CLINICAL STUDY

MEN1 gene mutation analysis in Italian patients with multiple endocrine neoplasia type 1A Morelli, A Falchetti, V Martinetti, L Becherini, M Mark¹, E Friedman¹ and M L Brandi*Endocrine Unit, Department of Clinical Physiopathology, University of Florence, Viale Pieraccini 6, 50139, Florence, Italy and ¹Susanne-Levy-Gertner Oncogenetics Unit, Chaim Sheba Medical Center, Tel Hashomer, 52621, Israel**(Correspondence should be addressed to Maria Luisa Brandi; Email: m.brandi@dfc.unifi.it)***Abstract**

Multiple endocrine neoplasia type 1 (MEN 1) is a familial syndrome characterized by parathyroid, enteropancreatic and pituitary tumors. The gene responsible for this syndrome is localized at chromosomal 11q13 region and DNA markers from this region cosegregate with the disease. The recent identification of the *MEN1* gene, encoding for a protein termed menin of 610 amino acids, allowed mutational screening to be performed both in affected families and sporadic cases. To date many different heterozygous mutations, spreading across all the encoding sequence, have been identified in MEN 1 patients with no apparent mutational hot spots or genotype–phenotype correlation. To analyze the genetic alterations of the *MEN1* gene occurring in Italian patients we performed mutational screening by Denaturant Gradient Gel Electrophoresis followed by sequencing of exons 2–10 of the *MEN1* gene in 27 Italian MEN 1 families and in five sporadic cases. We identified 17 different heterozygous mutations in 60% of analyzed cases. Twelve of these mutations are novel. Two mutations each occurred twice in unrelated families but no evidence of genotype–phenotype correlation can be established for these families. The extension of genetic diagnosis to asymptomatic family members allowed the identification of 10 *MEN1* mutant gene carriers, one newly described and nine previously detected by linkage analysis with DNA markers from the 11q13 region. Our findings add new information to the diversity of mutations occurring in the *MEN1* gene and confirm that the mutational screening of MEN 1 is a useful approach to detect individuals at higher risk of developing MEN 1-associated tumors.

*European Journal of Endocrinology 142 131–137***Introduction**

Multiple endocrine neoplasia type 1 (MEN 1) syndrome is characterized by the combined occurrence of neoplastic lesions of the parathyroid glands, the neuroendocrine cells of the gastroenteric tract, and the anterior pituitary. To a lesser extent, other endocrine and non-endocrine tissues can be affected: carcinoids, lipomas, pinealomas, adrenocortical and thyroid follicular tumors and spinal cord ependymomas are also associated with MEN 1 (1, 2).

The clinical picture for MEN 1 syndrome is largely dependent on the involved glands and on the presence of the type of hormonal hypersecretion. With increasing age all of the three tissues can be affected, but primary hyperparathyroidism is the most common endocrinopathy (87–97%) in MEN 1 and often the first manifestation (1). Hypersecretion of other involved organs, such as the endocrine pancreas and the anterior pituitary are respectively present in 32–75% and 16–40% of cases (3, 4). However, any of the MEN 1 lesions

could well be the first one to become clinically detectable (1). The prevalence of MEN 1 has been estimated as 1/30 000–1/50 000 on the basis of autopsy and epidemiological studies (5), but it might be underestimated because of the possibility of misdiagnosis. Thus MEN 1 syndrome may be defined as an unrecognized more than an uncommon disease (6).

MEN 1 tumors may either be inherited or they may occur sporadically (i.e. without an apparent family history), even though distinction between sporadic and familial cases may sometimes be difficult. The disorder is inherited with an equal sex distribution according to an autosomal pattern of inheritance and the offspring of a MEN 1 patient have a 50% of chance of inheriting the disease. The age of onset ranges from 6 to 81 years and most individuals carrying the predisposing genetic defect may escape clinical manifestations for several decades. Thus, when only clinical screening is performed, it is difficult to exclude family members from being at risk and children of unaffected individuals at risk should be included in the screening programs.

