Letters to the Editor

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Can hair be used to screen for breast cancer?

EDITOR—The use of hair as a biopsy tissue has been considered for some time. For instance, in the case of breast cancer, raised zinc levels in head hair have been reported.[1] Besides, *x* ray diffraction patterns of hair are rich and have attracted much attention for 70 years.[2] However, its potential use as a diagnostic indicator of disease was only suggested a short time ago.[3] Most recently, James *et al*[4] reported that *x* ray diffraction of hair taken from women diagnosed with breast cancer (and those at high risk by virtue of a proven *BRCA1*/*BRCA2* mutation) showed a diffuse ring. They claimed a 100% correlation with the disease, advocating the use of pubic hair as a simple non-invasive screening method for breast cancer. The use of pubic hair was suggested in view of possible damage to the head hair from cosmetic treatments. Despite this note of caution, the study of James *et al*[4] was based on 12 pubic hair samples with only eight from cancer affected subjects. Here, we report a detailed double blind study from 109 women belonging to five clinically distinct groups as well as a normal population group and show that there is no correlation between the diffuse ring and breast cancer or breast cancer predisposition..

The present work was initiated in November 1998 to provide an independent double blind study on clinically well controlled samples because of our concerns with the study of James *et al*[4] for which a major proportion of the samples was provided by two of us (AH and DGRE). Both diffraction and *x* ray fluorescence data have been obtained. Diffraction data were collected on station 2.1 with the multiwire area detector and the fluorescence data on station 7.1 of the UK Synchrotron Radiation Source (SRS). The use of a multiwire detector allowed on line alignment of hair within seconds and enabled efficient diffraction data collection from a large number of samples. Six groups of subjects were selected including a normal population group. For each subject, head and pubic hair were collected and reference numbers assigned in a random manner. The identities of samples were kept blinded until all data were analysed. Each woman was asked to provide information on whether they had had any hair treatment (perm, dye, etc) or were on medication. No hair treatment was reported for pubic hair and thus here only results from pubic hair (108 samples) are discussed. Results of head hair are included in table 1 for completeness. The groups consisted of 27 unaffected controls aged 26-60 years, 21 isolated cases of breast cancer (<31 years) who had been screened negative for *BRCA1*/*BRCA2* mutations, and three cases aged over 60 years not so tested. The remaining groups came from a set of 43 families with proven *BRCA1*/*BRCA2* mutations: 25 affected mutation carriers, 10 unaffected mutation carriers, and 23 unaffected close female relatives who tested negative for the known mutations in the family.

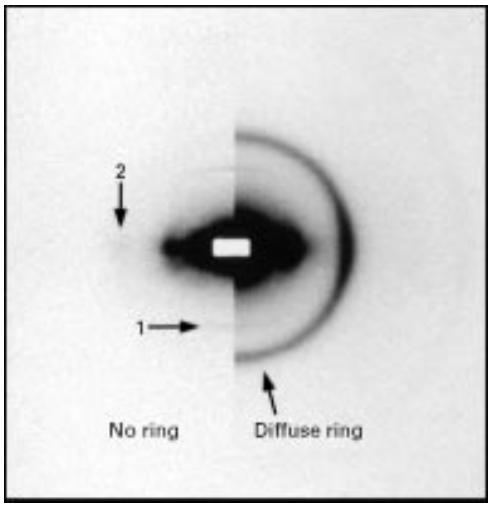

Figure 1 X ray diffraction patterns from the pubic hair of women diagnosed with breast cancer. The left half represents the pattern with no ring, the right half shows the diffuse ring. The first strong meridional reflection (arrow 1) is used for normalising the patterns, arrow 2 shows the equatorial reflection, which gets intensified in cases when the diffuse ring appears.

Diffraction patterns could be grouped into two categories: one characterised by a diffuse ring at 4.78 ± 0.10 nm and one with no ring present (fig 1). The occurrence of this ring is well known from *x* ray patterns of both keratins and muscle and is ascribed to lipid crystals resulting from degradation processes.[5] Contrary to the observation of James *et al*,[4] the pubic hair of only 12 of 49 (25%) women with breast cancer showed the diffuse ring. This is only slightly larger than in the normal population group, where about 20% of the pubic hair samples showed the ring. In the case of the affected group who tested positive for a *BRCA1* or *BRCA2* mutation, 56% of the pubic hair samples showed no ring and only 24% of the pubic hair samples showed the ring. Table 1 provides a detailed summary of diffraction results for all six groups. A statistical analysis for pubic hair samples was performed to determine

Table 1 Proportion of subjects in each of six groups whose diffraction patterns show or do not show the diffuse ring

	Pubic hair						*Head hair*					
	N	*U−*	*U+*	*A−*	*A+*	*Anh*	*N*	*U−*	*U+*	*A−*	*A+*	*Anh*
Ring* (%)	5 (19)	6 (26)	6 (67)	5 (24)	6 (24)	1 (33)	15 (56)	15 (65)	7 (70)	10 (48)	7 (28)	1 (33)
No ring (%)	22 (81)	17 (74)	3 (33)	16 (76)	19 (76)	2 (67)	12 (44)	8 (35)	3 (30)	11 (52)	18 (72)	2 (67)
Total = 217	27	23	9†	21	25	3	27	23	10	21	25	3

