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Mismatch repair defective breast cancer in the hereditary nonpolyposis colorectal cancer syndrome

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Abstract Whether or not breast cancer can be a feature of the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome has been debated. In order to clarify if defective mismatch repair (MMR) may indeed play a role in breast cancer, we used the Danish HNPCC register to identify all breast cancers that occurred in MMR gene mutation carriers. In total, 20 female mutation carriers were diagnosed with breast cancer at mean 50 years of age. These tumors were predominantly ductal carcinomas with extensive lymphocytic reactions in 8/14 evaluated tumors. MMR protein immunostaining showed loss of expression of MLH1, MSH2 or MSH6 corresponding to the mutations identified in 7 of the 16 cases investigated, and these tumors were diagnosed at mean 50 (33–66) years of age. The demonstration of defective MMR in a substantial proportion of the breast cancers studied links yet another tumor type to HNPCC. Though the low number do not motivate surveillance, our observation supports a role for defective MMR in breast cancer progression in HNPCC,

presumably through accelerated accumulation of mutations in breast cancer-associated genes.

Keywords HNPCC · MMR · MLH1 · MSH2 · MSH6 · Hereditary cancer

Introduction

Heredity has been recognized as a major cause of breast cancer with twin studies suggesting that hereditary components cause up to one-third of the cases [1]. The complexity of the genetic background of hereditary breast cancer is increasingly recognized, and the susceptibility alleles may broadly be classified into three groups with respect to their frequency in the population and the associated risk of cancer [2, 3]. Rare high-penetrance alleles, e.g., *BRCA1*, *BRCA2*, *TP53*, *PTEN*, *CDH1*, and *STK11* are estimated to be responsible for 2–3% of the cases. Moderate-penetrance alleles that confer a 1.5- to 2-fold risk increase have been found in *CHEK2*, *ATM*, *BRIPI*, and *PALB2* and could through interaction with other environmental or genetic factors contribute to a substantial proportion of the hereditary cases. Finally, an increasing number of low-penetrance alleles have been identified and could through multiplicative effects confer high absolute risk in individuals with inappropriate combinations of such alleles [4].

The hereditary nonpolyposis colorectal cancer (HNPCC) syndrome is a major cause of hereditary colorectal cancer, predominantly within the proximal colon, with 50–80% cumulative life time risk in mutation carriers, and in addition female carriers are at a 40–60% risk of endometrial cancer and a 10–15% risk of ovarian cancer [5–7]. A number of other tumors types, i.e., gastric cancer, cancer of

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the small intestine and the upper urinary tract, skin tumors, brain tumors, and soft tissue sarcomas, have also been linked to HNPCC, albeit at lower frequencies. Clinical criteria, the so-called Amsterdam criteria, consider development of colon cancer, endometrial cancer, cancer of the small intestine, and cancer of the upper urinary tract and are used for standardized classification of these families [8]. Defective mismatch repair (MMR) has been identified as causative and families in which mutations have been identified are referred to as having Lynch syndrome. Identification of HNPCC/Lynch syndrome families is crucial since surveillance programs, primarily for colorectal tumors, have proven highly effective.

There are conflicting reports on a possible increased risk of breast cancer in families with HNPCC/Lynch syndrome [6, 9–14]. Though breast cancer is not considered part of the tumor spectrum, MMR defective breast cancer has been

described in a number of mutation carriers [10, 11, 15–19]. In order to characterize the role of MMR in breast cancers that have developed in HNPCC families, we identified such cases in the national Danish HNPCC register and herein evaluated tumor morphology and MMR status.

