Molecular pathology of colorectal cancer predisposing syndromes

Puijenbroek, M. van

Citation

Puijenbroek, M. van. (2008, November 27). *Molecular pathology of colorectal cancer predisposing syndromes*. Retrieved from https://hdl.handle.net/1887/13286

Version: Corrected Publisher's Version

License: Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden

Downloaded from: https://hdl.handle.net/1887/13286

Note: To cite this publication please use the final published version (if applicable).

CHAPTER 9

Concluding remarks and implications for the future

In this thesis, we evaluate the use of molecular pathology for identifying individuals with an increased risk for colorectal cancer (CRC) based on their genetic makeup, and for generating insight into the tumorigenesis of familial CRC.

The described work can be divided into:

1) The use of reliable methods that are applicable in formalin-fixed paraffin-embedded (FFPE) tissues, which is of utmost importance since the majority of tumor tissue from familial CRC is only available as FFPE tissue.

2) Tumor profiling to guide genetic testing strategies and clinical genetic decision making, to gain insight on tumorigenesis in familial CRC [including Lynch syndrome and *MUTYH*-associated polyposis (MAP)], and to study the role of *CHEK2* and *PTPRJ*.

1) The use of reliable methods to test FFPE tissues

We used high-throughput methods suitable for FFPE materials to study the characteristics of colorectal tumors. One of these techniques, the MassEXTEND loss of heterozygosity (LOH) analysis (using Sequenom's MassARRAY RT software) is a sensitive, high-throughput, and cost-effective method for genotyping large series of cases for a limited number of single nucleotide polymorphisms (SNP). Moreover, LOH at a particular SNP can be studied in FFPE tumor tissues (Chapter 7). Shortly after our study, Ollikainen *et al*. used the same method to detect LOH in tumors from patients with a mismatch repair (MMR) defect [1]. In the classical LOH analysis using microsatellite repeat markers, the applicability is impaired in mismatch repair deficient tumors due to the intrinsic instability of these markers. In a later phase, we showed that SNP arrays can also be an excellent way to genotype archival tissues and to identify copy neutral LOH (cnLOH) in mismatch repair deficient tumors (Chapter 3). The introduction of this whole genome SNP array analysis enabled the detection of distinct small regions of cnLOH as well as the identification of copy number alterations in FFPE tumor tissues [2,3]. We used this platform to investigate chromosomal instability (CIN) in microsatellite unstable (MSI-high) carcinomas and MAP carcinomas (Chapter 3 and 5). We also suggest that the SNP array platform may be an important tool for finding the genetic cause of unexplained familial CRC.

Another method that we applied was immunohistochemical (IHC) pre-screening of several hundred familial CRC cases that were compacted into tissue micro arrays. This approach was used to screen for loss of CHEK2 expression in familial CRC and also to identify several unexplained MSI-high cases with loss of PMS2 expression in which later germline mutations were identified (Chapter 8 and Hendriks *et al*. (2006) Gastroenterology 130:312-322. list of additional publications).

2) Tumor profiling

Microsatellite instability (MSI) analysis

MSI analysis and IHC of MMR proteins (including PMS2) in CRC from index patients fulfilling the Amsterdam II or (modified) Bethesda criteria have now become a cost effective approach to identify Lynch syndrome patients and to direct germline MMR testing. The presence of a MSI-high phenotype (sporadic and hereditary) has also been associated with an improved prognosis and altered responses to various chemotherapies when compared to microsatellite stable (MSS) tumors [4-6]. There is now debate as to whether to refrain from 5-FU compounds in cases of CRC with MSI-high phenotypes [7].