The *MEN1* gene has been localized by genetic-mapping studies to chromosome 11q13 (7, 8) and loss of the wild-type copy of the inherited allele at this chromosomal region in the majority of MEN 1-associated tumors had implied that the *MEN1* gene represents a putative tumor suppressor gene (7). Linkage analysis in MEN 1 families using DNA markers from the 11q12–13 region has been an extremely important tool for many years in order to perform genetic screening on each first degree family member.

Recently, the *MEN1* gene was identified by positional cloning. It contains one untranslated exon and nine exons encoding a 610 amino acid protein, termed menin (9). This protein, which exhibits no apparent similarities to any already known protein, shows a nuclear localization (10). Regarding its function, very recent studies identified the transcription factor JunD as a direct menin-interacting partner, the menin protein being a tumor suppressor via direct binding to JunD and inhibition of JunD activated transcription (11). Germ-line mutations of the *MEN1* gene was identified in most of the MEN 1 families tested. Evidence is accumulating that suggests that menin function is likely lost in some sporadic neoplasias involving the same tissues affected in the hereditary MEN 1 syndrome (12–14). All the observed mutations spread across the entire coding region, suggesting the lack of obvious mutational hot spot or correlation between genotype and clinical phenotypes (9, 15–21). The majority of mutations predict premature protein truncation due to either nonsense or frameshifting type mutations. The frequent protein truncation mutations likely cause gene inactivation, consistent with the first mutational event according to the 'two hits' hypothesis for tumorigenic mechanism by a tumor suppressor gene (7).

Here we report the results of mutational analysis of the *MEN1* gene in 27 Italian unrelated MEN 1 families and in five isolated MEN 1 patients, all collected through the Italian Register of the Multiple Endocrine Neoplasias (RINEM) (22).

Materials and methods

Patients

Familial and sporadic cases were identified through RINEM including to date 27 MEN 1 families and 24 sporadic MEN 1 patients without apparent family history of the disease. The familial form of MEN 1 was defined as that with at least two members demonstrating hypercalcemia in addition to at least one member with evidence of pituitary or endocrine pancreatic–duodenal tumor. Informed consent was obtained from all the patients. Mutational analysis was performed in a total of 56 individuals from the 27 MEN 1 families: 36 affected subjects and 20 unaffected relatives. Additionally, five of the 24 sporadic cases were available for mutational screening. Thirteen of the 27 families,

including the single Italian MEN 1 family in which both parents are affected, had previously been analyzed by genetic linkage using polymorphic DNA markers from the *MEN1* chromosomal 11q12–13 region (23, 24).

Polymerase chain reaction

Genomic DNA was extracted from peripheral blood samples using standard methods. For each analyzed patient 0.2–0.5 µg of DNA was used as a PCR template for the amplification of the entire *MEN1* coding sequence using the Expand Long Template PCR system of Boehringer Mannheim (Indianapolis, IN, USA) according to the manufacturer's instructions. The following forward and reverse primers were used for Long-PCR: F, 5'-CTCATAACTTGCCGACCGACCCGTGACAG-3'; R, 5'-GAGGTGAGGCCTGTCCCCTTTGGGCTGGGG-3'. The PCR product was used in a series of nested PCRs to obtain 14 different fragments containing the region of interest (i.e. the exon and its flanking intronic sequences). For the specific nested fragments thermal cycling was performed in a Helix thermal cycler (Diatech, Ancona, Italy). An initial 94 °C denaturation step for 5 min was followed by 35 cycles of denaturation at 94 °C (1 min), annealing step at the specified temperature (Table 1) and extension step at 72 °C (1 min 30 s), with a final extension step of 5 min at 72 °C. PCR primer sequences and annealing temperature are shown in Table 1. For all PCRs the reaction volume was 50 µl containing 50 mmol/l KCl, 10 mmol/l Tris–HCl (pH 8.3), 1.5 or 2.5 mmol/l MgCl₂, 0.2 mmol/l of each deoxynucleotide, 30 pmoles of each upstream and downstream primer, 1 unit of Taq DNA polymerase (Boehringer Mannheim).

