Two nonsense somatic mutations in *MEN1* identified in sporadic insulinomas

Cheng Qi[1], Jiayue Duan[2], Qingfeng Shi[2], Mingguang Wang[2] and Changqing Yan[2]

1 Department of Surgical Oncology, The Second Hospital of Hebei Medical University, Shijiazhuang, China
2 Department of Hepatobiiary Surgery, The Second Hospital of Hebei Medical University, Shijiazhuang, China

Insulinomas are functional pancreatic neuroendocrine tumors that cause hypoglycemia and severe morbidity. The aim of our study was to identify gene mutations responsible for tumorigenesis of sporadic insulinoma. Whole exome sequencing analysis was performed on tumors and paired peripheral blood from three patients with insulinomas. After initial analysis, somatic mutations were obtained and a deleterious protein product was further predicted by various bioinformatic programs. Whole exome sequencing identified 55 rare somatic mutations among three insulinoma patients, including *MEN1* gene nonsense mutations (c. 681C>G; p.Tyr227* in exon 4 of *MEN1* and c. 346G>T; p.Glu116* in exon 2 of *MEN1*) in two different tumor samples. The mutations resulted in a significant truncation of the protein and a non-functional gene product, which was involved in defective binding of menin to proteins implicated in genetic and epigenetic mechanisms. Our results extend the growing list of pathogenic *MEN1* mutations in sporadic cases of insulinoma.

Insulinoma is a rare and sporadically occurring neuroendocrine tumor that secretes an excess of insulin, resulting in symptoms of hypoglycemia in patients [1]. Additionally, the catecholamines released from insulinoma produce several symptoms including sweating, nausea, weakness, anxiety and palpitation [2]. Insulinoma is usually a benign neoplasm that is smaller than 2 cm in diameter, without signs of angiogenesis or metastases, and is easily curable by surgical resection [2]. However, an understanding of the mechanism of the pathology of insulinoma is still needed. The small number of disease cases and lack of suitable animal models and cell strains have limited the study of the pathogenesis of insulinoma. The risk factors and related molecular mechanisms of insulinoma remain unclear.

Exome sequencing, which allows study of the complete protein-coding regions in the genome, is valuable in searching for underlying genetic variation in disease. Moreover, extensive exome sequencing studies from human tumors have indicated that there are a large number of mutations in each tumor [3]. Accumulating evidence suggests that genetic change is specific for insulinomas, such as high loss of heterozygosity rates on chromosome 22q and gain of 9q34 [4,5]. Additionally, exome sequencing has revealed that there was a somatic mutation in the DNA-binding zinc finger of the transcription factor Yin Yang 1 in insulinoma [6–8].

In order to further screen for potential genetic alterations in insulinoma, we selected the tumor tissue and matched blood of patients with insulinoma and performed exome sequencing and analysis of deleterious effects on the protein. In this study, we obtained a total of 55 gene mutations in insulinoma. Among them, mutations in *MEN1* were most related to the

Abbreviations

MEN-1, multiple endocrine neoplasia type 1; SNV, single nucleotide variant.

pathology of insulinoma, which provides evidence for use in early disease screening and target treatment of insulinoma.

Materials and methods

Insulinoma patient samples

In our present study, three patients (INS1, INS2 and INS3) with primary insulinoma were enrolled from the Second Hospital of Hebei Medical University. The paired tumor and a peripheral blood sample for DNA extraction were collected from the patients after surgical removal. The insulinoma was diagnosed depending on current clinical guidelines and histopathological confirmation [9]. The diagnosis was established using the following six tight criteria: (a) documented blood glucose levels ≤ 2.2 mmol·L^{-1} (≤ 40 mg·dL^{-1}); (b) concomitant insulin levels ≥ 66 μU·L^{-1} (≥ 36 pmol·L^{-1}; ≥ 3 μU·L^{-1}); (c) C-peptide levels ≥ 200 pmol·L^{-1}; (d) proinsulin levels ≥ 5 pmol·L^{-1}; (e) β-hydroxybutyrate levels ≤ 2.7 mmol·L^{-1}; (f) absence of sulfonylurea (metabolites) in the plasma and/or urine. Written consent forms were obtained from the enrolled participants, and the research protocol was approved by the ethics committee of the Second Hospital of Hebei Medical University (2017-R086) and complied with the principles of the Declaration of Helsinki. Clinical information for the patients is shown in Table 1.