Materials

The national Danish HNPCC-registry was initiated in 1991, covers a population of 5 million and contains clinical, molecular, and surveillance information on families diagnosed with HNPCC. Pedigree information is collected via the family history, and family members are traced by their unique civic registration numbers in the central population registry. Malignancies are verified in the national pathology register, in medical records and death certificates

Table 1 Summary of breast cancers in the Danish Lynch syndrome cohort

Individual number	Age at diagnosis	Histopathology	Tumor-infiltrating lymphocytes	Peritumoral lymphocytes	Gene mutated	Mutation	Immunohistochemical expression	Other cancer type/age
B3-14	33	Ductal	–	–	<i>MLH1</i>	c.1732-2A>T	mlh1-/pms2-	Colon/36
B1036-8	47	Medullary	+	+	<i>MSH2</i> ^b	c.942+3A>T	msh2-/msh6-	Endometrial/44
B501-8	66	Ductal	+	+	<i>MSH2</i>	c.942+3A>T	msh2-/msh6-	Endometrial/43
B1905-8	39	Ductal	NA	NA	<i>MSH2</i>	c.942+3A>T	msh2-/msh6-	–
B748-36	44	Ductal	–	–	<i>MSH6</i>	c.4001+2T>C	msh2-/msh6-	–
B748-28	58/63	Ductal ^a	–	–	<i>MSH6</i>	c.4001+2T>C	msh6-	–
B748-30	66	Ductal	+	+	<i>MSH6</i> ^b	c.4001+2T>C	msh2-	Ureter/66, cardia/68
B13-4	52/67	Ductal ^a	+	+	<i>MLH1</i>	c.1276C>T	4 MMR proteins normal	–
B19-46	59	Ductal/ mucinous	–	–	<i>MLH1</i>	c.1165C>T	4 MMR proteins normal	Colon/50
B19-144	40	Ductal	–	–	<i>MLH1</i>	c.1165C>T	4 MMR proteins normal	Colon/59
B992-80	45	Ductal	+	+	<i>MLH1</i>	c.1852_1854delAAG	4 MMR proteins normal	–
B8-14	45	DCIS	+	+	<i>MSH2</i>	c.1387-?_1661+?del	4 MMR proteins normal	Colon/35, ovarian/44
B669-8	53	Ductal	+	+	<i>MSH2</i>	c.3647-1G>A	4 MMR proteins normal	–
B172-10	48	Ductal	+	–	<i>MSH2</i>	c.888_889delC	4 MMR proteins normal	Rectal/20, colon/38
B678-60	60	Ductal	–	–	<i>MSH6</i>	c.3647-1G>A	4 MMR proteins normal	Endometrial/58
B1338-54	52	Ductal	NA	NA	<i>MSH6</i>	c.3647-1G>A	4 MMR proteins normal	–
B259-8	58	NA	NA	NA	<i>MSH2</i>	c.367-?_1076+?del	NA	–
B748-12	59/61	Ductal ^a	NA	NA	<i>MSH6</i>	c.4001+2T>C	NA	Endometrial/55, urethral/79
B1480-60	45	Ductal	NA	NA	<i>MSH6</i>	c.1444C>T	NA	Endometrial/45, urethral/69
B1388-8	53	Ductal	NA	NA	<i>MSH6</i>	c.3647-1G>A	NA	–

^a Refers to the second primary cancer, which was studied

^b MSI-high phenotype demonstrated in breast cancer tissue

with a >90% of the diagnoses confirmed. A number of families are identified today and a selection bias toward the most explicit families registered. The register contains 164 families with disease-predisposing MMR gene mutations, 97 of which fulfill the Amsterdam I/II criteria [20]. Totally, 47 breast cancers were diagnosed in mutation carriers ($n = 20$) and first-degree relatives ($n = 27$), but due to uncertain carrier status the latter were not investigated. Of the 20 mutation carriers, 11 developed a metachronous, HNPCC-associated cancer, most commonly colon cancer and/or endometrial cancer. In order to assess MMR function, we retrieved breast cancer specimens from proven mutation carriers and could hereby study 16/20 tumors.