N = control from normal population. U = unaffected (known not to have breast cancer). A = affected (known to have breast cancer). +/− = tested positive/negative for mutations in *BRCA1* and *BRCA2* genes. nh = no family history.
*Includes complete and partial ring. James *et al*[4] suggest that pubic hair should be used for diagnostic purposes; 50% of all head hair samples show the ring in contrast to only 27% of pubic hair samples. This difference may arise from hair treatment among other factors.
†One woman supplied only head hair.

whether there was an association between the presence of the diffuse ring and breast cancer. There were 49 samples from women with breast cancer and 59 from unaffected women. A χ^2 value of 0.86 was obtained. For 1% significance, a value of 6.63 and for 5% significance, a value of 3.84 is required. Thus, it can be concluded that there is no measurable association between the diffuse ring and breast cancer. The trace element (Zn, Cu, Fe, and S) analysis of intact hair showed no correlation with the ring structure in the diffraction pattern or with the subjects' group. The women in the normal population group whose hair had shown the diffuse ring were examined and shown not to have breast cancer.

Our *x* ray diffraction data do not support the recent claim that hair from breast cancer patients or those at high risk (*BRCA1*/*BRCA2* mutation carriers) show a distinct diffuse ring. This conclusion for breast cancer diagnosis was also reached on a much smaller study of head hair only.[6] In our study, diffraction patterns from 75% (37 of 49) of the breast cancer patients do not show this ring. Moreover, the χ^2 test shows no association between the diffuse ring and breast cancer and, as such, the claim that *x* ray diffraction of pubic hair can be used as a screening method for breast cancer or breast cancer predisposition is invalid.

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Mutation analysis of *SMAD2*, *SMAD3*, and *SMAD4* genes in hereditary non-polyposis colorectal cancer

EDITOR—Transforming growth factor-β (TGF-β) family members are known to be involved in the regulation of cell proliferation, differentiation, and apoptosis.[1] Members of the TGF-β family include TGF-βs, activins, and bone morphogenetic proteins (BMPs). Their signals are mediated to the cell nucleus by a network of transmembrane serine/threonine kinase receptors and their downstream effectors, the SMAD proteins.[2] SMAD proteins play a key role in intracellular TGF-β signalling and inactivating mutations of *SMAD*s, such as *SMAD2*, *SMAD3*, and *SMAD4,* provide resistance of cells to TGF-β induced growth inhibition.

To date, eight human *SMAD*s have been identified. Two of them, *SMAD2* and *SMAD4*, have been reported to be mutated in a subset of colorectal carcinomas.[3–6] Germline mutations of *SMAD4* have been found in patients with juvenile polyposis, a condition predisposing to colorectal cancer.[7–10]

SMAD3 mutations have not been reported in human cancers. In a recent study by Arai *et al*,[11] *SMAD3* mutations were analysed in 35 sporadic colorectal and 15 HNPCC cancers and no mutations were found. Targeted disruption of the *SMAD3* gene in mice has been reported to lead to development of colorectal cancer,[12] though other studies have not detected a clear association.[13 14] No genetic alterations in other *SMAD*s have been reported in malignancy.

Hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal dominantly inherited cancer susceptibility syndrome, associated with germline mutations in five DNA mismatch repair genes: *MLH1*, *PMS1, PMS2, MSH2*, and *MSH6*.[15–19] Inactivation of both alleles of a mismatch repair gene results in microsatellite instability (MSI) that is a hallmark of HNPCC tumours.[20–23] The genes responsible for microsatellite stable (MSS) HNPCC are still unknown.

Loss of growth inhibition by TGF-β is an important step in colon tumorigenesis and in HNPCC tumours with MSI this is mainly the result of frameshift mutations within a polyadenine sequence repeat in the TGF-β type II receptor (*TGFβRII*) gene.[24] It has been proposed that mutations in *TGFβRII* could underlie the cancer predisposition in MSS HNPCC,[25] and also that other genes involved in the TGF-β pathway are candidates for MSS HNPCC.[26]

Chromosomal deletions are common genetic alterations in cancer and they are targeted at tumour suppressor loci.[27 28] Previous studies have shown that one copy of chromosome 18q is lost in over 70% of sporadic colorectal cancers.[29–32] The *DCC* (deleted in colorectal cancer) gene has been suggested as a candidate target gene in this region and loss of expression of *DCC* has also been reported in colorectal cancers.[33] However, mutations in the coding region of *DCC* seem to be rare[34] and the position of *DCC* as a candidate tumour suppressor is not clear. Two other candidate genes, *SMAD4* and *SMAD2*, have recently been identified at the same 18q region[3 35] emphasising the possible role of the *SMAD* genes in colorectal tumorigenesis. The aim of the present study was to investigate whether germline mutations in *SMAD2, SMAD3*, and *SMAD4* underlie microsatellite stable HNPCC.

Mutation screening was performed in 14 Finnish HNPCC kindreds from which lymphoblastoid cell lines were available. Based on genealogical evidence the families are unrelated, though the existence of early common ancestors cannot be excluded. One affected subject per family was included in the study. Of the kindreds, six fulfilled the Amsterdam criteria for HNPCC.[36] Other patients represent familial HNPCC-like colorectal cancer (CRC); the number of patients with CRC or endometrial cancer ranged from two to six per family (average three) (table 1). All kindreds selected for this study have previously been shown to be *MLH1* and *MSH2* mutation negative.[37] In three kindreds, DNA from tumour tissue had not been available. From 10 families one and in one family two colorectal cancer samples were available and no evidence of MSI had been detected (table 1). The study