MMR unclassified variant (MMR-UV)

The identification of pathogenic MMR mutations in Lynch syndrome can be used to offer pre-symptomatic testing in currently unaffected family members. However, in the case of finding only an MMR-UV, the uncertainty about the contribution of such UV to the risk of developing cancer is a major problem, as these UVs could also represent rare variants without increased risk of cancer. Furthermore, the possibility remains that a true pathogenic mutation has been missed. Besides the existing test modalities (segregation assays, MSI status, IHC of MMR proteins, functional testing, etc.), additional proof is still needed [8]. Therefore, it is of great importance to search for additional tools that can provide insight on pathogenicity. We performed whole genome SNP arrays in MSI-high tumor materials from MMR-UV carriers. In five out of eight MMR-UV cases, additional chromosomal instability (although subtle) was found in comparison with tumors from true pathogenic mutation carriers (Chapter 3). This suggests that such additional CIN was necessary for tumorigenesis in cases with *a priori* weak mutator effects and that another mutation has not been missed. The validity of this observation should now be confirmed in a large series of MMR-UV cases. We recommend the collection of tumors from patients with the same UVs and the study of the patterns of genomic abnormalities in those tumors. Depending on the results, it might be useful to add genome-wide SNP array profiling of tumors from MMR-UV carriers to the existing tools to further elucidate the pathogenicity of the MMR-UVs.

KRAS2 pre-screening in familial CRC

General practitioners and medical specialists should be alert to recognize cases suspected for a hereditary cause of CRC. Several guidelines are available for this purpose; these include a positive family history, the age of onset, or the number and nature of polyps, e.g., adenomatous, hyperplastic, or hamartomatous (www.nav-vkgn.nl). As a supportive test, we studied the presence of the *MUTYH*-specific *KRAS2* c.34 G>T transversion in colon tumors in positive cases, followed by *MUTYH* hotspot analysis in FFPE tissues. The

sensitivity and specificity of the *KRAS2* c.34 G>T test combined with the *MUTYH* hotspot analysis is high for the detection of bi-allelic mutation carriers, although the exact figures cannot be calculated because we do not have access to the complete *MUTYH* sequence of all patients. We concluded that this test can reliably identify patients with (atypical) MAP. Therefore, we recommend *KRAS2* c.34G>T somatic pre-screening, followed by *MUTYH* hotspot mutation analysis if the result of the former is positive. If heterozygous hotspot *MUTYH* mutations are identified, a complete germline *MUTYH* mutation screening should be carried out if possible. Immediate *MUTYH* hotspot mutation analysis is a practical alternative in patients with >10 adenomas, or in cases of multiple CRCs in one generation for which only FFPE tissue is available (Chapter4). To this end, we developed a simplified *KRAS2* mutation detection procedure in archival tissue for codons 12 and 13. Furthermore, this *KRAS2* mutation analysis might be rather beneficial as it was recently shown that the presence of somatic *KRAS2* mutations leads to a negative response upon treatment with EGFR inhibitors in colon and lung cancers. This highlights the need for *KRAS2* mutation analysis to predict the response to treatment [9,10].

CHEK2

The *CHEK**1100delC allele has been proposed as a low-penetrance cancer susceptibility allele for breast cancer, and carriers appear to have a twofold increase in breast cancer risk [11]. The incidence of the 1100delC mutation was suggested to be higher among breast cancer families with CRC than in those without CRC, identifying a hereditary breast and colorectal cancer (HBCC) phenotype [12]. The incidence of the *CHEK2**1100delC mutation in familial and non familial CRC patients was 1.3% and 2.9%, respectively, which is not significantly higher than the European population frequency of 1.1–1.4%. With an estimated range of 1.3–1.6%, the frequency in the Dutch population seems similar [12]. Results suggest that the *CHEK2**1100delC mutation may not be significantly associated with familial CRC or with CRC risk in the population, although a very low-penetrance effect on CRC could not be excluded [13,14]. From our study, we conclude that homozygosity for the *CHEK2**1100delC mutation is not lethal in humans and does not lead to a severe clinical phenotype. CHEK2 protein abrogation is seen in cases with the *CHEK2**1100delC allele but not with the *CHEK2* germline variants, R117G, R137Q, R145W, I157T, and R180H in familial CRC. Other studies reported the correlation between two *CHEK2* variants (1100delC and I157T) and CRC; Sanchez *et al*. demonstrated that *CHEK2**1100delC is not of clinical relevance for Lynch syndrome and HBCC Spanish families [15], and de Jong *et al*. concluded that the frequency of the *CHEK2**1100delC genotype was not significantly increased in unselected CRC patients or in selected CRC patients diagnosed before age 50. However, after stratifying unselected CRC patients according to defined genetic risk, a significant trend of increasing frequency was observed [16]. In a study of Swedish CRC patients, the frequency of *CHEK2**1100delC was not significantly increased [17]. Based