Exome sequencing and data analysis

Genomic DNA was isolated from blood and tissue and was controlled for quality by measuring its concentration using a Nanodrop 2000 (Illumina, San Diego, CA, USA) and measuring fragmentation by agarose gel electrophoresis. Qualified Genomic DNA was prepared for exome sequencing with an Agilent SureSelect Human All Exon 50 Mb Exon Kit (Agilent Technologies, Santa Clara, CA, USA). The genomic DNA of each sample was fragmented and captured for exome sequencing with the Illumina HiSeq 2500 Sequencer platform (Illumina). For each sample, sequencing reads with 125-bp paired-end and $Q_{30} > 92\%$ were generated.

After filtering the low quality and contaminating reads, sequence reads were mapped to the human genome sequence (hg19) using the Burrows–Wheeler alignment tool (http://bio-bwa.sourceforge.net/), which generated the sequence alignment/map file. The PCR duplicate reads were further removed using the PICARD software program.

Single nucleotide variant detection and annotation

To obtain the important candidate genes, the MUTECT software [10] was used to detect single nucleotide variants (SNVs). Variants were filtered for minimum genotype quality of 50 and minimum coverage depths of 10. Then, the software ANNOVAR (http://www.openbioinformatics.org/annovar/) was applied to annotate the qualified variants. Finally, the variants were obtained and the deleteriousness of variants was subsequently predicted by various bioinformatics programs (e.g. SIFT, POLYPHEN2, LRT, MUTATIONTASTER, MUTATIONASSESSOR, FATHMM, RADIALSVM, LR).

Results

General characteristics of the patients

We studied three patients with insulinoma, two female and one male. None had a family history of insulinoma. Moreover, we also excluded multiple endocrine neoplasia type 1 (MEN-1 syndrome) from non-tumor tissue. The WHO grading classification of pancreatic neuroendocrine tumors updated in 2010 includes neuroendocrine tumor G1, neuroendocrine tumor G2, neuroendocrine carcinoma G3 and mixed adenoneuroendocrine carcinoma [11]. Depending on the classification system [12], two patients (INS1 and INS3) were classified as Grade II and one was classified as Grade I in this study. All presented with signs and symptoms of hypoglycemia. The hypoglycemia was corrected in all cases after surgical removal. General pathological and demographic characteristics for the three patients are shown in Table 1.

Genetic analysis

Genomic DNA from insulinomas and matched blood samples was subjected to whole exome sequencing. After mapping of the human genome sequence (hg19), a total of $> 86\%$ of the exome region was covered

Table 1. Clinical information for the patients with insulinoma. INS1, INS2 and INS3 represent the three patients with insulinoma.

Sample	Gender	Age at diagnosis (years)	Grade	Metastatic disease	Tumor size (cm)	Ki67
INS1	Male	64	G2	No	0.6	2%
INS2	Female	75	G1	No	0.8	2%
INS3	Female	57	G2	No	1.0	2%

(Table 2). Exome sequencing analysis overall identified 40 210, 40 272 and 41 910 SNVs for tumor tissue, and 41 106, 40 050 and 41 451 SNVs for the matched blood samples. We identified 55 rare somatic mutations among the three patients, of which 39 were nonsynonymous, four were nonsense and 12 were synonymous. An overview of detected somatic mutations after exome sequencing is provided in Table 3. MEN1 gene nonsense mutations occurred in two different tumor samples. A c. 681C>G; p.Tyr227* mutation was found in exon 4 of the MEN1 gene in INS1, and c. 346G>T; p.Glu116* mutation was found in exon 2 of the MEN1 gene in INS2. The mutations were not present in the corresponding leukocyte DNA. Additionally, these mutations were predicted to be damaging by SIFT or LRT.

