MEDICAL GENETICS IN PRACTICE

A report of a national mutation testing service for the *MEN1* gene: clinical presentations and implications for mutation testing

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Introduction: Mutation testing for the *MEN1* gene is a useful method to diagnose and predict individuals who either have or will develop multiple endocrine neoplasia type 1 (MEN 1). Clinical selection criteria to identify patients who should be tested are needed, as mutation analysis is costly and time consuming. This study is a report of an Australian national mutation testing service for the *MEN1* gene from referred patients with classical MEN 1 and various MEN 1-like conditions.
Results: All *55 MEN1* mutation positive patients had a family history of hyperparathyroidism, had hyperparathyroidism with one other MEN1 related tumour, or had hyperparathyroidism with multiglandular hyperplasia at a young age. We found 42 separate mutations and six recurring mutations from unrelated families, and evidence for a founder effect in five families with the same mutation.
Discussion: Our results indicate that mutations in genes other than *MEN1* may cause familial isolated hyperparathyroidism and familial isolated pituitary tumours.
Conclusions: We therefore suggest that routine germline *MEN1* mutation testing of all cases of ''classical'' MEN1, familial hyperparathyroidism, and sporadic hyperparathyroidism with one other MEN1 related condition is justified by national testing services. We do not recommend routine sequencing of the promoter region between nucleotides 1234 and 1758 (Genbank accession no. U93237) as we could not detect any sequence variations within this region in any familial or sporadic cases of MEN1 related conditions lacking a *MEN1* mutation. We also suggest that testing be considered for patients <30 years old with sporadic hyperparathyroidism and multigland hyperplasia.

MEN1 is a relatively rare autosomal dominant disorder, typically characterised by hyperplasia or tumours of the parathyroid, endocrine pancreas, anterior pituitary, gastrin cells, and neuroendocrine cells. Other less common sites affected include adrenal and adipose tissue.[1 2] The prevalence of MEN 1 has been estimated to be 1/30 000 to 1/50 000.[3] The disease has been reported to be 52% and 100% penetrant by the ages of 20 and 60 years respectively.[4] However, the clinical presentations are varied and depend largely on the hormones being overexpressed. Mutations in the *MEN1* gene have been shown to be associated with multiple endocrine neoplasia type 1 (MEN 1) and to cause a very similar clinical presentation to MEN 1 in a mouse model.[5] Thus, as the clinical features of this disease are diverse, genetic screening is a useful method to diagnose patients and to predict the family members who will develop the disease in later life.

MEN 1 predisposing mutations have been demonstrated over the entire nine exons of coding sequence of the *MEN1* gene, making mutation detection an expensive and time consuming process, albeit less expensive than annually screening entire families using biochemical and radiological tests.[2 6] Moreover, the clinically heterogeneous nature of the disease makes it difficult to determine appropriate testing criteria, especially for newly presenting patients. Hyperparathyroidism is the most common condition seen in MEN 1, but it is also a common condition generally, with a reported prevalence rate of 0.43%.[7] ''Classical'' MEN 1 presents with a family history of neoplasia of at least two different endocrine cell types, but *MEN1* mutations have been shown in patients with familial isolated hyperparathyroidism (FIHP).[8–10] In addition, *MEN1* mutations have been shown to have occurred de novo in approximately 10% of patients.[2 9] Because of the costs involved, routinely testing all patients with sporadic tumours in MEN1 related tissues is inappropriate; however, testing only ''classical'' MEN1 cases is clearly insufficient.

In this study we report the findings of an Australian mutation testing service after testing for *MEN1* mutations in 150 index cases with MEN1 related conditions. From these data, we suggest clinical criteria to identify patients most suitable for *MEN1* mutation testing.

METHODS

Patients

The index cases included in this report were referred from the Diabetes and Endocrinology Clinic of the Princess Alexandra Hospital or from clinicians throughout Australia and New Zealand. Clinical information was accessed from medical charts with informed consent or confirmed by the referring clinician after the appropriate radiological, biochemical, or histological analysis. Patients were categorised as having persistently elevated hormone levels and tumours of the parathyroid, pituitary, endocrine pancreas, gastrin cells, thymic or bronchial carcinoids, or other miscellaneous tumours of adrenal, adipose, or thyroid origin. Patient consent and ethics approval were obtained locally by the referring clinician.

