Oncological implications of RET gene mutations in Hirschsprung's disease

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Abstract

Background—Germline mutations of the RET proto-oncogene identical to those found in the tumour predisposition syndrome multiple endocrine neoplasia type 2A (MEN2A), were detected in 2.5–5% of sporadic and familial cases of Hirschsprung's disease. Some patients with Hirschsprung's disease may therefore be exposed to a highly increased risk of tumours.

Aims—To define clinical use of RET gene testing in Hirschsprung's disease and related patient management from an oncological point of view.

Methods—Sixty patients with Hirschsprung's disease were screened for RET mutations. In three, MEN2A type RET mutations were detected. Case reports for these three patients are presented.

Results and conclusions—Only 22 families or sporadic patients with Hirschsprung's disease and MEN2A type RET mutations have been reported. Therefore, it is difficult to predict tumour risk for patients with familial or sporadic Hirschsprung's disease, and their relatives, who carry these mutations. For these mutation carriers, periodic screening for tumours as in MEN2A is advised, but prophylactic thyroidectomy is offered hesitantly. RET gene testing in familial or sporadic Hirschsprung's disease is not recommended at present outside a complete clinical research setting. In combined MEN2A/Hirschsprung's disease families RET gene testing, tumour screening, and prophylactic thyroidectomy are indicated as in MEN2A.
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Keywords: DNA analysis; Hirschsprung's disease; multiple endocrine neoplasia type 2A; RET

Hirschsprung's disease (HD), which occurs in approximately 1 out of every 5000 newborns, is characterised by a congenital absence of enteric neurones in the distal colon and rectum.[1] Most cases are sporadic, but familial HD has been reported.[2][3] Hirschsprung's disease is associated with various chromosomal abnormalities and inherited disorders.[4][5] Two of these inherited disorders are the tumour predisposition syndromes, multiple endocrine neoplasia type 2A (MEN2A) and familial medullary thyroid cancer (FMTC). MEN2A is an autosomal dominant disorder characterised by medullary carcinoma of the thyroid (which may present as early as in the third year), phaeochromocytoma in 50% of cases, and parathyroid hyperplasia or adenoma in 20–25% of cases.[6][7] Familial medullary thyroid cancer is characterised by the familial occurrence of medullary carcinoma of the thyroid in the absence of other MEN2 tumours.

Germline mutations of the RET (REarranged during Transfection) proto-oncogene can be detected in virtually all families with MEN2A, FMTC,[8][9] and combined MEN2A/HD or FMTC/HD.[10–15] Germline RET mutations also occur in both familial and sporadic cases of HD without manifestations of either MEN2A or FMTC.[16–21] RET mutations were detected in 11–49% of familial HD cases and 9–35% of sporadic HD cases.[17][21–24] Different types of RET mutation can be found in these families. The mutations identical to those found in MEN2A or FMTC are of oncological interest. For the purpose of this article we will refer to these mutations as MEN2A type RET mutations. This type of RET mutation was found in 2.5–5% of the patients with HD[10][13][21][22][24] and these patients may have a high risk of developing MEN 2 tumours. Therefore, the question arises whether patients with HD and their relatives should be tested for MEN2A type RET mutations and, in case mutations are found, be screened for possible MEN 2 tumours.

While screening a series of 60 patients with HD for RET mutations,[23] we found three patients with a MEN2A type RET mutation. Using these three patients and their families as a clinical introduction to the subject, the aim of this study was to define the use of RET testing and related patient management in HD from an oncological point of view.

