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Tumor Characteristics as an Analytic Tool for Classifying Genetic Variants of Uncertain Clinical Significance

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Abstract

It is important to identify a germline mutation in a patient with an inherited cancer syndrome to allow mutation carriers to be included in cancer surveillance programs, which have been proven to save lives. Many of the mutations identified result in premature termination of translation, and thus in loss-of-function of the encoded mutated protein. However, the significance of a large proportion of the sequence changes reported is unknown. Some of these variants will be associated with a high risk of cancer and have direct clinical consequence. Many criteria can be used to classify variants with unknown significance; most criteria are based on the characteristics of the amino acid change, on segregation data and appearance of the variant, on the presence of the variant in controls, or on functional assays. In inherited cancers, tumor characteristics can also be used to classify variants. It is worthwhile to examine the clinical, morphological and molecular features of a patient, and his or her family, when assessing whether the role of a variant is likely to be neutral or pathogenic. Here we describe the advantages and disadvantages of using the tumor characteristics of patients carrying germline variants of uncertain significance (VUS) in *BRCA1*, *BRCA2*, or in one of the mismatch repair (MMR) genes, *MLH1*, *MSH2*,or *MSH6*, to infer pathogenicity.

Keywords

unclassified variants; VUS; UV; classification criteria; breast cancer; Lynch syndrome; *BRCA1*; *BRCA2*; *MLH1*; *MSH2*; *MSH6*

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INTRODUCTION

It is important to identify a germline mutation in a patient with an inherited cancer syndrome, such as hereditary breast (MIM# 114480) and ovarian cancer or Lynch syndrome (MIM# 120435), also referred to as hereditary nonpolyposis colorectal cancer (HNPCC), to allow mutation carriers to be included in cancer surveillance programs. Such programs are proven to be life-saving. Furthermore, family members not carrying the mutation could be safely treated as low risk, saving on unnecessary screening and anxiety. Many of the mutations identified result in premature termination of translation, and thus in loss-of-function of the encoded mutated protein. However, if the inclusion criteria are less strict it is estimated that between one-third and one-half of the sequence changes reported for *BRCA1* (MIM# 113705) and *BRCA2* (MIM# 600185), respectively (and the same holds true for the mismatch repair (MMR) genes) are variants of unknown clinical significance [Barnetson et al., 2008; Chao et al., 2008; Gomez-Garcia et al., 2005; Nyström-Lahti et al., 2002]. These variants, also termed unclassified variants (UVs), or variants of uncertain significance (VUS) are not predicted to lead to a premature termination of translation and, in general, it is not known whether they contribute to the disease phenotype or merely represent rare variants associated with no or little cancer risk. Some of these rare variants will be associated with a high risk of cancer of direct clinical consequence (see companion work by Plon et al. [2008]).

Many criteria can be used to classify such rare variants and some are based on tumor characteristics. Nontumor characteristics include: (1) de novo appearance of the mutation in a clinically affected individual; (2) segregation with the disease within pedigrees; (3) a variant is considered nonpathogenic when on the other allele (in *trans*) a known deleterious mutation is present (for genes where biallelic mutations are lethal, or have very obvious clinical phenotypes); (4) absence in control individuals; (5) a change of amino acid polarity or size; (6) occurrence of the amino acid change in a domain that is evolutionarily conserved between species and/or shared between proteins belonging to the same protein family [Tavtigian et al., 2008a,b]; and (7) deleterious effect in a functional assay or in an animal model [Couch et al., 2008]. In the case of high-penetrance cancer predisposition genes, it is assumed that loss of function will occur for both alleles of the disease-causing gene (for tumor suppressor genes or MMR genes) in tumor tissue. For cancer genes, therefore, another criterion that can be used to infer pathogenicity of a mutation is: (8) loss of function of the nonmutated allele by various mechanisms, including somatic mutation, deletion of the gene (and usually a large part of the surrounding chromosome [Spurdle et al., 2008b]) and hypermethylation of the promoter region of the gene. In some conditions, the resulting loss of protein may be detectable in tumor tissue by comparing it to normal tissue, such as: (9) loss of protein expression in tumors, which can be detected by immunohistochemistry (e.g., the MMR gene proteins) but can also be manifest by abnormally stable protein expression for proteins with rapid turnover; e.g., TP53 gene (MIM# 191170) mutation. Furthermore, morphological (10) or histopathological (11) features of tumors, shown to be associated with pathogenic mutations in previous studies, can also be used as evidence to evaluate the likely clinical significance of variants. Previous inclusion of an unclassified sequence variant in the increasing number of disease-specific mutation databases should not be considered sufficient evidence for pathogenicity [Greenblatt et al., 2008]. Evidence for a designation as pathogenic is not usually included in the database, and even if included, the evidence is often indirect and therefore prone to artifacts. Indirect evidence might also come from for instance, segregation analysis. Segregation of a mutation with the disease can be caused by the variant being in linkage disequilibrium with the true, as yet unidentified, mutation. Likewise, loss of heterozygosity (LOH), absence of staining for the protein (“negative immunohistochemistry”), or mutation-associated tumor features could all be due to an unidentified mutation in the same gene or in its regulatory sequences. Therefore, knowledge of the sensitivity and extent of mutation testing is crucial to the interpretation of pathogenicity.

Here we describe the advantages and disadvantages of using tumor characteristics to infer pathogenicity of the variant in patients carrying a germline variant of unknown clinical significance in the *BRCA1*, *BRCA2*, or in one of the MMR genes, *MLH1* (MIM# 120436), *MSH2* (MIM# 609309), or *MSH6* (MIM# 600678). We discuss the specificity and sensitivity of tumor characteristics of known disease-causing pathogenic mutations, and extrapolate their use in classifying rare sequence variants.

FAMILIAL BREAST CANCER

Molecular Basis of Familial Breast Cancer

Breast cancer is one of the most common cancers affecting women in the Western world and its incidence is rising. As for many cancers, there is a large environmental component to risk and a significant genetic component, with 20 to 30% of cases occurring against a family history of the disease. However among the 20 to 30% of familial cases, only 2 to 3% overall are due to known, high-risk genes, with *BRCA1* and *BRCA2* being the most frequently implicated [Pharoah et al., 2002]. Younger onset of disease than in the general population is a typical feature seen in the presence of inherited, high-penetrance genetic susceptibility. Much of the remaining genetic risk is likely to be accounted for by frequent, low-penetrance genetic polymorphisms that are now being identified through whole genome association studies [Easton et al., 2007; Hunter et al., 2007].

The two most important breast cancer susceptibility genes are *BRCA1* and *BRCA2*. Together, they account for about 30% of the familial risk for breast cancer. In most populations, mutations are rare (an estimated population carrier rate of the order of 1/400 for each gene), but in some populations, such as the Ashkenazim, deleterious mutations are seen much more frequently—about 2.5% of Ashkenazi Jews carry 1 out of 3 founder mutations in *BRCA1* or *BRCA2*. Both *BRCA1* and *BRCA2* function as tumor suppressors, as the wild-type alleles are often lost in tumors from heterozygote carriers.

BRCA1 acts as a hub protein and is involved in genomic stability, DNA repair, DNA damage response and cell-cycle checkpoint control, chromatin remodeling, transcriptional regulation, and protein ubiquitination. BRCA1 facilitates DNA repair by its involvement in homologous recombination and DNA damage response [reviewed in Narod and Foulkes, 2004; Wang, 2007; Yoshida and Miki, 2004]. Its precise functions and their relationship to cancer pathogenesis continue to be investigated; notable recent findings include the further refinement of the BRCA1 double-strand break recognition mechanism [Greenberg, 2008; Chen et al., 2008]. BRCA1-related tumors are often “basal-like” (described below), and it seems likely that this is related to functions independent of those implicated in DNA repair, since BRCA2-related breast cancers are not often basal-like, but BRCA2 is intimately involved in the repair of both single- and double-strand DNA breaks [reviewed in Narod and Foulkes, 2004]. In addition to DNA repair functions, there is evidence that BRCA2 also participates in cytokinesis. Daniels et al. [2004] demonstrated that dividing cells with BRCA2 mutations, although riddled with DNA breaks and chromosomal abnormalities, are less able to separate because of failed homologous-strand repair. Thus the abnormalities of chromosome number seen in BRCA2-deficient cells are, at least in part, a direct consequence of BRCA2 dysfunction.

Breast tumor pathology—BRCA1 and BRCA2—Breast cancer is a heterogeneous disease, with distinct types being recognized with respect to morphology, immunohistochemistry, and, more recently, by molecular markers. It has been established that breast tumors arising in carriers of a pathogenic *BRCA1* mutation tend to have a distinctive tumor phenotype. This was first noted on the basis of simple pathology features [Armes et al., 1998; Lakhani et al., 1998, 2000].

BRCA1-associated breast cancers are usually of high histological grade, with exceptionally high mean mitotic counts, a syncytial growth pattern, pushing margins, confluent necrosis, and a high degree of aneuploidy. Invasive *BRCA1* tumors do not usually coexist with ductal in situ carcinoma (DCIS), but DCIS can be a presenting feature [Hoogerbrugge et al., 2003, 2006; Hwang et al., 2007; Lakhani et al., 1998, 2000]. The atypical medullary carcinoma histological subtype has been shown to be overrepresented in *BRCA1* mutation carriers (see Table 1) and this classification probably reflects the frequently observed heavy lymphocytic infiltrate in BRCA1-associated tumors [Honrado et al., 2005].

BRCA2-associated breast cancers arise at a slightly later age than *BRCA1*-related tumors (average age around 48 years compared to 43 years for *BRCA1*) and they are morphologically somewhat less distinct from sporadic breast cancers than *BRCA1*-associated tumors. There have been only two studies reporting independent predictors of *BRCA2* mutation status. The large Breast Cancer Linkage Consortium study [Lakhani et al., 1998], reported tubule formation, mitotic count, and continuous pushing margins to be significant predictors of *BRCA2* mutation status, and confirmed that the tubule formation was significantly different to non-*BRCA1/2* tumors using multiple regression analysis [Lakhani et al., 2000]. In addition, Bane et al. [2007] reported that *BRCA2* mutation carriers are significantly more likely to develop estrogen-positive higher-grade tumors than are controls, and that in situ cancers are more common.

Breast tumor pathology—non-BRCA1 and BRCA2—Familial non-*BRCA1*/*BRCA2* breast cancers tend, on average, to have a somewhat lower grade, with possibly a more frequent reporting of lobular morphology than breast cancers in general and BRCA1/2 tumors, in particular [Lakhani et al., 2000; Oldenburg et al., 2006]. However, there are no “typical” appearances that allow a familial non-*BRCA1/2* breast tumor to be recognized, as they are fairly similar to breast cancers seen in the general population.