Abbreviations: FHPT-JT, familial hyperparathyroidism and jaw tumour syndrome; FIHP, familial isolated hyperparathyroidism; MEN 1, multiple endocrine neoplasia type 1

Data for this report was obtained retrospectively, and as such is subject to the biases of the referring clinicians. Samples from patients with sporadic tumours without any other indication of a germline mutation (such as parathyroid tumours presenting at a young age or multigland involvement) were not received. This report does not include results of predictive testing performed in families after a *MEN1* mutation was found in an index case, as it does not add to the aim of this report.

Mutation analysis

DNA extraction and MEN1 gene amplification

DNA was extracted from peripheral blood leucocytes using the method of Miller *et al*.[11] The nine exons of the coding sequence of menin were amplified using PCR as described previously.[10] The primers for the promoter region and each of the exons have been published previously.[8 12] Reactions were performed in a volume of 50 µl and contained 10 mmol/l Tris-HCl, 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 250 µmol/l dNTPs, 20 pmol of each primer, 5% DMSO, 0.5 U Ampli*Taq* Gold (Perkin Elmer) and approximately 50 ng of template DNA. Cycling conditions were: 94°C for 10 minutes, followed by 35 cycles of 94°C for 1 minute, 62°C for 1 minute, (the annealing temperature for the promoter region and exon 10 was 60°C, for exon 3 was 58°C, and for exon 2 was 64°C) and 72°C for 1.5 minutes. PCR products were purified using High Pure PCR Product Purification kit (Roche), following the manufacturer's instructions.

DNA sequencing

Reaction conditions for cycle sequencing were as follows: 200–300 ng of PCR product, 2.5 pmol primer and 4 µl of Big Dye Terminator (version 2) mix or 2 µl of Big Dye Terminator (version 3.1) (both PE Applied Biosystems) were combined in a 10 µl reaction volume. The primers used were the same as for PCR. Cycling conditions were 25 cycles of 96°C for 30 seconds, 55°C for 15 seconds, and 60°C for 4 minutes. Amplification products were precipitated with 120 µl 70% isopropanol, washed with 70% isopropanol, and allowed to dry before being gel separated on an ABI377 or a ABI3730 automated sequencer at the Australian Genome Research Facility, Brisbane. Heterozygous base changes were confirmed by resequencing an independent PCR product. Sequencing chromatographs were confirmed by two independent researchers.

Microsatellite genotyping

Genotyping using the microsatellites PYGM, D11S4909, D11S4938, and D11S4946 was performed using PCR in a total volume of 10 µl containing 50–100 ng genomic DNA, 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 250 µmol/l each dATP, dTTP, and dGTP, 25 µmol/l dCTP, 1 µCi α-^{32}P dCTP, 5 pmol of each primer, and 0.5 U Ampli*Taq* Gold (Perkin Elmer). Microsatellite primers have been previously described.[12 13] Reactions were amplified using the following conditions: 94°C for 10 minutes, followed by 30 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1.5 minutes. PCR products were separated by polyacrylamide gel electrophoresis, followed by autoradiography. In addition, the single nucleotide polymorphism D418D in exon 9 of the *MEN1* gene was used to exclude a founder effect in some families.

RESULTS

MEN1 mutation analysis

Of the 150 patients analysed by our service, we found 55 had *MEN1* mutations; 42 of the mutations were distinct and six occurred more than once (tables 1, 2). We found that 249–253delGTCT occurred three times, 1268G→A and 1378C→T both occurred twice, 1546–1547insC occurred seven times, 1546–1547delC occurred three times, and 1548–1549insG occurred twice. In order to determine if there were any common founders in the remaining families, we looked for allele sharing at the *MEN1* locus in families that shared the same mutation (table 2). Using the microsatellite markers D11S4909, PYGM, D11S4946, and D11S4938, and the D418D single nucleotide polymorphism, we were unable to exclude the possibility of a founder effect in five of the families with a 1546–1547insC mutation and two families with the 1548–1549insG mutation. Upon further investigation we were able to trace all of the five families that shared the 1546–1547insC mutation back to a common founder, resulting in a pedigree of eight generations. This extended family is now being phenotypically characterised.