on the research to date, the role of the *CHEK**1100delC allele in familiar CRC seems to be limited, which excludes it as a candidate allele for testing in the clinical genetic context in families with clustering of CRC. Two studies showed that *CHEK2* I157T is associated with an increased risk of CRC. Kilpivaara *et al*. observed the association in both familial and sporadic CRC patients. Furthermore, they found support for the role of *CHEK2* I157T as a susceptibility allele for multiple cancer types [18]. Cybulski *et al*. concluded that the I157T mutation increased the risk of CRC in the population. In addition, they suggested that truncating mutations may confer a lower risk or no increase in risk for CRC [19].

PTPRJ

PTPRJ is one of the colon cancer susceptibility alleles identified in mouse studies. Frequent LOH of the *PTPRJ**1176 A>C allele was shown in human sporadic colorectal, breast [20], and lung tumors [21], and in human thyroid carcinomas [22]. Additionally, Ruivenkamp *et al*. concluded that LOH of the *PTPRJ**1176 A>C allele frequently occurs in the adenoma stage of sporadic human CRC [23]. Our study revealed that the importance of the newly identified *PTPRJ*- c.827A>C SNP appears to be limited in familial CRC. In 2006, one published report suggested that *PTPRJ* plays a role in early colon neoplasia by studying two *PTPRJ* microsatellite markers in 32 aberrant crypt foci [24]. In the recent association studies, the *PTPRJ*- c.827A>C SNP was not identified as a cancer susceptibility allele for CRC. However, this does not exclude the possibility that this SNP functions as a low-penetrant allele.

SNP typing of Lynch syndrome, MMR-UV, sporadic MSI-high and MAP tumors

We characterized chromosomal instability (physical loss, gain, and cnLOH) and microsatellite instability in carcinomas from Lynch syndrome patients with pathogenic MMR mutations, MMR-UV carriers, MAP patients, and patients with sporadic *MLH1* promoter hypermethylation. The profiles were distinct; in MSI-high carcinomas from Lynch syndrome patients with pathogenic mutations, copy number variation is rare. Genome-wide copy neutral LOH is also rare, and the only cnLOH detected is usually confined to the locus harboring pathogenic mutations in *MLH1, MSH2,* or *PMS2*. In MMR-UV cases and sporadic MMR deficiency, there is often a slight increase in chromosomal instability [25,26] (Chapters 3 and 4), whereas MAP carcinomas show many aberrant chromosomal regions. Interestingly, these regions are mostly affected by cnLOH. The latter is in contrast to sporadic colon cancer, where physical chromosomal loss is the main characteristic. The percentages of chromosomal amplifications in MAP and sporadic microsatellite stable colorectal carcinomas are comparable.

Co-segregation of *MSH6* and *MUTYH* germline mutations

The *MSH6* Lynch syndrome family in which family members are heterozygous or compound heterozygous for *MUTYH* germline mutations showed a remarkably mild clinical phenotype of an *MSH6/MUTYH* compound heterozygote mutation carrier (Chapter 6). Selection against MSH6 mismatch repair deficient cells might, at least in part, explain this phenotype, which is in line with Kambare *et al*., who suggested that BER and DNA MMR pathways are mutually exclusive. This suggestion implies that cells with abrogation of both pathways are not viable and undergo apoptosis [27]. We observed only one patient with the above-mentioned genotype. In the literature, combined germline defects such as *APC* plus an MMR mutation are described to be associated with an increased cancer risk or accelerated tumorigenesis [28,29]. *MUTYH* in addition to missense *MSH6* mutations are hypothesized to increase cancer risk [28]. A recent study does not find this association between *MUTYH* and *MSH6* UV and pathogenic germline mutations [30]. The number of patients is relatively low in the latter two studies, and no additional analysis was done in the family members of the identified patients. Additional experiments are now necessary to gain more understanding on the interaction of *MUTYH* and *MSH6*. We therefore obtained primary skin fibroblast cultures from the *MSH6/MUTYH* compound heterozygote mutation carrier and from her relatives carrying different combinations of *MSH6* and *MUTYH* mutations. In these cultures, DNA repair mechanisms will be analyzed for apoptotic responses, cell viability, and clonal survival in order to find support for the notion that abrogation of both MSH6 DNA mismatch and base excision repair in a cell can lead to apoptosis and a milder clinical phenotype. Furthermore, it would be of great value to study the fibroblasts from patients and their family members identified in the other studies to compare the difference between *MSH6*-UVs and pathogenic *MSH6* mutations in combination with mono-allelic *MUTYH* mutations.