Methods

For MMR protein immunostaining, 4- μ m sections were deparaffinized in Tissue-Clear[®] (Sakura Finetek, Hoge Rijndijk, the Netherlands) and rehydrated through decreasing

concentrations of ethanol. Antigen retrieval was achieved by microwave treatment in 1 mM EDTA/10 mM Tris buffer (pH 9.0) boiling for 15 min followed by a cooling for 15 min. Immunohistochemical staining was performed manually using antibodies to MLH1 (G168-15, dilution 1:100) and PMS2 (A16-4, 1:500) from BD Biosciences (San Jose, CA, USA), MSH2 (25D12, 1:200) from Novocastra (Newcastle, UK), and MSH6 (Clone 44, 1:200) from BD Biosciences. The EnVision[™] detection kit (DAKO Cytomation) was used and modified using an extra enhancing step after diaminobenzidine treatment and 0.1% hematoxyline staining. Retained staining was defined as nuclear staining in neoplastic cells. Nuclear staining in stromal components and lymphocytes served as internal controls and was required in order to classify a tumor as having loss of one or several MMR proteins in the tumor cells.

The microsatellite instability (MSI) analysis included the mononucleotide repeats BAT25, BAT26, BAT40 and the dinucleotide repeats D2S123, D5S346, BAT34, and D17S250 and was performed according to standard

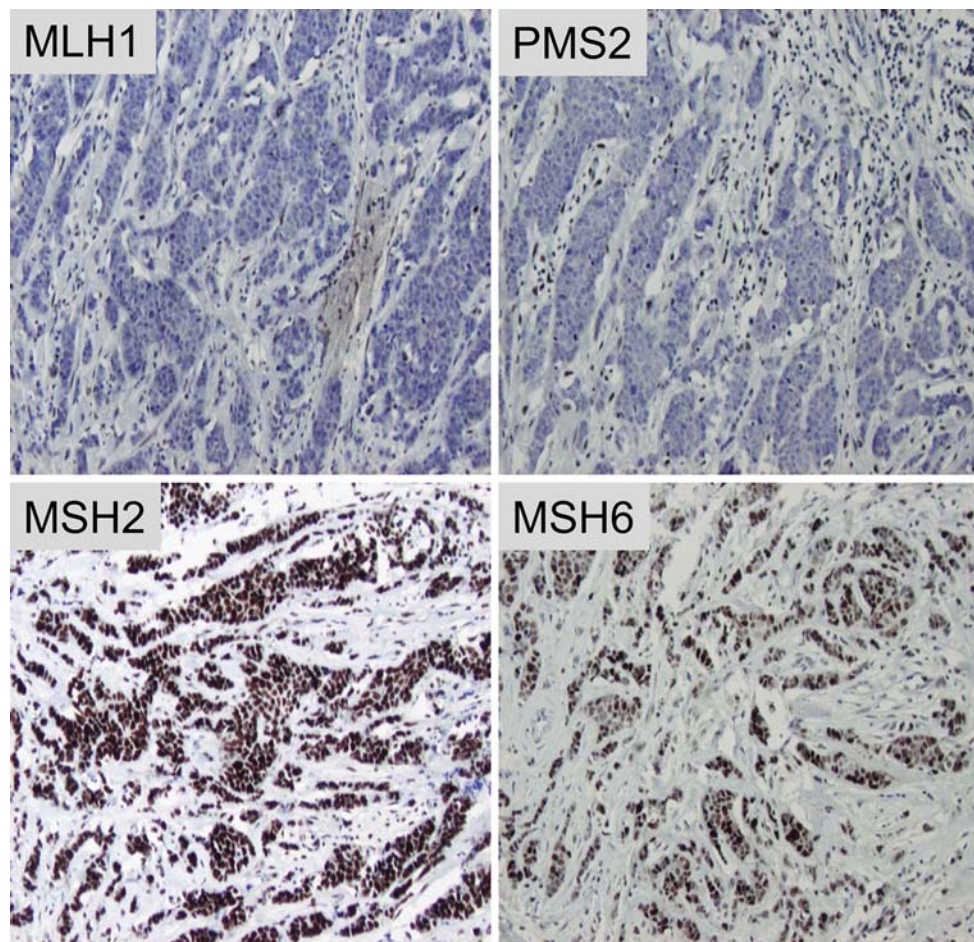


Fig. 1 MMR protein immunostaining of a breast cancer (case 3–14) demonstrating loss of expression for MLH1 and PMS2 in the tumor cells with retained staining in the stroma (*top*). The staining for MSH2

and MSH6 was retained in both components (*bottom*). In concordance with this observation, the patient carries a germline mutation in the MLH1 gene

procedures using fluorescently labeled primers and size separation on an ABI310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). A detailed protocol is available from the authors upon request.

