LETTER TO JMG

Genetic testing in hereditary non-polyposis colorectal cancer families with a *MSH2*, *MLH1*, or *MSH6* mutation

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About 5% of colorectal cancers are associated with the autosomal dominantly inherited cancer susceptibility syndrome hereditary non-polyposis colorectal cancer (HNPCC).[1 2] HNPCC is characterised by a high risk of developing colorectal cancer and endometrial cancer at a young age (cumulative lifetime risk 80-90% and 30-40%, respectively), and by an increased risk of developing various other tumour types, such as ovarian, uroepithelial, small intestine, biliary tract, stomach, brain, and skin cancers.[2–5] Germline mutations in one of three mismatch repair genes (*MSH2*, *MLH1*, and *MSH6*) were found to be responsible for a majority of HNPCC families.[6–9]

Knowledge of the causative mutation in a particular HNPCC family enables the identification of at risk family members by genetic testing. Clearly, the absence or presence of a mutation is of considerable medical and psychological significance. Subjects not carrying the mutation are relieved from a continuous anxiety and can be dismissed from medical surveillance, saving them trouble and reducing health care costs.[10] Importantly, subjects with the mutation can benefit from a medical surveillance programme. For HNPCC, colonoscopy has been shown to be a potent tool for the detection and treatment of premalignant adenomas or early colorectal carcinomas in at risk subjects, reducing the risk of developing colorectal cancer and decreasing the overall mortality by about 65%.[11 12] The possibility of early detection of colorectal cancer by stool analysis using the genetic markers TP53, BAT26, and K-RAS raises expectations for the development of less invasive surveillance procedures.[13] Furthermore, intervention trials with non-steroidal anti-inflammatory drugs (NSAID) in subjects at risk for developing colorectal cancer are in progress.[14 15]

So far, studies on the use of genetic testing in HNPCC families have used families or subjects who had been registered for research purposes.[10 16 17] It is conceivable, however, that these research families represent a selected group of HNPCC families where decision making processes are different from those in families in a clinical setting. Here, we report the use of genetic testing in 18 clinically ascertained HNPCC families with a known mutation in *MSH2, MLH1*, or *MSH6*.

Key points

- In a clinical setting, considerable interest was observed for genetic testing in HNPCC families with a known germline mutation.
- Testing was used more frequently by subjects with a higher pre-test genetic risk for the mutation, by women, and by subjects with children.
- Genetic testing has earned a place in the standard medical care for subjects at risk for HNPCC.

Table 1 Mutations in the *MLH1*, *MSH2*, and *MSH6* genes in the HNPCC families studied

Gene	Nature of the mutation (nucleotide change)	Families (n=18)
MSH2	Genomic deletion exon 3	1
	Splice acceptor site intron 9 (IVS9_2A>G)	1
	Frame shift mutation exon 2 (229_230delAG)	1
	Nonsense mutation exon 13 (2038C>T)	1
	Genomic deletion exon 1	1
	Nonsense mutation exon 8 (1285C>T)	2
	Frameshift mutation exon 2 (1705_1706delGA)	1
	Frameshift mutation exon 14 (2347delC)	1
MLH1	Splice donor site intron 8 (IVS8+1delG)	1
	In frame deletion exon 16 (1852_1854delAAG)	5
	Splice donor site exon 16 (1896G>A)	1
	Splice acceptor site intron 9 (IVS9-1G>C)	1
MSH6	Frameshift mutation exon 4 (1784delT)	1

MATERIAL AND METHODS

Patients

Eligible HNPCC families have been referred to the Department of Clinical Genetics of the Erasmus University Medical Centre Rotterdam for oncogenetic counselling by general practitioners and medical specialists since 1992. DNA analysis of the *MSH2*, *MLH1*, and *MSH6* genes was performed at the Department of Human and Clinical Genetics, Leiden University Medical Centre, as described previously.[18–20] Families were included in the study when a mutation in either of these three mismatch repair genes was identified before 2000. Subjects of these families were included when they were aged 18 years and over at the time of molecular diagnosis in the family and when they had a pre-test genetic risk for carrying the mutation of 100%, 50%, or 25% (see results).