We tested 85 index cases with a family history of MEN1 related disease including 23 cases with FIHP, 49 cases with a family history of hyperparathyroidism and one other MEN1 related tumour, 7 cases with a family history of pituitary tumour and neuroendocrine tumours, and 6 cases with familial isolated pituitary tumours (fig 1). We found a *MEN1* mutation in 41/49 (84%) of patients with a family history of hyperparathyroidism with at least one other MEN1 related condition. Family members of the eight MEN1 families without *MEN1* mutations are now being recruited so that haplotyping of the MEN1 locus can be performed. All the cases with a family history of MEN1 related disease and a *MEN1* mutation had a family history of hyperparathyroidism. We found *MEN1* mutations in five of the 23 (22%) FIHP families. Multiglandular parathyroid hyperplasia was reported in all five FIHP cases with *MEN1* and 11 of the 18 FIHP cases without *MEN1* mutations. Of the remaining seven cases, a single adenoma was reported in six cases and in one case, two adenomata were reported. We did not find *MEN1* mutations in the 13 cases with a family history of MEN1 related tumours without familial hyperparathyroidism.

Of the 65 patients tested who lacked any family history of MEN1 related disease, 50 had hyperparathyroidism and at least one other MEN1 related condition, and 11 had sporadic hyperparathyroidism. We found that 8/50 patients (16%) with sporadic hyperparathyroidism and one other MEN1 related tumour had *MEN1* mutations. In one of these cases we tested both parents and could not find a mutation in either parent. We found that 1/11 cases (9%) with sporadic hyperparathyroidism had a *MEN1* mutation only. This patient was diagnosed at 20 years of age. *MEN1* mutation testing on both parents of this individual proved negative. Of the 10 cases of sporadic hyperparathyroidism without *MEN1* mutations, four cases were diagnosed before the age of 20 years and the remainder were cases with multiglandular parathyroid tumours (age range 50–66 years).

Analysis of patients without MEN1 coding region mutations

It is conceivable that mutations within the promoter could alter expression of the *MEN1* gene, so we analysed 524 bp of the promoter and 5'UTR (corresponding to the sequence between nucleotide 1234 and 1758 of Genbank accession no. U93237) in all patients who tested negative for mutations in the coding region. We found no sequence variations within this portion of the *MEN1* gene promoter in any patient.

DISCUSSION

The data presented in this report support other studies showing that mutations within the *MEN1* gene are scattered throughout the gene and in some cases have not been previously described.[2 4 6 8 10 32–38] Consequently, testing for *MEN1* mutations is a costly and time consuming process. In order to establish reasonable mutation testing criteria, we reviewed clinical features of all the index cases with *MEN1*

