Table 2 Results of multivariate Cox proportional hazards model

Covariate	Odds of death (95% CI)	p
Type of treatment centre (specialty compared to non-specialty)	0.05 (0.00, 0.45)	0.008
Age at diagnosis (per year)	0.84 (0.78, 0.90)	<0.001
Intracranial meningiomas (per tumour)	1.27 (1.06, 1.51)	0.008

Age at diagnosis was used instead of age at onset because tumour burden was usually evaluated at diagnosis.

by mode of inheritance ($p=0.03$); in particular, patients with maternal inheritance had a lower proportion of missense mutations (7% versus 24%). Patients with maternal inheritance had significantly higher mortality ($p=0.02$).

In the multiple linear regression analyses, age at onset and age at diagnosis did not vary significantly with mode of inheritance (table 1). Patients with missense mutations and large deletions had significantly higher ages at onset and diagnosis than patients with nonsense or frameshift mutations. Patients treated at specialty centres had lower age at onset and significantly lower age at diagnosis than patients treated at non-specialty centres. In the multivariate Cox proportional hazards model, the odds of death did not vary significantly with mode of inheritance or type of constitutional *NF2* mutation (table 2). The odds of death varied significantly with type of treatment centre, age at diagnosis, and number of intracranial meningiomas.

The results of this study, the largest to date, are consistent with previous genotype-phenotype correlation studies in *NF2*,⁸⁻¹¹ but do not support maternal inheritance as an independent correlate of disease severity in *NF2*. The effects of constitutional *NF2* mutation type and treatment in specialty centres could not be examined in the earlier, more limited data of Kanter *et al.*,⁴ Evans *et al.*,⁵ and Parry

*et al.*⁶ Potential confounders should be routinely examined when outcomes that can be affected by multiple factors are being studied.

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Familial medullary thyroid carcinoma and prominent corneal nerves associated with the germline V804M and V778I mutations on the same allele of *RET*

Lidia Kasprzak, Serge Nolet, Louis Gaboury, Carles Pavia, Carles Villabona, Francisca Rivera-Fillat, Josep Oriola, William D Foulkes

EDITOR—Germline mutations in the *RET* proto-oncogene are associated with multiple endocrine neoplasia (MEN) type 2A, 2B, and familial medullary thyroid carcinoma (FMTc). MEN 2A is characterised primarily by medullary thyroid carcinoma (MTC), parathyroid hyperplasia, and pheochromocytoma. MEN 2B has additional stigmata including a marfanoid habitus, mucosal neuromas, and corneal nerve thickening. In FMTc, the

only lesion present is MTC. Although characteristic of MEN 2B, prominent corneal nerves have been noted in subjects with MEN 2A.

Case reports

We have studied a family presenting with MTC and corneal nerve thickening (CNT), but no evidence of MEN 2 related disease outside the thyroid gland (fig 1). The proband (III.1) was found to have prominent corneal nerves on

routine ophthalmological examination in his late 30s. No further action was taken and he presented with a palpable thyroid mass at the age of 53.

MEN 2B was suspected and he was referred for laboratory screening tests for MTC and pheochromocytoma. Serum calcitonin was raised at 4090 ng/l (normal range <60 ng/l). Serum calcium and phosphorus were normal. Twenty four hour urinary total catecholamine, metanephrine, and vanillylmandelic acid levels were within normal limits. A thoracic and abdominal CT scan performed before surgery was normal and scanning of the neck confirmed the presence of the right thyroid mass. The patient underwent a total thyroidectomy and pathological examination indicated bilateral MTC with extension beyond the capsule. His endocrinologist referred him for a genetic evaluation with a diagnosis of MEN 2B. However, other than CNT, he and his relatives did not have any of the other stigmata of this subtype. Family history was notable for thyroid carcinoma in the maternal cousin (III.4) and her son (IV.2). Interestingly, his mother (II.1), who appeared to be an obligate carrier, remained unaffected and died at 93 years of age related causes. The proband was encouraged to inform his family in Spain about the option of genetic counselling through the centre in Barcelona. Subsequently, IV.2 and all of his children were assessed and offered genetic testing. Patient IV.2 first noticed a right cervical enlargement at the age of 35 and later was diagnosed with multiple nodules and subjected to total thyroidectomy. The histological examination showed a multicentric medullary thyroid carcinoma with bilateral thyroid involvement. Lymph node and bone

metastases were identified. Catecholamines and metanephrines were within normal limits. He was also found to have prominent corneal nerves. His children were screened for serum calcitonin. One of the three offspring (V.2) had raised basal and pentagastrin stimulated levels (CT 15 pg/ml, basal and 23 pg/ml, five minutes).

