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Breast cancer immunohistochemistry can be useful in triage of some HNPCC families

S. Shanley · C. Fung · J. Milliken · J. Leary · R. Barnetson · M. Schnitzler · J. Kirk

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Abstract Immunohistochemistry of tumour samples is increasingly used in the triage of families where hereditary non-polyposis colorectal cancer (HNPCC) due to mismatch repair defects is suspected. Usually, this is undertaken in tumours that are a recognised part of the spectrum of HNPCC-related cancers e.g. colon or endometrial cancers. Although breast cancers are not classed as part of this spectrum, this study examined the extent to which some breast tumours do arise by the mismatch repair pathway in these families. This may have clinical utility in families where an individual with a 'classic HNPPC-related' tumour is not available for evaluation. Immunohistochemistry of a breast tumour may identify an individual in whom germline mutation testing is worthwhile.

S. Shanley · J. Leary · J. Kirk
Familial Cancer Service, Westmead Hospital, Westmead, Sydney, Australia

S. Shanley (✉)
Cancer Genetics Unit, Royal Marsden Hospital NHS Foundation Trust, Downs Rd, Sutton SM2 5PT, UK
e-mail: Susan.Shanley@rmh.nhs.uk

C. Fung · J. Milliken
Department of Pathology, Westmead Hospital, Westmead, Sydney, Australia

R. Barnetson
Colon Cancer Genetics Group, University of Edinburgh Cancer Research Centre and MRC Human Genetics Unit, Western General Hospital, Edinburgh EH4 2XU, UK

M. Schnitzler
Department of Surgery, Royal North Shore Hospital, St Leonards, Sydney, Australia

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Introduction

Breast cancer is not generally regarded as one of the tumours that forms part of the spectrum of features in hereditary non-polyposis colorectal cancer (HNPCC). It has been seen at increased rates in some families [1] although not in others [2, 3]. Recently the issue has been re-addressed by Barrow et al. [4] who report a moderate increase in risk for *hMLH1* carriers. Molecular studies in HNPCC families have demonstrated that microsatellite instability (MSI) is a feature of some breast cancers that arise in family members [2, 5–8] and that this is probably a rare phenomenon in sporadic breast cancers [9]. However, microsatellite stable bowel cancer phenocopies may also occur in HNPCC families[10]. Given that the overall incidence of breast cancer is not established as high across HNPCC families, it has been postulated that the mismatch repair (MMR) genes are unlikely to play a role in initiation of breast cancer. However, it has been suggested that MMR mutations promote the progression of genetic events once tumorigenesis has commenced. No specific histological phenotype is recognised in those breast cancers seen to date in HNPCC families although a report by Westenend et al. [11] does note a remarkably dense lymphocytic infiltrate associated with a breast cancer which has loss of hMSH2 in an *hMSH2* mutation carrier with a grade 2 oestrogen and progesterone receptor positive breast cancer.

In the analysis of colorectal tumours in HNPCC families, the evaluation of MMR protein expression by

immunohistochemistry (IHC) is being increasingly utilised as a means of triaging families to determine eligibility for germline analysis of MMR genes [12]. As this technique is less costly and more widely available than microsatellite analysis, albeit with slight reduction in sensitivity for identifying HNPCC mutation carriers, it is in some centres the primary means of triage. In some families, where there is no living affected member with a recognised HNPCC spectrum tumour to test, IHC of other tumours in family members could pinpoint alternative candidate members for germline analysis. Where an HNPCC-type family member has breast cancer, it may be possible to assess MMR protein expression in breast tumour material by IHC analysis to predict germline mutation status (at least in families where breast cancer may be part of the HNPCC spectrum) and therefore identify candidates to be offered mutation analysis.

Methods

The study was approved by the ethics committee of the Western Sydney Area Health Service. Twenty-nine families with a family history consistent with the Amsterdam I or II criteria [13] for HNPCC were identified from the records of the Familial Cancer Service at Westmead Hospital between 1995 and 2000. Of these, eight families had at least one family member affected with breast cancer. Germline MMR gene mutations were identified in a proband (affected by an HNPCC-related cancer) from four of the eight families by denaturing high performance liquid chromatography (dHPLC), two in *hMLH1* and two in *hMSH2*. Three other families did not undergo mutation screening as no living affected person with a recognised HNPCC spectrum tumour was available. In one family, MMR gene mutation searching was undertaken but was uninformative.

Paraffin blocks were obtained from a breast tumour case in each of the four mutation-positive families. Blocks from a colorectal cancer in a separate family member were obtained in three of these kindreds. DNA was extracted [14] from breast and colon cancer samples and was examined for MSI using a panel of five markers [15]. Microsatellite scoring was undertaken by two reviewers independently.

