

Analysis of MEN1 c.482G>A (p.Gly161Asp) mutation in a pedigree with familial multiple endocrine neoplasia type 1

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Received March 31, 2017; Accepted September 25, 2017

DOI: 10.3892/mmr.2017.7749

Abstract. Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder characterized by the development of neuroendocrine tumors, which in turn are caused by mutations in the *MEN1* gene. In the present study, a case of a 46-year-old woman who was clinically diagnosed with MEN1 based on the presence of prolactinoma and bilateral parathyroid adenoma was reported. The patient's serum prolactin (PRL) levels were successfully controlled via bromocriptine therapy, and the serum levels of calcium and intact parathyroid hormone (PTH) reduced one day following parathyroidectomy. Genetic testing revealed a missense mutation c.482G>A (p.Gly161Asp) in exon 3 of the *MEN1* gene, and it led to the identification of two carriers in the pedigree (patient's elder sister and brother). Both of the carriers revealed to have high blood calcium, PTH and PRL. The mutation identified in this pedigree has never been reported in China. The sequence alignments and tertiary structure of menin protein were made by Polyphen2, SNPs3D, and SIFT, which were used to predict the function of mutant menin. Since the mutant menin may interfere with the menin-JunD or menin-Smad3 interactions, further investigations are necessary to explore the function of mutant protein. In view of that, identification of mutations and longtime follow-up are important for patients with a pedigree clearly indicating MEN1.

Introduction

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder characterized by the occurrence of tumors of the parathyroids, anterior pituitary and neuroendocrine tumors of the pancreas and duodenum. It is furthermore associated with germline mutations of the *MEN1* tumor suppressor gene. Parathyroid tumors, which lead to hypercalcemia are the most common feature of MEN1 occurring in about 95% of patients. Pancreatic islet cell tumors occur in approximately 40% of patients, while anterior pituitary tumors occur in around 30% of patients (1). The *MEN1* gene, which was identified in 1997 by Chandrasekharappa *et al* (2), consists of 10 exons that encode the protein referred to as menin. A comprehensive review and analysis of 38 years of data related to 613 *MEN1* mutations found that frameshift mutations represent the highest rate (42%), while missense mutations show a frequency of 25.5%. Nonsense mutations represent 14%, splice-site mutations 10.5%; in-frame del/ins 5.5% and gross deletions represent the remaining 2.5% (3). Furthermore, the most mutations resulted in the 2, 9 and 10 exons, but thus far the genotype-phenotype correlation has not been established (4). Therefore, DNA testing for *MEN1* gene is an established and useful tool for the diagnosis of MEN1. In the present study we report a familial MEN1 case with prolactinoma and parathyroid adenoma, and a missense mutation c.482G>A in exon 3 of *MEN1* gene, which has been shown to change the 161st amino acid from glycine to aspartate.

Case report

A 46-year-old woman was admitted to our hospital because of malaise and gastrointestinal discomfort. For the past 6 years she has been receiving bromocriptine treatment for prolactinoma, and she had a month old fracture in the left leg. Her mother died of 'gastric cancer' 6 years ago, and her brother was diagnosed with multiple kidney calculus 4 years ago. The rest of the family history was noncontributory. Her body mass index (BMI) was 31.1 kg/m². The patient's initial laboratory examinations are listed in Table I. Pituitary magnetic resonance imaging (MRI) scanning showed an adenoma in the pituitary (Fig. 1A). Abdominal computed tomography (CT) revealed multiple small calculi in the left kidney (Fig. 1B), while bone

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Abbreviations: MEN1, multiple endocrine neoplasia type 1; BMI, body mass index; MRI, magnetic resonance imaging; CT, computed tomography; BMD, bone mineral density

Key words: multiple endocrine neoplasia type 1, *MEN1* gene, missense mutation, mutant menin

Table I. Laboratory results of the proband.

Variable	Laboratory data	Normal range
PTH (ng/ml)	426.6	15-65
Calcium (mmol/l)	3.08	2.2-2.7
24-h urine Ca (mmol/day)	8.9	2.5-7.5
Phosphate (mmol/l)	0.68	0.81-1.9
PRL (ng/ml)	35.7	5.18-26.53
GH (ng/ml)	0.5	0.06-5
IGF-1 (ng/ml)	166	94-252
HbA1c (%)	5.1	4.0-6.5
ACTH (8:00 am) (pg/ml)	45.6	7.2-63.3
Cortisol (8:00 am) (ng/ml)	511	171-536

PTH, intact parathyroid hormone; Ca, calcium; PRL, prolactin; GH, growth hormone; IGF-1, insulin-like growth factor 1; HbA1c, haemoglobin A1C; ACTH, adrenocorticotrophic hormone.