The proband's genomic DNA was screened for mutations in exons 10, 11, and 13-16 of the *RET* gene. Direct sequencing identified two heterozygous missense mutations. Analysis of exon 13 showed a single base pair substitution of G to A at codon 778 resulting in a change from valine to isoleucine in the predicted amino acid sequence. Sequence analysis of exon 14 showed a heterozygous single base pair substitution of G to A at codon 804 resulting in a change from valine to methionine. Only exons 13 and 14 were analysed in the proband's mother, sister, and niece. Both mutations were subsequently identified in II.1 (an obligate carrier) and neither was found in III.2 or IV.1. Testing in Spain was performed concurrently on the affected subject IV.2, who was found to be positive for both V778I and V804M. All of his children were tested and one had the same genotype as her father while two were negative for both exon 13 and 14 missense mutations. Of note, the previously undescribed variant in exon 13 was present only in subjects carrying the V804M mutation. No mutation was found in *RET* codon 778 in four unaffected (V804M negative) family members, or in 73 and 20 controls from Montreal and Barcelona, respectively. Furthermore, we have amplified the proband's DNA by PCR, encompassing exon 13/intron 13/exon 14 of *RET*, by using an upstream primer localised at

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**Program in Cancer
Genetics, Departments
of Oncology and Human
Genetics, McGill
University, Montreal,
Quebec, Canada**
L Kasprzak
W D Foulkes

**Département de
Pathologie, Centre
Hospitalier de
l'Université de
Montréal, Montréal,
Quebec, Canada**
S Nolet
L Gaboury

**Servei
d'Hormonologia,
(IDIBAPS), Hospital
Clínic, Barcelona, Spain**
C Pavia

**Secció de
Endocrinologia,
Hospital Universitari de
Sant Joan de Deu,
Barcelona, Spain**
C Villabona

**Servei
d'Endocrinologia, CSU
Bellvitge, Hospital
Principes de España,
Barcelona, Spain**
F Rivera-Fillat
J Oriola

**Department of
Medicine, Research
Institute of the McGill
University Health
Centre, Montreal
General Hospital, Room
L10-116, 1650 Cedar
Avenue, Montreal,
Quebec H3G 1A4,
Canada**
W D Foulkes

Correspondence to:
Dr Foulkes,
WILLIAM.FOULKES@
MCGILL.CA

Figure 1 A truncated pedigree of the family is shown. Filled symbols indicate affected subjects. The letters below the symbols refer to the site of cancer, the numbers following the abbreviations indicate age at diagnosis. MTC, medullary thyroid cancer; PSU, primary site unknown; d, age at death; CNT, corneal nerve thickening.

the end of intron 12 and a downstream primer localised at the beginning of intron 14. The PCR product is 1497 bp long. Since the G to A at position 778 destroys a single *MaeII* restriction site (ACGT) on the PCR product, only the normal homozygous allele is cut by the *MaeII* enzyme. After an overnight incubation with *MaeII* to assure complete digestion, we performed a second round PCR on the *MaeII* digestion product. Only an allele with the A (rather than the G) nucleotide in codon 778 can be amplified since this amplified PCR product is left intact by *MaeII*. Another *MaeII* digestion on this second PCR product confirmed the absence of a *MaeII* site. Restriction analysis with *NlaIII* (CATG), which cuts the mutated allele (G to A) at codon 804, indicated that the 778I variant and the 804M mutation are on the same allele. Moreover, we performed direct sequencing of the second PCR amplification product and we were able to confirm the presence of the 778I variant on the same 804M mutated allele.

Discussion

The point mutations in exon 14 leading to a substitution of valine 804 by either leucine or methionine have been reported in several families with FMTC.¹⁻³ The prevalence of this particular alteration in codon 804 seems to be quite low in FMTC families (frequency of 0.03 according to Hansford and Mulligan⁴) and there have been only three reports of the germline V804M in apparently sporadic MTC cases.⁵⁻⁷ Nilsson *et al*⁸ showed that the V804L mutation is not exclusively associated with familial MTC as it has been identified in the MEN 2A pedigree. It appears that certain germline sequence variants identified in exons 13 and 14 are seen at a higher frequency in patients with sporadic MTC as compared with that of the general population. Two of the reported polymorphic variants (L769L in exon 13 and S836S in exon 14) have been implicated as having a potentially promoting effect in the aetiology of MTC.^{9,10} The restriction of the V778I mutation to V804M carriers was noted and we have confirmed that V778I is in *cis* with the disease allele in the proband. The novel V778I germline alteration may be a low penetrance allele contributing to the development of the CNT in this pedigree. Notwithstanding, codon 804 mutations have been initially associated with a later onset and less aggressive disease phenotype suggesting lower penetrance⁷; however, there are several reports indicating a quite variable clinical picture including anticipation.^{3,11} The specific V778I sequence variant may be responsible for significantly earlier age of onset of MTC and more aggressive disease course in this family. Even though the replacement of valine by isoleucine in the predicted amino acid sequence results in a rather benign change (hydrophobic, branched chain to hydrophobic, branched chain amino acid) at the level of primary protein structure, it is not clear how it might alter the tertiary conformation. In addition, it should be noted that both exon 13 and 14 mutations affect the intracellular domain of the receptor tyrosine kinase encoded by the