Denaturant gradient gel electrophoresis analysis

The theoretical melting profiles of the 14 amplified fragments were generated using the computer algorithm of Lerman and co-workers (25). The melting map predicted that in all cases the region of interest of the amplified PCR product would be contained in a single, low-melting domain. Hence, mutations that occur within those regions are likely to be detected by denaturant gradient gel electrophoresis (DGGE). Denaturant gradients for each fragment were empirically optimized (Table 1). Parallel denaturant gradient gels were cast and run using the D-Gene apparatus (Bio-Rad, Hercules, CA, USA) according to the method of Myers and co-workers (26). Six to 20 of the exon specific PCR were denatured at 95 °C for 3 min, allowed to form heteroduplexes for 1 h at room temperature and loaded onto the gel. Electrophoresis was achieved for 15 h at 44 V or for 4 h at 160 V in 58 °C on a 9% acrylamide gel with increasing denaturant concentrations (Table 1). Gels were stained with ethidium bromide and visualized under u.v. light.

Table 1 PCR primers and DGGE conditions.

Exon	PCR		DGGE conditions	
	Primer sequences	Annealing (°C)	Denaturant gradient (%)	Time (h)
2.1	F: GTGGAACCTTAGCGGACCCT R: GCclamp-AAGGAAAGGAGCACCAGGTC	58	50–80	4
2.2	F: CGCCTGTTTGCTGCCGAGCT R: GCclamp-TTCTTCACCAGCTCACGGCT	58	50–80	15
2.3	F: CGACCTGTCCCTCTATCCTC R: GCclamp-GGGTTTTGAAGAAGTGGGAG	62	40–70	15
3	F: GCclamp-CTGTTAAAGCACAGAGGACC R: CAAGGCTGGGGGGAGGGAAAC	60	50–80	15
4	F: GCclamp-GAGACATAATGATCTCATCC R: AAGTCTGGCCAGCCCAGTC	64	40–70	15
5	F: GCclamp-CGCTCATAACTCTCTCCTTC R: AGCCCCCGGCCTCACCA	58	40–70	15
6	F: GCclamp-GGGTGGCAGCCTGAATTATG R: CACTGTTAGGGTCTCCCTTC	58	50–80	15
7	F: GCclamp-GATCCTCTGCCTCACCTCCA R: GAGGGGAAGAAAGGACAGGC	62	40–70	15
8.1 ^a	F: GCclamp-CGACCCTACAGAGACCCAC R: GCCCGCTCCAGCAAGCTGG	60	40–85	4
8.2 ^a	F: CAACCTGCTGAAGGAGGCAG R: GCclamp-CCATCCCTAATCCCGTAC	60	30–70	4
9	F: ATCTGTGCCCTCCCTTCCCC R: GCclamp-CGCTCACCACCTGTAGTGCC	58	50–80	15
10.1 ^a	F: GCclamp-GCAACCTTGCTCTACCTTG R: TCCAGTGCTGGCTTCTTGGG	64	50–80	4
10.2	F: GCclamp-CGGCCGCTAGTGAGCCGAGA R: GAAAGTGAGCACTGGACCCCTC	58	60–90	15
10.3	F: AGCACCCGCAGCATCACAC R: GCclamp-CCCACAAGCGGTCCAAGAAGTC	60	50–80	15

^aDirect sequencing of this fragment is more reliable.

DNA sequencing

PCR products exhibiting an abnormal electrophoretical run and therefore suspected of harboring mutations were sequenced. One microliter of the long-range PCR product was amplified by nested PCR using primer pairs containing a tail complementary to the labeled primers used for the sequencing reaction. Sequencing standard primers M13universalCS(-43) and M13reverse(-49) were labeled by fluorochrome IRD-800 (BioSense, Milan, Italy). The sequencing reactions were performed according to the cycling system protocol on LI-COR DNA Sequencer 4200 (LI-COR Inc., Lincoln, NE, USA) using the SequiTherm Excel II DNA sequencing kit (Epicentre Technologies, Biospa, Milan, Italy). Confirmation of the sequence data was carried out in the families to demonstrate segregation of the mutation with the disease. Whenever a mutation resulted in an altered restriction site, the appropriate enzyme was used. Ten microliters of the specific PCR product were digested by the appropriate restriction endonuclease (Boehringer Mannheim) according to

the manufacturer's recommendations, run on a 2% agarose gel at 100 V for 2–3 h and visualized with ethidium bromide. In the cases where the mutation did not affect a restriction site the mutated fragment was sequenced in both affected and unaffected family members.