Immunohistochemical characterization of BRCA1/2-associated tumors—Showing loss of the BRCA1 or BRCA2 proteins by immunohistochemical staining (IHC) has not proven to be useful in selecting tumors in which mutation of either gene underlies the tumor development. However, based on protein expression of proteins other than BRCA1/2, the *BRCA1*- and *BRCA2*-associated tumors do show differences (Table 1). *BRCA1* tumors show no or only weak (<10% of cells) expression of estrogen (ER-negative) or progesterone receptors (PR-negative). Tumors are usually high-grade and high tumor grade correlates strongly with ER negativity, but in addition the BRCA1 tumors rarely overexpress the oncogene ERBB-2 (HER2-negative); this is commonly referred to as “triple negative” breast cancer and is typical of the basal-like subtype [Turner et al., 2004]. In a study of 72 breast cancers diagnosed in Ashkenazi Jewish women under 65 years of age, *BRCA1* tumors were nine times more likely to express the basal marker CK5/6 than tumors in non-*BRCA1* or *BRCA2* mutation carriers (P = 0.002) [Foulkes et al., 2003]. In a much larger series of cases (182 *BRCA1* carriers, 63 *BRCA2* carriers, and 109 sporadic breast cancer patients), the Breast Cancer Linkage Consortium showed that ER-negative, CK5/6-positive, and CK14-positive breast tumors were approximately 27 times more likely to have a *BRCA1* mutation compared to controls; nearly one-half (43%) of all *BRCA1* tumors had this phenotype, whereas it was seen in only 1.6% of the *BRCA1/2*-negative controls [Lakhani et al., 2005].

A recent study of basal cytokeratin expression in a smaller study of Finnish breast cancer families [Eerola et al., 2008] showed that, compared to sporadic tumors, CK14 expression was significantly increased in *BRCA1* mutation carriers particularly (n = 46; P = 0.0005), but also in *BRCA2* carriers (n = 40; P = 0.011) and non-*BRCA1/2* familial cases (n = 358; P = 0.005).

Expression of the basal marker epidermal growth factor receptor (EGFR) has also been shown to be an indicator of *BRCA1* mutation status [Lakhani et al., 2005]. It is important to note, however, that among series of sporadic (nonfamilial) breast tumors, about 15% will be classified as basal type or triple-negative tumors and this subtype is more frequent in young breast cancer cases [Bauer et al., 2007]; i.e., these features are not exclusive to tumors in *BRCA1* mutation carriers. Furthermore, there is a difference in the breast cancer pathology developing in younger and older BRCA1 gene carriers (the average age for BRCA1 breast cancer is about 42 years). Older BRCA1 gene carriers in particular exhibit less striking differences in phenotype [Eerola et al., 2008].

Like *BRCA1* breast tumors, *BRCA2* tumors tend to be of higher histological grade and are rarely HER2+, but unlike *BRCA1* tumors they are often ER-positive [Bane et al., 2007; Foulkes et al., 2004; Lakhani et al., 2005]. The observed relationship between expression of ER in *BRCA1/2* tumors seems specific to each gene, as ER-negative staining is seen more often in BRCA1 tumors compared to BRCA2 or sporadic tumors at all ages [Foulkes et al., 2004].

The identification of immunohistochemical markers for *BRCA2* mutation status is desirable, and is theoretically possible given the evidence that other gene expression profiles delineate breast tumors by *BRCA1* and *BRCA2* mutation status [Hedenfalk et al., 2001; Sorlie et al., 2003; van't Veer et al., 2002]. There is suggestive evidence that such markers of *BRCA2* mutation status may be found in E-cadherin expression [Palacios et al., 2005a], MYC duplication/amplification [Adem et al., 2004; Palacios et al., 2005b], *TBX2* duplication [Adem et al., 2004], RAD51 nuclear expression and increased CHEK2 expression [Honrado et al., 2005], and increased cyclin D1 expression [Colombo et al., 2008]. However, the results from these studies should be viewed with caution due to their relatively small sample sizes, ranging from only eight to 24 carriers of a *BRCA2* mutation.

LOH in familial breast cancer—Consistent with evidence that BRCA1/2 act as tumor suppressors, reports on largely truncating mutations indicate that ~80% of tumors from *BRCA1* and *BRCA2* mutation carriers undergo inactivation of the wild-type allele by LOH [Collins et al., 1995; Cornelis et al., 1995]. Likelihood ratios for use of LOH data have been derived and used for assessing causality of *BRCA1/2* sequence variants [Chenevix-Trench et al., 2006]. These likelihood ratios assumed 30% LOH of *BRCA1*/*BRCA2* in sporadic tumors, with random allele loss such that 15% would lose wild-type and 15% would lose the variant allele. However, analysis by the same group of an expanded dataset of 63 different variants showed more loss of the variant than expected based on the underlying assumptions [Spurdle et al., 2008a]. This increased loss was observed for variants ultimately classified as pathogenic or as neutral/low clinical significance. There was no evidence that this was a technical issue, and at present these authors advise that LOH data be excluded from multifactorial analysis until adequate reference data can be accumulated on the role of LOH in the development of breast cancer in carriers of *BRCA1/2* missense mutations. One explanation for their observation is that the course of tumor development, with respect to somatic mutation, may differ according to mutation type and/or degree of risk associated with the mutation. Indeed, data published some years ago on the role of different adenomatosis polyposis coli (*APC*) gene alleles in the course of this disease [Spirio et al., 1998] led the authors to postulate that less penetrant APC mutations undergo a somatic inactivating point mutation in the wild-type allele and then LOH of the mutant occurs as a third hit as the tumor develops.

Other tumor features that may prove useful for classification include chromosomal rearrangements and mRNA expression. A class predictor has been made that allows a given breast tumor to be classified based on its genomic comparative genomic hybridization (CGH) profile compared to the *BRCA1*-like class or sporadic-like class [Joosse et al., in press; Jönsson et al., 2005; van Beers, 2005; Wessels et al., 2002]. Analysis of almost 50 breast tumors from

hereditary breast and ovarian cancer families, in whom no *BRCA1/2* mutations had been identified in routine diagnostic screening, showed that only a few (4%) show a *BRCA1*-like profile [Joosse et al., in press]. The array CGH (aCGH) approach could also be useful in investigating the role of *BRCA1* variants. We are currently assessing whether the presence of a *BRCA1*-like profile may add proof of clinical significance for a given germline alteration [P. Nederlof, F.B.L. Hogervorst et al., unpublished data]. An example of such a variant analysis has recently been published by Tischkowitz et al. [2008]; they suggest, by several lines of evidence, including the presence of a *BRCA1* aCGH profile, that the M1775 K variant is pathogenic. A defect in *BRCA1/2* may lead to a specific tumor development pathway that results in the accumulation of specific genomic aberrations. As such, genomic profiles do not provide direct functional proof of the significance of a given variant, but a profile does indicate the involvement of dysfunctional BRCA1 for which the variant may be responsible.

Likelihood Ratios for Assessing Pathogenicity of *BRCA1* and *BRCA2* Rare SequenceVariants

The tumor features described above are not exclusive to *BRCA1* or *BRCA2* mutation carriers, and it is known that somatically-acquired BRCA1 rearrangements may be associated with BRCA1-like tumor morphology [van der Looij et al., 2000]. The increased proportion of particular features seen in *BRCA1* mutation carriers relative to other familial breast cancers and/or sporadic breast cancers can nevertheless be used to derive likelihood ratios. These likelihood ratios can be used in multifactorial likelihood analysis (see companion work by Goldgar et al. [2008]) to evaluate *BRCA1* sequence variants of unknown clinical significance. For instance, based on the observation of these characteristics in 80% of BRCA1 tumors compared to 27% of sporadic tumors [Lakhani et al., 2000] an ER-negative and high-grade tumor phenotype provides a 3:1 likelihood of the tumor having arisen in a *BRCA1* mutation carrier [Chenevix-Trench et al., 2006]. Similarly, as mentioned from the observed frequency of IHC phenotypes described in the previous section [Lakhani et al., 2005] it is estimated that an ER-negative, CK5/6-positive, and CK14-positive breast tumor has a 27:1 likelihood of having arisen in a *BRCA1* mutation carrier [Spurdle et al., 2008a].

Source data from two publications describing large series of *BRCA2* tumors can be used to derive likelihood ratios based on tubule formation [Lakhani et al., 2000] or ER status and grade [Bane et al., 2007] for use in multifactorial likelihood analysis. The less distinctive nature of *BRCA2* tumors means that these ratios will naturally carry less weight for prediction than the histopathological features of *BRCA1*, with a maximum likelihood ratio of approximately two-fold using either data source. Tubule formation is considered the preferred feature for grading since scoring for tumor grade relies on the three parameters of tubule formation, meiotic counts, and pleomorphism, with high interobserver variability recorded for the pleomorphism component. Tubule formation has been used to assist in variant classification in several studies [Chenevix-Trench et al., 2006; Spurdle et al., 2008].

The use of such likelihood ratios in the clinical setting comes with the standard caveats associated with the multifactorial likelihood analysis approach, principally that likelihood estimates are defined by the source data on which they were based. The tumor pathology likelihood ratios above were derived from analyzing a single dataset [Bane et al., 2007; Lakhani et al., 1998, 2000, 2005], and further large studies would no doubt provide more robust estimates of tumor features in *BRCA1/2* mutation carriers and other breast cancer groups. Likelihoods are valid only for patient populations similar to those from which such data were derived—in this instance familial breast cancer patients, generally ascertained via counseling clinics, and not preselected for *BRCA1/2* mutation testing on the basis of their tumor status. Another issue is whether mutation type affects phenotype, and while at least some functionally abrogated missense mutations have been shown to display the histopathological features

commonly observed for truncating mutations [Lovelock et al., 2007], the tumor features of a large series of missense mutations would need to be assessed before this issue could be evaluated with any confidence. This will require large-scale collaborative studies given the paucity of proven missense mutations, apart from BRCA1 RING and some BRCT domain mutations. Last, these modeling methods are designed to test if variants are of similar high risk as the average *BRCA1/2* mutation [Antoniou et al., 2003], and they cannot yet be used to assess whether variants are associated with a lower or more moderate risk of cancer.