RET proto-oncogene. Miyauchi *et al*¹² reported a patient with a MEN 2B phenotype owing to a germline V804M mutation and a novel Y806C mutation occurring on the same allele of the *RET* gene. The authors speculated that the presence of the apparently non-pathogenic Y806C mutation in the *cis* position might have resulted in a structural change of the *RET* protein potentiating the significance of V804M alone. This particular case presented at the age of 23 with bilateral MTC with multiple foci and lymph node metastasis. Alternatively, Y806C could represent a low penetrance variant which affects the phenotype because of an additive effect in the presence of the second mutation. Subsequent comparison of the biological activity of the V804M/Y806C combination with that of the MEN 2B exclusive mutations M918T or A883F suggests similar biological properties of these three mutant proteins.¹³ Furthermore, the transforming activity of *RET* with the V804/Y806C compound was apparently eight to 13 times that of *RET* with each mutation analysed separately. Further studies of the *RET* alleles in this and other kindreds may identify a unique effect of the known disease causing mutations in the presence of rare variants on the same chromosome 10, resulting in a different phenotype than observed in the presence of the mutation alone. Prominent corneal nerves which can be visualised by slit lamp examination are present in a large proportion of the MEN 2B patients¹⁴ and are less frequently observed in those with MEN 2A.¹⁵ As there are few such cases, it is not clear whether CNT in association with MEN 2A can be correlated with specific mutations in the *RET* gene. The significance of CNT was overlooked in our proband whose affected status might have been established earlier if genetic consultation had been considered by the ophthalmologist.

- A family with MTC and CNT was assessed through a 53 year old affected subject.
- *RET* exon 13 and 14 mutations in *cis* (V778I and V804M) were identified in four family members.
- A novel variant V778I was found, potentially affecting the phenotype through an additive effect in the presence of V804M.

We thank Dr W Gregory for referring the proband to the Hereditary Cancer Clinic at McGill University and the family for their continued cooperation.

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Comprehension of cancer risk one and 12 months after predictive genetic testing for hereditary non-polyposis colorectal cancer

Katja Aktan-Collan, Ari Haukkala, Jukka-Pekka Mecklin, Antti Uutela, Helena Kääriäinen

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Department of Medical Genetics, Haartman Institute, University of Helsinki, Helsinki, Finland
K Aktan-Collan

Department of Medical Genetics, Family Federation of Finland, POB 849, FIN-00101 Helsinki, Finland
K Aktan-Collan
H Kääriäinen

Department of Social Psychology, University of Helsinki, Helsinki, Finland
A Haukkala

Department of Surgery, Jyväskylä Central Hospital, Jyväskylä, Finland
J-P Mecklin

Department of Epidemiology and Health Promotion, National Public Health Institute, Helsinki, Finland
A Uutela

Correspondence to:
Dr Aktan-Collan,
katja.aktan@vaestoliitto.fi

EDITOR—The main purpose of offering predictive genetic testing for hereditary cancer is to reduce unnecessary worry among those with a low risk of cancer (mutation negative) and to recognise those with a high risk (mutation positive), so as to promote preventive measures.^{1,2} Ideally, those shown to be at high risk would understand this, would learn to live with the knowledge, and, most importantly, would attend cancer surveillance programmes regularly. Those at low risk would feel relieved and no physically and emotionally uncomfortable surveillance would be needed. This result would be seriously hampered if those tested did not fully understand the meaning of the test results, which could lead to unnecessary worry or failure to adhere to surveillance. At present, most predictive tests are performed in carefully organised settings, which include comprehensive pre- and post-test counselling that would be expected to minimise the risk of misunderstanding the test result. However, commercial tests predicting cancer are already available,³ and this has raised concerns about predictive testing with minimal counselling or even without personal contact with a health care professional.⁴

The impact of genetic counselling on risk perception and impact of risk perception on genetic testing intentions has been studied previously.⁵ However, this is the first report on the comprehension of test results (perception of cancer risk) after predictive genetic testing for cancer, in this case hereditary non-polyposis colorectal cancer (HNPCC), which is the most common form of hereditary colon cancer.

HNPCC is an autosomal dominant disease of adulthood with an 80-90% lifetime risk for colorectal cancer and a lesser risk of extracolonic cancers, the most common of which are endometrial and gastric cancer.⁶ Hereditary colorectal cancer differs essentially from other hereditary cancers, of which hereditary breast cancer is a good example. Firstly, in HNPCC, the life long risk of developing colorectal cancer among mutation positive subjects is uniformly very high (80-90%),^{7,8} while in breast cancer the corresponding risk varies between 40 and 80%.⁹⁻¹¹ Secondly, for HNPCC, in contrast to breast cancer, clinical surveillance among those at high risk has been shown to reduce mortality from colorectal cancer substantially.^{12,13} Predictive genetic testing is now possible in all the families with HNPCC in which the predisposing germline mutation is known. Previous studies among first degree relatives of patients with colorectal cancer have suggested a great interest in possible testing and intention to learn the results.^{14,15} Reports on the actual offering of predictive genetic tests to HNPCC families have shown that the acceptance rates have varied greatly (14-81%).¹⁶⁻¹⁹ This study describes how the members of HNPCC families comprehended their predictive test results in terms of their risk of developing colorectal cancer and discusses what may have influenced this.

Methods

During 1995-1997, we offered counselling about predictive genetic testing to adults at 50% risk in 36 HNPCC families in which mutations in the *MLH1* gene had previously