Mutation of MEN1 gene

The p.Tyr227* mutation (SWLYLKGSYMRCDRK MEV) and p.Glu116* mutation (VSSRELVKKVSD VIWNSL) both resulted in a significant truncation of menin, which may destroy the functional domain and affect its function. Amino acids of p.Tyr227* and p.Glu116* mutations are highly conserved across multiple species (Fig. 1A,B). Moreover, two nonsense mutations locate in the functional domain of MEN1, indicating that it may affect the binding of lysine methyltransferase 2A (Fig. 1C). In the current study, the nonsense mutations happened early in the sequence of MEN1, which may obviously result in a non-functional gene product (Fig. 1D).

Discussion

Insulinoma is a common neuroendocrine tumor with an incidence of four in every 1 million persons annually [13]. Moreover, most of the insulinomas arise sporadically. Although rare, it has the potential to produce profound metabolic derangements that require

Table 2. Summary of sequencing data. C1, C2 and C3: tissues of three patients with insulinoma; N1, N2 and N3: blood of three patients with insulinoma.

Sample	Reads	Read length (bp)	$> Q_{30}$ (%)	Exome size (bp)	Exome coverage
C1	104 472 186	125	94.82	74 856 280	87.36%
C2	107 751 402	125	92.34	74 856 280	87.79%
C3	112 709 600	125	94.69	74 856 280	88.19%
N1	110 592 066	125	94.5	74 856 280	87.90%
N2	98 905 302	125	94.49	74 856 280	86.08%
N3	109 660 740	125	94.58	74 856 280	88.44%

early recognition and treatment. In this study, exome sequencing was performed on three sporadic insulinoma cases to delineate genetic contributors to this rare endocrine tumor. Although the overall frequency of somatic mutations was low and predicted to be damaging, there were two nonsense variants that occurred in MEN1 in two of the three patients, namely a c. 681C>G; p.Tyr227* mutation in exon 4 of MEN1 and a c. 346G>T; p.Glu116* mutation in exon 2 of MEN1. Our result showed that the mutations in MEN1 may play an important role in the development of insulinoma. However, further functional research is needed to validate their roles.

MEN1 consists of 10 exons [9] and encodes a protein with 615 amino acids [14]; it is considered a putative tumor suppressor gene associated with neuroendocrine tumors [15]. Menin, the encoded protein of MEN1, is a typical GTPase stimulated by nm23. It is mostly found in the nucleus and can regulate gene expression in a positive or negative way, and it has been demonstrated to interact with transcription activators, transcription repressors, cell signaling proteins and various other proteins. In addition, it plays major roles in DNA repair, cell cycle regulation and chromatin remodeling. Generally, menin is considered as a transcriptional regulator and interacts with a number of nuclear and cytosolic proteins, which indicates that it may participate in various biological pathways of tumor formation [16–18]. Additionally, a number of sporadic endocrine tumors, including parathyroid adenomas, pancreatic insulinomas and pituitary prolactinomas, have somatic mutations of MEN1 alleles, suggesting that MEN1 may play a role in non-hereditary endocrine tumors [15,19,20]. Our results showed that MEN1 mutations were found in two of the three insulinoma patients, which provided further evidence that MEN1 might be an important factor in the pathological process of insulinoma.

It is noted that insulinomas can occur sporadically or in combination with MEN-1 syndrome. Moreover, the MEN1 gene is the first gene that has been identified as a candidate gene in the tumorigenesis of insulinoma. MEN-1 syndrome represents an autosomal dominant disorder related to mutations in the MEN1 gene mapped to chromosome 11q13 [21,22]. Generally, simple and local tumor enucleation of MEN-1 syndrome-associated insulinomas is not likely to be curative. Although genetic testing for MEN1 fails to detect mutation rate of 10–25%, it plays a vital role in identifying patients with hereditary insulinomas [23,24]. Therefore, genetic testing for MEN-1 syndrome is beneficial to clinical diagnosis. Okamoto *et al.* [25] suggested that a novel six-nucleotide insertion in exon 4