LYNCH SYNDROME

Molecular Basis of Intestinal Tumorigenesis in Lynch Syndrome

Lynch syndrome is caused by a germline mutation in one allele of any of four DNA MMR genes, *MLH1*, *MSH2*, *MSH6*, or *PMS2* (reviewed in de la Chapelle [2004]). Inadvertent somatic loss of the wild-type allele results in MMR deficiency. The MMR pathway corrects replication errors such as mispaired nucleotides but also small insertion/deletion loops resulting from slippage of the polymerases during replication of simple-sequence repeats (also called microsatellites; e.g., A_n or $[CA_n]$). MMR deficiency therefore results in a generalized spontaneous mutator phenotype that is easily recognized by increased or (more often) decreased microsatellite lengths. This phenomenon is referred to as microsatellite instability (MSI).

MMR is a ubiquitous DNA repair process and is active in all dividing cells. It is, therefore, paradoxical that in Lynch syndrome the spectrum of cancer is largely confined to the colon and, to a lesser extent, the endometrium. Insights into the factors that determine the tumor spectrum in Lynch syndrome can be derived from characteristics of MMR heterozygous and MMR-deficient cells and from the phenotypes of Lynch syndrome tumors. Conversely, these insights may be used to help predict whether UVS in MMR genes confer functional MMR defects. Multiple properties of MMR-deficient cells combined with the properties of the specific microenvironment of the colon may be responsible for both the location and phenotype of tumors [Chao and Lipkin, 2006]. MMR-deficient cells accumulate both spontaneous frameshifts and nucleotide substitution mutations at an up to 1,000-fold increased rate compared to normal cells [Li, 2008]. In addition to this spontaneous mutability, it is probable that exposure to mutagenic compounds also plays an important role in tumorigenesis in Lynch syndrome. The proximal colon is a genotoxic environment containing mutagenic agents derived from endogenous sources, such as bile, which induces reactive DNA-methylating nitrosamines [Bernstein et al., 2005], combined with exogenous mutagens from food (such as heterocyclic amines). In addition to its proximal location with respect to the bile duct, the glutinous composition of the stool in this part of the colon may allow increased contact of these mutagens with the lumen. These mutagens probably greatly increase loss, by mutation or LOH, of the wild-type allele of the germline-mutated MMR gene [Borgdorff et al., 2005], resulting in complete loss of MMR in a relatively large fraction of the enterocytes in Lynch syndrome patients. Two intrinsic properties of MMR-deficient cells contribute to their subsequent malignant transformation. First, MMR-deficient cells have acquired tolerance to the toxicity of nitrosamines that methylate the O^6 position of guanosines and have aberrant DNA damage responses to other mutagenic agents [Li, 2008]. Therefore, the MMR-deficient cells in the proximal colon of a Lynch syndrome patient may have a direct proliferative advantage that facilitates their clonal outgrowth. Moreover, MMR-deficient cells are more mutable than MMR-proficient cells by many different mutagenic compounds, including nitrosamines and food-derived heterocyclic amines, and other mutagens [Andrew et al., 1998; Borgdorff et al., 2006; Sansom et al., 2003; Smith-Roe et al., 2006]. For this reason, bile-induced and food-derived mutagens probably not only induce somatic mutation of the wild-type MMR allele and enhance clonal outgrowth of the resulting MMR-deficient enterocytes but, in addition, induce more mutations in oncogenes and tumor suppressor genes in these cells.

The risk of malignant transformation of MMR-deficient cells will also be relatively high in the gastrointestinal epithelium (and also endometrium) since this tissue contains high quantities of cells with a high proliferation rate, resulting in the rapid accumulation of mutations. There may also be yet other intrinsic properties of colonic enterocytes that favor their malignant transformation. Tumorigenesis in Lynch syndrome may partly be caused by mutations in a restricted set of tumor-related genes that are prone to inactivation by frameshifting within an intragenic microsatellite [Li, 2008]. The transforming growth factor β type II receptor (TGFβR2) is one such gene. Specifically in enterocytes in the colon, mutations in a microsatellite within the coding region of TGFβR2 contribute to malignant transformation. Furthermore, the presence of tumor-infiltrating lymphocytes in Lynch syndrome–related colonic tumors (see below) is compatible with an enhanced immunogenicity of these cancers. There is evidence that this immune response is induced by the presence of novel immunogenic peptides in the tumor cells as a consequence of the many frameshift mutations in cellular genes [Schwitalle et al., 2008]. The loss of human leukocyte antigen (HLA) components that are essential for triggering the cellular immune response in a large fraction of MSI-H colon cancers [Kloor et al., 2005;Dierssen et al., 2007] supports the protective activity of the immune system against MSI-high (MSI-H) colorectal tumors as well. Nevertheless, in contrast to other organs, tumors in the lumen of the colon might be relatively shielded from this immune response—a phenomenon that may further aid in restricting tumorigenesis to the colon in Lynch syndrome.

Pathological Features of Lynch Syndrome–Associated Colorectal and Endometrial Cancer

Some distinct pathological features of MMR-deficient colon cancers are associated with Lynch syndrome, including poor differentiation, increased signet cells, medullary features, peritumoral lymphocytic infiltration, a Crohn-like reaction, and tumor infiltrating lymphocytes mixed with tumor cells [Jass, 2004]. However, these histology features are neither pathognomonic nor highly specific for Lynch syndrome, and sporadic colorectal cancers may also show these features, especially when those sporadic cancers have MSI (Table 2). The sensitivity of eight CD3+ tumor infiltrating lymphocytes (TILs) per high-power field has been reported to have 75% sensitivity and 67% specificity for diagnosing MSI-H cancers—be they Lynch colorectal cancer or sporadic MSI-H colorectal cancer [Alexander et al., 2001]. Use of additional variables has been reported to improve MSI-H status prediction, with 90% sensitivity and 77% specificity using a cutoff of > 2 TILs per high-power field, and observation of any mucinous differentiation and absence of “dirty necrosis” within glandular lumina [Greenson et al., 2003]. There is some evidence to suggest that there are features (apart from age at onset) to distinguish MSI-H sporadic cancers from MSI-H Lynch cancers, with mucinous differentiation observed in only 19 to 22% of Lynch cancers vs. 31 to 43% in sporadic MSI-H cancers.

Furthermore, tumors of patients with mutations in *MLH1* and *MSH2* are mostly located proximal to the splenic flexure (right-sided), in contrast to sporadic microsatellite-stable colon tumors that are largely located more distally (left-sided) [Jass, 2004]. It might be anticipated that the subset of pathogenic MMR variants will give rise to tumors with similar characteristics. It has, however, been suggested that an exception to this are tumors from patients with pathogenic *MSH6* mutations, as it was shown that two-thirds of tumors from patients with a pathogenic *MSH6* mutations are left-sided (14/20 tumors). However, most of these tumors were microsatellite-stable, so it cannot be excluded that some were unrelated to the MSH6 germline mutation [Berends et al., 2002].

The largest study assessing MMR mutation-related pathology features of endometrial tumors, the second most common tumor type in Lynch syndrome, examined 47 *MSH2* and only 3 *MLH1* mutation carriers, and 26 women with MSI-H tumors due to epigenetic *MLH1* methylation [Broaddus et al., 2006]. This study provided suggestive evidence that tumors of

MSH2 mutation carriers were more likely to be of nonendometrioid histology, and *MLH1*-methylated "sporadic" tumors were more likely to be high-grade and endometrioid. However, it should be emphasized that *MLH1*-methylated tumors likely have a very different etiology from tumors in Lynch syndrome patients with an *MLH1* germline mutation. Evidence for mutation-associated features was suggested by a study of six Lynch-related endometrial cancers [van den Bos et al., 2004], which reported that poor differentiation, presence of Crohn-like lymphoid reaction, and lymphangioinvasive growth were increased for Lynch cases. This finding is supported by a large study of 146 early-onset endometrial cancer cases by Walsh et al. [2008]. The latter study showed that presumptive Lynch syndrome endometrial cancer cases were significantly more likely than non-Lynch cases to present with tumors that are poorly differentiated (18% vs. 5% grade 3), higher stage (45% vs. 21% stage II-IV), and with tumor-infiltrating lymphocytes (68% vs. 34%), higher mitotic rate (mean per 10 high-power field, 21.7 vs. 11.4) and deeper myometrial invasion (10.2 mm vs. 4.9 mm) [Walsh et al., 2008]. While it was not possible to verify mutation status for this dataset, the results show great promise for using endometrial pathology markers to identify MMR gene mutation carriers, and, indeed, results are supported by a recent study reporting tumor-infiltrating lymphocyte counts and peritumoral lymphocytes as independent predictors of MSI-H status in endometrial tumors [Shia et al., 2008].

There are no large series describing specific pathological features for other Lynch syndrome–associated malignancies in proven mutation carriers, apart from colorectal and endometrial cancer. There is a small study comparing molecular features of 13 gastric cancers from MMR mutation carriers to 46 sporadic gastric cancers. The study suggests that gastric cancers are true Lynch spectrum malignancies, with significantly lower methylation index, and absent MLH1 promoter methylation [Gylling et al., 2007].

Studies describing MSI and IHC in other Lynch syndrome–associated tumors in patients not tested for mutations do exist (e.g., Hartmann et al. [2003] and Catto et al. [2003]). However, as the mutation status is unknown in these studies, they cannot be used for the prediction analysis of VUSs.

MSI in tumors of Lynch syndrome– suspected cases—As determining MSI is simple and because the large majority of tumors from Lynch syndrome families show MSI (mostly called MSI-high or MSI-H), MSI testing has been put forward as the selection criterion for mutational analysis [Boland et al., 1998].

Several studies have determined the sensitivity and specificity of MSI in colon cancers for detecting a pathogenic MMR mutation in one of the MMR genes. The sensitivity of MSI for a mutation is over 80%, with a specificity of approximately 70%. The positive predictive value for a pathogenic mutation is, however, much lower, between 20% and 25% (e.g. [Kets et al., 2008; Kievit et al., 2004; Niessen et al., 2006; Overbeek et al., 2008]). These numbers are, of course, highly dependent on the patient populations studied (these data are based on MSI data collected in colon cancers). Data on endometrial tumors suggests that while MSI informativeness is slightly lower, no tumors are missed using the consensus set, which is based on screening larger sets of markers (R.M.W. Hofstra et al., unpublished data) [de Leeuw et al., 2000].