Case reports

PATIENT 1

This three year old girl was diagnosed with long segment HD at the age of eight days, on clinical and histological grounds. Preoperative ultrasound examination of the abdomen revealed right sided renal aplasia and during the operation a small cyst-like structure near the bifurcation of the aorta was removed. Histological examination showed renal tissue. The 31 year old mother of this patient had presented with the combination of medullary thyroid carcinoma and phaeochromocytoma at the age of 28 and been diagnosed as having MEN2A. No other cases of HD or MEN2A were diagnosed in the family (fig 1A). MEN2A might, however, have occurred in a brother who died suddenly at the age of 25 (no further
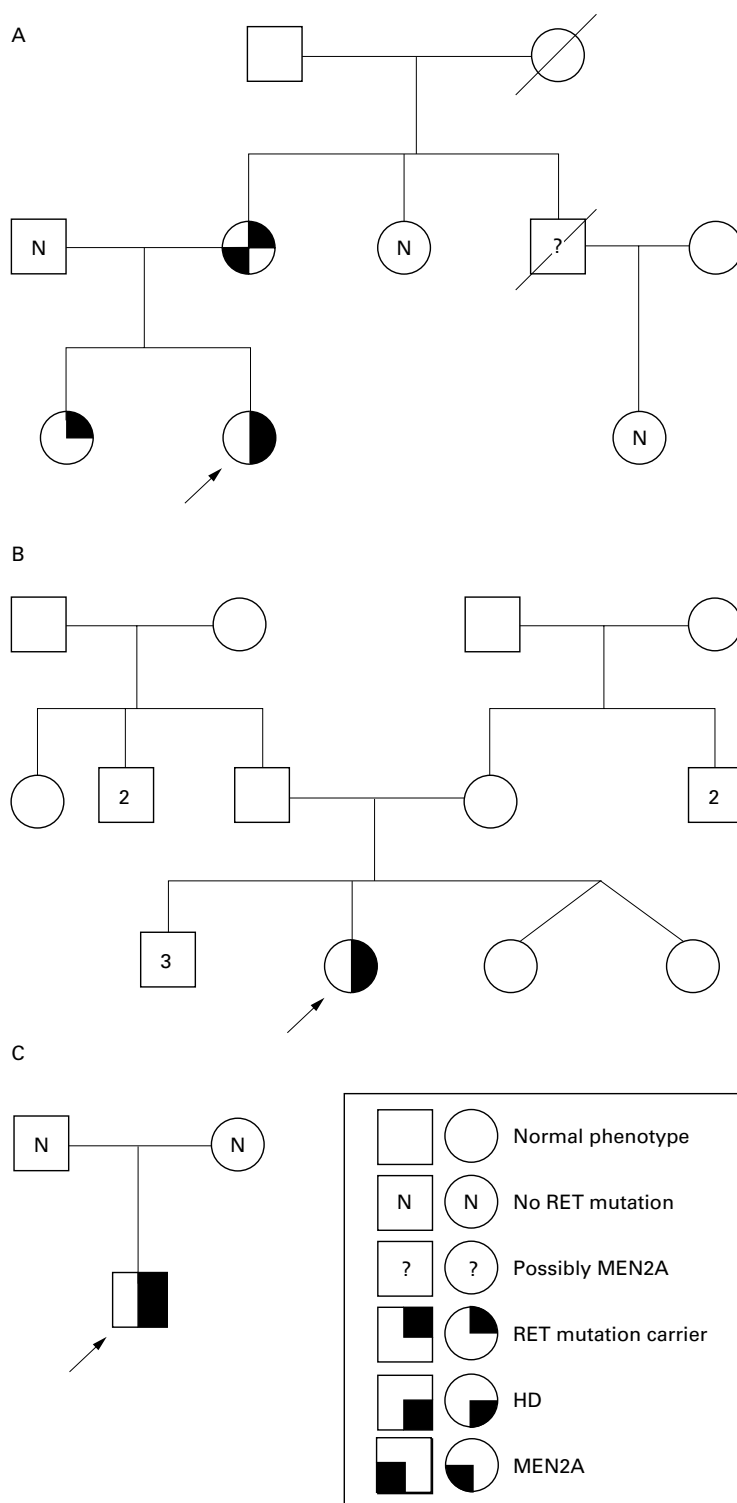
A

B

C

Figure 1   Pedigrees of the families of the index patients. In (A) patient 1 is indicated with an arrow. In (B) patient 2 is indicated with an arrow; she probably has combined MEN2A/HD. Her relatives could not be tested for the RET mutation or screened for MEN 2 tumours. Numbers within squares represent the additional number of asymptomatic males in that generation. In (C) patient 3 is indicated with an arrow; the mutation was not shown in his parents. HD, Hirschsprung's disease.

