HNPCC, molecular and clinical dilemmas

Wagner, A.

Chapter 4.

Discussion

The diagnosis of HNPCC represents a major challenge for geneticists and clinicians because of its geno- and phenotypic heterogeneity[191]. In my PhD thesis, I have attempted to define the spectrum of mutations at the main MMR genes causing HNPCC, and the establishment of genotype-phenotype correlations to allow clinical selection of families, guide their mutation analysis, and delineate surveillance protocols based on the specific genetic lesion.

In **chapter 2**, we addressed the molecular genetic analysis of HNPCC. We showed that the vast majority of 'classical' HNPCC families is due to *MLH1* or *MSH2* mutations. *MSH6* and possibly *PMS2* mutations cause a more atypical phenotype and are responsible for only a small proportion of classical families. Based on our observations, it is unlikely that other major HNPCC genes will be found in the future. Failure of mutation detection in the major MMR genes in Amsterdam criteria positive families is likely to be due to: 1. marginal fulfilment of the clinical criteria in colorectal cancer only, true mutation-negative families; 2. wrong selection of patients to be tested from otherwise mutation-positive families (e.g. phenocopies); 3. failure of mutation detection techniques in mutation-positive families.

Colorectal cancer is a relatively common tumour type. Families may therefore fulfil the Amsterdam criteria by chance or because of additional susceptibility factors. Also, within families with a *MLH1*, *MSH2*, or *MSH6* mutation, "sporadic" colorectal cancer cases may occur. In my opinion, extensive and detailed pedigree analysis is still the cornerstone for the selection of individuals for genetic testing for HNPCC. The establishment of a detailed family history is a time consuming and painstaking exercise, but it additionally allows the selection for testing for other susceptibility factors for colorectal cancer. In classical HNPCC families mutation analysis is indicated and does not require preselection by microsatellite instability tests (MSI) or immunohistochemical (IHC) analysis, although IHC analysis can direct mutation analysis. MSI and IHC analyses of tumours are in particular helpful for selection for mutation analysis within families not or marginally fulfilling the Amsterdam criteria. For selection for MSI and IHC analysis the revised Bethesda guidelines are very useful. However, I advocate liberal inclusion for MSI and IHC analysis. Some practical guidelines for the management of HNPCC and HNPCC-like families based on our own experience and on the literature are formulated in Figure 10. IHC and/or MSI analysis as a pre-screen tool for MMR gene analysis can also be routinely offered to certain patient-groups like colorectal patients diagnosed under the age of 50 years or patients with multiple tumours. However, some form of pre-test counselling of patients is requisite, since absent staining of MSH2 and/or MSH6 are strongly indicative for the presence of a germline defect and patients should be made aware of this before testing.

Our discovery of large genomic rearrangements in *MLH1* and *MSH2* dramatically increased mutation detection rate at these major HNPCC genes. These findings underscore the need to implement molecular approaches capable to detect large genomic rearrangements. To date, many DNA-diagnostic laboratories have added PCR-based mutation techniques for large genomic deletions, e.g. MLPA[77], to their routine mutation detection techniques[35, 232, 279]. Notably, MLPA likely would have missed the 10Mb paracentric inversion of chromosome 2p we detected by Southern blot analysis in a classical HNPCC kindred, as reported in **chapter 2.3**. The identification of this inactivating *MSH2* mutation within this family was of medical benefit, as it enabled the selection of a suitable kidney donor for one of the relatives[189]. I advocate that DNA-diagnostic laboratories refine their search for atypical mutations (genomic deletion, inversions, and insertions) within families with a high chance of carrying a *MLH1*, *MSH2* and/or *MSH6* mutation which tested negative by conventional mutational analysis.

In our search for genomic rearrangements, we also identified a common North-American deletion in *MSH2*, accounting for as much as ~10% of our cohort (**chapter 2.1 and 2.2**). The combination of clinical and molecular genetic data indicated that all families carrying this *MSH2* exon 1-6 deletion descend from one ancestor who lived in Germany in the 18th century. The identification of this founder mutation enabled a conclusive genetic test in large numbers of HNPCC risk carriers in North America.

The second main diagnostic challenge of HNPCC is represented by its clinical variability. As reported in **chapter 3.1 and 3.2**, the cancer phenotype of *MSH6* mutation carriers differs from that of *MLH1* and *MSH2*. *MSH6* mutations are associated with a delayed age of onset of colorectal and endometrial cancer (55-57 years vs. 43-44 years, and 54 years vs. 48-49 years, respectively). Moreover, the cumulative lifetime risk of endometrial cancer in female mutation carriers is increased compared to *MLH1* and *MSH2* mutation carriers (71% vs. 27-40% at 70 years of age), while the risk of colorectal cancer is reduced (30% vs. 53-68% at 70 years). These observations will aid the clinical recognition of *MSH6* mutation positive families, and contribute to the development of *MSH6*-tailored cancer recommendations for screening and preventative options.

Above all, HNPCC challenges families who have to live with the disease and its threats. The availability of a genetic test for HNPCC adds to this complexity, but also offers hope for interference.