There are no published data on the predictive value of MSI testing for the presence of a germline mutation in MMR genes in Lynch syndrome–associated malignancies other than colorectal or endometrial cancer. However, there are indications that this value may be much lower in gastric and ovarian cancers [Kanemitsu et al., 2007; Khaliqhe et al., 2007]. The lower predictive value may be explained by methylation of the *MLH1* promoter. Combining MSI and *MLH1* promoter methylation testing shows that 7 out of 10 sporadic MSI gastric cancers have promoter

methylation in contrast to 0 out of 10 Lynch MSI gastric cancers [Gylling et al., 2007]. Loss of MMR protein immunostaining is detected at a lower rate in colorectal adenomas than in colorectal carcinomas associated with Lynch syndrome. This finding might be explained by biclonality of the adenoma (see, e.g. [de Wind et al., 1998; Giuffrè et al., 2005; Greenspan et al., 2007; Lino et al., 1999, 2000]). It indicates that the predictive value of MSI testing in colorectal adenomas for the presence of MMR genes is lower than in colorectal carcinomas.

IHC in tumors of Lynch syndrome– suspected cases—The examination of tumor tissues for the loss of expression of an MMR protein by IHC is a proven and effective method to prescreen tumors for a defective MMR phenotype. Tumor tissues that demonstrate an absence of nuclear staining in the presence of a positive staining in the surrounding normal cells are presumed to have biallelic loss (mutation) of the corresponding MMR gene. IHC analysis is now routinely applied for the detection of MSH2, MLH1, MSH6, and PMS2 tumor protein expression. Interestingly, loss of only the MSH6 protein and loss of only the PMS2 protein can be observed, whereas loss of the MSH2 protein is always accompanied by loss of the MSH6 protein, and, similarly, loss of the MLH1 protein is mostly accompanied by loss of the PMS2 protein [Jass 2007b]. It is widely accepted that this concomitant loss of MMR proteins is related to the stability of the proteins, a stability that is dependent on heterodimer formation. Loss of the MSH6 protein alone or loss of the PMS2 protein alone is an indication for the presence of a germline mutation in the respective gene. Loss of the MLH1 protein detected by IHC can be due to a *MLH1* germline mutation plus a second somatic *MLH1* mutation on the other alleles, or, rarely, germline hypermethylation of one allele plus somatic hypermethylation of the second allele (in the tumor), or biallelic somatic *MLH1* promoter hypermethylation in the tumor, as seen in most sporadic cases [Jass, 2007a,b].

The findings described above are based on tumors in patients carrying clearly pathogenic mutations only, the large majority of which resulted in protein truncation or instability. Calculations on the sensitivity and specificity of IHC have been conducted for clearly pathogenic mutations and they show that the sensitivity of IHC for the presence of a mutation was almost 70%, the specificity over 80%, and the positive predictive value almost 40% (see for instance [Piñol et al., 2005; Southey et al., 2005; Stormorken et al., 2005; Engel et al., 2006; Niessen et al. 2006]). It has been postulated that IHC is the best, single-selection criterion for detecting MMR mutations. However, most literature on IHC is based on data from dedicated and experienced research labs/pathologists. A validation study showed that 82% of the pathologists reached the same interpretation of IHC [Overbeek et al., 2008], but consensus was less frequent in MSI-positive tumors (75%), while only highly experienced pathologists did not miss an MSI-positive tumor by IHC. Therefore IHC for MMR gene expression using antibodies for the MLH1, PMS2, MSH2, and MSH6 proteins can serve as a reliable alternative to MSI testing, provided it is performed in a center with ample experience and provided that absent staining is confirmed by MSI testing. Most rare sequence variants considered to be of unknown clinical significance give rise to a single amino acid change, and is it is thus possible that tumors of patients carrying such a variant will not always show complete absence of the mutated protein (see for instance [Mangold et al., 2005; Raevaara et al., 2005]). Furthermore, Raevaara et al. [2005] have shown that IHC in different tumors from patients with the same UV is not consistent.

IHC markers apart from the MMR proteins themselves have been little explored in Lynch syndrome endometrial tumors. Limited evidence suggests they show higher cyclin B1 expression, loss of BAX expression, and nuclear Cdk2 expression [Fridrichova, 2006; Jass, 2004; Rijcken et al., 2006].

LOH in tumors of Lynch syndrome– suspected cases—Initial data showed frequent loss of the wild-type allele in tumors from patients with pathogenic MMR mutations,

suggesting that MMR genes resemble tumor suppressor genes in that two hits are required to cause a phenotype [Hemminki et al., 1994]. There are, however, several studies describing the loss of the variant allele of the MMR genes [Sanchez de Abajo et al., 2006; Wu et al., 2001]. A dual role of LOH at MMR loci in Lynch syndrome is suggested [Sanchez de Abajo et al., 2006]. This model suggests that LOH can serve as a second hit in certain tumors whereas in others it should be regarded as a sign of progression. In the latter cases, the LOH results not only in the loss of the MMR gene but also in the loss of other tumor progression genes. This theory is supported by some [Ollikainen et al., 2007; Yan et al., 2007] but rejected by others [Tuupanen et al., 2007]. A possible difference has been postulated between mutation type and tumor type [Ollikainen et al., 2007]. Another reason for the observed differences might be the detection method (using the mutation as the marker for LOH and using sequencing vs. microsatellite markers surrounding the gene) or the DNA quality. When microsatellite markers are used we should also keep in mind that MSI makes LOH interpretation more difficult, as MSI can cause the (partial) loss of one or both of the alleles of the markers used. As the data for the pathogenic mutations is inconsistent, we believe that LOH cannot currently be used for evaluating the clinical significance of sequence variants in MMR genes (see also the “LOH in familial breast cancer” section).

Exclusion criteria in Lynch syndrome—The presence of a clearly pathogenic germline mutation in trans with a UV is often used as an exclusion criterion for pathogenicity of a variant, under the assumption that compound heterozygotes for pathogenic variants in the same gene have a recognizable phenotype. Homozygosity for MMR deficiency is not lethal in either the mouse [Baker et al., 1996; Prolla et al., 1998] or in humans. The phenotype resulting from homozygosity for a pathogenic mutation in any of the Lynch genes is of a young-onset neurofibromatosis-like disorder often associated with hematologic, cerebral, and gastrointestinal malignancies [Felton et al., 2007]. Compound heterozygotes with mutations in two different MMR genes also display a recognizable phenotype [Poley et al., 2007]. It is thus assumed that for most high-risk families, only a single pathogenic mutation accounts for disease in the family, and variants detected in high-risk cases with a known or suspected mutation (due to IHC results, and family profile) in genes other than the target gene would be considered unlikely pathogenic.

The same can be concluded for hypermethylation of both alleles of a gene in the tumor, as the mechanism of biallelic inactivation by promoter hypermethylation in *MLH1* is well known in sporadic colorectal cancer cases, with absence of the variant in another affected family member [Jass, 2007a].

The detection of a positive BRAF-V600E mutation in a MSI-H colorectal cancer suggests a sporadic origin of the disease, as it correlates with *MLH1* promoter hypermethylation and the absence of germline alterations of *MLH1*, *MSH2*, and *MSH6* by Domingo et al. [2004, 2005]. These authors found no BRAF mutation in the 125 tested, proven Lynch syndrome tumors nor in 45 cases showing abnormal MSH2 immunostaining, indicating that this hotspot mutation is very rarely if ever involved in the tumorigenesis of colorectal cancer linked to HNPCC ($P<0.001$). These findings suggest the use of BRAF as an exclusion criteria for Lynch syndrome or as a molecular marker of sporadic colorectal cancer. However, BRAF mutation analysis is less sensitive for this purpose than hypermethylation analysis. BRAF is not informative (mutated) in endometrial tumors (R.M.W. Hofstra et al., unpublished results) [Moreno-Bueno et al., 2006; Pappa et al., 2006].

Likelihood ratios for assessing pathogenicity of MMR rare sequence variants—Given the examples provided by BRCA1/2 regarding derivation of likelihood ratios and their application in multifactorial likelihood models, it is likely that similar data on tumor pathology features of MMR mutation carriers could be applied for evaluating the clinical significance of

sequence variants in the MMR genes. Given the use of MMR protein IHC and MSI (see below) as a prioritization tool for preselecting a subset of likely mutation carriers for MMR gene screening, there would be an increased likelihood of detecting a causative mutation, but also of categorizing a variant as causal when it is in cis with an undetected mutation. However, this bias could be counteracted for by calculating the likely mutation status based on IHC and/or MSI status, using this factor as a prior probability of pathogenicity, and adequately accounting for the extent of mutation screening in the calculation of prior probabilities. Additional pathology features that are not in use as selection criteria for mutation screening could then be included in the evaluation of variant pathogenicity as additional multifactorial likelihood scores (see companion work by Goldgar et al. [2008]).

CONCLUDING REMARKS

Currently, recognized breast tumor features associated with pathogenic *BRCA1* mutations can be helpful in the descriptive interpretation of the pathogenicity of *BRCA1* UVs and ER expression and grade, or ER, CK5/6, and CK14 expression can be included as predictive features in multifactorial modeling. Tumor features of *BRCA2* tumors are far less distinct, but the available data suggest that there is value to assessing tubule formation, and ER expression and grade, in the evaluation of *BRCA2* UVs.

Assuming that the subset of disease-associated MMR gene variants of unknown clinical significance has the same characteristics as proven pathogenic MMR gene mutations, the best predictive pathological features in colorectal or endometrial cancer is MSI. The probability that a germline variant is likely to be pathogenic can be further supported by the absence of a *BRAF* mutation, the absence of methylation of the promoter of MLH1, and the absence of another pathogenic mutation in trans.

When using these pathological features one should always consider that the predictive values depend on the sensitivity and specificity of the test, on the prevalence of the condition in the population being tested (prior probability), and on several prior probabilities such as family history and the presence of second primary tumors.

Overall, it is often necessary to examine clinical, morphological, and molecular features of tumors of variant carriers in high-risk families to assess the role of a variant in predisposition to disease. Such tumor investigation might be seen as the perfect in vivo study. As discussed elsewhere in this issue, all possible characteristics must be used for determining the likelihood ratios, and pathological characteristics can strongly influence these ratios. The use of likelihood ratios, as describe above and in the accompanying work by Goldgar et al. [2008], comes with the standard caveats associated with the multifactorial likelihood analysis approach, principally that likelihood estimates are defined by the source data on which they were based. One of the basic assumptions that might influence the analysis is the fact that most studies on variants hypothesize that these largely missense alterations have the same (pathological) characteristics as proven pathogenic mutations. This is defendable but has hardly ever been proven. Moreover, if such rare variants are associated with only a moderate or low risk of disease, they might not closely resemble the known pathological characteristics of pathogenic mutations. It is therefore important to do further research on this. Further study is also required to better assess the association of mutation status with other tumor features, including clinical presentation and pathology, as defined by a broad range of molecular markers. Large multidisciplinary studies are needed to build reliable multifactorial likelihood prediction models, and we believe that the pathological characteristics of tumors will play an important role in building these reliable models.