Results

The 20 breast cancers in mutation carriers were diagnosed at mean 50 (range 33–67) years of age. All but one of the diagnoses were histopathologically confirmed and revealed ductal carcinomas in 16 cases, medullary differentiation in one case and mucinous differentiation in one, whereas the last tumor was classified as ductal carcinoma in situ (DCIS). The disease-predisposing MMR gene mutations affected *MSH6* in eight cases, *MSH2* in seven cases, and *MLH1* in five cases. Lymphocytic reactions were classified by pathologic review of 15 tumors and identified tumor-infiltrating lymphocytes in eight breast cancers and peritumoral lymphocytes in seven (Table 1; Fig. 2). MMR protein immunostaining was performed in 16 tumors and showed normal, retained expression in nine tumors and loss of at least one MMR protein in seven tumors. The losses affected *MLH1/PMS2* in one case, *MSH2/MSH6* in four, *MSH2* only in one, and *MSH6* only in one tumor. The losses were in all cases consistent with the underlying germline MMR gene mutation (Table 1; Fig. 1). The seven MMR defective tumors were diagnosed at mean age 50 (33–66) years and included five ductal carcinomas and one medullary cancer (Fig. 2).

Discussion

Studies on the role of MMR defects in breast cancer have reached different conclusions regarding a possible link to HNPCC/Lynch syndrome. Since no increased risk has been demonstrated, breast cancer is not considered part of the HNPCC/Lynch syndrome, and surveillance for breast cancer is not called for. A number of case reports have, however, described breast cancer in individuals with HNPCC (Table 2). This observation led us to investigate the frequency of breast cancer in the national Danish HNPCC register and to assess MMR status in tumors from mutation carriers. In the 164 families with disease-predisposing MMR gene mutations, we identified 20 mutation carriers affected with breast cancer at mean 51 years of age. Loss of MMR protein expression was found in 7/16 breast cancers evaluated, which demonstrates that the defective MMR plays a role in a substantial proportion of these cases. These seven tumors were linked to three MMR gene mutations, *MLH1* c.1732-2A>T, *MSH2* c.942+3A>T, and *MSH6* c.4001+2T>C. The *MSH2* mutation is

