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Report

Analysis of Aneuploidy Frequencies in Sperm from Patients with Hereditary Nonpolyposis Colon Cancer and an hMSH2 Mutation

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Hereditary nonpolyposis colon cancer (HNPCC) has been shown to be caused by mutations in the mismatch repair genes hMSH2, hMLH1, hPMS1, and hPMS2. Recent evidence has demonstrated that mutations in mismatch repair genes disrupt meiosis in mice. A large HNPCC kindred in Newfoundland, Canada, has an hMSH2 mutation—an A→T transversion at the +3 position of the splice-donor site of exon 5. We have studied sperm from men with this hMSH2 mutation, since it is possible that mismatch repair mutations in humans might also have an effect on meiosis and normal segregation of chromosomes. The frequencies of aneuploid and diploid sperm were determined in 10 men with the hMSH2 mutation, by use of multicolor FISH analysis for chromosomes 13, 21, X, and Y. A minimum of 10,000 sperm per man was studied per chromosome probe. Control individuals consisted of men in the same kindred with HNPCC who did not carry the mutation and of other normal men from Newfoundland. A total of 321,663 sperm were analyzed: 200,905 sperm were from men carrying the hMSH2 mutation and 120,758 sperm were from control men. There was a significantly increased frequency of disomy 13, disomy 21, XX, and diploidy in mutation carriers compared with control men. These results suggest that the hMSH2 mutation may affect meiosis in humans.

Hereditary nonpolyposis colon cancer (HNPCC [MIM 120436]) has been shown to be caused by mutations in the mismatch repair genes hMSH2, hMLH1, hPMS1, and hPMS2 (Papadopoulos et al. 1994). Mice engineered to carry mutations at mismatch repair loci also have an increased susceptibility to tumors (de Wind et al. 1995). Mismatch repair has an important function in meiotic cells as well as in somatic cells: repair of mismatches during homologous recombination is essential to meiosis, and mutations in yeast mismatch repair homologues have been associated with aberrant recombination and abnormal segregation of chromosomes (Hollingsworth et al. 1995). Attempts are being made to unravel the specific features of each mismatch repair gene, and it appears that, in yeast, pms1 and msh2 function to prevent genetic exchange between diverged DNA sequences, whereas mlh1 is required for both crossover and gene-conversion pathways of meiotic recombination (Hunter and Borts 1997). Recent evidence has demonstrated that mutations in mismatch repair genes also disrupt meiosis in mice. Mice that were homozygous for an Mlh1 mutation were infertile because of a 10–100-fold reduction in chiasma frequency leading to meiotic arrest (Baker et al. 1996). Mice that were homozygous for Pms2 had abnormalities in chromosome synapsis and produced few abnormal sperm (Baker et al. 1995). There has been one report (Hackman et al. 1997) of a woman who is a compound heterozygote for two MLH1 mutations and is fertile; thus, it appears that effects on fertility may differ in mice and humans or between the sexes.

The present study was initiated to determine whether men that are heterozygous for a mismatch repair gene demonstrate any effect on meiosis and chromosome seg-

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Table 1

Individual Frequencies of Disomic and Diploid Sperm in hMSH2 Mutation Heterozygotes and in Control Donors

Sperm-Donor Type and No.	Age (years)	Frequencies (%) of Disomic and Diploid Sperm						
		Disomy 13	Disomy 21	XX	YY	XY	Diploid	X-Bearing
Mutation carriers:								
1	42	.21	.28	.07	.03	.39	.56	50.4
2	35	.04	.07	.10	.09	.09	.06	49.3
3	22	.11	.23	.21	.13	.56	.38	49.9
4	48	.04	.15	.10	.08	.35	.18	50.0
5	50	.17	.18	.09	.03	.63	.26	50.1
6	22	.09	.26	.12	.05	.27	.28	49.4
7	45	.06	.13	.16	.08	.24	.31	49.6
8	36	.04	.17	.04	.07	.14	.62	50.1
9	39	.08	.13	.01	.05	.18	.25	49.6
10	52	.11	.12	.03	.03	.10	.12	49.0
Control donors:								
1[a]	57	.03	.08	.05	.05	.15	.24	49.5
2[a]	48	.06	.28	.08	.10	.64	.31	49.2
3	47	.03	.11	.06	.07	.54	.21	49.9
4	31	.11	.12	.05	.06	.22	.10	49.3
5	40	.06	.04	.03	.05	.32	.16	50.1
6	28	.08	.12	.05	.05	.55	.32	49.6