Table 1 Clinical features of probands and families found to have MEN1 mutations

Family	Clinical risk features† of proband	MEN1related tumours† of family members	Mutation	Effect on protein	Reference
1	P, PI, FH	P, PA	Deletion of exon 1 and 2	Loss of start site	8
2	P, FH	P	13insACGCTdelGCC	Frameshift	
3	P, G, FH	PI, G	74del15	Frameshift	
4	P, PI, PA, FH	P, PI, PA, C	249–253delGTCT	Frameshift	8, 10, 4
5	P, FH	P, PI, PA	249–253delGTCT	Frameshift	8, 10, 4
6	P, PI, FH	P, PI, PA, G	249–253delGTCT	Frameshift	8, 10, 4
7	P, FH	P	255ins19	Frameshift	8§
8	P, PI, FH	P, PI.PA, C	269–271delAT	Frameshift	4
9	P, G, C, FH	P, G, C	322C→T	R108X	4
10	P, PA, FH	P, PA	404–405delA	Frameshift	
11	P, PI, PA, FH	P, PI, PA, G, C, L	IVS2–3C→G	Splicing error	10
12	P, PI, PA		571G→T	E191X	4
13	P, FH	P	590C→T	T197I	
14	P, PI		625–629delCAGA	Frameshift	
15	P, FH	P, PI	631–635delGTCA	Frameshift	
16	P, PI, FH	P, PI, PA, C	IVS3+1G→T	Splicing error	10
17	P, C, FH	P, PA, C, L	660G→A	W220X	8
18	P, FH	G, C	686G→T	R229L	4, 8, 10
19	P, PI, PA, G, FH	P, G	IVS4+1G→T	Splicing error	10
20	P, PA, G		772C→T	Q258X	9
21	P, C, FH	P, G	851C→A	A284E	
22	P, PI, PA		IVS5+1G→A	Splicing error	33
23	P, PI, FH	P, G	1010C→A	A337D	34
24	P, G, A, FH	P, G	1056T→G	Y353D	
25	P, FH	P	1057–1060delACT	Y353del	
26†	P, PI, G, FH	P	1117C→T	P373S	8
27	P, PI, G, FH	P, PI	1227C→A	C409X	
28	P, PI, G, C, FH	P, PI	1244G→C	R415P	8
29	P, FH	P, PI, G, C	1252G→C	D419H	
30	P, C, FH	P, PI, PA, C	1268G→A	W423X	8
31	P, PA, C, FH	P	1268G→A	W423X	8
32	P, PA, FH	P, C	1304–1305delG	Frameshift	35
33	P, PI, G, C, FH	P, G, L	1342-IVS9+2del	Frameshift	36
34	P, PI, PA, A		1378C→T	R460X	4, 6, 8
35	P, PA, G, C		1378C→T	R460X	4, 6, 8
36	P, PI, G, C, FH	?	1410C→T	Q450X	37
37	P, G, FH	P, G	IVS9del-13-+1	Splicing error	
38	P, PI, PA, FH	P, PI	1546–1547delC	Frameshift	4,6,34
39	P		1546–1547delC	Frameshift	4,6,34
40	P, PA, PI, FH	P, PI	1546–1547delC	Frameshift	4,6,34
41‡	P, PI, PA, FH	P, PI, PA, G, C, L	1546–1547insC	Frameshift	4,6,8,10,34
42	P, FH	P	1546–1547insC	Frameshift	4,6,8,10,34
43	P, PI, PA, FH	P	1546–1547insC	Frameshift	4,6,8,10,34
44	P, FH	P, G	1548–1549insG	Frameshift	
45	P, PA, FH	P	1548–1549insG	Frameshift	
46	P, PA		1556–1557insT	Frameshift	37
47	P, PI, PA		1579C→T	R527X	10
48	P, PI, FH	P, PA	1590delA, 1592G→C	Frameshift	38
49	P, PA, G, L, FH	P, G, L	1688A→T, 1693–1694delCT	Frameshift	
50	P, L, FH	P, PI, G, L	1701delC	Frameshift	

P, hyperparathyroidism; PI, pituitary tumour; PA, endocrine pancreatic tumour; G, gastrinoma or Zollinger-Ellison syndrome; C, carcinoid; L, lipoma; A, adrenal tumour/hyperplasia. The nucleotide numbering begins at the A of the ATG of the initiator Met codon as per recommendations of the Nomenclature Working Group.[39] †Family 26 was originally found as two separate families but subsequently found to be related. ‡Family 41 was originally found as five separate families but subsequently found to be related. §This mutation was previously incorrectly reported as 256ins18.[8]

mutations and found that they all had hyperparathyroidism or a family history of hyperparathyroidism. In addition, 22% of FIHP index cases had a *MEN1* mutation. We did not detect any MEN1 mutations in seven cases with a family history of pituitary tumour and neuroendocrine tumours nor in six cases with familial isolated pituitary tumours. This finding supports previous studies showing a lack of *MEN1* mutations in cases with familial isolated pituitary tumours.[10] From these data, we conclude that all patients with familial hyperparathyroidism should be tested for *MEN1* mutations.