The detection of a pathogenic *MLH1*, *MSH2* or *MSH6* mutation within a family enables relatives conclusive genetic testing, allowing them to obtain certainty about their cancer risks. The use of predictive genetic testing is influenced by several factors, among which

the availability of effective and acceptable risk-reducing interventions. Former studies reported a high uptake for predictive genetic testing for HNPCC (75-81%)[12, 272]. However, we believe that these studies are likely to be biased in favour of a high uptake as only individuals who had agreed to register for research purposes were included. It is conceivable that these individuals are also willing to undertake genetic testing, and are thus not representative for HNPCC patients at large. We studied the use of genetic testing in families with a known *MLH1*, *MSH2* or *MSH6* mutation in a clinical setting (**chapter 3.3**). Fifty-seven percent of 308 individuals with an *a priori* 50% risk of carrying the germline mutation opted for genetic testing, which is approximately 20% lower than in the Finnish study. Hence, in our study a considerable portion of risk carriers refrained from genetic testing. This was confirmed by Hadley et al.[85] and Ponz de Leon et al.[235], who found a use of genetic testing of respectively 51% and 44% among 50% risk carriers. Reasons for not testing may include lack of information, fear for cancer for themselves or their children, fear for screening procedures or fear for social discrimination. In the Netherlands, knowledge of a genetic predisposition for cancer is passed among relatives. In our study, the vast majority of gene carriers appreciated the way they were informed about the possibility of genetic testing, and the information they received during counselling (88% and 97%, respectively; **chapter 3.4**). Of note, little is known about the views and feelings of relatives who refrain from counselling and testing. Both a very high and a very low cancer concern may prevent individuals from seeking genetic advice. In risk carriers for Hereditary Breast Ovarian Cancer (caused by BRCA1 or BRCA2 gene mutations), Lerman et al.[154] found that individuals who refrained from obtaining their personal genetic test result had higher depression levels than those who chose to learn their personal test result (irrespective of outcome). However, Lodder et al.[173] found no difference in anxiety and depression levels between healthy women who opted for testing for the family-specific BRCA1 or BRCA2 mutation and women who refrained from testing. The latter were higher educated, more often childless and more reluctant towards prophylactic surgery. Having children was also positively correlated with testing for HNPCC in our study, indicating that other factors than cancer fear are also important in the decision to opt for genetic testing.

Colonoscopy is experienced as a burdensome screening technique among HNPCC mutation carriers diagnosed at our department (**chapter 3.4**). The development of less invasive screening and preventative options is clearly needed. The availability of less repulsive preparation fluid would already represent a significant improvement. In the future, testing for genetic markers in stool or other non-invasive approaches such as virtual colonoscopy may become alternatives for colonoscopy[9, 50, 79, 157, 292, 293]. Nonetheless, the

above mentioned techniques share the drawback that once a polyp or a given genetic marker is detected, conventional colonoscopy has to be performed after all to allow polypectomy. In the future, significant advances in *in vivo* optical imaging based on specific molecular markers will allow the selective identification of dysplastic lesions to guide polypectomy[194]. The elucidation of the molecular mechanisms underlying tumour formation and progression will also result in the development of tailor-made pharmacological intervention. However, availability of such 'smart drugs' may not be expected in the near future. Also, the characterisation of peptides specific for MSI-positive colorectal cancer cells, that can be recognised by T cells, fuels hope for the development of a prophylactic vaccine for HNPCC carriers on the long term[250].

Finally, fear for financial and social discrimination is an important reason for not opting for genetic testing[85]. In the Netherlands, health insurance companies fund genetic counselling, genetic testing and the various screening strategies. The Dutch law prohibits discrimination of employees and exclusion from health insurance on grounds of genetic susceptibility for cancer. Since 1995 the Association of Insurance Companies (The Hague) has agreed on a moratorium which ensures that a clients' genetic susceptibility is not taken into account in case of insurances for disablement up to €32.000 in the first year and €22.000 in the following years, and life-insurances up to €160.000. As a result of this policy, no problems with health insurance or employment were reported among Dutch MMR gene mutation carriers, though 4 out of 10 healthy carriers who opted for life insurance or mortgage experienced some kind of restraint (**chapter 3.4**).

Additional research on the reasons for and psychosocial impact of refraining from genetic testing is needed. The more since, 88% of proven Dutch HNPCC carriers diagnosed at our department opted for regular colonoscopic screening after genetic testing, compared with 33% before testing (**chapter 3.4**). The significant impact of genetic testing on surveillance will lead to a considerable decrease of HNPCC-related cancer morbidity and mortality. Also, we showed that most tested individuals are able to cope with their cancer predisposition at a mean follow-up of three and a half years (**chapter 3.4**). Ten percent of the studied mutation carriers had high cancer concerns and 9% felt regret of testing. This was associated with a high-perceived colorectal cancer risk, possibly induced by recent cancer related events in the family. Years after genetic testing, some mutation carriers may thus need additional counselling and psychological support. Based on our findings and experience, I advocate that from the moment a family is identified as at risk for HNPCC, ongoing psychological support and counselling facilities should be available to the individual family members. This can easily be implemented in a multidisciplinary family cancer clinic facility.

In conclusion, future challenges for clinical geneticists and other professionals active in HNPCC diagnosis and clinical management include: further unravelling of the molecular mechanisms underlying this susceptibility, translation of this 'molecular knowledge' into preventative tools, the development of less burdensome screenings options, the delivery of optimal multidisciplinary care, and the contribution (by education and counselling) to a social climate that enables individuals at risk to make an informed and free choices about genetic testing.