Letters to the Editor

Is Breast Cancer Part of the Tumor Spectrum of Hereditary Nonpolyposis Colorectal Cancer?

To the Editor:

In the January issue of the *Journal,* Scott et al. (2001) reported the incidence of various types of cancer in 95 families with hereditary nonpolyposis colorectal cancer (HNPCC [MIM 120435 and 120436]). The patients in these families were categorized according to their mismatch-repair (MMR) profiles. Of these families, 12 were identified as having an *hMSH2* mutation, 22 families had an *hMLH1* mutation, and, in 61 families, no mutation was identified. To our surprise, a remarkably high incidence of breast cancer was found in the family members with HNPCC, particularly in the *hMLH1* (standardized incidence rate [SIR] 14.77 [95% confidence interval {CI} 6.2–35]) and the mutation-negative (SIR 18.03 [95% CI 12.2–26.7]) groups. No increased incidence was found in the *hMSH2* mutation carriers (SIR 2.02 [95% CI 0.3–12.7]). Previous studies reported much lower incidences of breast cancer in HNPCC. For instance, Watson and Lynch (1993) identified only 19 cases of breast cancer in 23 families with HNPCC, occurring at a median age of 51 years. The observed:expected (O:E) ratio in the Watson and Lynch (1993) study was 0.9. Another recent study of 183 MMR-mutation carriers by Aarnio et al. (1999) reported an incidence (SIR) of 1.4 (95% CI 0.4–3.7). These contradictory data prompted us to evaluate the risk of developing breast cancer in the families registered at the Dutch HNPCC registry.

Almost 200 families suspected of HNPCC are currently known at our registry. A total of 138 families either meet the "Amsterdam criteria" or harbor a germline mutation in one of the MMR genes. In 79 families, a germline mutation has been identified (34 *hMLH1,* 40 *hMSH2,* and 5 *hMSH6*). Only 4 of 187 proven or putative *hMLH1* mutation carriers and 3 of 141 *hMSH2* mutation carriers developed breast cancer. The O:E ratio of breast cancer in *hMLH1* mutation carriers was 0.6 (95% CI 0.2–1.5); in *hMSH2* mutation carriers, the O:E ratio was 0.6 (95% CI 0.2–1.7). The mean age at diagnosis was 46 years (range 32–59 years).

One of these patients, a 32-year-old *hMSH2* mutation carrier, underwent periodic examination of the colon and rectum, endometrium, ovaries, and stomach at the Department of Gastroenterology and Gynaecology, Leiden University Medical Center. Recently, she presented with an enlarged lymph node (5 cm in diameter) in the right axilla. On further analysis, a very small tumor in the upper lateral quadrant of the right breast was discovered. Fine-needle aspiration of the axillary tumor revealed an adenocarcinoma. The patient subsequently underwent a modified radical mastectomy. Histological examination of the surgical specimen demonstrated poorly differentiated adenocarcinoma in the breast and a metastasis in the axilla. Sixteen other lymph nodes were free of cancer. The estrogen and progesterone receptors were negative. The tumor exhibited widespread microsatellite instability (MSI).

In 1996, Risinger et al. performed molecular genetic studies in five patients with breast cancer from families with HNPCC. In three of the five tumors, MSI was observed. In one family with a known mutation, expression of only the mutant allele was identified in the breast cancer tissue. In 1999, Boyd et al. described a male patient, in a large family with HNPCC, affected by breast and colorectal cancer. This patient had an *hMLH1* germline mutation, and the breast tumor exhibited MSI and reduction to homozygosity for the *hMLH1* mutation.

Our study, like most studies reported in the literature, suggests that the relative risk of developing breast cancer is not increased in HNPCC. On the other hand, these studies show that breast tumors in families with HNPCC may present at an unusually early age, as illustrated by our patient. In addition, molecular genetic studies reveal MSI in breast tumors identified in families with HNPCC.