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APPENDIX

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REFERENCES

Adem C, Soderberg CL, Hafner K, Reynolds C, Slezak JM, Sinclair CS, Sellers TA, Schaid DJ, Couch F, Hartmann LC, Jenkins RB. ERBB2, TBX2, RPS6KB1, and MYC alterations in breast tissues of BRCA1 and BRCA2 mutation carriers. Genes Chromosomes Cancer 2004;41:1–11. [PubMed: 15236312]

Alexander J, Watanabe T, Wu TT, Rashid A, Li S, Hamilton SR. Histopathological identification of colon cancer with microsatellite instability. Am J Pathol 2001;158:527–535. [PubMed: 11159189]

Andrew SE, McKinnon M, Cheng BS, Francis A, Penney J, Reitmair AH, Mak TW, Jirik FR. Tissues of MSH2-deficient mice demonstrate hypermutability on exposure to a DNA methylating agent. Proc Natl Acad Sci USA 1998;95:1126–1130. [PubMed: 9448296]

Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, Loman N, Olsson H, Johannsson O, Borg A, Pasini B, Radice P, Manoukian S, Eccles DM, Tang N, Olah E, Anton-Culver H, Warner E, Lubinski J, Gronwald J, Gorski B, Tulinius H, Thorlacius S, Eerola H, Nevanlinna H, Syrjakoski K, Kallioniemi OP, Thompson D, Evens C, Peto J, Lalloo F, Evens DG, Easton DF. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003;72:1117–1130. [PubMed: 12677558]

Armes JE, Egan AJ, Southey MC, Dite GS, McCredie MR, Giles GG, Hopper JL, Venter DJ. The histologic phenotypes of breast carcinoma occurring before age 40 years in women with and without BRCA1 or BRCA2 germline mutations: a population-based study. Cancer 1998;83:2335–2345. [PubMed: 9840533]

Baker SM, Plug AW, Prolla TA, Bronner CE, Harris AC, Yao X, Christie DM, Monell C, Arnheim N, Bradley A, Ashley T, Liskay RM. Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. Nat Genet 1996;13:336–342. [PubMed: 8673133]

Bane AL, Beck JC, Bleiweiss I, Buys SS, Catalano F, Daly MB, Giles G, Godwin AK, Hibshoosh H, Hopper JL, John FM, Layfield L, Longacre T, Miron A, Senie R, Southey MC, West DW, Whittemore AS, Wu H, Andrulis IL, O'Malley FP. BRCA2 mutation-associated breast cancers exhibit a distinguishing phenotype based on morphology and molecular profiles from tissue microarrays. Am J Surg Pathol 2007;31:121–128. [PubMed: 17197928]

Barnetson RA, Cartwright N, van Vliet A, Haq N, Drew K, Farrington S, William N, Warner J, Campbell H, Porteous MF, Dunlop MG. Classification of ambiguous mutations in DNA mismatch repair genes identified in a population-based study of colorectal cancer. Hum Mutat 2008;29:367–374. [PubMed: 18033691]

Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California Cancer Registry. Cancer 2007;109:1721–1728. [PubMed: 17387718]

Berends MJ, Wu Y, Sijmons RH, Mensink RG, van der Sluis T, Hordijk-Hos JM, de Vries EG, Hollema H, Karrenbeld A, Buys CH, van der Zee AG, Hofstra RM, Kleibeuker JH. Molecular and clinical characteristics of MSH6 variants: an analysis of 25 index carriers of a germline variant. Am J Hum Genet 2002;70:26–37. [PubMed: 11709755]

Bernstein H, Bernstein C, Payne CM, Dvorakova K, Garewal H. Bile acids as carcinogens in human gastrointestinal cancers. Mutat Res 2005;589:47–65. [PubMed: 15652226]

Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58:5248–5257. [PubMed: 9823339]

Borgdorff V, van Hees-Stuivenberg S, Meijers CM, de Wind N. Spontaneous and mutagen-induced loss of DNA mismatch repair in Msh2-heterozygous mammalian cells. Mutat Res 2005;574:50–57. [PubMed: 15914206]

Borgdorff V, Pauw B, van Hees-Stuivenberg S, de Wind N. DNA mismatch repair mediates protection from mutagenesis induced by short-wave ultraviolet light. DNA Repair 2006;5:1364–1372. [PubMed: 16880010]

Broaddus RR, Lynch HT, Chen LM, Daniels MS, Conrad P, Munsell MF, White KG, Luthra R, Lu KH. Pathologic features of endometrial carcinoma associated with HNPCC: a comparison with sporadic endometrial carcinoma. Cancer 2006;106:87–94. [PubMed: 16323174]

Catto JW, Azzouzi AR, Amira N, Rehman I, Feeley KM, Cross SS, Fromont G, Sibony M, Hamdy FC, Cussenot O, Meuth M. Distinct patterns of microsatellite instability are seen in tumours of the urinary tract. Oncogene 2003;22:8699–8706. [PubMed: 14647464]

Chao EC, Lipkin SM. Molecular models for the tissue specificity of DNA mismatch repair-deficient carcinogenesis. Nucleic Acids Res 2006;34:840–852. [PubMed: 16464822]

Chao EC, Velasquez JL, Witherspoon MS, Rozek LS, Peel D, Ng P, Gruber SB, Watson P, Rennert G, Anton-Culver H, Lynch H, Lipkin SM. Accurate classification of MLH1/MSH2 missense variants with multi-variate analysis of protein polymorphisms-mismatch repair (MAPP-MMR). Hum Mutat 2008;29:852–860. [PubMed: 18383312]

Chen L, Nievera CJ, Lee AY, Wu X. Cell cycle-dependent complex formation of BRCA1.CtIP.MRN is important for DNA double-strand break repair. J Biol Chem 2008;283:7713–7720. [PubMed: 18171670]

Chenevix-Trench G, Healey S, Lakhani S, Waring P, Cummings M, Brinkworth R, Deffenbaugh AM, Burbidge LA, Pruss D, Judkins T, Scholl T, Bekessy A, Marsh A, Lovelock P, Wong M, Tesoriero A, Renard H, Southey M, Hopper JL, Yannoukakos K, Brown M, kConFab Investigators. Easton D, Tavtigian SV, Goldgar D, Spurdle AB. Genetic and histopathologic evaluation of BRCA1 and BRCA2 DNA sequence variants of unknown clinical significance. Cancer Res 2006;66:2019–2027. [PubMed: 16489001]

Collins N, McManus R, Wooster R, Mangion J, Seal S, Lakhani SR, Ormiston W, Daly PA, Ford D, Easton DF. Consistent loss of the wild type allele in breast cancers from a family linked to the BRCA2 gene on chromosome 13q12- 13. Oncogene 1995;10:1673–1675. [PubMed: 7731724]

Colombo M, Giarola M, Mariani L, Ripamonti CB, De Benedetti V, Sardella M, Losa M, Manoukian S, Peissel B, Pierotti MA, Pilotti S, Radice P. Cyclin D1 expression analysis in familial breast cancers may discriminate BRCAX from BRCA2-linked cases. Mod Pathol 2008;21:1262–1270. [PubMed: 18327210]

Cornelis RS, Neuhausen SL, Johansson O, Arason A, Kelsell D, Ponder BA, Tonin P, Hamann U, Lindblom A, Lalle P, The Breast Cancer Linkage Consortium. High allele loss rates at 17q12-21 in breast and ovarian tumors from BRCAl-linked families. Genes Chromosomes Cancer 1995;13:203–210. [PubMed: 7669740]

Couch FJ, Rasmussen L, Hofstra RMW, Monteiro AANM, Greenblatt MS, de Wind N, IARC Unclassified Genetic Variants Working Group. Assessment of functional effects of unclassified genetic variants. Hum Mutat 2008;29:1314–1326. [PubMed: 18951449]

Daniels MJ, Wang Y, Lee M, Venkitaraman AR. Abnormal cytokinesis in cells deficient in the breast cancer susceptibility protein BRCA2. Science 2004;306:876–879. [PubMed: 15375219]

de la Chapelle. Genetic predisposition to colorectal cancer. Nat Rev Cancer 2004;4:769–780. [PubMed: 15510158]

de Leeuw WJ, Dierssen J, Vasen HF, Wijnen JT, Kenter GG, Meijers-Heijboer H, Brocker-Vriends A, Stormorken A, Moller P, Menko F, Cornelisse CJ, Morreau H. Prediction of a mismatch repair gene defect by microsatellite instability and immunohistochemical analysis in endometrial tumours from HNPCC patients. J Pathol 2000;192:328–335. [PubMed: 11054716]

de Wind N, Dekker M, van Rossum A, van der Valk M, te Riele H. Mouse models for hereditary nonpolyposis colorectal cancer. Cancer Res 1998;58:248–255. [PubMed: 9443401]

Dierssen JW, de Miranda NF, Ferrone S, van Puijenbroek M, Cornelisse CJ, Fleuren GJ, van Wezel T, Morreau H. HNPCC versus sporadic microsatellite-unstable colon cancers follow different routes toward loss of HLA class I expression. BMC Cancer 2007;7:33. [PubMed: 17316446]

Domingo E, Laiho P, Ollikainen M, Pinto M, Wang L, French AJ, Westra JL, Frebourg T, Espín E, Armengol M, Hamelin R, Yamamoto H, Hofstra RMW, Seruca R, Lindblom A, Peltomäki P, Thibodeau SN, Aaltonen LA, Schwartz S. BRAF screening as a low-cost effective strategy for simplifying HNPCC genetic testing. J Med Genet 2004;41:664–668. [PubMed: 15342696]

Domingo E, Niessen RC, Oliveira C, Alhopuro P, Moutinho C, Espín E, Armengol M, Sijmons RH, Kleibeuker JH, Seruca R, Aaltonen L, AImai K, Yamamoto H, Schwartz S Jr, Hofstra RMW. BRAF-V600E is not involved in the colorectal tumorigenesis of HNPCC patients with functional MLH1 and MSH2 genes. Oncogene 2005;24:3995–3998. [PubMed: 15782118]

Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, Struewing JP, Morrison J, Field H, Luben R, Wareham N, Ahmed S, Healey CS, Bowman R, SEARCH collaborators; Meyer KB, Haiman CA, Kolonel LK, Henderson BE, Le Marchand L, Brennan P, Sangrajrang S, Gaborieau V, Odefrey F, Shen CY, Wu PE, Wang HC, Eccles D, Evans DG, Peto J, Fletcher O, Johnson N, Seal S, Stratton MR, Rahman N, Chenevix-Trench G, Bojesen SE, Nordestgaard BG, Axelsson CK, Garcia-Closas M, Brinton L, Chanock S, Lissowska J, Peplonska B, Nevanlinna H, Fagerholm R, Eerola H, Kang D, Yoo KY, Noh DY, Ahn SH, Hunter DJ, Hankinson SE, Cox DG, Hall P, Wedren S, Liu J, Low YL, Bogdanova N, Schürmann P, Dörk T, Tollenaar RA, Jacobi CE, Devilee P, Klijn JG, Sigurdson AJ, Doody MM, Alexander BH, Zhang J, Cox A, Brock IW, MacPherson G, Reed MW, Couch FJ, Goode EL, Olson JE, Meijers-Heijboer H, van den Ouweland A, Uitterlinden A, Rivadeneira F, Milne RL, Ribas G, Gonzalez-Neira A, Benitez J, Hopper JL, McCredie M, Southey M, Giles GG, Schroen C, Justenhoven C, Brauch H, Hamann U, Ko YD, Spurdle AB, Beesley J, Chen X, kConFab; AOCS Management Group. Mannermaa A, Kosma VM, Kataja V, Hartikainen J, Day NE, Cox DR, Ponder BA. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007;447:1087–1093. [PubMed: 17529967]

Eerola H, Heinonen M, Heikkila P, Kilpivaara O, Tamminen A, Aittomaki K, Blomqvist C, Ristimaki A, Nervanlinna H. Basal cytokeratins in breast tumors among BRCA1, BRCA2 and mutation-negative breast cancer families. Breast Cancer Res 2008;10:R17. [PubMed: 18275599]

Engel C, Forberg J, Holinski-Feder E, Pagenstecher C, Plaschke J, Kloor M, Poremba C, Pox CP, Rüschoff J, Keller G, Dietmaier W, Rümmele P, Friedrichs N, Mangold E, Buettner R, Schackert HK, Kienle P, Stemmler S, Moeslein G, Loeffler M, German HNPCC Consortium. Novel strategy for optimal sequential application of clinical criteria, immunohistochemistry and microsatellite

analysis in the diagnosis of hereditary nonpolyposis colorectal cancer. Int J Cancer 2006;118:115–122. [PubMed: 16003745]

Felton KEA, Gilchrist DM, Andrew SE. Constitutive deficiency in DNA mismatch repair. Clin Genet 2007;71:483–498. [PubMed: 17539897]

Foulkes WD, Stefansson IM, Chappuis PO, Bégin LR, Goffin JR, Wong N, Trudel M, Akslen LA. Germline BRCA1 mutations and a basal epithelial phenotype in breast cancer. J Natl Cancer Inst 2003;95:1482–1485. [PubMed: 14519755]

Foulkes WD, Metcalfe K, Sun P, Hanna WM, Lynch HT, Ghadirian P, Tung N, Olopade OI, Weber BL, McLennan J, Olivotto IA, Bégin LR, Narod SA. Estrogen receptor status in BRCA1- and BRCA2-related breast cancer: the influence of age, grade, and histological type. Clin Cancer Res 2004;10:2029–2034. 2004. 10. [PubMed: 15041722]

Fridrichova I. New aspects in molecular diagnosis of Lynch syndrome (HNPCC). Cancer Biomark 2006;2:37–49. [PubMed: 17192058]

Giuffrè G, Müller A, Brodegger T, Bocker-Edmonston T, Gebert J, Kloor M, Dietmaier W, Kullmann F, Büttner R, Tuccari G, Rüschoff J, German HNPCC Consortium; German Cancer Aid (Deutsche Krebshilfe). Microsatellite analysis of hereditary nonpolyposis colorectal cancer-associated colorectal adenomas by laser-assisted microdissection: correlation with mismatch repair protein expression provides new insights in early steps of tumorigenesis. J Mol Diagn 2005;7:160–170. [PubMed: 15858139]

Goldgar DE, Easton DF, Byrnes GB, Spurdle AB, Iversen ES, Greenblatt MS, IARC Unclassified Genetic Variants Working Group. Integration of various data sources for classifying uncertain variants into a single model. Hum Mutat 2008;29:1265–1272. [PubMed: 18951437]

Gomez-Garcia FB, Ambergen T, Blok MJ, van der Wijngaard A. Patients with an unclassified genetic variant in the BRCA1 or BRCA2 genes show different clinical features from those with a mutation. J Clin Oncol 2005;23:2185–2190. [PubMed: 15800311]

Greenberg RA. Recognition of DNA double strand breaks by the BRCA1 tumor suppressor network. Chromosoma 2008;117:305–317. [PubMed: 18369654]

Greenblatt MS, Brody LC, Foulkes W, Genuardi M, Hofstra R, Plon S, Sijmons RH, Sinilnikova OM, Spurdle AB, IARC Unclassified Genetic Variants Working Group. Locus-specific databases and recommendations to strengthen their contribution to the classification of variants in cancer susceptibility genes. Hum Mutat 2008;29:1273–1281. [PubMed: 18951438]

Greenson JK, Bonner JD, Ben-Yzhak O, Cohen HI, Miselevich I, Resnick MB, Trougouboff P, Tomsho LD, Kim E, Low M, Almog R, Rennert G, Gruber SB. Phenotype of microsatellite unstable colorectal carcinomas: well-differentiated and focally mucinous tumors and the absence of dirty necrosis correlate with microsatellite instability. Am J Surg Pathol 2003;27:563–570. [PubMed: 12717242]

Greenspan EJ, Cyr JL, Pleau DC, Levine J, Rajan TV, Rosenberg DW, Heinen CD. Microsatellite instability in aberrant crypt foci from patients without concurrent colon cancer. Carcinogenesis 2007;28:769–776. [PubMed: 17088260]

Gylling A, Abdel-Rahman WM, Juhola M, Nuorva K, Hautala E, Järvinen HJ, Mecklin JP, Aarnio M, Peltomäki P. Is gastric cancer part of the tumour spectrum of hereditary non-polyposis colorectal cancer? A molecular genetic study. Gut 2007;56:926–933. [PubMed: 17267619]

Hartmann A, Dietmaier W, Hofstädter F, Burgart LJ, Cheville JC, Blaszyk H. Urothelial carcinoma of the upper urinary tract: inverted growth pattern is predictive of microsatellite instability. Hum Pathol 2003;34:222–227. [PubMed: 12673555]

Hedenfalk I, Duggan D, Chen Y, Radmacher M, Bittner M, Simon R, Meltzer P, Gusterson B, Esteller M, Kallioniemi OP, Wilfond B, Borg A, Trent J, Raffeld M, Yakhini Z, Ben-Dor A, Dougherty E, Kononen J, Bubendorf L, Fehrle W, Pittaluga S, Gruvberger S, Loman N, Johannsson O, Olsson H, Sauter G. Gene-expression profiles in hereditary breast cancer. N Engl J Med 2001;344:539–548. [PubMed: 11207349]

Hemminki A, Peltomäki P, Mecklin JP, Järvinen H, Salovaara R, Nyström-Lahti M, de la Chapelle A, Aaltonen LA. Loss of the wild type MLH1 gene is a feature of hereditary nonpolyposis colorectal cancer. Nat Genet 1994;8:405–410. [PubMed: 7894494]

Honrado E, Osorio A, Palacios J, Milne RL, Sánchez L, Díez O, Cazorla A, Syrjakoski K, Huntsman D, Heikkilä P, Lerma E, Kallioniemi A, Rivas C, Foulkes WD, Nevanlinna H, Benítez J.

Immunohistochemical expression of DNA repair proteins in familial breast cancer differentiate BRCA2-associated tumors. J Clin Oncol 2005;23:7503–7511. [PubMed: 16234517]

Hoogerbrugge N, Bult P, de Widt-Levert LM, Beex LV, Kiemeney LA, Ligtenberg MJ, Massuger LF, Boetes C, Manders P, Brunner HG. High prevalence of premalignant lesions in prophylactically removed breasts from women at hereditary risk for breast cancer. J Clin Oncol 2003;21:41–45. [PubMed: 12506168]

Hoogerbrugge N, Bult P, Bonenkamp JJ, Ligtenberg MJ, Kiemeney LA, de Hullu JA, Boetes C, Niermeijer MF, Brunner HG. Numerous high-risk epithelial lesions in familial breast cancer. Eur J Cancer 2006;42:2492–2498. [PubMed: 16908132]

Hunter DJ, Kraft P, Jacobs KB, Cox DG, Yeager M, Hankinson SE, Wacholder S, Wang Z, Welch R, Hutchinson A, Wang J, Yu K, Chatterjee N, Orr N, Willett WC, Colditz GA, Ziegler RG, Berg CD, Buys SS, McCarty CA, Feigelson HS, Calle EE, Thun MJ, Hayes RB, Tucker M, Gerhard DS, Fraumeni JF Jr, Hoover RN, Thomas G, Chanock SJ. A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet 2007;39:870–874. [PubMed: 17529973]

Hwang ES, McLennan JL, Moore DH, Crawford BB, Esserman LJ, Ziegler JL. Ductal carcinoma in situ in BRCA mutation carriers. J Clin Oncol 2007;20(25):642–647. 25. [PubMed: 17210933]

Jass JR. Role of the pathologist in the diagnosis of hereditary non-polyposis colorectal cancer. Dis Markers 2004;20:215–224. [PubMed: 15528787]

Jass JR. Heredity and DNA methylation in colorectal cancer. Gut 2007a;56:154–155. [PubMed: 17172593]

Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. Histopathology 2007b;50:113–130. [PubMed: 17204026]

Jönsson G, Naylor TL, Vallon-Christersson J, Staaf J, Huang J, Ward MR, Greshock JD, Luts L, Olsson H, Rahman N, Stratton M, Ringnér M, Borg A, Weber BL. Distinct genomic profiles in hereditary breast tumors identified by array-based comparative genomic hybridization. Cancer Res 2005;65:7612–7621. [PubMed: 16140926]

Joosse SA, van Beers EH, Tielen IHG, Horlings H, Peterse JL, Hoogerbrugge N, Ligtenberg MJ, Wessels LFA, Axwijk P, Verhoef S, Hogervorst FBL, Nederlof PM. Prediction of *BRCA1*-association in hereditary non-*BRCA1/2* breast carcinomas with array-CGH. Breast Cancer Res Treat. in press.