Procedure

In general, the initial search for the causative mutation had been performed on blood DNA of the youngest colorectal cancer patient in the family (the index subject). Identified mutations were confirmed in all relatives affected with an HNPCC related tumour from which DNA samples were available. The initial counsellee and index subjects were asked to inform all the adult first and second degree relatives of patients with an HNPCC related tumour about the genetic predisposition to cancer in their family. Written information to distribute among their family members was made available to them. This information included facts on the inheritance of the cancer susceptibility in their family, the

Table 2 Characteristics of the families studied

Gene	Families (n=18)	Mean number CRC/family	Mean age CRC (range)	Mean number EC/family	Mean age EC (range)
MSH2	9	4.8	43.8 y (23–75)	1	46.4 y (30–54)
MLH1	8	5.4	43.7 y (27–72)	0.5	46.3 y (41–82)
MSH6	1	7	60.4 y (32–84)	5	55 y (50–60)

CRC, colorectal cancer. EC, endometrial cancer.

possibility of genetic testing, the risks of developing cancer, and the options for intervention. Relatives opting for genetic testing received one or more individual pre-test counselling sessions according to the recommendations of the American Society of Clinical Oncology,[21] including the discussion of medical, genetic, and psychosocial aspects of genetic testing. Psychological support was offered to all subjects throughout the testing procedure. Disclosure of the test results followed within 6-12 weeks after blood sampling. Mutation carriers were referred to local specialists for follow up and surveillance. In The Netherlands, this surveillance comprises colonoscopy and gynaecological examination every one to two years. In this country, prophylactic colectomy is currently not offered to unaffected mutation carriers.

Data collection and statistical analysis

All data were collected from medical records. Descriptive statistics were used to establish test rates of genetic testing. The influence of pre-test genetic risk, gender, parenthood, and age on the use of genetic testing was first assessed by univariate analysis. Subjects were categorised into subjects younger than 50 years and subjects 50 years and older. The simultaneous influence of gender, parenthood, and age was also assessed by multivariate logistic regression analysis in the 50% risk subjects. Pre-test genetic risk had been excluded from the multivariate analysis, as about half of the data on parenthood and age were missing for the 25% risk subjects.

To assess the time dependent rate of genetic testing, Kaplan-Meier survival probabilities were calculated for 50% risk subjects with a first degree relative with an HNPCC related tumour. By doing so, we avoided including the time 25% risk subjects had to wait for the genetic test result of their parent.

RESULTS

A cohort of 18 consecutive HNPCC families was selected that had a known mutation in *MSH2* (n=9), *MLH1* (n=8), or *MSH6* (n=1) (table 1). All families were of European origin. Five apparently unrelated families had an identical *MLH1* mutation and two apparently unrelated families had an identical *MSH2* mutation (table 1). At the time of clinical ascertainment, 15 of the 18 families fulfilled the Amsterdam II criteria.[22] Of the other three families, two families presented with a single patient with colorectal cancer under the age of 40 years and one family had three patients with endometrial cancer and one patient with ovarian cancer, all diagnosed over the age of 50 years.[23] Specific details of the number of colorectal and endometrial cancers per family and ages of onset are listed in table 2.

The 18 selected families consisted of 523 living subjects with a 100% (n=60), 50% (n=308), or 25% (n=155) pre-test genetic risk of carrying the family specific mutation (table 3). For practical reasons, the subjects diagnosed with an HNPCC related tumour (n=56) and obligate carriers (n=4) were designated as having a 100% pre-test genetic risk. The subjects with a 50% risk had a first degree relative with an HNPCC related tumour (n=267) or a first degree relative who was a mutation carrier (n=41). The subjects with a 25% risk had a living unaffected parent with a 50% risk (n=64) or a parent with a 50% risk who had died without evidence of an HNPCC related tumour (n=91).