[a] Control donors 1 and 2 were part of the kindred with HNPCC.

regation. A large HNPCC kindred in Newfoundland, Canada, was selected, to test 10 men with an hMSH2 mutation.

The kindred with HNPCC has an hMSH2 mutation—an A→T transversion at the +3 position of the splice-donor site of exon 5. Mutation carriers in this family (Liu et al. 1994) exhibit malignancies commonly observed in Lynch (type 2) syndrome. Mutation carriers (members of the study group) and noncarriers (members of the control group) were asked to donate sperm for the present study. Normal, healthy men from Newfoundland who lived in regions similar to those of the men in the kindred and who had no chronic illness or history of chemotherapy, radiotherapy, or exposure to a known mutagen were also asked to be part of the control group. Semen was airfreighted in cold packs from Newfoundland to Calgary. Informed consent was obtained from the sperm donors, and the study was approved by the institutional review board of the University of Calgary.

The frequencies of aneuploid and diploid sperm were determined, by means of multicolor FISH analysis, for chromosomes 13, 21, X, and Y. A minimum of 10,000 sperm was studied per man per chromosome. Details of the technique used are provided elsewhere (McInnes et al. 1998).

To compare results between mutation carriers and control donors, the proportion of abnormal sperm and the standard error of proportion were estimated, taking the clustering within individuals into account. A two-tailed Z statistic (standard normal) was calculated (Rosner 1995).

A total of 321,663 sperm were analyzed: 200,905 sperm were from 10 men carrying the hMSH2 mutation and 120,758 sperm from six control men. We initially tried to obtain all control samples from those men in the kindred with HNPCC who did not carry the mutation; however, since only two men (donor 1 and donor 2 in table 1) were willing to be donors, we used four other men from Newfoundland as control individuals. The 10 men that were mutation heterozygotes had a mean age of 39.1 years (range 22–52 years); six of the men had children and four did not. None of the wives of these men had experienced any spontaneous abortions. The six control donors had a mean age of 41.8 years (range 28–57 years); four of the control donors had fathered children and two had not. There was no history of spontaneous abortion among the wives of the control individuals. One of the men that was a mutation heterozygote (donor 8) and one of the control donors (donor 5) were smokers.

Individual frequencies of sperm disomic for chromosomes 13, 21, XX, YY, and XY as well as of diploidy and sex ratio (the percentage of X-bearing sperm) from mutation carriers and from control individuals are presented in table 1. A summary of the mean values is provided in table 2.

There was no significant difference between mutation carriers and control individuals, with regard to the frequency of disomic YY sperm (0.064% vs. 0.063%) or to the proportion of X-bearing sperm (50.0% vs. 50.1%). There was a significantly lower frequency of disomic XY sperm in mutation carriers (0.30%) com-

Table 2

Mean Frequencies of Disomic and Diploid Sperm in hMSH2 Mutation Heterozygotes and Control Donors

	Mean Frequencies (%) of Disomic and Diploid Sperm						
Sample Type	Disomy 13	Disomy 21	XX	YY	XY	Diploid	X-Bearing
Mutation carriers	.09*	.17**	.09***	.06	.30***	.30***	50.0
Control donors	.06	.12	.05	.06	.40	.22	50.1

* $P = .02$, by two-tailed Z test.
** $P = .01$, by two-tailed Z test
*** $P = .005$, by two-tailed Z test.

pared with control donors (0.40%; $P = .0005$, two-tailed Z test). There was a significantly increased frequency of disomic sperm in mutation heterozygotes, compared with control donors, for chromosomes 13 (0.10% vs. 0.06%; $P = .02$, two-tailed Z test), 21 (0.17% vs. 0.12%; $P = .01$, two-tailed Z test), and XX (0.09% vs. 0.05%; $P = .003$, two-tailed Z test). There was also a significant increase in the frequency of diploid sperm in mutation carriers (0.30%) compared with that of the diploid sperm in control donors (0.22%; $P = .002$, two-tailed Z test).