Kanemitsu K, Kawasaki K, Nakamura M, Li D, Yasuda T, Kuroda D, Yokozaki H, Kamigaki T, Kuroda Y. MSI is frequently recognized among gastric cancer patients with a family history of cancer. Hepatogastroenterology 2007;4:2410–2414. [PubMed: 18265677]

Kets CM, van Krieken JH, van Erp PE, Feuth T, Jacobs YH, Brunner HG, Ligtenberg MJ, Hoogerbrugge N. Is early-onset microsatellite and chromosomally stable colorectal cancer a hallmark of a genetic susceptibility syndrome? Int J Cancer 2008;122:796–801. [PubMed: 17957798]

Khaliqhe L, Ayhan A, Weale ME, Jacobs IJ, Ramus SJ, Gayther SA. Genetic intra-tumor heterogeneity in epithelial ovarian cancer and its implications for molecular diagnosis of tumors. J Pathol 2007;211:286–295. [PubMed: 17154249]

Kievit W, de Bruin JH, Adang EM, Ligtenberg MJ, Nagengast FM, van Krieken JH, Hoogerbrugge N. Current clinical selection strategies for identification of hereditary non-polyposis colorectal cancer families are inadequate: a meta-analysis. Clin Genet 2004;65:308–316. [PubMed: 15025724]

Kloor M, Becker C, Benner A, Woerner SM, Gebert J, Ferrone S, von Knebel Doeberitz M. Immunoselective pressure and human leukocyte antigen class I antigen machinery defects in microsatellite unstable colorectal cancers. Cancer Res 2005;65:6418–6424. [PubMed: 16024646]

Lakhani SR, Jacquemier J, Sloane JP, Gusterson BA, Anderson TJ, van de Vijver MJ, Farid LM, Venter D, Antoniou A, Storfer-Isser A, Smyth E, Steel CM, Haites N, Scott RJ, Goldgar D, Neuhausen S, Daly PA, Ormiston W, McManus R, Scherneck S, Ponder BA, Ford D, Peto J, Stoppa-Lyonnet D, Bignon YJ, Struewing JP, Spurr NK, Bishop DT, Klijn JG, Devilee P, Cornelisse CJ, Lasset C, Lenoir G, Barkardottir RB, Egilsson V, Hamann U, Chang-Claude J, Sobol H, Weber B, Stratton MR, Easton DF. Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA1 and BRCA2 mutations. J Natl Cancer Inst 1998;90:1138–1145. [PubMed: 9701363]

Lakhani SR, Gusterson BA, Jacquemier J, Sloane JP, Anderson TJ, van de Vijver MJ, Venter D, Freeman A, Antoniou A, McGuffog L, Smyth E, Steel CM, Haites N, Scott RJ, Goldgar D, Neuhausen S, Daly

PA, Ormiston W, McManus R, Scherneck S, Ponder BA, Futreal PA, Peto J, Stoppa-Lyonnet D, Bignon YJ, Stratton MR. The pathology of familial breast cancer: histological features of cancers in families not attributable to mutations in BRCA1 or BRCA2. Clin Cancer Res 2000;6:782–789. [PubMed: 10741697]

Lakhani SR, Reis-Filho JS, Fulford L, Penault-Llorca F, van der Vijver M, Parry S, Bishop T, Benitez J, Rivas C, Bignon YJ, Chang-Claude J, Hamann U, Cornelisse CJ, Devilee P, Beckmann MW, Nestle-Krämling C, Daly PA, Haites N, Varley J, Lalloo F, Evans G, Maugard C, Meijers-Heijboer H, Klijn JG, Olah E, Gusterson BA, Pilotti S, Radice P, Scherneck S, Sobol H, Jacquemier J, Wagner T, Peto J, Stratton MR, McGuffog L, Easton DF, Breast Cancer Linkage Consortium. Prediction of BRCA1 status in patients with breast cancer using estrogen receptor and basal phenotype. Clin Cancer Res 2005;11:5175–5180. [PubMed: 16033833]

Li G-M. Mechanisms and functions of DNA mismatch repair. Cell Res 2008;18:85–98. [PubMed: 18157157]

Lino H, Jass JR, Simms LA, Young J, Leggett B, Ajioka Y, Watanabe H. DNA microsatellite instability in hyperplastic polyps, serrated adenoma, and mixed polyps: a mild mutator pathway for colorectal cancer? J Clin Pathol 1999;52:455–460. [PubMed: 10562815]

Lino H, Simms L, Young J, Arnold J, Winship IM, Webb SI, Furlong KL, Leggett B, Jass JR. DNA microsatellite instability and mismatch repair protein loss in adenomas presenting in hereditary non-polyposis colorectal cancer. Gut 2000;47:37–42. [PubMed: 10861262]

Lovelock PK, Spurdle AB, Mok MT, Farrugia DJ, Lakahani SR, Healey S, Arnold S, Buchana D, kConFab Investigtiors. Couch FJ, Henderson BR, Goldgar DE, Tavtigian SV, Chenevix-Trench G, Brown MA. Identification of BRCA1 missense substitutions that confer partial functional activity: potential moderate risk variants? Breast Cancer Res 2007;9:R82. [PubMed: 18036263]

Mangold E, Pagenstecher C, Friedl W, Fischer HP, Merkelbach-Bruse S, Ohlendorf M, Friedrichs N, Aretz S, Buettner R, Propping P, Mathiak M. Tumours from MSH2 mutation carriers show loss of MSH2 expression but many tumours from MLH1 mutation carriers exhibit weak positive MLH1 staining. J Pathol 2005;207:385–395. [PubMed: 16216036]

Moreno-Bueno G, Sanchez-Estevez C, Palacios J. Low frequency of BRAF mutations in endometrial and cervical carcinomas. Clin Cancer Res 2006;12:3865. [PubMed: 16778116]

Narod SA, Foulkes WD. BRCA1 and BRCA2: 1994 and beyond. Nat Rev Cancer 2004;4:665–676. [PubMed: 15343273]

Niessen RC, Berends MJ, Wu Y, Sijmons RH, Hollema H, Ligtenberg MJ, de Walle HE, de Vries EG, Karrenbeld A, Buys CH, van der Zee AG, Hofstra RM, Kleibeuker JH. Identification of mismatch repair gene mutations in young patients with colorectal cancer and in patients with multiple tumors associated with hereditary non-polyposis colorectal cancer. Gut 2006;55:1781–1788. [PubMed: 16636019]

Nyström-Lahti M, Perrera C, Räschle M, Panyushkina-Seiler E, Marra G, Curci A, Quaresima B, Costanzo F, D'Urso M, Venuta S, Jiricny J. Functional analysis of MLH1 mutations linked to hereditary nonpolyposis colon cancer. Genes Chromosomes Cancer 2002;33:160–167. [PubMed: 11793442]

Oldenburg RA, Kroeze-Jansema K, Meijers-Heijboer H, van Asperen CJ, Hoogerbrugge N, van Leeuwen I, Vasen HF, Cleton-Jansen AM, Kraan J, Houwing-Duistermaat JJ, Morreau H, Cornelisse CJ, Devilee P. Characterization of familial non-BRCA1/2 breast tumors by loss of heterozygosity and immunophenotyping. Clin Cancer Res 2006;12:1693–1700. [PubMed: 16551851]

Ollikainen M, Hannelius U, Lindgren CM, Abdel-Rahman WM, Kere J, Peltomäki P. Mechanisms of inactivation of MLH1 in hereditary nonpolyposis colorectal carcinoma: a novel approach. Oncogene 2007;26:4541–4549. [PubMed: 17260015]

Overbeek LI, Ligtenberg MJL, Willems RW, Blokx WA, Dubois SV, van der Linden H, Meijer JW, Mlynek-Kersjes ML, Hoogerbrugge N, Hebeda KM, van Krieken JH. Interpretation of immunohistochemistry for mismatch repair proteins is only reliable in a specialized setting. Am J Surg Pathol 32:1246–1251. [PubMed: 18677806]

Palacios J, Honrado F, Osorio A, Cazorla A, Sarrio D, Barroso A, Rodrigues S, Cigudosa JC, Diez O, Alonso C, Lerma E, Dopazo J, Rivas C, Benitez J. Phenotypic characterization of BRCA1 and BRCA2 tumors based in a tissue microarray study with 37 immunohistochemical markers. Breast Cancer Res Treat 2005a;90:5–14. [PubMed: 15770521]

Palacios J, Honrado E, Cigudosa JC, Benítez J. ERBB2 and MYC alterations in BRCA1- and BRCA2-associated cancers. Genes Chromosomes Cancer 2005b;42:204–205. [PubMed: 15543618]

Pappa KI, Choleza M, Markaki S, Giannikaki E, Kyroudi A, Vlachos G, Voulgaris Z, Anagnou NP. Consistent absence of BRAF mutations in cervical and endometrial cancer despite KRAS mutation status. Gynecol Oncol 2006;100:596–600. [PubMed: 16256179]

Pharoah PD, Antoniou A, Bobrow M, Zimmern RL, Easton DF, Ponder BA. Polygenic susceptibility to breast cancer and implications for prevention. Nat Genet 2002;31:33–36. [PubMed: 11984562]

Piñol V, Castells A, Andreu M, Castellví-Bel S, Alenda C, Llor X, Xicola RM, Rodríguez-Moranta F, Payá A, Jover R, Bessa X, Gastrointestinal Oncology Group of the Spanish Gastroenterological Association. Accuracy of revised Bethesda guidelines, microsatellite instability, and immunohistochemistry for the identification of patients with hereditary nonpolyposis colorectal cancer. JAMA 2005;293:1986–1994. [PubMed: 15855432]

Plon SE, Eccles DM, Easton DF, Foulkes W, Genuardi M, Greenblatt MS, Hogervorst FBL, Hoogerbrugge N, Spurdle AB, Tavtigian S, IARC Unclassified Genetic Variants Working Group. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. Hum Mutat 2008;29:1282–1291. [PubMed: 18951446]

Poley JW, Wagner A, Hoogmans MM, Menko FH, Tops C, Kros JM, Reddingius RE, Meijers-Heijboer H, Kuipers EJ, Dinjens WN, Rotterdam Initiative on Gastrointestinal Hereditary Tumors. Biallelic germline mutations of mismatch-repair genes: a possible cause for multiple pediatric malignancies. Cancer 2007;109:2349–2356. [PubMed: 17440981]

Prolla TA, Baker SM, Harris AC, Tsao JL, Yao X, Bronner CE, Zheng B, Gordon M, Reneker J, Arnheim N, Shibata D, Bradley A, Liskay RM. Tumour susceptibility and spontaneous mutation in mice deficient in Mlh1, Pms1 and Pms2 DNA mismatch repair. Nat Genet 1998;18:276–279. [PubMed: 9500552]