Immunohistochemistry was performed manually. Paraffin sections were heated in an oven at 60°C for 1 h prior to dewaxing. Antigen retrieval was performed using Universal Decloaker (Biocare UD1000 M) in a pressure cooker at full pressure for 3 min. Sections were cooled for 20 min prior to washing in phosphate-buffered saline/Tween 20 buffer for 5 min. Antibody hybridisation used 100 µl of primary antibody-hMSH2 diluted 1 in 200 (Zymed 18-2343) for 1 h at room temperature or hMLH1 (Biocare CM220C clone G 168-15) diluted 1 in 50 overnight. After washing, labelling was undertaken with IDS labs Superstain system HRP-IDST1007 biotin and streptavidin conjugates sequentially, each for 10 min at room temperature, prior to DAB hydrogen peroxide for 5 min at room temperature. Counterstaining was undertaken with haematoxylin. hMSH6 staining was not routinely available during the study period. Scoring of immunohistochemistry was done by a single observer who was blinded to the mutation status of the families.

Results

The four mutation-positive families are represented in Fig. 1a–d. Loss of MMR protein staining was observed in three of the four breast cancers tested (Table 1). These IHC results correlate with the known mutation status of each of the three families (two having an *hMLH1* mutation, and one an *hMSH2* mutation). In two of these families, the individual with breast cancer was a confirmed mutation carrier, but in the third, germline DNA was not available. Microsatellite instability was demonstrated in two of these three breast cancers but analysis failed in the third case. The fourth breast cancer (which had normal IHC) was microsatellite stable.

Notably, the ages of onset (56–79 years) of the breast cancers in these three cases with loss of staining are no younger than the average age of sporadic breast cancer. Limited data were available on histology other than their being ductal carcinomas. One was reported as grade 2/3 (receptor status not available), but details on grade and receptor status were not available for the other tumours.

Colorectal cancers were available from one family member in three of the four families. The loss of staining in two of these colorectal tumours (one with hMLH1 loss and one with hMSH2 loss) correlated with the loss of staining seen in the breast cancer in the family. In the third colorectal cancer (where loss of hMSH2 was present), the corresponding breast cancer had normal IHC. All three colon cancers with IHC loss were microsatellite unstable.

Discussion

A proportion of breast cancers from germline MMR mutation carriers exhibit DNA mismatch repair defects. This is supported by the finding of the lack of IHC staining for an MMR protein in three breast cancers from HNPCC family members. In two of these three families, the underlying mutation was in the *hMLH1* gene and in the third family it was present in the *hMSH2* gene. Not only was the mutation demonstrated in the family, but also in