Genetic testing was used by 260 of 523 (50%) eligible subjects (table 3). A mutation was detected in 133 (51%) subjects, of whom 83 were unaffected. Of the subjects with a pre-test genetic risk of 100%, 50%, and 25% for carrying the mutation, 87%, 57%, and 21% respectively used genetic testing ($p<0.0001$ for 100% *v* 50%; $p<0.0001$ for 50% *v* 25%) (tables 3 and 4). Of the 25% risk subjects who had an unaffected 50% risk parent who did not opt for testing, only three of 64 (5%) subjects used genetic testing. In contrast, of the 25% risk subjects with a dead unaffected 50% risk parent, 30 of 91 (33%) subjects used genetic testing (table 4). The test rate among the 308 50% risk subjects was 62% in women versus 51% in men, suggesting a small but significant preference for women to use genetic testing ($p=0.041$, table 4). The test rate in 50% risk subjects with children was 70% versus 45% for those without children, indicating parenthood as a stronger positive predictor towards testing ($p<0.001$, table 4). The age of the subjects did not influence test rates (table 4). Multivariate analysis of the data was consistent with the univariate analysis, again indicating gender and parenthood as significant parameters for genetic test usage (table 4).The mean time of follow up after identification of the mutation in the family was 42 months (range 12-74 months). Forty-one percent of 50% risk subjects decided for genetic testing within one year. At a

Table 3 Genetic testing and outcome in 100%, 50%, and 25% risk carriers in the 18 HNPCC families

			25%			
Pre-test risk	100%	50%	Parent alive	Parent dead	Total	Total
Number	60	308	64	91	155	523
Tested	52 (87%)	175 (57%)	3 (5%)	30 (33%)	33 (21%)	260 (50%)
Mutation carrier	50 (96%)	80 (46%)	0	3 (10%)	3 (9%)	133 (51%)

Table 4 Genetic test use in relation to gender, parenthood, age, and pre-test genetic risk

	Total	Tested (%)	Univariate	Multivariate
Gender*				
Females	156	97 (62)	p=0.041	p=0.045
Males	152	78 (51)		
Children*				
Yes	182	127 (70)	p<0.001	p=0.013
No	107	48 (45)		
Age*				
<50 y	191	104 (55)	p=0.24	p=0.39
>50 y	117	72 (62)		
Pre-test risk				
50%	308	175 (57)	p<0.0001	-
25%	155	33 (21)		
25% risk carriers				
Parent alive	64	3 (5)	p<0.0001	-
Parent dead	91	30 (33)		

*In subjects with a pre-test genetic risk of 50%.

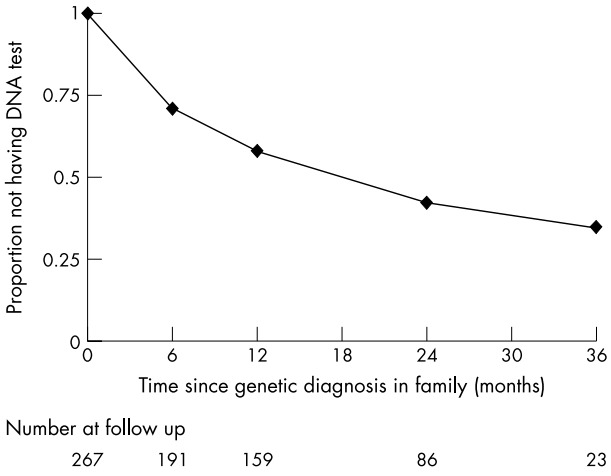

Figure 1 Time dependent rate of genetic testing in subjects with a 50% pre-test genetic risk. The proportion of 50% risk carriers not having the genetic test is shown at 0, 6, 12, 24, and 36 months after the identification of the familial mutation. Only 50% risk carriers with a first degree relative with an HNPCC related tumour are included.

follow up at two years and three years after molecular diagnosis, 58% and 65% respectively of the 50% risk subjects were tested (fig 1).

DISCUSSION

To our knowledge, the present study is the first to evaluate the use of genetic testing for HNPCC in a clinical setting. Importantly, we determined the use of genetic testing in complete pedigrees, including all affected family members and their unaffected first and second degree relatives. This was done in 18 HNPCC families with an identified pathogenic mutation in *MSH2*, *MLH1*, or *MSH6*. These families were quite extensive, with an average of 29 study subjects per family. The use of genetic testing by 50% risk carriers in our families was 57%. This test rate was lower than the 75% test rate in a Finnish cohort of 446 subjects at similar risk for an HNPCC germline mutation.[16] The Finnish subjects, however, had consented to registration and participation in research, which is likely to be positively correlated with interest in genetic testing. Also, the Finnish population is known for its positive attitude towards genetic testing.[24] In a Northern American study only 90 out of 208 subjects (43%) from four extended HNPCC research families were tested.[17] As the pre-test genetic risks of the study subjects were not specified, we cannot compare their data with ours.