In this report we found that 16% of apparently sporadic cases of hyperparathyroidism and one other tumour had a *MEN1* germline mutation. In one of these cases we were able to demonstrate that the mutation was de novo. However as MEN1 is reported to be 100% penetrant by 60 years of age, it is likely that the remaining cases are also de novo mutations. Previous studies have reported a de novo rate of approximately 10%.[4 9] As the numbers reported in this study are subject to the biases of the referring clinicians, we cannot compare our findings with others.

Interestingly, we found a *MEN1* mutation in 1/11 cases (9%) with sporadic hyperparathyroidism. A recent study reported *MEN1* germline mutations in 5% of apparently sporadic hyperparathyroidism patients.[14] Although our data support this finding, the numbers tested in this report are not enough to make a definite conclusion with regard to testing criteria. In addition, the patients included in our report had either multiglandular disease or hyperparathy-roidism at a young age and thus are a biased sample population of patients with sporadic hyperparathyroidism. Further investigations are needed to determine the *MEN1* mutation detection rate of patients with sporadic hyperparathyroidism.

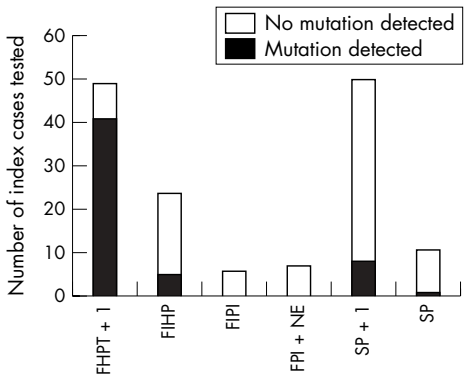

Figure 1 *MEN1* mutations detected in index cases with MEN1 related conditions. The index cases tested are grouped as familial hyperparathyroidism plus one other MEN1 related condition (FHPT+1), familial isolated hyperparathyroidism (FIHP), familial isolated pituitary tumours (FIPI), family history of pituitary tumour and a neuroendocrine tumour (FPI+NE), sporadic hyperparathyroidism plus one other MEN1 related condition (SP+1), and sporadic hyperparathyroidism (SP).

Because of the costs involved, MEN1 mutation testing on all cases of primary hyperparathyroidism is impractical. We therefore suggest that *MEN1* mutation testing be performed on patients with multiglandular parathyroid hyperplasia that present at a young age, as the typical age of presentation of hyperparathyroidism in MEN1 patients is 20–25 years old, 30 years younger than the majority of patients with hyperparathyroidism.[15 16] All FIHP patients with a *MEN1* mutation in this report had multiglandular disease, and the age of diagnosis for the single case of primary hyperparathyroidism with a germline MEN1 mutation was 20 years old. In support of this, Langer *et al*[17] recently reported a similar finding with 2/15 cases of apparently sporadic multiglandular hyperparathyroidism having *MEN1* germline mutations. We also recommend that serum calcium and parathyroid hormone be routinely measured in the families of all primary hyperparathyroidism patients that have multiglandular disease, as a number of our familial hyperparathyroidism probands presented without apparent family history. However, upon further investigation by the referring clinician, a family history of hyperparathyroidism was established. Therefore we suggest that biochemical studies of additional family members in apparently sporadic cases of hyperparathyroidism should always precede more expensive *MEN1* mutation testing.

This is the first large study to investigate the involvement of a region reported[18] to be the *MEN1* promoter in *MEN1* gene mutation negative patients with MEN1-like syndromes. To date, no germline or somatic *MEN1* promoter mutations have been reported.[19–21] This finding does not exclude mutations within other untranscribed or untranslated regions of the gene or large deletions of entire exons contributing to the disease. We report eight families with a family history of hyperparathyroidism and one other MEN1 related tumour. Future haplotype studies in these families will be of interest.