Table 3. Overview of the gene mutations found by exome sequencing in the three patients with insulinoma.

| Sample	Chr	Start	Ref	Alt	Gene	Mutation type	AA change
INS1	1	1.1×10^8	G	A	PSRC1	Nonsynonymous	p.Ala227Val
INS1	2	1 926 848	A	G	MYT1L	Synonymous	p.Asn231Asn
INS1	2	2.38×10^8	T	C	COL6A3	Nonsynonymous	p.Glu1565Gly
INS1	3	1.47×10^8	C	T	ZIC4	Nonsynonymous	p.Arg246His
INS1	3	1.89×10^8	C	T	TPRG1	Synonymous	p.Leu120Leu
INS1	5	1.46×10^8	G	T	PPP2R2B	Nonsynonymous	p.Pro235Thr
INS1	6	32 548 632	T	A	HLA-DRB1	Nonsynonymous	p.Arg218Ser
INS1	7	1 542 657	G	A	INTS1	Nonsynonymous	p.Arg77Cys
INS1	7	4 249 780	T	A	SDK1	Nonsense	p.Leu329*
INS1	7	1.48×10^8	A	G	CNTNAP2	Nonsynonymous	p.Lys1156Glu
INS1	7	1.57×10^8	G	T	PTPRN2	Nonsynonymous	p.Ser827Tyr
INS1	9	99 157 190	A	G	ZNF367	Synonymous	p.Cys202Cys
INS1	10	99 153 502	C	A	RRP12	Nonsynonymous	p.Ala157 Ser
INS1	10	1.3×10^8	C	G	MKI67	Nonsynonymous	p.Gly477Ala
INS1	11	64 575 141	G	C	MEN1	Nonsense	p.Tyr227*
INS1	11	1.02×10^8	A	G	CEP126	Nonsynonymous	p.Ile674Val
INS1	13	95 887 080	G	A	ABCC4	Synonymous	p.Ala105Ala
INS1	14	74 968 287	G	T	LTBP2	Nonsynonymous	p.Pro1726Gln
INS1	16	28 943 787	G	C	CD19	Nonsynonymous	p.Gly70Ala
INS1	16	31 405 651	C	A	ITGAD	Nonsynonymous	p.Phe42Leu
INS1	17	21 318 727	A	T	KCNJ12	Nonsynonymous	p.Met25Leu
INS1	17	57 761 285	A	G	CLTC	Nonsynonymous	p.His1462Arg
INS1	19	10 799 330	G	A	ILF3	Nonsynonymous	p.Gly843Ser
INS2	1	22 332 008	T	C	CELA3A	Synonymous	p.Asp66Asp
INS2	1	40 229 393	G	A	BMP8B	Synonymous	p.Leu313Leu
INS2	6	32 007 839	G	T	CYP21A2	Nonsynonymous	p.Ala236Ser
INS2	8	52 733 231	G	A	PCMTD1	Nonsense	p.Arg176*
INS2	8	88 298 821	T	A	CNBD1	Nonsynonymous	p.Tyr322Asn
INS2	8	1.25×10^8	G	A	TMEM65	Nonsynonymous	p.Ala179Val
INS2	8	1.25×10^8	T	C	TMEM65	Nonsynonymous	p.Tyr176Cys