We found that the magnitude of pre-test genetic risk for carrying the mutation was strongly correlated with test rates (87%, 57%, and 21% for 100%, 50%, and 25% risk subjects, table 3). This phenomenon has also been observed in other inherited diseases.[25–27] Interestingly, 25% risk subjects with an unaffected 50% risk parent who was alive rarely opted for genetic testing (5%), whereas subjects with the same pre-test genetic risk but with a dead unaffected 50% risk parent used genetic testing in one-third of the cases (tables 3 and 4). This may be because the identification of the mutation in a child designates the parent as an obligate carrier and children from a living unaffected 50% risk subject may therefore be more likely to refrain from genetic testing in order not to overrule their parents' preference for "not knowing". Also, children may share strategies to cope with genetic risks with their parents. The slightly higher genetic test rates in women than men (62% *v* 51%) was also seen in another late onset inherited disease.[26] In HNPCC, however, the additional risk for endometrial cancer in female mutation carriers probably also influences the use of genetic testing. The significantly higher test rates among subjects with children compared to subjects without children (70% *v* 45%) has also been observed in families with inherited breast and ovarian cancer (HBOC).[25] These findings emphasise that knowledge of the cancer risks for offspring is a major reason for subjects to opt for genetic testing. The high test rate in the affected 100% risk subjects also seems to reflect this phenomenon, since their participation is pivotal especially for the identification and conformation of the pathogenic mutation in the family, but generally has few medical implications for themselves.

A significant proportion of subjects was tested more than one year after the identification of the family specific mutation (65% of the 50% risk carriers at a follow up of three years) (fig 1). Interestingly, at our institute, the time period for deciding for genetic testing was strikingly longer for subjects at risk for HNPCC than for subjects at risk for HBOC (50% at 18 months versus 9 months).[25] The differences in time needed for decision making may perhaps be related to the one to two years interval for colonoscopy in HNPCC families versus the six months interval for breast surveillance in HBOC families.

Genetic testing rates depend on the natural history of the disease concerned, the success of treatment, the efficacy and acceptability of surveillance and prevention, and the costs involved.[28] The use of genetic testing in 50% risk subjects varies from about 20% in Huntington's disease (no interventions available, but relief of uncertainty and relevance for, for example, reproductive choices)[26 29] to ~55% in hereditary breast and ovarian cancer (*BRCA1* and *BRCA2* genes, surveillance and prophylactic mastectomy, prophylactic oophorectomy, and chemoprevention available for female carriers),[26 30] to ~90% in familial hypercholesterolaemia (lipid lowering treatment available).[31] The 57% HNPCC genetic testing rate in 50% risk carriers reported here is similar to that of women with the same genetic risk for carrying a *BRCA1* or *BRCA2* mutation. This suggests that the total load of factors that influence the choice to opt for genetic testing in HNPCC equals that in HBOC. The efficacy of regular colonoscopy in HNPCC mutation carriers is more favourable compared to regular mammography in young women with a *BRCA1* or *BRCA2* mutation.[11 32] This limited efficacy of mammography causes some women with a *BRCA1* or *BRCA2* mutation to opt for prophylactic mastectomy.[25 30] Though colonoscopy is not as invasive as the prophylactic mastectomy in HBOC, the burden and violation of physical integrity of this surveillance may be an underestimated factor. Also, the additional risks for other HNPCC related cancers, like brain tumours, for which no

options for surveillance and prevention are available, can cause subjects to refrain from testing. Other reasons not to opt for testing might be fear of financial and social discrimination and inability to cope with a positive test result. Notably, in The Netherlands, cancer predisposition is no reason for exclusion by the health insurance companies, nor for denial of access to employment. It was shown that most tested subjects are able to cope with genetic testing for cancer predisposition in the short term, particularly also for HNPCC.[16 33–36] More data on the long term psychosocial impact of genetic testing for HNPCC as well as data on the reasons and psychosocial impact of refraining from genetic testing are needed.

From the data reported here, however, it can be concluded that there is considerable interest in genetic testing in subjects from HNPCC families with a known mutation. Also, in view of the reduction in morbidity and mortality upon surveillance of identified mutation carriers, genetic testing has earned a place in the standard medical care for people at risk for HNPCC.

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