Raevaara TE, Korhonen MK, Lohi H, Hampel H, Lynch E, Lönnqvist KE, Holinski-Feder E, Sutter C, McKinnon W, Duraisamy S, Gerdes AM, Peltomäki P, Kohonen-Ccorish M, Mangold E, Macrae F, Greenblatt M, de la Chapelle A, Nyström M. Functional significance and clinical phenotype of nontruncating mismatch repair variants of MLH1. Gastroenterology 2005;129:537–549. [PubMed: 16083711]

Rijcken F, van der Zee A, van der Sluis T, Boersma-van Ek W, Kleibeuker J, Hollema H. Cell cycle regulators and apoptosis-associated proteins in relation to proliferative activity and degree of apoptosis in HNPCC versus sporadic endometrial carcinoma. Histopathology 2006;48:275–285. [PubMed: 16430474]

Sanchez de Abajo A, de la Hoya M, van Puijenbroek M, Godino J, Diaz-Rubio E, Morreau H, Caldes T. Dual role of LOH at MMR loci in hereditary non-polyposis colorectal cancer. Oncogene 2006;25:2124–2130. [PubMed: 16288214]

Sansom OJ, Bishop SM, Court H, Dudley S, Liskay RM, Clarke AR. Apoptosis and mutation in the murine small intestine: loss of Mlh1- and Pms2-dependent apoptosis leads to increased mutation in vivo. DNA Repair (Amst) 2003;2:1029–1039. [PubMed: 12967659]

Schwitalle Y, Kloor M, Eiermann S, Linnebacher M, Kienle P, Knaebel H, Tariverdian M, Benner A, Von Knebel Doeberitz M. Immune response against frameshift-induced neopeptides in HNPCC patients and healthy HNPCC mutation carriers. Gastroenterology 2008;134:988–997. [PubMed: 18395080]

Shia J, Black D, Hummer AJ, Boyd J, Soslow RA. Routinely assessed morphological features correlate with microsatellite instability status in endometrial cancer. Hum Pathol 2008;39:116–125. [PubMed: 17949789]

Smith-Roe SL, Hegan DC, Glazer PM, Buermeyer AB. Mlh1-dependent suppression of specific mutations induced in vivo by the food-borne carcinogen 2-amino-1-methyl-6-phenylimidazo (4,5-b) pyridine (PhIP). Mutat Res 2006;594:101–112. [PubMed: 16256150]

Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lønning PE, Brown PO, Børresen-Dale AL, Botstein D. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci USA 2003;100:8418–8423. [PubMed: 12829800]

Southey MC, Jenkins MA, Mead L, Whitty J, Trivett M, Tesoriero AA, Smith LD, Jennings K, Grubb G, Royce SG, Walsh MD, Barker MA, Young JP, Jass JR, St John DJ, Macrae FA, Giles GG, Hopper

JL. Use of molecular tumor characteristics to prioritize mismatch repair gene testing in early-onset colorectal cancer. J Clin Oncol 2005;23:6524–6532. [PubMed: 16116158]

Spirio LN, Samowitz W, Robertson J, Robertson M, Burt RW, Leppert M, White R. Alleles of APC modulate the frequency and classes of mutations that lead to colon polyps. Nat Genet 1998;20:385–388. [PubMed: 9843214]

Spurdle AB, Lakhani S, Healey S, Parry S, Deffenbaugh AM, Pruss D, Da Silva L, Brinkworth R, Hopper JL, kConFab Investigators. Brown MA, Babikyan D, Chenevix-Trench G, Tavtigian SV, Goldgar D. Clinical classification of *BRCA1* and *BRCA2* DNA sequence variants: the value of cytokeratin profiles and evolutionary analysis. J Clin Oncol 2008a;26:1657–1663. [PubMed: 18375895]

Spurdle AB, Couch FJ, Hogervorst FBL, Radice P, Sinilnikova OM, IARC Unclassified Genetic Variants Working Group. Prediction and assessment of splicing alterations. Hum Mutat 2008b;29:1304–1313. [PubMed: 18951448]

Stormorken AT, Bowitz-Lothe IM, Norèn T, Kure E, Aase S, Wijnen J, Apold J, Heimdal K, Møller P. Immunohistochemistry identifies carriers of mismatch repair gene defects causing hereditary nonpolyposis colorectal cancer. J Clin Oncol 2005;23:4705–4712. [PubMed: 16034045]

Tavtigian SV, Byrnes GB, Goldgar DE, Thomas A. Classification of rare missense substitutions, using risk surfaces, with genetic- and molecular-epidemiology applications. Hum Mutat 2008a;29:1342–1354. [PubMed: 18951461]

Tavtigian SV, Greenblatt MS, Lesueur F, Byrnes GB, IARC Unclassified Genetic Variants Working Group. In silico analysis of missense substitutions using sequence-alignment based methods. Hum Mutat 2008b;29:1327–1336. [PubMed: 18951440]

Tischkowitz M, Hamel N, Carvalho MA, Birrane G, Soni A, van Beers EH, Joosse SA, Wong N, Novak D, Quenneville LA, Grist SA, kConFab10. Nederlof PM, Goldgar DE, Tavtigian SV, Monteiro AN, Ladias JA, Foulkes WD. Pathogenicity of the BRCA1 missense variant M1775 K is determined by the disruption of the BRCT phosphopeptide-binding pocket: a multi-modal approach. Eur J Hum Genet 2008;16:820–832. [PubMed: 18285836]

Turner N, Tutt A, Ashworth A. Hallmarks of “BRCAness” in sporadic cancers. Nat Rev Cancer 2004;4:814–819. [PubMed: 15510162]

Tuupanen S, Karhu A, Järvinen H, Mecklin JP, Launonen V, Aaltonen LA. No evidence for dual role of loss of heterozygosity in hereditary non-polyposis colorectal cancer. Oncogene 2007;26:2513–2517. [PubMed: 17043646]

van Beers EH, van Welsem T, Wessels LF, Li Y, Oldenburg RA, Devilee P, Cornelisse CJ, Verhoef S, Hogervorst FB, van’t Veer LJ, Nederlof PM. Comparative genomic hybridization profiles in human BRCA1 and BRCA2 breast tumors highlight differential sets of genomic aberrations. Cancer Res 2005;65:822–827. [PubMed: 15705879]

van den Bos M, van den Hoven M, Jongejan E, van der Leij F, Michels M, Schakenraad S, Aben K, Hoogerbrugge N, Ligtenberg M, van Krieken JH. More differences between HNPCC-related and sporadic carcinomas from the endometrium as compared to the colon. Am J Surg Pathol 2004;28:706–711. [PubMed: 15166662]

van der Looij M, Cleton-Jansen AM, van Eijk R, Morreau H, van Vliet M, Kuipers-Dijkshoorn N, Oláh E, Cornelisse CJ, Devilee P. A sporadic breast tumor with a somatically acquired complex genomic rearrangement in BRCA1. Genes Chromosomes Cancer 2000;27:295–302. [PubMed: 10679919]

van’t Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530–536. [PubMed: 11823860]

Walsh MD, Cummings MC, Buchanan DD, Dambacher WM, Arnold S, McKeone D, Byrnes R, Barker MA, Leggett BA, Gattas M, Jass JR, Spurdle AB, Young J, Obermair A. Molecular, pathologic, and clinical features of early-onset endometrial cancer: identifying presumptive Lynch syndrome patients. Clin Cancer Res 2008;14:1692–1700. [PubMed: 18310315]

Wang W. Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. Nat Rev Genet 2007;8:735–748. [PubMed: 17768402]

Wessels LF, van Welsem T, Hart AA, van't Veer LJ, Reinders MJ, Nederlof PM. Molecular classification of breast carcinomas by comparative genomic hybridization: a specific somatic genetic profile for BRCA1 tumors. Cancer Res 2002;62:7110–7117. [PubMed: 12460933]

Wu Y, Berends MJW, Post JG, Mensink RGJ, Verlind E, Sijmons RH, van der Zee AGJ, Hollema H, Kleibeuker JH, Buys CHCM, Hofstra RMW. Germline variants of EXO1 gene in patients with atypical hereditary nonpolyposis colorectal cancer. Gastroenterology 2001;120:1580–1587. [PubMed: 11375940]

Yan H, Jin H, Xue G, Mei Q, Ding F, Hao L, Sun SH. Germline hMSH2 promoter mutation in a Chinese HNPCC kindred: evidence for dual role of LOH. Clin Genet 2007;72:556–561. [PubMed: 17894833]

Yoshida K, Miki Y. Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. Cancer Sci 2004;95:866–871. [PubMed: 15546503]

TABLE 1

Differences and Similarities Between *BRCA1*- and *BRCA2*-Related Breast Cancers: A Brief Overview*

	BRCA1	BRCA2
Morphology	Ductal, no special type (75%), typical medullary ~5%, atypical medullary 10–30%	Ductal, no special type (75%), atypical medullary <5%, lobular/ductal with lobular features more common than in BRCA1 (~10%)
Grade	High (grade 3,75)	Medium/high (grade 2,45%; grade 3,45)
ER (%)	Negative (75)	Positive (75)
HER2 (%)	Negative (95)	Negative (95)
P53 (%)[a]	Positive (50)	Positive (40)
CK5 (%)	Positive (50)	Negative (90)
BCL2[b]	Low	High
Triple negative phenotype (%)[c,d]	~50	0–10
Core basal phenotype (%)[e,d]	~60	0–10
CDKN2A[b]	Low	High
Cyclin D1 (%)	Negative (90)	Positive (60)

*Adapted from Narod and Foulkes [2004]. Note the phenotype of *BRCA2*-related breast cancers is somewhat similar to sporadic, nonhereditary breast cancer.

[a]Based on immunohistochemistry.

[b]Three studies or less, or differing criteria, so no percentage added.

[c]Triple negative phenotype (TNP): ER-, PR- and HER2-negative.

[d]Based on unpublished data fromW.D. Foulkes, L.A. Akslen, and J.-S. Brunet; P value for TNP association with *BRCA1*: 6.7×10^{-5}, for CBP association with *BRCA1*: 4.1×10^{-7}.

[e]Core basal phenotype (CBP): ER- and HER2-negative, CK5/6- and/or EGFR-positive.

TABLE 2

Difference and Similarities Between Lynch Syndrome and Sporadic MSI-H Colorectal Tumors

	Lynch syndrome	Sporadic MSI-H tumor
Morphology	Lymphocytic infiltration	Lymphocytic infiltration
Ploidy	Diploid	Diploid
MSI	MSI-H	MSI-H
IHC	Protein absent for the mutated gene	MLH1 absent
Methylation	No methylation	Methylation MLH1 promoter
BRAF	No BRAF mutation	BRAF mutation
Localization	Right-sided > left-sided	Right-sided > left-sided
Precursor	Adenoma	Adenoma