INS2	9	1.13×10^8	C	A	SVEP1	Nonsynonymous	p.Arg902Leu
INS2	11	64 577 236	C	A	MEN1	Nonsense	p.Glu116*
INS2	11	89 018 006	C	A	TYR	Nonsynonymous	p. Pro417His
INS2	12	6 787 522	G	A	ZNF384	Nonsynonymous	p.Pro153Ser
INS2	12	9 243 947	A	G	A2M	Synonymous	p.Ser773Ser
INS2	12	1.25×10^8	A	G	UBC	Synonymous	p.Asp647Asp
INS2	17	7 671 513	G	A	DNAH2	Nonsynonymous	p.Arg1290His
INS2	17	7 834 438	C	G	TRAPPC1	Nonsynonymous	p. Ser67Thr
INS2	17	34 797 666	G	A	TBC1D3B	Synonymous	p.Ala490Ala
INS2	19	41 355 849	A	G	CYP2A6	Synonymous	p. Leu73Leu
INS2	X	37 027 691	T	C	FAM47C	Nonsynonymous	p.Phe403Ser
INS3	1	12 854 188	T	C	PRAMEF1	Nonsynonymous	p.Cys138Arg
INS3	2	1.28×10^8	G	A	MYO7B	Nonsynonymous	p.Gly163Asp
INS3	4	1.52×10^8	C	A	RPS3A	Nonsynonymous	p.Asn147Lys
INS3	4	1.52×10^8	A	C	RPS3A	Nonsynonymous	p.Gln157Pro
INS3	5	1.4×10^8	T	C	PCDHA5	Nonsynonymous	p.Val596Ala
INS3	6	99 850 428	T	C	PNISR	Nonsynonymous	p.Lys439Glu
INS3	6	1.38×10^8	C	T	OLIG3	Nonsynonymous	p.Ala175Thr
INS3	8	33 449 689	C	A	DUSP26	Nonsynonymous	p.Ala160Ser
INS3	10	21 903 830	T	G	MLLT10	Nonsynonymous	p.Cys194Gly
INS3	11	1 093 437	G	C	MUC2	Synonymous	p.Thr1752Thr
INS3	11	1.26×10^8	C	T	PUS3	Nonsynonymous	p.Glu143Lys
INS3	16	7 629 904	C	T	RBFOX1	Synonymous	p.Asp152Asp
INS3	17	79 667 512	G	A	HGS	Nonsynonymous	p.Ser633Asn
INS3	19	19 030 142	G	A	COPE	Nonsynonymous	p.Pro6Ser
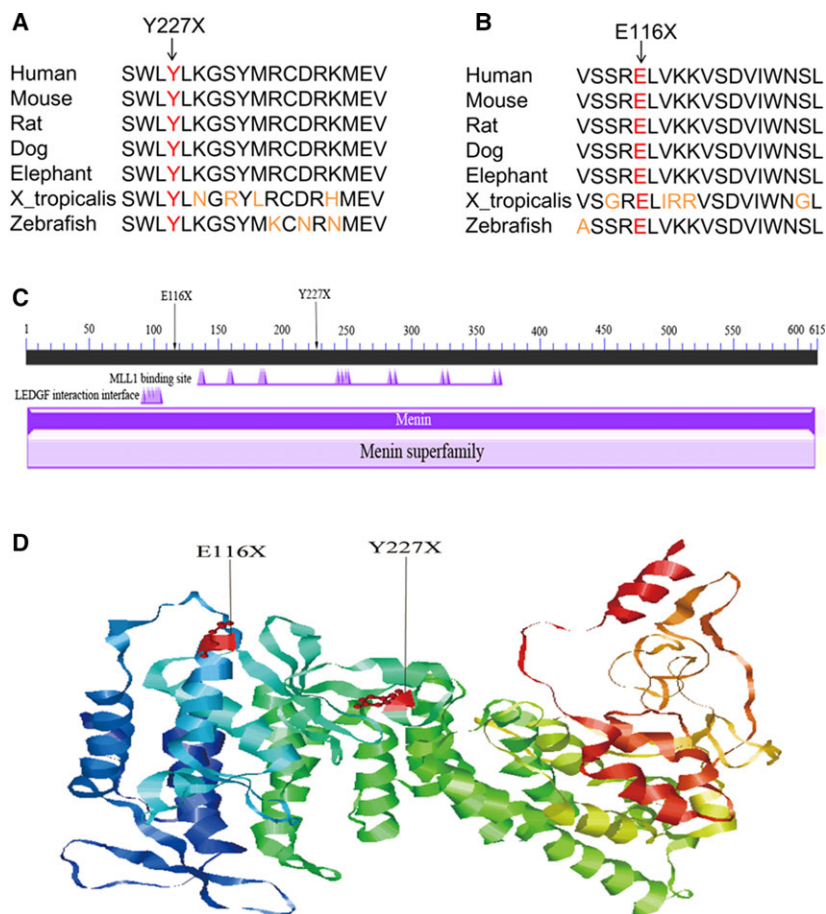
Fig. 1. Nonsense mutation in MEN1. (A) Conservation of p.Tyr227* in the MEN1 protein. (B) Conservation of p.Glu116* in the MEN1 protein. (C) Locations of p.Tyr227* and p.Glu116* mutations in the conserved domain in the MEN1 protein. (D) Location of p.Tyr227* and p.Glu116* mutations on the crystal structure of the MEN1 protein.