Implications for the future

The contribution of molecular pathology in the identification of familial causes of CRC in the near future will be dual; it will play a role in diagnostic as well as research settings.

Tests that are readily applicable and straightforward (for example, MSI, additional MMR IHC, and *KRAS2* mutation analyses) will be extensively used in molecular pathology diagnostics.

In a research setting, molecular pathology will be an important player in determining the contribution to an increased cancer risk of the alleles that are presently identified with the analysis of disease susceptibility through whole genome association studies. In these studies, dedicated SNP profiles are identified that can predict higher chances

for certain disorders in individuals. Examples are the recent identification of susceptibility SNPs for breast cancer, CRC, and prostate cancer [31-38]. The CRC susceptibility SNPs published so far all have significant p values ($p < 10^{-7}$) although the odds ratio for each individual SNP is low. Based on a recently published paper on prostate cancer, one can speculate that a combination of five of these variants within one individual leads to an increased relative risk, although severe concerns were recently raised about the statistical analysis of these data [39-46]. The identification of these susceptibility SNPs is based on the common disease-common variant theory. Therefore, this approach is still unable to find rare susceptibility alleles in populations that include recessive alleles. Furthermore, the biological role of the now identified alleles is mostly unknown because these SNPs are most likely just tagging the true gene variants responsible. For all these reasons, the regions of interest are now sequenced for such causal variants [47]. How these responsible SNP variants contribute to an increased CRC risk should also be studied for example through molecular pathology in well-defined patient/tumor and control cohorts with available follow-up data.

The latest screening strategies for CRC in the general population focus on endoscopic surveillance above the age of 50, possibly in combination with fecal occult blood or fecal DNA testing. It will be interesting to see if and when the recently identified common disease/common variant SNPs and the true genetic variants that are linked to them will be implemented in CRC screening in the general population. If successful, generating these types of profiles for CRC susceptibility in the general population might be a very beneficial screening method, although ethical problems may be encountered.

Our recent experience showed that the role of tumor profiling in the search for as yet unidentified genetic causes of CRC is often met with skepticism. We now argue that the distinct tumor profiles that are found (chapters 3 and 5) are convincing examples that molecular pathology approaches might also be crucial for the characterization and possible elucidation of unresolved familial causes of CRC. We should not forget this critical example: the identification of *MUTYH* mutations in 2002 as the genetic cause for many unexplained polyposis patients, later named *MUTYH*-associated polyposis, came initially from tumor analysis.