We propose a possible explanation for the contrasting observations of, on one hand, an unusually early age at diagnosis of breast cancer and, on the other hand, a normal (or even decreased) risk of developing breast cancer. It has generally been accepted that the development of breast cancer in the general population takes, on average, ~20 years. This suggests that, like non–mutation carriers in the general population, a carrier of an MMR gene mutation may develop the first stages of a tumor at age 40 years. However, because of the defect in the MMR system, mutations may accumulate in genes involved in the progression of breast cancer. The accu-

mulation of mutations may lead to acceleration of tumor development and to presentation of breast cancer at a much earlier age. The normal lifetime risk of developing breast cancer in HNPCC patients may indicate that the MMR defect is not involved in the initiation of breast cancer.

The answer to the question whether breast cancer is part of the tumor spectrum of HNPCC should be "no" if we consider the absence of an increased lifetime risk. Yet this question should be answered with "yes" if we take into account the possible role of the MMR defect in the progression of a breast tumor. Application of the latter criterion implies that a large variety of tumor types should in fact be regarded as part of the tumor spectrum of HNPCC. We believe that decisions as to whether surveillance should be advised for a specific type of cancer should be based on the age-specific cancer risk and the availability of sensitive and specific screening tools. Many cancers that are currently not included in the surveillance program may develop at an early age in patients with HNPCC. Therefore, we urge clinicians managing HNPCC to be especially alert when the patient presents with unusual symptoms.

H. F. A. VASEN,[1] H. MORREAU,[2]
AND J. W. R. NORTIER[1]
Departments of [1]Clinical Oncology and [2]Pathology
Leiden University Medical Center
Leiden, The Netherlands

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Address for correspondence and reprints: Dr. H. F. A. Vasen, The Netherlands Foundation for the Detection of Hereditary Tumours, Leiden University Medical Center, Rijnsburgerweg 10, "Poortgebouw Zuid," 2333 AA Leiden, The Netherlands. E-mail: nfdht@xs4all.nl

Reply to Vasen et al.

To the Editor:
Hereditary nonpolyposis colorectal cancer (HNPCC) is associated, at least in part, with germline mutations in genes involved in DNA mismatch repair. Two genes, termed "hMSH2" and "hMLH1," account for HNPCC in ~60% of families whose symptoms adhere to the Amsterdam Criteria (Syngal et al. 2000). Three other genes—hPMS1, hPMS2, and hMSH6—account for an additional 5%–10%, the exact percentage not being known at this time. There remains a significant proportion of families, ~30%, in which HNPCC does not appear to be accounted for by these genes, suggesting that additional genes, which may or may not have anything to do with DNA mismatch repair, are involved. Given that errors in DNA mismatch repair result in the characteristic signature of microsatellite instability (MSI), it should be relatively straightforward to determine whether families whose symptoms adhere to the Amsterdam Criteria but who do not harbor changes in known DNA mismatch-repair genes display MSI. To our knowledge, little information exists that indicates which of these two scenarios is most likely.

The letter by Vasen et al. (2001) questions the association between mutations in the DNA mismatch-repair gene hMLH1 and breast cancer, which we identified in a report published at the beginning of this year (Scott et al. 2001). In our report, we presented data that indicated a statistically significant difference between the likelihood of developing breast cancer in the hMLH1 mutation–positive group and the mutation-negative group compared with the likelihood in hMSH2 mutation–positive families. One of the reasons we focused on breast cancer was precisely because there was little or no agreement as to whether it was part of the disease spectrum of HNPCC. Furthermore, there were sufficient anecdotal reports of breast cancer occurring at an earlier

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 re-analyze 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.

RODNEY J. SCOTT

Discipline of Medical Genetics
Faculty of Medicine and Health Sciences
University of Newcastle
New Lambton, New South Wales
Australia

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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 $16,569 \text{ bp} \times 50 \times 100 = 83 \text{ Mb}$ 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.

FRANCESCO GIANNELLI
Division of Medical & Molecular Genetics
Guy's, King's, and St. Thomas' School of Medicine
London

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Address for correspondence and reprints: Professor F. Giannelli, Division of Medical & Molecular Genetics, GKT School of Medicine, 8th floor, Guy's Hospital Tower, London Bridge, London SE1 9RT, United Kingdom. E-mail: adrienne.knight@kcl.ac.uk