age within the context of HNPCC to suggest that it is part of the disease spectrum.

The results that were obtained reflect breast cancer incidence observed in our population. We cannot explain why the findings for our population differ from those observed in the Dutch families with HNPCC or those reported by Watson et al. (1993) or Aarnio et al. (1999), who showed that there was no increased risk of breast cancer in HNPCC. In the analysis of Dutch families with HNPCC, either no association or indeed a slight protective effect of DNA mismatch-repair errors was reported.

There are several interesting differences between our population and the Dutch population. The most interesting is the relative percentage of families with linkage to *hMSH2* and *hMLH1*. In Holland, the ratio of *hMSH2* to *hMLH1* mutation carriers is ~1:1, compared with our findings, which suggest a 1:2 ratio of *hMSH2* to *hMLH1* mutations. This difference does not account for the discrepancy seen between our population and the Dutch population, but it does suggests that there are significant differences between the two. We are currently accumulating more HNPCC families (>230) and will reanalyze the data when mutation analysis is complete, to determine whether the results of our initial analysis of the first 95 families hold true or were a result of a bias within our population.

Finally, we agree with the notion put forward by Vasen et al. (2001) that breast cancer development may be accelerated in persons who are deficient in DNA mismatch repair.

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Mitochondria and the Quality of Human Gametes

To the Editor:

Ruiz-Pesini et al. (2000) cleverly show that extant human mtDNA variation affects sperm function. They find that mitochondrial haplogroup T is overrepresented in asthenozoospermic populations and shows reduced sperm oxidative phosphorylation pathway (OXPHOS) activity, relative to the H haplogroup that is overrepresented in nonasthenozoospermic populations. These authors—as well as Moore and Reijo-Pera (2000), in the accompanying invited editorial—stress that, because of the exclusive matrilinear inheritance of mitochondria, mutations of mtDNA purely affecting male fertility are not selected against and therefore can become fixed. The absence of a direct check against mitochondrial mutations that affect male fertility is unfortunate and begs the question of why such a pattern became established.

In keeping with an earlier suggestion (Giannelli 1986), I propose that the exclusion of sperm mitochondria from the zygote is part of a scheme enabling mitochondria to provide an indirect measure of sperm quality and, hence, to favor fertilization by optimal spermatozoa while avoiding the risk of passing on mtDNA exposed to high physiological stress and, hence, potential damage. This would clearly have adaptive value and could help justify the establishment of matrilineal mitochondrial inheritance.

There is evidence that mitochondria have a role in germ-cell selection. Krakauer and Mira (1999), in a phylogenetic study, note that species producing fewer offspring have fewer egg mitochondria and experience greater ovarian atresia, and these authors conclude that lower numbers of mitochondria offer greater opportunities for variation in mitochondrial function and, thus, for elimination of eggs with poor mitochondria. This results in purifying selective pressure on mitochondrial genomes. Some proof of a mitochondrial role in ovarian

atresia exists, as microinjection of 5×10^3 mitochondria from nonapoptotic follicular granulosa rescues the oocytes from FVB-strain mice that undergo an inherently high rate of apoptosis in vitro (Perez et al. 2000). With regard to spermatozoa, Ruiz-Pesini et al. (2000) provide direct evidence that mitochondrial function is critical to their motility and hence presumably to their success in fertilization.

Nevertheless, egg and sperm differ dramatically in their mitochondrial complement, since the human oocyte has 10^6 mtDNA molecules, whereas mature spermatozoa have only 100 (Cummings 1998). It follows that spermatozoa should be exquisitely sensitive to mitochondrial malfunction.

I suggest that this allows mitochondria to become both a sensitive meter of genetic damage to sperm and the means of selecting, at fertilization, spermatozoa derived from the germ-cell lines that have best preserved the quality of their genome during postzygotic life. However, reliance on a small complement of mitochondria to produce very high levels of kinetic energy in conditions of unusually high oxygen tension in the female genital tract exposes sperm mtDNA to extraordinary risks from oxygen radicals, and, therefore, the disposal of mtDNA at zygote formation also seems advantageous. To provide a sensitive, indirect measure of the quality of the sperm genome, the mitochondrial complement of a spermatozoon must meet two conditions: it should not afford a high degree of functional redundancy, and it must offer a target for deleterious mutations larger than the nuclear genome.

The results of Ruiz-Pesini et al. (2000) suggest that the former is true, because a mitochondrial haplogroup associated with modest OXPHOS deficit is associated with asthenozoospermia. More-direct experimental evidence on this point would require the inactivation of a proportion of the mitochondria and subsequent examination of sperm function. Such evidence is not available, but, since each human spermatozoon competes with 10^8 colleagues for a single oocyte, it seems probable that a sperm needs all of the energy its 100 mitochondria can produce.

It is reasonable to conclude that the mitochondrial complement of a spermatozoon offers a sufficiently large target for deleterious mutations, for the following reasons. The germinal mutation rate for mtDNA is 50-fold greater than that for genomic DNA. Human mitochondria have no introns, intergenic sequences, or complex, large centromeres and telomeres, and they have nonredundant gene sequences that even show some overlap. In contrast, nuclear coding sequences are highly dispersed (International Human Genome Sequencing Consortium 2001) and show some degree of functional redundancy. Therefore, the essential information content of mtDNA can be considered to be at least 100-fold greater than that of a nuclear DNA segment of similar length. It follows that the effective target for deleterious mutations presented by a mtDNA molecule should be equivalent to 16,569 bp $\times$ 50 $\times$ 100 = 83 Mb of genomic DNA. This is ~1/36 of the haploid genome. Therefore, the 100 mtDNA molecules of a spermatozoon offer a target for deleterious mutation equivalent to 2.8 haploid genomes. Thus, if the functional redundancy of the mitochondrial complement of a spermatozoon, in terms of its competition with 10^8 other spermatozoa, is <2.5-fold, the mitochondrial complement of the male gametes can provide a sensitive, indirect measure of their genetic well-being and an effective means of sperm selection at fertilization.

This may be important in the moderation of the genetic risks resulting from the production of a huge number of male gametes by large numbers of cell divisions. Of course, the scope and consequences of sperm selection should be even greater in species with common polyandry, where sperm from different males may compete at fertilization.

Experimental evidence in favor of my hypothesis could be sought by investigation of whether spermatozoa in the top grade for a relevant phenotype—for example, mobility—show less mitochondrial damage than those in lower grades. This would require analysis of individual, phenotypically selected spermatozoa by means capable of revealing mutations affecting as little as 1 mtDNA molecule in 100. This is a hard, but not impossible, task. It does require the mtDNA content of a single spermatozoon to be diluted to obtain pools of ~10 mtDNA molecules. Then efficient and fast screening methods, such as fluorescent solid phase mismatch cleavage or denaturing high-performance liquid chromatography (DHPLC), can be used, after PCR amplification, to detect a single mutant mtDNA molecule among the other 10.

I hope that the above comments will stimulate interest in the potential role of mitochondria as guardians of the quality of the male contribution to the human zygote.

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