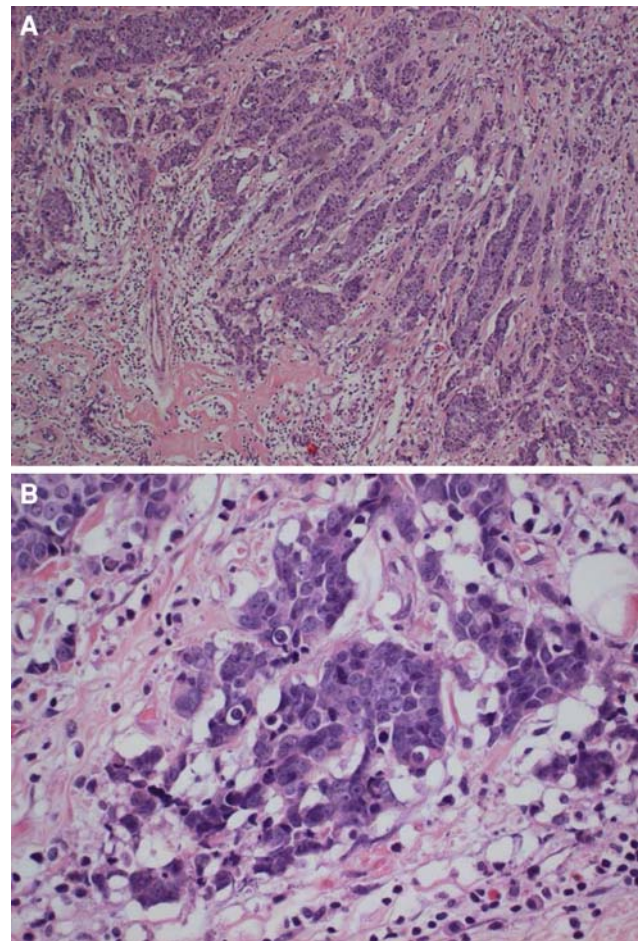
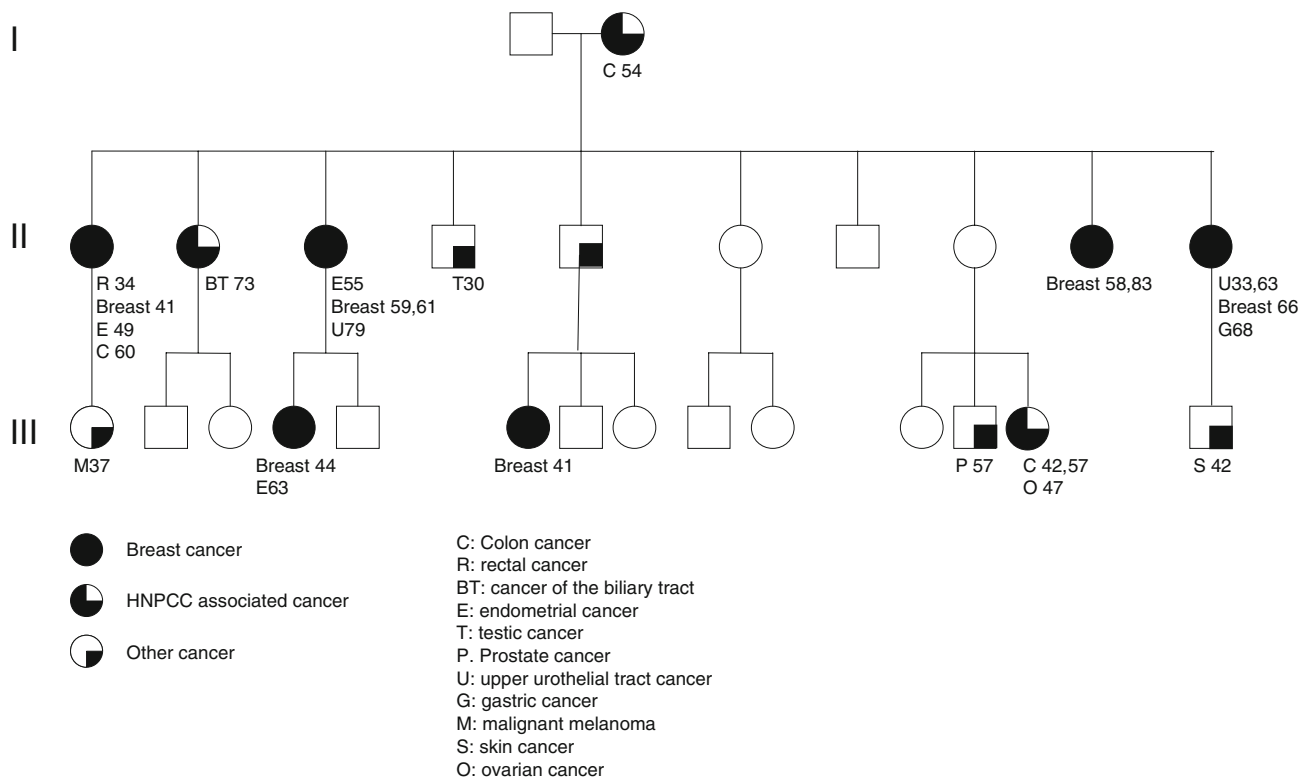