Results

A total of 17 different heterozygous germ-line mutations were identified in 15 of 27 (56%) MEN 1 families and in four of five (80%) sporadic cases. These different mutations spread across most of the nine translated exons (Fig. 1) and consisted of three nonsense, three missense, two splicing, two insertional and six deletion frameshift mutations, and one intronic deletion (Table 2). Twelve of the 17 described mutations are novel, the remaining five (359del4, Q450X, 738del4, T344R, R415X) have been previously described in MEN 1 patients from other countries (9, 15, 17, 20, 21). Regarding the two insertional frameshift, 311ins5

Figure 1 Schematic representation of the mutations identified in the *MEN1* gene. Exon 1, the 5' part of exon 2 and the 3' part of exon 10 are untranslated (black boxes). The start (ATG) and the stop (TGA) codons are indicated within exon 2 and exon 10 respectively. The locations of the mutations are shown above (deletions and insertion) and under (missense, nonsense and splicing) the gene structure. Mutation nomenclature is the same as that in Table 2.

Table 2 *MEN1* mutations from Italian *MEN 1* families and sporadic cases.

Family (F)/ Individual (S)	Exon	Codon	Mutation ^a	Description of mutation	Method of detection
S03	2	45	E45K ^b	Missense, Glu → Lys, GAG → AAG	DGGE
F22 F26	2	68,69	311ins5(GCCCC) ^b	Frameshift, 52 missense aa stop	DGGE, 4% Agarose
F13	2	83,84	359del4(GTCT)	Frameshift, 32 missense aa stop	DGGE, 4% Agarose
F05	3	210,211	738del4(ACAG)	Frameshift, 12 missense aa stop	DGGE, 4% agarose
F19	3	215	V215M ^b	Missense, Val → Met, GTG → ATG	DGGE
S02	Intron 3		765-4delT ^b	Intronic mutation	DGGE
F02	4		765-1(G → C) ^b	Splice acceptor site	DGGE
F10	4		893 + 1(G → A) ^b	Splice donor site	DGGE
S05b	7	317	1059delC ^b	Frameshift, 50 missense aa stop	DGGE
F28	7	321	1071delT ^b	Frameshift, 46 missense aa stop	DGGE
F21	7	344	T344R	Missense, Thr → Arg, ACG → AGG	DGGE, Cfr I
F01	8	385	1264delC ^b	Frameshift, 59 missense aa stop	Sequencing
S04	8	386	1267delG ^b	Frameshift, 58 missense aa stop	Sequencing
F07	9	415	R415X	Nonsense, Arg → Stop, CGA → TGA	DGGE
F27	9	450	Q450X	Nonsense, Gln → Stop, CAG → TAG	DGGE
F04 F17	10	482	1555insG ^b	Frameshift, 45 missense aa stop	Sequencing
F23	10	508	Q508X ^b	Nonsense, Gln → Stop, CAG → TAG	DGGE, Mae I

^a Mutation nomenclature follows standard criteria (27).

^b Novel mutations.

(exon 2) and 1555insG (exon10), each occurred twice in different unrelated families. The other mutations were unique and involved 6/9 of the translated exons, and splice-junction sites (donor and acceptor) flanking exon 4. No mutation was found in 12 families and in one sporadic case neither by DGGE screening nor full sequencing.

Previous genetic linkage studies performed in 13 of the 27 MEN 1 families analyzed here showed cosegregation of DNA markers from the 11q12–13 region with the disease (23, 24). In ten of these families mutation analysis confirmed the presence of mutation in the affected members as well as confirmed the genetic diagnosis of ten asymptomatic mutated gene carriers. In the remaining three families no mutation was found, although linkage to the 11q13 region could not be excluded. In addition to these linkage data, one asymptomatic carrier was identified by direct mutational screening in one family (F23) where linkage analysis has not been performed as only DNA from one affected individual was available. By DGGE followed by sequencing we identified a novel nonsense mutation in exon 10 for this patient. The mutation truncates the protein at 508 amino acid and creates a new Mae I restriction site. Enzymatic digestion of the available DNA from other family members allowed identification of one asymptomatic *MEN1* mutant gene carrier. Mutation analysis was also performed in the single Italian MEN 1 family (F05) in which both parents are affected (23). We confirmed the presence of the previously described

frameshift mutation 738del4ACAG (21) in the affected mother and in the other affected members on her side in the pedigree and in all of three siblings using heteroduplex analysis on 4% agarose gel (Fig. 2). No mutation was found in the affected father and in his affected sisters, neither by DGGE nor full sequencing, although linkage to the *MEN1* region has been clearly established allowing identification among the three siblings of one heterozygote and two homozygote individuals for the disease gene (23).