Reference list

1. Ollikainen M, Hannelius U, Lindgren CM, *et al*. (2007) Mechanisms of inactivation of MLH1 in hereditary nonpolyposis colorectal carcinoma: a novel approach. Oncogene 26:4541-4549.
2. Lips EH, Dierssen JWF, van Eijk R, *et al*. (2005) Reliable high-throughput genotyping and loss-of-heterozygosity detection in formalin-fixed, paraffin-embedded tumors using single nucleotide polymorphism arrays. Cancer Research 65:10188-10191.
3. Oosting J, Lips EH, van Eijk R, *et al*. (2007) High-resolution copy number analysis of paraffin-embedded archival tissue using SNP BeadArrays. Genome Res 17:368-376.
4. Sinicrope FA, Rego RL, Halling KC, *et al*. (2006) Prognostic impact of microsatellite instability and DNA ploidy in human colon carcinoma patients. Gastroenterology 131:729-737.
5. Sinicrope FA, Rego RL, Foster N, *et al*. (2006) Microsatellite instability accounts for tumor site-related differences in clinicopathologic variables and prognosis in human colon cancers. Am J Gastroenterol 101:2818-2825.
6. Warusavitarne J, Schnitzler M. (2007) The role of chemotherapy in microsatellite unstable (MSI-H) colorectal cancer. Int J Colorectal Dis 22:739-748.
7. Jo WS, Carethers JM. (2006) Chemotherapeutic implications in microsatellite unstable colorectal cancer. Cancer Biomark 2:51-60.
8. Niessen RC, Sijmons RH, Berends MJW, *et al*. (2004) Hereditary non-polyposis colorectal cancer: Identification of mutation carriers and assessing pathogenicity of mutations. Scand J Gastroenterol 39:70-77.
9. van ZN, Mathy A, Boerrigter L, *et al*. (2007) EGFR and KRAS mutations as criteria for treatment with tyrosine kinase inhibitors: retro- and prospective observations in non-small-cell lung cancer. Ann Oncol 18:99-103.
10. Amado RG, Wolf M, Peeters M, *et al*. (2008) Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 26:1626-1634.
11. Meijers-Heijboer H, van den OA, Klijn J, *et al*. (2002) Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. Nat Genet 31:55-59.
12. Meijers-Heijboer H, Wijnen J, Vasen H, *et al*. (2003) The CHEK2 1100delC mutation identifies families with a hereditary breast and colorectal cancer phenotype. Am J Hum Genet 72:1308-1314.
13. Kilpivaara O, Laiho P, Aaltonen LA, *et al*. (2003) CHEK2 1100delC and colorectal cancer. J Med Genet 40:e110.
14. Cybulski C, Gorski B, Huzarski T, *et al*. (2004) CHEK2 is a multiorgan cancer susceptibility gene. Am J Hum Genet 75:1131-1135.
15. Sanchez de AA, de la HM, Godino J, *et al*. (2005) The CHEK2 1100delC allele is not relevant for risk assessment in HNPCC and HBCC Spanish families. Fam Cancer 4:183-186.
16. de Jong MM, Nolte IM, Te Meerman GJ, *et al*. (2005) Colorectal cancer and the CHEK2 1100delC mutation. Genes Chromosomes Cancer 43:377-382.
17. Djureinovic T, Lindblom A, Dalen J, *et al*. (2006) The CHEK2 1100delC variant in Swedish colorectal cancer. Anticancer Res 26:4885-4888.
18. Kilpivaara O, Alhopuro P, Vahteristo P, *et al*. (2006) CHEK2 I157T associates with familial and sporadic colorectal cancer. J Med Genet 43:e34.
19. Cybulski C, Wokolorczyk D, Kladny J, *et al*. (2007) Germline CHEK2 mutations and colorectal cancer risk: different effects of a missense and truncating mutations? Eur J Hum Genet 15:237-241.
20. Lesueur F, Pharoah PD, Laing S, *et al*. (2005) Allelic association of the human homologue of the mouse modifier Ptprj with breast cancer. Hum Mol Genet 14:2349-2356.