mineral density showed severe osteoporosis (Hologic Inc., Waltham, MA, USA). Dual-phase ^{99m}Tc -Sestamibi (^{99m}Tc -MIBI) scintigraphy revealed a significant uptake in the parathyroid gland located in the inferior side of bilateral thyroid, which was suggestive of parathyroid adenoma (Fig. 1C). The blood calcium decreased gradually after using bisphosphonate and salmon calcitonin. The postoperative pathology confirmed the bilateral parathyroid adenoma (Fig. 1D), and on day after parathyroidectomy, the levels of serum calcium (2.4 mmol/l)

and parathyroid hormone (PTH) (92.2 ng/ml) were immediately reduced.

DNA was extracted from peripheral blood and resected parathyroid tissue samples of the proband. The samples were taken from her father, two elder sisters, brother and son after they signed a written informed consent. Simultaneously, their serum calcium, PTH and prolactin (PRL) were tested. We found the mutation c.482G>A (p.Gly161Asp) in the third exon of *MEN1* gene in both peripheral blood and resected parathyroid tissue of the proband (Fig. 2A). Patient's brother and one of the elder sisters revealed the same mutation (Fig. 2B), as well as high blood calcium, PTH and PRL. Other family members tested normal, in both gene testing and related laboratory examination (Fig. 2C and D). The identified mutation has previously been reported (5); nevertheless previous studies did not clearly illustrate pathogenicity of this mutation and the function of the mutant protein. Polyphen2, SNPs3D and SIFT were used to predict the function of the 161st amino acid substitute from glycine to aspartate. Polyphen2 predicted result score was 1.00 (i.e., score closer to 1.00 was interpreted as the greater mutation damage; score closer to 0 was interpreted as the smaller damage); SNPs3D predicted result score was -1.97 (positive score was interpreted as harmless; negative score was interpreted as harmful); SIFT predicted result score was 0.00 (<0.05 was interpreted as having the ability to affect protein function). All the obtained results revealed that mutation may be harmful for the menin protein. Gene sequencing was performed by Sangon Biotech Co., Ltd. (Shanghai, China). The seqman of DNASTAR package was used for alignment of sequenced and normal genes. The name of mutation