Discussion

We report the screening for germ-line mutations of the *MEN1* gene in 27 MEN 1 families and five isolated MEN 1 cases with no apparent family history of the disease. We detected mutations in about 60% of all cases (15 families and four sporadic cases). When the DGGE screening was negative we performed full sequencing in order to eliminate mistakes due to an insufficient DGGE sensitivity. Only three additional mutations were so identified while 14 mutations were detected by DGGE analysis. On the basis of these data the method described here for mutation screening shows sufficient accuracy and reliability in order to perform genetic tests in MEN 1 families (Fig. 3). However negative DGGE cases should undergo direct sequencing. Mutations were also found in four of five isolated cases in which no apparent family history of MEN 1 could be found. This rate of detection makes mutation analysis of the *MEN1*

Figure 2 Heteroduplex analysis in the single MEN 1 family (F05) where both parents are affected (23). Electrophoresis on 4% agarose gel of exon 3 PCR product for each family member is shown. Filled circles and boxes indicate affected individuals. Lanes 6–10 exhibit the presence of heteroduplex DNA fragments caused by the 738del4 mutation (Table 2).

Figure 3 Examples of six *MEN1* mutations detected by DGGE analysis. Each lane represents the electrophoretical migration of different samples. Four exons from the *MEN1* gene are shown: (a) exon 4, heteroduplexes are visible in lanes 2 and 5, sequencing of these two samples confirmed the presence of two different splice site mutations (Table 2); (b) exon 2, one heteroduplex in lane 7, sequencing revealed the mutation E45K (Table 2); (c) exon 3, one heteroduplex in lane 1 caused by mutation V215 M (Table 2); (d) exon 9, this image shows the known polymorphism D418D due to a C → T change (15), homoduplex C/C in lanes 1, 3, 6, 8, 11, 15 and 16; homoduplex T/T in lanes 2, 7, 12 and 17; heteroduplex C/T in lanes 5, 9, 10, 13 and 14; samples in lanes 4 and 18 were suspected to harbor mutation since they show a distinguishable electrophoretical pattern, sequencing confirmed the presence of two different nonsense mutations (Table 2). DGGE analysis allowed detection of 82% of the *MEN1* mutations.

gene worthwhile in isolated cases. All the isolated cases had two or more of the MEN 1-related features. DNA from parents of the analyzed sporadic cases were not available, so we cannot confirm the existence of *de novo* mutations for these patients.

Previously published data described that between 5 and 20% of clinically proven MEN 1 patients may not harbor identifiable mutations in *MEN1* coding sequence (9, 15, 19–21). In our study no mutation was found in 12 families (40%) and in one of the affected parents for the F05 family where a different mutation in each parent could be expected. It is possible that the mutations in all these cases lay in regulatory or untranscribed regions of the *MEN1* gene which are undetectable by our methods of screening. Another possibility is the occurrence of germ-line heterozygous large deletion of the entire *MEN1* gene. More likely, different genetic defects other than those of the *MEN1* gene might be responsible. In three of these 12 mutation-negative families, as well as in the F05 family, previous linkage studies have demonstrated cosegregation between *MEN1* and closely flanking polymorphic markers from the 11q13 region. This makes it difficult to exclude the involvement of the *MEN1* gene. Recent studies identified the transcription factor JunD as a direct menin-interacting partner. Menin's tumor suppressor function involves direct

binding to JunD and inhibition of JunD-activated transcription (11). We can speculate that alterations in the pathway activated by the menin–JunD interaction may be responsible for the same effects of an altered *MEN1* product, suggesting the hypothesis of the existence of phenocopies. Alternatively, alterations of the transcription factor JunD may interfere with the normal tumor suppressor function of the *MEN1* gene. Finally, the possibility of incorrect clinical diagnosis has to be taken into account.

