2 = 0.16; NS
22	2	13	1	χ^2 = 1.56; NS
Total (n = 154)	31 (20.1)	87 (56.5)	36 (23.4)	χ^2 = 0.29; NS
Controls (n = 46)	11 (23.9)	26 (56.5)	9 (19.6)	…

[a] NS = not significant.

mechanism of origin of nondisjunction among human acrocentric trisomies (Hassold and Chiu 1985; Zaragoza et al. 1994). However, for both MTHFR and MTRR, the pooled data from trisomies 13, 14, 15, and 22 were virtually identical to those from controls (tables 3 and 4). Thus, if there is an effect of folate polymorphisms on acrocentric trisomies, it may well be restricted to trisomy 21.

Fourth, it could be that the effect of the MTHFR and/or MTRR polymorphisms is linked to the survival of the trisomic conceptus. For example, the MTHFR 677C→T allele might be in linkage disequilibrium with alleles that promote fetal survival (e.g., involving a nearby locus that encodes a placental protein). If so, we might expect increased survival of T allele–carrying trisomy 21 fetuses, with CC homozygotes being overrepresented among spontaneously aborted trisomy 21 conceptuses. The present data set does not allow us to formally examine the possibility of differential selection for the trisomies that we investigated. That is, whereas we studied both spontaneously aborted and live-born trisomies, for most categories of trisomy, only live-born conceptuses (e.g., for the sex-chromosome trisomies) or spontaneous abortions (e.g., for all autosomal trisomies other than trisomy 18) were available for analysis. Nevertheless, it should be relatively easy to examine this question for trisomy 21, for which it is possible to obtain case material from both live-born and spontaneously aborted conceptuses.

Finally, it could be that the previous results on trisomy 21 were fortuitous and that maternal folate polymorphisms have little, if any, independent effect on meiotic nondisjunction in humans. The absence of a demonstrable familial component to human nondisjunction (e.g., Sayee and Thomas 1998)—expected if folate variants are a significant causative agent—and the absence of significant racial variation in nondisjunction frequencies (Hassold et al. 1980) are consistent with this conclusion. Further, two recent analyses of trisomy 21 suggest that this may, indeed, be the case. That is, in an analysis of 177 mothers of trisomy 21 conceptuses in which the extra chromosome 21 was known to be maternally derived, Petersen et al. (2000) were unable to demonstrate an increase in MTHFR mutations by comparison with controls. Further, in a similar analysis of MTHFR in 85 mothers of individuals with DS, Chadefaux-Vekemans et al. (in press) found no difference between case and control subjects. Thus, the results of these analyses provide little evidence that maternal folate-pathway polymorphisms influence the risk of chromosome 21 nondisjunction. Nevertheless, our observation of a significant association between MTHFR variants and trisomy 18 and the previous links between MTHFR and/or MTRR and trisomy 21 cannot be summarily dismissed and may indicate chromosome-specific effects. The extraordinary clinical importance of such effects, if proven, make it essential that additional analyses now be conducted to confirm or refute the association.

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Electronic-Database Information

Accession numbers and the URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for MTHFR [MIM 236250] and MTRR [MIM 602568])

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