phaeochromocytoma. The RET gene was screened for mutations by means of denaturing gradient gel electrophoresis followed by direct sequencing of aberrant DNA fragments.[23 25] The girl exhibited the same germline mutation as was later found to have been previously detected in her mother in another laboratory. A TTC-TGC to TTT-CGC change was observed in codons 619–620, changing the corresponding amino acids phenylalanine-cysteine in the RET protein to phenylalanine-arginine. Cys620Arg mutations have been reported previously in MEN2A and MEN2A/HD kindreds.[10 26] Therefore, periodic screening and prophylactic thyroidectomy at the age of three to six years were recommended for this patient. The five year old sister of the HD patient was found to be an asymptomatic carrier of the RET mutation. Recently, she underwent prophylactic thyroidectomy and central node dissection; the surgical specimens revealed normal histology. Two other family members underwent DNA analysis and did not show the mutation.

PATIENT 2

This 34 year old woman was diagnosed with short segment HD at the age of eight weeks, on clinical and histological grounds. At the age of 33 she was tested for germline RET mutations (techniques as in patient 1) after an informed consent procedure including discussion of possible oncological aspects. A TGC to TAC mutation was detected in codon 609, changing its code for cysteine to one for tyrosine. Cys609Tyr mutations have previously been reported in MEN2A.[26 27] The family history was negative for HD and bowel diseases in general, and MEN 2 tumours and cancer in general (fig 1B). Relatives of the patient could not be approached for DNA analysis. After the RET mutation was found, the patient was screened biochemically for MEN 2 tumours. Her basal calcitonin level was normal: 3.88 ng/l (normal range 3.00–29.00). However, after pentagastrin stimulation, the calcitonin levels were clearly abnormal, with a peak value of 1290 ng/l (normal peak value is up to three times the normal unstimulated level) which is indicative of thyroid (C cell) pathology. Urinary screening for phaeochromocytomas was normal. The possibility of thyroid pathology was discussed with the patient and repeat pentagastrin testing (with subsequent thyroidectomy if the high calcitonin levels were confirmed) offered. However, the patient did not wish to undergo any further testing at that time because she perceived this as a direct threat to the success of a child adoption procedure she had recently started. She was offered psychosocial support.

PATIENT 3

This four year old boy was diagnosed with short segment HD at the age of six weeks, on clinical and histological grounds. Family histories of HD and congenital abnormalities in general were negative (fig 1C). A germline RET mutation was detected in this patient (techniques as in patient 1). In codon 620 the

details available). The mother of the MEN2A patient died at the age of 55 from ovarian cancer; biochemical screening of her father at the age of 59 revealed no abnormalities. Biochemical screening of the little girl provided no evidence for C cell hyperplasia, medullary thyroid carcinoma, parathyroid involvement, or

Table 1   MEN2A type germline mutations of the RET gene found in pateints/families with Hirschsprung's disease (HD)

Mutation	Phenotype†	HD/RET carriers††	HD + MEN2A/HD†††	Reference
Cys609Trp	HD (familial)	3/7	NA	10
Cys609Tyr	HD (familial)	NR	NA	22
Cys609Tyr	HD/MEN2A	1/7	1/1	33
Cys609Tyr	HD/MEN2A	1/7	1/1	33
Cys609Tyr	HD (sporadic)	NA	NA	Current report
Cys618Arg	HD/MEN2A	2/6	2/2	13
Cys618Arg	HD/MEN2A	2/5	2/2	10
Cys618Arg	HD/FMTC	2**/3	0/3	15
Cys618Arg	HD/FMTC	2***/29	2/2***	15
Cys618Ser	HD/MEN2A	3/at least 42‡	2/3	12, 33
Cys618Ser	HD/MEN2A	4/at least 83‡	3/4	12, 33, 34
Cys618Ser	HD/MEN2A	1/3	1/1	33
Cys620Trp	HD/MEN2A	1/6	0/1	33
Cys620Arg	HD/MEN2A	2/4	1/2	33
Cys620Arg	HD/MEN2A	5/10	4/5	10
Cys620Arg	HD/MEN2A	2/2	1/2	10
Cys620Arg	HD/FMTC*	NA	NA	10
Cys620Arg	HD/MEN2A	1**/6	1/1**	14
Cys620Arg	HD/MEN2A	1/3	0/1	Current report
Cys620Arg	HD (sporadic)	NR	NA	22
Cys620Arg	HD (sporadic)	NR	NA	24
Cys620Arg§	HD (sporadic)	NA	NA	Current report