of the MEN1 gene might contribute to the familial insulinoma. A prior study indicated that MEN1 gene mutations were lacking in 27 sporadic insulinomas [26]. By evaluating a large family with malignant insulinoma and hyperparathyroidism, Hasani-Ranjbar et al. [27] found a novel MEN1 gene frameshift germ-line mutation, which was associated with malignant insulinoma. Moreover, a recent study found several novel pathogenic MEN1 mutations in sporadic cases of insulinoma [28]. Herein, we found mutations in MEN1 on chromosome 11 in patients with insulinoma, which had a damaging role in the function of the encoded protein. Our finding was consistent with other studies indicating the role of MEN1 mutation in human sporadic insulinomas [26,29–32], which provided a crucial clue in the treatment of insulinoma. Interestingly, Waldmann et al. [33] found the p.E116X mutation in exon 2 of the MEN1 gene in 21 patients with MEN-1 syndrome and adrenal lesions. In addition, Turner et al. [34] identified the p.Y227X mutation in exon 4 of the MEN1 gene in multiple endocrine neoplasia type 1. This further suggested that MEN1 was significantly associated with insulinoma.

Conclusions

In the current study, exome sequencing for three sporadic insulinomas identified two somatic nonsense mutations in MEN1 (c. 681C>G; p.Tyr227* and c. 346G>T; p.Glu116*), which might induce a non-functional gene product and contribute to the oncogenesis of sporadic insulinoma. However, there were some limitations in this study. The samples selected were small and larger samples are further needed to validate their roles in insulinoma. Further studies are needed to precisely explore the role of genetic mutations of MEN1 in clinical manifestations of these patients. In addition, validation of MEN1 mutations (such as by Sanger sequencing) and study of the underlying biological function of the MEN1 mutations is needed in a further study.

Author contributions

JD, QS and MW analyzed and interpreted the data. CQ was the major contributor in writing the manuscript. CY designed the project. All authors read and approved the final manuscript.

References

1 Shin JJ, Gorden P and Libutti SK (2010) Insulinoma: pathophysiology, localization and management. *Future Oncol* **6**, 229–237.

2 Fendrich V, Waldmann J, Bartsch DK and Langer P (2009) Surgical management of pancreatic endocrine tumors. *Nat Rev Clin Oncol* **6**, 419–428.

3 Kennedy SR, Loeb LA and Herr AJ (2012) Somatic mutations in aging, cancer and neurodegeneration. *Mech Ageing Dev* **133**, 118–126.

4 Wild A, Langer P, Ramaswamy A, Chaloupka B and Bartsch DK (2001) A novel insulinoma tumor suppressor gene locus on chromosome 22q with potential prognostic implications. *J Clin Endocrinol Metab* **86**, 5782–5787.

5 Speel EJ, Scheidweiler AF, Zhao J, Matter C, Saremaslani P, Roth J, Heitz PU and Komminoth P (2001) Genetic evidence for early divergence of small functioning and nonfunctioning endocrine pancreatic tumors: gain of 9Q34 is an early event in insulinomas. *Cancer Res* **61**, 5186–5192.