Chromosome abnormalities have a huge impact on human health and well-being. They are responsible for a great number of pregnancy losses and for the birth of children with physical and mental handicaps. Estimates of the frequency of chromosome anomalies at conception vary from 5% (Hook 1981) to 50% (Boué et al. 1975). These result from chromosome-segregation errors occurring at meiosis, yet we have very little information about the cause of these abnormalities and the factors that influence their production. It is certainly possible that the action of specific genes could effect the normal meiotic segregation of chromosomes in humans, since this has been demonstrated in other organisms (Moore et al. 1994; Roeder 1995). The genes involved in mismatch repair could have important meiotic consequences in humans. The effect of homozygous mutations in these genes in male mice is dramatic, with synaptic abnormalities, reduced numbers of chiasmata, and premature separation of bivalents (Baker et al. 1995, 1996). Chromosome pairing and recombination have been intimately linked with the fidelity of chromosome segregation in a number of organisms (Hollingsworth et al. 1995; Roeder 1995), and we have emerging evidence in humans that abnormalities in recombination are linked with nondisjunction. For example, Hassold et al. (1991) have determined that 47,XXY of paternal origin have a greatly decreased frequency of recombination in the pseudoautosomal region. Furthermore, recent evidence has demonstrated altered recombination in cases of trisomy 21 of paternal origin (Savage et al. 1997). We have demonstrated that sperm that has undergone XY nondisjunction has a markedly reduced recombination frequency in the pseudoautosomal region, compared with normal unisomic sperm (Martin et al. 1999). Thus, it is reasonable to assume that genes involved with chromosome pairing and recombination could affect chromosome segregation, even in the heterozygous state.

We studied 10 men that were heterozygous for a specific mutation of the hMSH2 gene and six other men from Newfoundland, for sperm-aneuploidy frequencies. The sperm-aneuploidy frequencies for chromosomes 13 and 21 were higher than the values observed in Calgary men studied by our laboratory at the University of Calgary (McInnes et al. 1998). The Newfoundland men were older (mean age 39.1 years for men with mutation heterozygotes and 41.8 years for control men, compared with 35.6 years for men from Calgary), but this is unlikely to be a cause of the difference, as we have previously examined an age effect in 18 men stratified into six age groups and have shown no effect of donor age on the frequency of disomy for chromosomes 13 or 21. The difference may reflect the fact that the men from Newfoundland were largely from rural areas, whereas the previously studied individuals from Calgary were from urban areas. There may be unknown lifestyle factors that cause a lower frequency of aneuploidy for the autosomes in these men. In any case, sperm from men from Newfoundland—whether control individuals or test subjects—were treated in an identical manner.

We found a significantly increased frequency of aneuploid sperm for chromosomes 13, 21, and X and of diploid sperm, in mutation heterozygotes compared with control donors. The magnitude of the increase was not great, varying from 1.4–1.8 times the incidence observed in control donors. This increased frequency of chromosomally abnormal sperm could manifest itself as infertility, or it could result in pregnancy loss or abnormal children. Of the heterozygous men we studied, 6/10 were fathers, and we are not aware of their wives having any cases of spontaneous abortion or giving birth to abnormal children. It would be interesting to study other men with mismatch repair mutations, to determine the frequency of sperm-cell aneuploidy. Also, if the rare possibility of a man homozygous for a mismatch repair gene is encountered, it would be most informative to deter-

mine his fertility status and, if possible, to analyze his meiotic cells.

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Electronic-Database Information

The accession number and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for HNPCC [MIM 120436])

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