Fig. 2 Hematoxylin and Eosin staining of a MMR defective breast cancer showing **a** $\times 10$ magnification with abundant peritumoral lymphocytes and **b** $\times 40$ magnification with tumor-infiltrating lymphocytes

recognized as internationally recurrent, and the breast cancers occurred in individuals from 3 of the 16 Danish families with this mutation. The *MSH6* mutation has been identified in four families in Denmark, but strikingly the breast cancers developed in three females from the same family (Fig. 3). MMR defective colorectal cancers are characterized by frequent lymphocytic reactions, most commonly expressed as peritumoral lymphocytes and as tumor-infiltrating lymphocytes [21]. Their overrepresentation in colorectal cancers in HNPCC has been clearly established, whereas a possible role in breast cancer remains to be clarified. Lymphocytic reactions in HNPCC-associated breast cancer have previously only been reported in one of the case studies, which identified extensive lymphocytic infiltrates [15]. Half of the cancers here investigated showed lymphocytic reactions and if indeed present at the high frequency suggested here, it could represent a potential marker for MMR defective breast cancer in individuals with HNPCC/Lynch syndrome.

Table 2 Summary of present and published breast cancers linked to HNPCC/Lynch syndrome

Author	Age at diagnosis	Gene mutated	Microsatellite instability/ MMR immunostaining	Other HNPCC-related cancer
Risinger et al. [16]	46	<i>MLH1</i>	MSI	+
Boyd et al. [19]	71 ^a	<i>MLH1</i>	MSI	+
de Leeuw et al. [18]	46	<i>MLH1</i>	MSI	+
	47	<i>MLH1</i>	MSI	+
	50	<i>MLH1</i>	MSI	+
	32	<i>MSH2</i>	MSI	–
	52	<i>MSH2</i>	MSI	+
	29	<i>MSH2</i>	MSI	–
	49	<i>MSH6</i>	MSI	+
	49	<i>MSH2</i>	MSI/msh2-/msh6-	–
Westenend et al. [15]	49	<i>MSH2</i>	MSI/msh2-/msh6-	–
Current study				
B3-14	33	<i>MLH1</i>	mlh1-/pms2-	+
B748-36	44	<i>MSH6</i>	msh2-/msh6-	–
B748-28	58	<i>MSH6</i>	msh6-	–
B748-30	66	<i>MSH6</i>	msh2-	+
B1036-8	47	<i>MSH2</i>	msh2-/msh6-	+
B501-8	66	<i>MSH2</i>	msh2-/msh6-	+
B1905-8	39	<i>MSH2</i>	msh2-/msh6-	–

^a Male breast cancer**Fig. 3** Pedigree from a Lynch syndrome family with a *MSH6* c.4001C>T mutation. The three females indicated with arrows developed breast cancers with immunohistochemical loss of MSH6

expression. Since several females developed breast cancer, mutation analysis of the *BRCA1* and *BRCA2* genes had been performed to exclude hereditary breast and ovarian cancer with normal result

Including the cases here identified, 17 breast cancers that developed in individuals with Lynch syndrome have demonstrated MMR defects (Table 2). These tumors were diagnosed at an early age, mean 50 years, which is 10–20 years earlier than the sporadic cases are diagnosed and at about the same age as the more common colorectal and endometrial cancers typically are diagnosed in HNPCC. This age also concurs with the mean age for breast cancer in BRCA mutation carriers [22]. Though the route for breast cancer development in HNPCC remains unknown, it is possible that the MMR defect may also accelerate the accumulation of mutations in breast cancer-associated genes, and thereby enhance tumor progression. In summary, the demonstration of defective MMR in concordance with the gene mutated in about half of the breast cancers from female HNPCC/Lynch syndrome patients suggests that this tumor type can be added to the list of tumors, e.g., hepatobiliary cancer, gliomas, sebaceous carcinomas of the skin, and soft tissue sarcomas that occur at low frequency in this syndrome. From a clinical perspective, this observation serves as a reminder not to dismiss breast cancers in these families as sporadic, but to consider that development hereof may indicate mutation carrier status.

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