6 Cromer MK, Choi M, Nelson-Williams C, Fonseca AL, Kunstman JW, Korah RM, Overton JD, Mane S, Kenney B, Malchoff CD *et al.* (2015) Neomorphic effects of recurrent somatic mutations in Yin Yang 1 in insulin-producing adenomas. *Proc Natl Acad Sci USA* **112**, 4062–4067.

7 Cao Y, Gao Z, Li L, Jiang X, Shan A, Cai J, Peng Y, Li Y, Jiang X, Huang X *et al.* (2013) Whole exome sequencing of insulinoma reveals recurrent T372R mutations in YY1. *Nat Commun* **4**, 2810.

8 Lichtenauer UD, Di Dalmazi G, Slater EP, Wieland T, Kuebart A, Schmittfull A, Schwarzmayr T, Diener S, Wiese D, Thasler WE *et al.* (2015) Frequency and clinical correlates of somatic Ying Yang 1 mutations in sporadic insulinomas. *J Clin Endocrinol Metab* **100**, E776–E782.

9 de Herder WW, Niederle B, Scoazec JY, Pauwels S, Kloppel G, Falconi M, Kwekkeboom DJ, Oberg K, Eriksson B, Wiedenmann B *et al.* (2006) Well-differentiated pancreatic tumor/carcinoma: insulinoma. *Neuroendocrinology* **84**, 183–188.

10 Cibulskis K, Lawrence MS, Carter SL, Sivachenko A, Jaffe D, Sougnez C, Gabriel S, Meyerson M, Lander ES and Getz G (2013) Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol* **31**, 213–219.

11 Bosman F, Carneiro F, Hruban R and Theise N (2010) *WHO Classification of Tumours of the Digestive System*, 4th edn. WHO, Geneva, Switzerland.

12 Sobin LH (1974) *Histological Classification of Tumours.* Springer, Berlin, Heidelberg, Germany.

13 Service FJ, McMahon MM, O'Brien PC and Ballard DJ (1991) Functioning insulinoma–incidence, recurrence, and long-term survival of patients: a 60-year study. *Mayo Clin Proc* **66**, 711–719.

14 Guru SC, Manickam P, Crabtree JS, Olufemi SE, Agarwal SK and Debelenko LV (1998) Identification and characterization of the multiple endocrine neoplasia type 1 (MEN1) gene. *J Intern Med* **243**, 433–439.

15 Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR, Debelenko LV, Zhuang Z, Lubensky IA, Liotta LA *et al.* (1997) Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science* **276**, 404–407.

16 Shen HC, He M, Powell A, Adem A, Lorang D, Heller C, Grover AC, Ylaya K, Hewitt SM, Marx SJ *et al.* (2009) Recapitulation of pancreatic neuroendocrine tumors in human multiple endocrine neoplasia type I syndrome via Pdx1-directed inactivation of Men1. *Cancer Res* **69**, 1858–1866.

17 Agarwal SK, Kennedy PA, Scacheri PC, Novotny EA, Hickman AB, Cerrato A, Rice TS, Moore JB, Rao S, Ji Y *et al.* (2005) Menin molecular interactions: insights into normal functions and tumorigenesis. *Horm Metab Res* **37**, 369–374.

18 Nikfarjam M, Warshaw AL, Axelrod L, Deshpande V, Thayer SP, Ferrone CR and Fernandez-del Castillo C (2008) Improved contemporary surgical management of insulinomas: a 25-year experience at the Massachusetts General Hospital. *Ann Surg* **247**, 165–172.

19 Ludwig L, Schleithoff L, Kessler H, Wagner PK, Boehm BO and Karges W (1999) Loss of wild-type MEN1 gene expression in multiple endocrine neoplasia type 1-associated parathyroid adenoma. *Endocr J* **46**, 539–544.

20 Libutti SK, Crabtree JS, Lorang D, Burns AL, Mazzanti C, Hewitt SM, O'Connor S, Ward JM, Emmert-Buck MR, Remaley A *et al.* (2003) Parathyroid gland-specific deletion of the mouse Men1 gene results in parathyroid neoplasia and hypercalcemic hyperparathyroidism. *Cancer Res* **63**, 8022–8028.