21. Ruivenkamp CA, van Wezel T, Zanon C, *et al*. (2002) Ptprj is a candidate for the mouse colon-cancer susceptibility locus Scc1 and is frequently deleted in human cancers. Nat Genet 31:295-300.
22. Iuliano R, Le P, I, Cristofaro C, *et al*. (2004) The tyrosine phosphatase PTPRJ/DEP-1 genotype affects thyroid carcinogenesis. Oncogene 23:8432-8438.
23. Ruivenkamp C, Hermsen M, Postma C, *et al*. (2003) LOH of PTPRJ occurs early in colorectal cancer and is associated with chromosomal loss of 18q12-21. Oncogene 22:3472-3474.
24. Luo L, Shen GQ, Stiffler KA, *et al*. (2006) Loss of heterozygosity in human aberrant crypt foci (ACF), a putative precursor of colon cancer. Carcinogenesis 27:1153-1159.
25. Douglas EJ, Fiegler H, Rowan A, *et al*. (2004) Array comparative genomic hybridization analysis of colorectal cancer cell lines and primary carcinomas. Cancer Research 64:4817-4825.
26. Trautmann K, Terdiman JP, French AJ, *et al*. (2006) Chromosomal instability in microsatellite-unstable and stable colon cancer. Clin Cancer Res 12:6379-6385.
27. Kambara T, Whitehall VL, Spring KJ, *et al*. (2004) Role of inherited defects of MYH in the development of sporadic colorectal cancer. Genes Chromosomes Cancer 40:1-9.
28. Niessen RC, Sijmons RH, Ou J, *et al*. (2006) MUTYH and the mismatch repair system: partners in crime? Hum Genet 119:206-211.
29. Soravia C, DeLozier CD, Dobbie Z, *et al*. (2005) Double frameshift mutations in APC and MSH2 in the same individual. Int J Colorectal Dis 20:466-470.
30. Steinke V, Rahner N, Morak M, *et al*. (2008) No association between MUTYH and MSH6 germline mutations in 64 HNPCC patients. Eur J Hum Genet 16:587-592.
31. Antoniou AC, Spurdle AB, Sinilnikova OM, *et al*. (2008) Common breast cancer-predisposition alleles are associated with breast cancer risk in BRCA1 and BRCA2 mutation carriers. Am J Hum Genet 82:937-948.
32. Gold B, Kirchhoff T, Stefanov S, *et al*. (2008) Genome-wide association study provides evidence for a breast cancer risk locus at 6q22.33. Proc Natl Acad Sci U S A 105:4340-4345.
33. Tomlinson IP, Webb E, Carvajal-Carmona L, *et al*. (2008) A genome-wide association study identifies colorectal cancer susceptibility loci on chromosomes 10p14 and 8q23.3. Nat Genet :
34. Tomlinson I, Webb E, Carvajal-Carmona L, *et al*. (2007) A genome-wide association scan of tag SNPs identifies a susceptibility variant for colorectal cancer at 8q24.21. Nat Genet :
35. Tenesa A, Farrington SM, Prendergast JG, *et al*. (2008) Genome-wide association scan identifies a colorectal cancer susceptibility locus on 11q23 and replicates risk loci at 8q24 and 18q21. Nat Genet :
36. Gudmundsson J, Sulem P, Rafnar T, *et al*. (2008) Common sequence variants on 2p15 and Xp11.22 confer susceptibility to prostate cancer. Nat Genet 40:281-283.
37. Eeles RA, Kote-Jarai Z, Giles GG, *et al*. (2008) Multiple newly identified loci associated with prostate cancer susceptibility. Nat Genet 40:316-321.
38. Thomas G, Jacobs KB, Yeager M, *et al*. (2008) Multiple loci identified in a genome-wide association study of prostate cancer. Nat Genet 40:310-315.
39. Coates RJ, Khoury MJ, Gwinn M. (2008) Five genetic variants associated with prostate cancer. N Engl J Med 358:2738.
40. Eisinger F. (2008) Five genetic variants associated with prostate cancer. N Engl J Med 358:2740-2741.
41. Gartner CE, Barendregt JJ, Hall WD. (2008) Five genetic variants associated with prostate cancer. N Engl J Med 358:2738-2739.
42. Janssens AC, van Duijn CM. (2008) Five genetic variants associated with prostate cancer. N Engl J Med 358:2739.

43. Severi G, Byrnes GB, Hopper JL. (2008) Five genetic variants associated with prostate cancer. N Engl J Med 358:2739-2740.
44. Thorat MA. (2008) Five genetic variants associated with prostate cancer. N Engl J Med 358:2740.
45. Vickers A, Lilja H, Scardino P. (2008) Five genetic variants associated with prostate cancer. N Engl J Med 358:2740.
46. Zheng SL, Sun J, Wiklund F, *et al*. (2008) Cumulative association of five genetic variants with prostate cancer. N Engl J Med 358:910-919.
47. Broderick P, Carvajal-Carmona L, Pittman AM, *et al*. (2007) A genome-wide association study shows that common alleles of SMAD7 influence colorectal cancer risk. Nat Genet 39:1315-1317.