For all 13 different mutations described in 15 MEN 1 families, we confirmed that they were inherited according to the MEN 1 phenotype, while none of these mutations were observed in an analysis of 50 normal DNA samples.

Two of the observed mutations occurred twice, each in two unrelated different families. The analysis of the clinical features in these families for both mutations did not suggest any correlation between genotype and phenotype. In general our data confirm those previously published about the apparent lack of genotype–phenotype correlation (15–21). Twelve of the mutations identified in this study are novel, adding new information to the well known diversity of *MEN1* mutations and confirming the absence of mutational hot spots for the *MEN1* gene. As recently shown, the central domain, a separate domain at amino acid

residues 139–242 and the N-terminus of menin could have a critical role in menin–JunD interactions (11). Looking at the position of the identified mutations on amino acid sequence, it is important to note that the majority of *MEN1* mutations identified in our analysis localizes in these domains of the protein. However, the importance of this observation will be confirmed by additional studies on menin function, considering that mutations falling outside these critical amino acid regions are disease-associated and, therefore, the disruption of menin–JunD binding may not be the critical feature for disease expression.

In conclusion, we propose the application of the mutational screening reported here using DGGE analysis followed by sequencing as a useful approach to detect individuals at higher risk of developing MEN 1-associated tumors. Future information on menin function will be fundamental for building up clinical counseling for gene carriers based on mutational analysis.