21 Wermer P (1963) Endocrine adenomatosis and peptic ulcer in a large kindred. Inherited multiple tumors and mosaic pleiotropism in man. *Am J Med* **35**, 205–212.

22 Larsson C, Skogseid B, Oberg K, Nakamura Y and Nordenskjold M (1988) Multiple endocrine neoplasia type 1 gene maps to chromosome 11 and is lost in insulinoma. *Nature* **332**, 85–87.

23 Callender GG, Rich TA and Perrier ND (2008) Multiple endocrine neoplasia syndromes. *Surg Clin North Am* **88**, 863–895.

24 Brandi ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C, Conte-Devolx B, Falchetti A, Gheri RG, Libroia A *et al.* (2001) Guidelines for diagnosis and therapy of MEN type 1 and type 2. *J Clin Endocrinol Metab* **86**, 5658–5671.

25 Okamoto H, Tamada A, Hai N, Doi M, Uchimura I, Hirata Y and Kosugi S (2002) A novel six-nucleotide

insertion in exon 4 of the MEN1 gene, 878insCTGCAG, in three patients with familial insulinoma and primary hyperparathyroidism. *Jpn J Clin Oncol* **32**, 368–370.

26 Cupisti K, Hoppner W, Dotzenrath C, Simon D, Berndt I, Roher HD and Goretzki PE (2000) Lack of MEN1 gene mutations in 27 sporadic insulinomas. *Eur J Clin Invest* **30**, 325–329.

27 Hasani-Ranjbar S, Amoli MM, Ebrahim-Habibi A, Gozashti MH, Khalili N, Sayyahpour FA, Hafeziyeh J, Soltani A and Larijani B (2011) A new frameshift MEN1 gene mutation associated with familial malignant insulinomas. *Fam Cancer* **10**, 343–348.

28 Jyotsna VP, Malik E, Birla S and Sharma A (2015) Novel MEN 1 gene findings in rare sporadic insulinoma–a case control study. *BMC Endocr Disord* **15**, 44.

29 Gortz B, Roth J, Krahenmann A, de Krijger RR, Muletta-Feurer S, Rutimann K, Saremaslani P, Speel EJ, Heitz PU and Komminoth P (1999) Mutations and allelic deletions of the MEN1 gene are associated with a subset of sporadic endocrine pancreatic and neuroendocrine tumors and not restricted to foregut neoplasms. *Am J Pathol* **154**, 429–436.

30 Zhuang Z, Vortmeyer AO, Pack S, Huang S, Pham TA, Wang C, Park WS, Agarwal SK, Debelenko LV, Kester M *et al.* (1997) Somatic mutations of the MEN1 tumor suppressor gene in sporadic gastrinomas and insulinomas. *Cancer Res* **57**, 4682–4686.

31 Moore PS, Beghelli S, Zamboni G and Scarpa A (2003) Genetic abnormalities in pancreatic cancer. *Mol Cancer* **2**, 7.

32 Gumbs AA, Moore PS, Falconi M, Bassi C, Beghelli S, Modlin I and Scarpa A (2002) Review of the clinical, histological, and molecular aspects of pancreatic endocrine neoplasms. *J Surg Oncol* **81**, 45–53; discussion 54.

33 Waldmann J, Bartsch DK, Kann PH, Fendrich V, Rothmund M and Langer P (2007) Adrenal involvement in multiple endocrine neoplasia type 1: results of 7 years prospective screening. *Langenbecks Arch Surg* **392**, 437–443.

34 Turner JJ, Leotlela PD, Pannett AA, Forbes SA, Bassett JH, Harding B, Christie PT, Bowen-Jones D, Ellard S, Hattersley A *et al.* (2002) Frequent occurrence of an intron 4 mutation in multiple endocrine neoplasia type 1. *J Clin Endocrinol Metab* **87**, 2688–2693.