*In this family HD segregates independently of the familial RET mutation. **In an additional relative with HD RET was not analysed.
***In three additional relatives with HD RET was not analysed.
†Phenotype refers to the classification of the family type prior to DNA analysis and (unpublished) follow up. ††HD/RET carriers refers to the number of family members diagnosed with HD out of the total number of family members with a proven RET mutation plus untested relatives with clinical MEN2A. These ratios are given mainly to show the incomplete penetrance of the mutations with regard to the HD phenotype, not to provide a precise figure. The ratios given are probably an overestimation of penetrance as not all unaffected relatives have been tested for RET mutations; uncertain cases were not included. †††HD + MEN2A/HD refers to the number of patients with both HD and MEN2A out of the total number of relatives with HD. Young relatives with HD may of course develop MEN2A tumours at a later age; uncertain cases were not included.
‡Exact numbers not published.
§De novo mutation.
NA, not applicable; NR, not reported.
The International RET Mutation Consortium recently published an update of their dataset[9] and report on five MEN2A/HD families and one FMTC/HD family with a Cys620Arg mutation in 5/6 cases and in the remaining case a Cys618Arg germline mutation of RET. It cannot be deduced from that report whether those families are identical to the families described in the reports listed in this table.

same TGC to CGC change as in patient 1 was observed, changing the corresponding amino acid in the RET protein from cysteine to arginine. This mutation was not detected in the peripheral blood lymphocytes of his parents, which implies that it is a de novo (spontaneous) mutation; however, germline mosaicism in one of the parents (presence of the mutation in only part of the germ cells), a phenomenon which has been reported in RET gene associated HD,[21] cannot be excluded. Periodic screening of the boy, but not prophylactic thyroidectomy, has been recommended.

Discussion

Given our present knowledge, we believe that patients with familial or sporadic HD, and their relatives, should only be tested for RET gene mutations in a complete clinical research setting. If MEN2A type RET mutations are found, then they should be screened for MEN 2 tumours. We considered the following.

Firstly, the molecular background should be taken into account. The RET proto-oncogene encodes a transmembrane receptor, which has glial cell line derived neurotrophic factor (GDNF) and neurturin (NTN) as its ligands.[28–31] This receptor is believed to transduce signals involved in cell growth and in differentiation of neural crest cell derived tissues, including thyroid C cells (involved in medullary thyroid carcinoma), the adrenal medulla (involved in phaeochromocytoma), enteric neurones, and also the parathyroid glands and kidneys which are not neural crest cell derived.

Different types of mutation of RET can have a strikingly different impact on the function of the receptor it codes for. On the one hand, mutations in MEN 2 and FMTC are presumably or have been proved to be of the activating type, whereby ligand binding is no longer necessary for substrate activation (phosphorylation).[32] Activating mutations are thought to predispose to tumour development as they provide the cells with a proliferative advantage. The RET mutations found in all combined MEN2A (or FMTC)/HD families and in 2.5–5% of sporadic and familial HD cases analysed to date are of this type[10 13 21 22] (table 1).

On the other hand, 95–97.5% of the mutations found in HD are presumably or have been proved to be of the inactivating type, which is not expected to predispose to tumour development.[35–38] Instead, these mutations are thought to cause defective migration, proliferation, differentiation, or survival of the enteric neuroblasts which form the enteric ganglia.[39] This hypothesis of a loss of RET function leading to HD is supported by the absence of enteric ganglia in so called knockout mice which lack both copies of the RET gene.[40] These mice also show renal agenesis or severe renal dysplasia.[40] Patient 1 is one of the few clinical examples of a proved (heterozygous) RET mutation associated with both HD and renal dysplasia. Similar cases have been reported by Attie and colleagues[21] and McGaughran et al,[41] respectively.