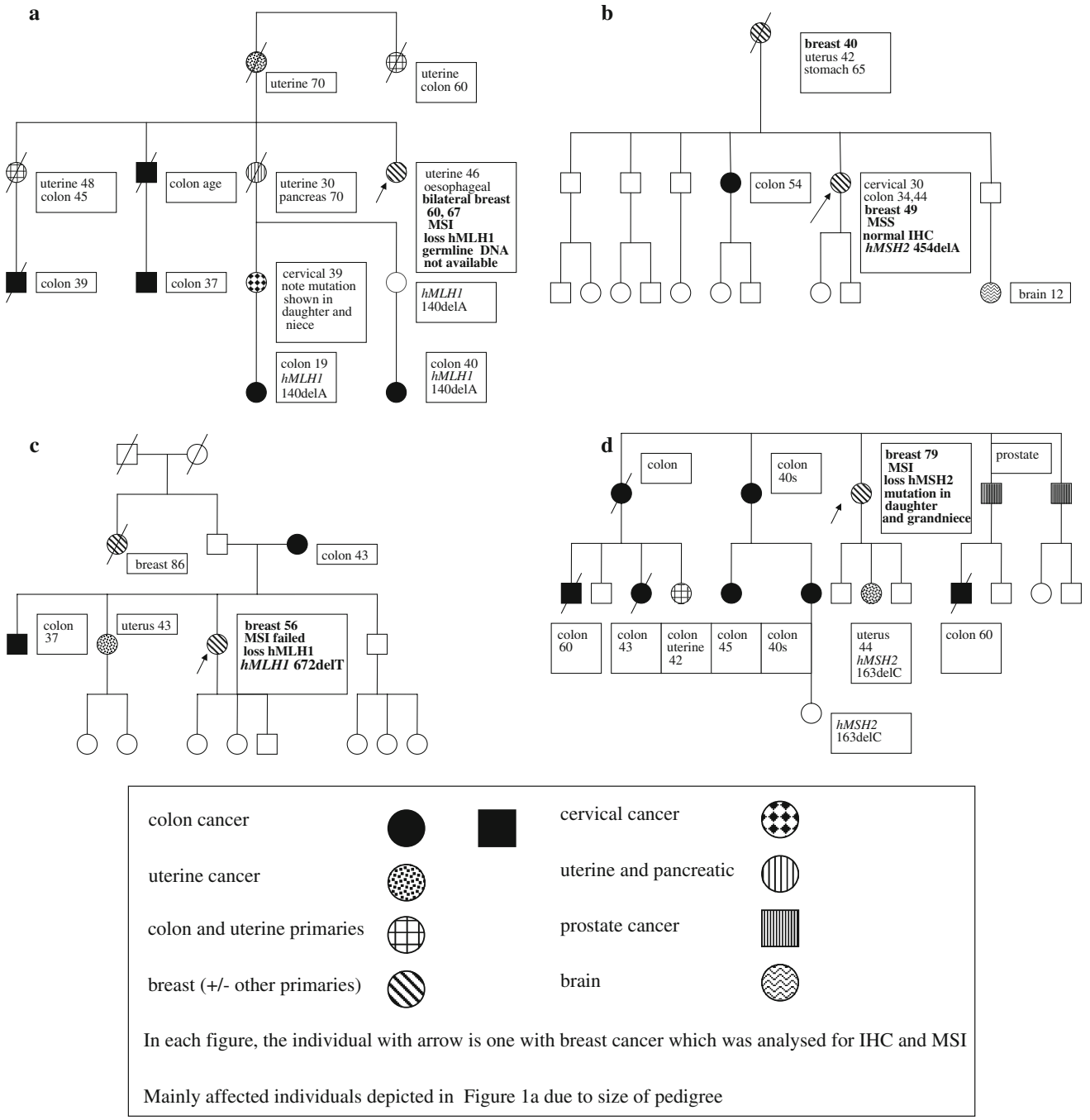

Fig. 1 Pedigrees of mutation-positive families. **a** *hMLH1* 140delA family. **b** *hMSH2* 454delA family. **c** *hMLH1* 672delT family. **d** *hMSH2* 163delC family

the individuals with breast cancer themselves in two of the three individuals where it was possible to assess this finding. A blood sample for DNA was not available from the third individual but she was noted to have had other HNPCC spectrum tumours that substantiate the interpretation that she had HNPCC (see Fig. 1a). In each, the IHC findings indicated loss of the appropriate protein encoded by the mutant gene in the family. The IHC results were more robust than the MSI analyses which failed in one breast cancer.

The finding of the MSS stable breast cancer with intact staining in Family 1b indicates that, as expected, some breast cancers arise by a pathway which appears to be separate from the mismatch repair pathway which gives rise to the colon cancers. Breast cancer is one of the most common types of cancer, arising in one in eight women in

Table 1 Microsatellite and immunohistochemical findings in breast cancers in families being assessed for HNPCC

Family categorisation	Age of breast cancer diagnosis	MMR and IHC findings in breast cancer	MMR and IHC findings in colorectal cancer	Mutation status in family	Mutation status in individual with breast cancer	Interpretation
No 1a Amsterdam II	Bilateral breast cancer at 60, 67	MSI loss of hMLH1	Not able to test specimen unavailable	*hMLH1 140delA*	Not tested but has other HNPCC spectrum tumour-see Fig. 1a	Breast cancer arisen by mismatch repair pathway
No 1b Amsterdam II	49	MSS no loss on IHC	MSI loss of hMSH2	*hMSH2 454delA*	Carrier of deleterious mutation	HNPCC family but breast cancer arisen by other pathways
No 1c Amsterdam II	56	Microsatellite analysis failed loss of hMLH1	MSI loss (patchy) of hMLH1	*hMLH1 672delT*	Carrier of deleterious mutation	Breast cancer arisen by mismatch repair pathway
No 1d Amsterdam II	79	MSI loss of hMSH2	MSI loss of hMSH2	*hMSH2 163delC*	Obligate carrier status due to mutation shown in daughter and sister's granddaughter- see Fig. 1d	Breast cancer arisen by mismatch repair pathway

MMR mismatch repair
MSI microsatellite unstable
MSS microsatellite stable
IHC immunohistochemistry

the Australian population [16]. Therefore, it is likely that a proportion of these cancers identified in family members from HNPCC families will have arisen independently and not as a result of a DNA mismatch repair defect. In such cases, germline analysis of MMR genes in the member with breast cancer is unlikely to be informative.

In summary, these results support the finding that although the risk of breast cancer may not be high in HNPCC families in general, some breast cancers do arise through mismatch repair defects in HNPCC kindreds. If there is no family member with a recognised HNPCC spectrum cancer to assess, then identifying an individual who has a breast tumour which has lost staining of an MMR protein is worthwhile so that mutation testing can be offered. Although our study did not examine hMSH6, others have reported loss of this protein in a breast cancer in an individual with HNPCC [8] and hMSH6 is now routinely included in tumour analyses where HNPCC is being considered. Where a breast tumour in a possible HNPCC family has normal MMR protein staining, it would appear that the breast cancer has arisen by other pathways and hence would not be informative of the individual's HNPCC mutation status.

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