Hyperparathyroidism is the most common and earliest presenting MEN 1 related condition. Over 95% of patients with *MEN1* gene mutations have hyperparathyroidism.[22 23] It is not surprising that we found that all of our mutation positive families involved multiple cases of hyperparathyroidism and that we were able to detect a *MEN1* gene mutation in 5/23 of patients with FIHP. Familial hyperparathyroidism is also a common syndrome in familial hyperparathyroidism and jaw tumour syndrome (FHPT-JT). Mutations in the *HRPT2* gene have recently been identified in a large percentage of families with FHPT-JT.[24 25] However, mutations in *HRPT2* appear to be uncommon in FIHP families.[27] In contrast, the presence of inactivating mutations of the calcium sensing receptor has been reported in 14–18% of familial FIHP cases.[26 27]

Approximately 25% of gastrinoma cases have MEN1 syndrome. To date there have been no large studies investigating the presence of germline *MEN1* mutations in familial isolated gastrinoma cases, which probably reflects the rare occurrence of this syndrome without the presence of hyperparathyroidism. Somatic *MEN1* mutations have been reported in approximately one third of sporadic gastrinomas, and there has been one report of a germline *MEN1* mutation in a patient with no family history of MEN1.[28–30] With the exception of insulinomas, many clinicians believe surgery is contraindicated in MEN1 patients with enteropancreatic lesions, and so the finding of a germline *MEN1* mutation can significantly change the surgical management of a patient. It has therefore been suggested that consideration should be given to *MEN1* mutation testing of sporadic cases of Zollinger-Ellison cases.[2] Further investigations of *MEN1* detection rates in sporadic Zollinger-Ellison syndrome are warranted.

Recently, in situ immunofluorescence analysis of neuroendocrine tumours showed a lack of menin expression in a case with a germline mutation in *MEN1* and loss of the wild type allele.[31] Although this was only a pilot study, it raises the possibility of an alternative and possibly more cost effective method of testing for *MEN1* mutations in sporadic cases of hyperparathyroidism and Zollinger-Ellison syndrome.

In conclusion, our data indicate that routine germline *MEN1* mutation testing by national testing services of all cases of ''classical'' MEN1, familial hyperparathyroidism, and sporadic hyperparathyroidism with one other MEN1 related condition is justified. However, routine testing of the promoter region between nucleotide 1234 and 1758

Table 2 *MEN1* gene locus haplotype analysis of families with recurrent *MEN1* gene mutations

	Haplotypes															
	249–253delTCT			1546–1547insC							1546–1547delC		1548–1549insG		1268G→A	
Family no.	4	5	6	41	41	43	41	41	41	42	38	40	44	45	30	31
D11S4909	5/9	1/3	8/8	9	9	5/7	9	9	9	3/3	4/8	9/9	4/9	4/4	7/9	1/10
PYGM	8/9	11	7/8	7	7	3/9	7	7	7	1/2	8/8	8/10	1/6	3/4	14/14	4/5
MEN1 D418D	T	C	–	–	–	–	–	–	–	–	–	–	T	C/T	–	–
D11S4946	4/5	4/5	2/2	4	4	2/6	4	4	4	4/4	2/2	5	4	4/5	1/3	5
D11S4938	1/3	3/3	3/3	1	1	1/3	1	1	1	2/3	1/1	1/5	3/3	1/3	1/3	3/3

The proband of each family was typed for the microsatellite markers D11S4909, PYGM, D11S4946, and D11S4938, and in the case of families 3 and 4, for the D419D single nucleotide polymorphism in exon 9 of *MEN1*. For probands sharing the same mutation and at least one microsatellite allele, only the affected allele is shown. In some cases, families with more than one affected family member were genotyped, allowing the identification of the linked allele. The linked allele was determined by looking for allele sharing between affected individuals within each of the families (data not shown). Patients with sporadic MEN1 were not genotyped, including one patient with 1546–1547delC and the two patients with 1378C→T.

(Genbank accession no. U93237) is not recommended, as this region does not account for the proportion of MEN1 cases that lack coding sequence and splice site mutations. Although our data are not conclusive and more investigations are warranted, we also suggest that testing of patients who develop sporadic hyperparathyroidism with multigland hyperplasia before the age of 30 years be considered. Further studies are recommended to assess the justification of screening patients with Zollinger-Ellison syndrome without a family history of MEN1.

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