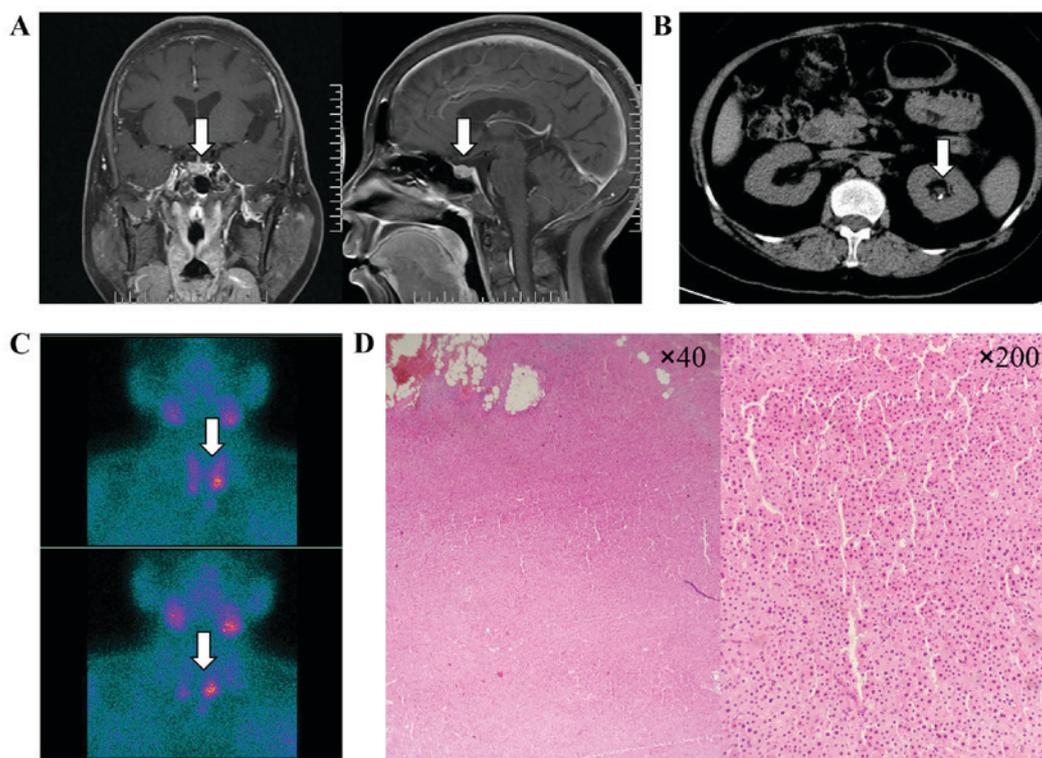


Figure 1. Real-time imaging and pathology examination of the proband. (A) Pituitary MRI showed adenoma in the pituitary. (B) Abdominal CT revealed multiple small calculi in the left kidney. (C) At early and late phase, dual-phase ^{99m}Tc -MIBI scintigraphy revealed a significant uptake in the parathyroid gland located in the both inferior thyroid (D) Postoperative pathology confirmed the bilateral parathyroid adenoma (H&E staining; $\times 40$ and $\times 200$). MRI, magnetic resonance imaging; CT, computed tomography; ^{99m}Tc -MIBI, ^{99m}Tc -Sestamibi.

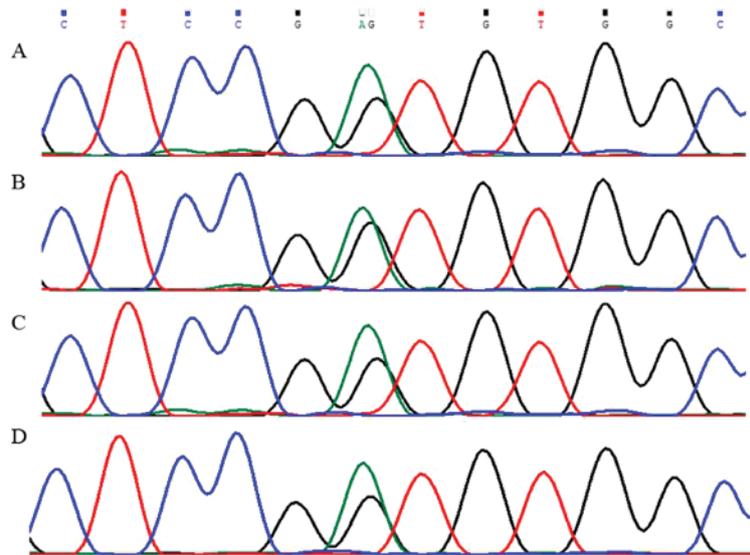


Figure 2. Genetic analysis in the pedigree. Heterozygous mutation c.482G>A (p.Gly161Asp) in the third exon of *MEN1* gene in both peripheral blood (A) and resected parathyroid tissue (B) of the proband. The patient's elder sister (C) and brother (D) had the same mutation. MEN1, multiple endocrine neoplasia type 1.

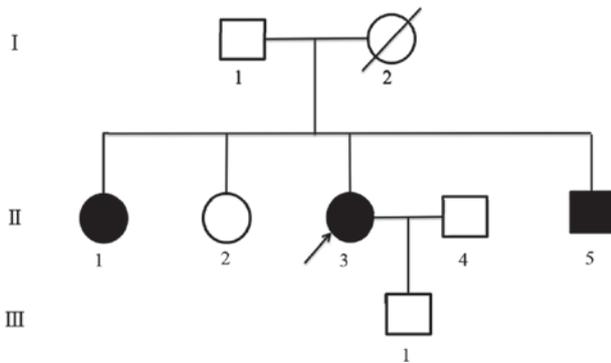


Figure 3. Pedigree diagram of the proband's family. Family members are indicated by generations (Roman numbers) and individuals (Arabic numbers). Circles indicate women and squares indicate men. Black symbol, mutation carriers and all heterozygous genotype, and white symbol, normal. Slash indicates a deceased individual at the time of the investigation, and the arrow indicates the presence of a proband.

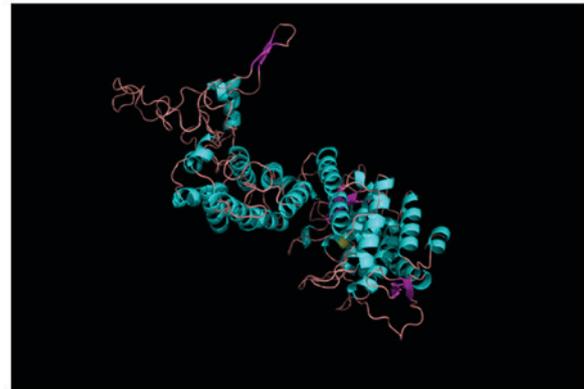


Figure 5. The tertiary structure of mutant menin was predicted using Swiss-model. Mutant amino acid p.Gly161Asp is presented in yellow.

The tertiary structure of menin protein was predicted using Swiss-model (6).