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References

- Brandi ML, Marx SJ, Aurbach GD & Fitzpatrick LA. Familial Multiple Endocrine Neoplasia type 1: a new look at pathophysiology. *Endocrine Review* 1987 **8** 391–405.
- Kato H, Uchimura I, Morohoshi M, Fujisawa K, Kobayashi Y, Numano F *et al.* Multiple Endocrine Neoplasia type 1 associated with spinal ependymoma. *Internal Medicine* 1996 **35** 285–289.
- Samaan NA, Ouais S, Ordóñez N, Choksi UA, Sellin RV & Hickey RC. Multiple Endocrine Syndrome type 1. *Cancer* 1989 **64** 741–752.
- Vasen HEA, Lamers CBHW & Lips CJM Screening for Multiple Endocrine Neoplasia Syndrome type 1. *Archives of Internal Medicine* 1989 **149** 2717–2722.
- Metz DC, Jensen RT, Bale AE, Skarulis MC, Eastman RC, Nieman L, Norton JA *et al.* Multiple endocrine neoplasia type 1: clinical features and management. In *The Parathyroids*, pp 591–645. Eds J-P Bilezikian, M-A Levine & R Marcus. New York: Raven Press, 1994.
- Shepherd JJ. The natural history of Multiple Endocrine Neoplasia Type 1. Highly uncommon or highly unrecognized. *Archives of Surgery* 1991 **126** 935–952.
- Larsson C, Skögl B, Öberg K, Nakamura Y & Nordenskjöld M. Multiple Endocrine Neoplasia type 1 gene maps to chromosome 11 and is lost in insulinomas. *Nature* 1988 **332** 85–87.
- Nakamura Y, Larsson C, Julier C, Bystrom C, Skögl B, Wells S *et al.* Localization of the genetic defect in Multiple Endocrine Neoplasia type 1 within a small region of chromosome 11. *American Journal of Human Genetics* 1989 **44** 751–755.
- Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR *et al.* Positional cloning of the gene for Multiple Endocrine Neoplasia-type 1. *Science* 1997 **276** 404–407.
- Guru SC, Goldsmith PK, Burns AL, Marx SJ, Spiegel AM, Collins F & Chandrasekharappa SC. Menin, the product of the *MEN1* gene, is a nuclear protein. *Proceedings of the National Academy of Sciences of the USA* 1998 **95** 1630–1634.
- Agarwal SK, Guru SC, Heppner C, Herdos MR, Collins RM, Park SY *et al.* Menin interacts with the AP1 transcription factor JunD and represses JunD-activated transcription. *Cell* 1999 **96** 143–152.
- Debelenko LV, Brambilla E, Agarwal SK, Swalwell JI, Kester MB, Lubensky IA *et al.* Identification of *MEN1* gene mutations in sporadic carcinoid tumors of the lung. *Human Molecular Genetics* 1997 **6** 2285–2290.
- Heppner C, Kester MB, Agarwal SK, Debelenko LV, Emmert-Buck MR, Guru SC *et al.* Somatic mutation of *MEN1* gene in parathyroid tumors. *Nature Genetics* 1997 **16** 375–378.
- Zhuang Z, Vortmeyer AO, Pack S, Huang S, Phan TA, Huang C *et al.* Somatic mutations of the *MEN1* tumor suppressor gene in sporadic gastrinomas and insulinomas. *Cancer Research* 1997 **57** 4682–4686.
- Agarwal SK, Kester MB, Debelenko LV, Heppner C, Emmert-Buck MR, Skarulis MC *et al.* Germline mutations of the *MEN1* gene in familial multiple endocrine neoplasia type 1 and related states. *Human Molecular Genetics* 1997 **6** 1177–1183.
- Lemmens I, Van de Ven WJ, Kas K, Zhang CX, Giraud S, Wautot V *et al.* Identification of the multiple endocrine neoplasia type 1 (*MEN1*) gene. The European Consortium on *MEN1*. *Human Molecular Genetics* 1997 **6** 1177–1183.
- Mayr B, Apenberg S, Rothämel T, von zur Mühlen A & Brabant G. Menin mutations in patients with multiple endocrine neoplasia type 1. *European Journal of Endocrinology* 1997 **137** 684–687.
- Shimizu S, Tsukada T, Futami H, Ui K, Kameya T, Kawanaka M *et al.* Germline mutations of the *MEN1* gene in Japanese kindred with multiple endocrine neoplasia type 1. *Japanese Journal of Cancer Research* 1997 **88** 1029–1032.
- Basset JHD, Forbes SA, Pannett AAJ, Lloyd SE, Christie PT, Wooding C *et al.* Characterization of mutations in patients with multiple endocrine neoplasia type 1. *American Journal of Human Genetics* 1998 **62** 232–244.
- Giraud S, Zhang CX, Serova-Sinilnikova O, Wautot V, Salandre J, Buisson N *et al.* Germ-line mutation analysis in patients with multiple endocrine neoplasia type 1 and related disorders. *American Journal of Human Genetics* 1998 **63** 455–467.
- Teh BT, Kytola S, Farnebo F, Bergman L, Wong K, Weber G *et al.* Mutation analysis of the *MEN1* gene in multiple endocrine neoplasia type 1, familial acromegaly and familial isolated hyperparathyroidism. *Journal of Endocrinology and Metabolism* 1998 **83** 2621–2626.
- Gheri RG, Brandi ML & the MEN Syndrome Study Group of the Italian Society of Endocrinology. The Italian Register of MEN syndromes. *Journal of Endocrinological Investigation* 1995 **18** 116–119.
- Brandi ML, Weber G, Svensson A, Falchetti A, Tonelli F, Castello R *et al.* Homozygotes for autosomal dominant neoplasia syndrome (*MEN 1*). *American Journal of Human Genetics* 1993 **53** 1167–1172.
- Morelli A, Falchetti A, Castello R, Furlani L, Tomassetti P, Tonelli F *et al.* Genetic screening to identify the gene carrier in Italian and German kindreds affected by multiple endocrine neoplasia type 1 (*MEN 1*) syndrome. *Journal of Endocrinological Investigation* 1995 **18** 329–335.
- Lerman LS, Fisher SG, Hurley I, Silverstein K & Lumelsky N. Sequence-determined DNA separation. *Annual Review of Biophysics and Bioengineering* 1984 **13** 399–423.
- Myers RM, Maniatis T & Lerman LS. Detection and localization of single base changes by denaturing gradient gel electrophoresis. In *Methods in Enzymology*, vol 155 pp 501–527. Ed R Wu. San Diego: Academic Press, 1987.
- Beaud AL & Tsui LC. A suggested nomenclature for designating mutations. *Human Mutation* 1993 **2** 245–248.

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