The occurrence of HD, generally associated with lack of RET function, in the presence of presumed activating tumour predisposing RET mutations seems to be a contradiction. Another puzzling finding is that, although in the majority

of MEN2A families RET mutations affect cysteine codon 634,[9] no such mutations have been found in MEN2A/HD families (table 1). Mutations of three other cysteine codons, namely 609, 618, and 620, account for all cases reported to date.

Recent findings by Ito and colleagues[42] may provide a clue to these apparent oddities. These authors showed that mutations of RET in cysteine codons 609, 618, and 620 were associated with a much lower expression of the RET protein on the cell surface than mutations of cysteine in codon 634. This is important because, in order to act properly, the receptor needs to be positioned within the cell membrane. One could therefore speculate that the decreased number of receptors at the cell surface falls below the critical threshold needed for successful development of the enteric ganglia. In the C cells, adrenal medulla, and parathyroid gland, however, intrinsic activation of the mutated receptor (though present in lower numbers) may lead to tumour formation. Ito *et al* speculate that the lower number of receptors at the cell surface will cause tumours which may differ in clinical behaviour from those seen in patients with codon 634 mutations,[42] but this hypothesis has yet to be verified.

Secondly, in addition to the molecular data, the predictive clinical value of finding RET mutations in patients with HD and their relatives has to be considered. Given a specific germline MEN2A type RET mutation, it is not yet possible to make an exact prediction of the actual risks of developing an MEN 2 tumour and HD. MEN2A, MEN2A/HD, and HD families may show explicit intrafamilial and interfamilial differences in the expression of their mutant genes. These differences relate to the age of onset of possible tumours, the presence or absence of phaeochromocytomas, and possible parathyroid involvement (table 1). Similar variation is seen with regard to HD: both short and long segment HD as well as the absence of HD may be associated with the same RET mutation.[10 12 21 33 43] This seriously limits the use of RET testing to support counselling for HD risks in offspring.

The phenotypic expression of germline RET mutations can apparently be influenced by additional genetic factors (modifying genes) or environmental factors. With regard to MEN2A, there are enough data available to make general estimates of tumour risks. In contrast, the total number of index patients with HD with MEN2A type mutations, observed to date is very small. Including our own three patients, 22 cases have been reported so far and only five of these belong to the most difficult category for risk estimation: those with no family history of MEN 2 tumours.

Although family history might be an indication of tumour risk, unfortunately, a negative family history of MEN 2 tumours for patients with HD and MEN2A type RET mutations does not mean that the tumour risk is not increased. Families may be small and MEN 2 tumours may be asymptomatic. After bio-

chemical screening for tumours and pedigree expansion and verification, at least some sporadic or familial patients with HD with MEN2A type mutations might actually turn out to be MEN2A/HD patients or families. Our patient 2 is a likely candidate for the combined MEN2A/HD disorder and it could be that other family members are at risk for MEN 2 tumours and should, if circumstances allow, be tested. In cases of de novo RET mutations, as in our patient 3, there is of course no way to deduce tumour risk from family history. In many sporadic or familial HD only cases it will be impossible to predict whether or not tumour risk is increased as substantially as it is in MEN2A. Only in exceptional cases, for example, the presence of many—especially older—relatives with a MEN 2 type mutation and a negative family history of MEN 2 tumours, do the data suggest that that particular family is not exposed to a substantially increased risk of these tumours.

Predicting tumour risk for RET mutation carriers from MEN2A (or FMTC)/HD families, such as patient 1, seems less difficult. The published cases indeed suggest that the risk is as high as for MEN2A (or FMTC) only families. This also applies to patients with HD in these families, as the large majority of family members with HD also developed MEN 2 tumours (table 1). Furthermore, some of the patients with HD without MEN 2 tumours are still young and may develop these tumours later in life.