Discussion

The clinical diagnosis of MEN1 depends on the identification of neoplastic disease in at least two of the commonly affected organs, i.e., the parathyroid gland, endocrine pancreas, and anterior pituitary (7). In the present case, the diagnosis of MEN1 was demonstrated by prolactinoma and primary hyperparathyroidism with bilateral parathyroid adenoma. Genetic testing of the patient and family members revealed a missense mutation c.482G>A (p.Gly161Asp) in patients and patient's elder sister and brother. The sequencing peak pattern revealed additional peak confirming the heterozygous mutation, and suggesting the patient's mother might had the same mutation in the pedigree, while other familiars tested normal (Fig. 3). Sequence alignments revealed that the glycine residues were highly conserved among menin proteins from different species (Fig. 4).

p.Gly161

Q503J8_DANRE	TKLDSSG	VAF	VVAAC	QVL	GLK	DVHL
MEN1_MOUSE	TKLDSSG	VAF	VVGAC	QAL	GLR	DVHL
MEN1_RAT	TKLDSSG	VAF	VVGAC	QAL	GLR	DVHL
MEN1_BOVIN	TKLDSSG	VAF	VVGAC	QAL	GLR	DVHL
MEN1_HUMAN	TKLDSSG	VAF	VVGAC	QAL	GLR	DVHL
MEN1_CANLF	TKLDSSG	VAF	VVGAC	QAL	GLR	DVHL
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Figure 4. Amino acid sequence alignments of human menin and other homologous proteins. The figure represents partial alignment of menin sequences of human, mouse, rat, bovine, dog and zebrafish around the position of missense mutation (p.Gly161), which is denoted by a frame (the asterisk revealed highly conserved from different mammalia species). MEN1, multiple endocrine neoplasia type 1.

referenced the cDNA sequence NM_00244.3 of Genbank.

The product of the *MEN1* gene, menin, is a nuclear protein (8) and it is believed to function as a tumor suppressor having a number of potential roles in maintaining normal cell function. Some of the more researched menin functions indicated by direct interactions with many proteins and other molecules are: 3 JunD-interacting domains at codons 1-40, 139-242, and 323-428, Smad family at codons 40-278, and 477-610, and 3 nuclear localization signals at codons 479-497, 546-572, and 588-608 (1). The missense mutation c.482G>A (p.Gly161Asp) was identified at codon 161 which involved menin-JunD or menin-Smad3 interactions regions. Menin contains a deep pocket that can bind mixed-lineage leukemia 1 (MLL1 or KMT2A) protein or the transcription factor (TF) JunD, with opposite effects on gene transcription (9). In transcriptional regulation, menin has been shown to interact with the activating protein-1 (AP-1) and TF JunD (10), and to suppress Jun-mediated transcriptional activation (11). Thevenon *et al* (12) reported that patients with mutations affecting the JunD interacting domain had a higher risk of death secondary to a MEN1 tumor. In addition, members of the Smad family, Smad3 and the Smad 1/5 complex have been shown to be involved in the transforming growth factor- β (TGF- β) (13) and the bone morphogenetic protein-2 (BMP-2) signaling pathways (14), respectively. For the tertiary structure, the mutation p.Gly161Asp changes the location of hydrogen bands (Fig. 5). The substitution of glycine, a neutral amino acid for aspartate, an acidic amino acid, might interfere with the menin-JunD or menin-Smad3 interactions. In the present case of the Gly161Asp mutation, the Polyphen2, SNPs3D and SIFT results, all supported the damage function of menin. Further studies are necessary to explore the function of mutant protein *in vivo* and *in vitro*.

Knudson's two-hit theory with a loss of heterozygosity (LOH) in the *MEN1* gene is commonly used to explain tumorigenesis in the MEN1 patients (15). In the present study, the identified miscellaneous peak of the proband's resected parathyroid tissue confirmed a heterozygous missense mutation without LOH, implying LOH might not be a unique condition. Luzzi *et al* (16) found that microRNA24-1 (miR-24-1) has ability to bind to the untranslated region (3'UTR) of MEN1 mRNA. Also, the negative feedback-loop between the oncomir miR-24-1 and menin, existing in MEN1 parathyroid adenoma without LOH, modulates the MEN1 tumorigenesis by mimicking the 'Knudson's second hit'.

In summary, we herein reported a case of MEN1 with a missense mutation c.482G>A (p.Gly161Asp) of the *MEN1* gene, which appeared to be responsible for MEN1. The proband and two family members had the same mutation and abnormal laboratory examinations. This suggests that identification of mutations might be very important for prompt diagnosis and monitoring of at-risk family members in MEN1 pedigrees (17).

Acknowledgements

This study was supported by the the Joint Project of Medical Science and Technology of Henan Province, China (grant no. 201201015).

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