Thirdly, the options for tumour prevention or early intervention should be taken into account. The prognosis for patients with MEN 2 tumours detected at the symptomatic stage is worse compared with that for patients with tumours detected by screening.[44 45] Therefore, although the risk of a tumour is not yet known, we think that patients and relatives of sporadic or familial HD families known to carry a MEN 2 type RET mutation should undergo biochemical screening as in MEN2A. Screening, which starts at the age of three to five years, includes a yearly basal calcitonin test, a calcitonin test under pentagastrin stimulation to detect C cell hyperplasia/medullary thyroid carcinoma, measurement of serum calcium levels to test for parathyroid hyperplasia/adenomas, and measurement of catecholamines in urine to detect phaeochromocytomas.[27 46–48]

As in MEN2A, one should be aware of the possibility that (moderately) raised calcitonin levels may simply reflect MEN 2 independent C cell hyperplasia, which is not a precursor of medullary thyroid carcinoma. This hyperplasia is found in approximately 5% of the general population.[49] A false positive response to pentagastrin stimulation in RET mutation negative relatives has also been reported when C cell hyperplasia was not shown.[50] The test for calcitonin levels may also give false negative results. In some MEN2A/FMTC families medullary thyroid carcinoma has been encountered in children with normal pentagastrin stimulated calcitonin levels who underwent thyroidectomy after DNA diagnosis.[48 51 52] For

this reason prophylactic thyroidectomy is performed as early as the age of five years in confirmed RET mutation carriers in MEN2A or FMTC families with normal (stimulated) plasma calcitonin levels, although some clinicians prefer to wait until the pentagastrin test results are abnormal.[47 48 52] As it is difficult to predict the actual tumour risk for patients with sporadic or familial HD associated with a MEN2A type RET mutation, we are hesitant to suggest prophylactic thyroidectomy (and central lymph node dissection) when calcitonin levels are normal.

Long term follow up of patients and families with HD with different types of RET mutations is needed to evaluate the clinical value of testing for the RET gene in HD. In the meantime, we propose, based on our review, that such testing should be limited to a complete clinical research setting in which clinical genetic assessment, screening, and treatment for MEN 2 tumours are available, and long term follow up of the families is carefully registered. In contrast, RET testing and clinical management in HD/MEN2A (or FMTC) families should follow the guidelines for MEN2A. In all cases, informed consent, including a discussion of possible consequences of DNA testing, should be obtained prior to testing.

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Call for Patients with Familial Pancreatic Disease: The EUROPAC Register

We are establishing a national UK register (EUROPAC) of families with hereditary pancreatitis, familial pancreatic cancer and where pancreatic cancer has occurred as part of a familial cancer syndrome. This collaboration in Liverpool is between the Department of Clinical Genetics (Dr Ian Ellis) and the Academic Department of Surgery (Professor John Neoptolemos). The data and samples are collected by behalf of ESPAC (the European Study Group for Pancreatic Cancer), Professor Markus Büchler, Berne, and Professor Hans Beger, Ulm. The study will collaborate with Dr David Whitcomb of the Midwest Multicenter Pancreatitis study group in the United States. We aim to recruit families who are prepared to donate blood for DNA studies. We hope to gain a clearer understanding of the genetic relationship between hereditary pancreatitis and familial pancreatic cancer, and develop screening protocols for individuals at risk.

Hereditary pancreatitis is associated with a mutation in the recently identified cationic trypsinogen gene. This mutation renders the enzyme active within the pancreas, leading to autodigestion. Individuals with recurrent pancreatitis have a greatly increased risk of developing pancreatic cancer, and there is some evidence that DNA analysis of cells from pancreatic fluid may be valuable in detecting premalignant changes which can predict the development of pancreatic adenocarcinoma.

The criteria for inclusion in the study are as follows:
- Hereditary pancreatitis: Three relatives with chronic pancreatitis in the absence of ethanol dependence, hypercalcaemia, or an obstructive cause.
- Familial pancreatic cancer: Two first degree relatives with pancreatic adenocarcinoma. Three or more relatives with pancreatic ductal adenocarcinoma. Pancreatic ductal adenocarcinoma in any two relatives where the sum of their ages is less than 110 years.
- Other familial cancer syndromes: A single documented pancreatic ductal adenocarcinoma in any family with an established familial cancer syndrome—for example, BRCA2, FAMMM, A-T, HNPCC, or FAP.

If you know of any suitable families who may be interested in joining the study, please contact: Fiona McRonald, Clinical Genetics, Alder Hey Children's Hospital, Eaton Road, Liverpool L12 2AP. Tel: 0151 252 5905.

Thank you for your help.