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ORIGINAL ARTICLE

Coexistence of multiple endocrine neoplasia type 1 and type 2 in a large Italian family

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Abstract To describe the coexistence of mutations of both the multiple endocrine neoplasia type 1 (*MEN1*) and type 2 (*MEN2*) genes in a large Italian family and evaluate if it could be associated with more aggressive clinical manifestations of the two syndromes. Blood samples were obtained for genetic and biochemical analyses. The *RET* gene exons (8, 10, 11, 13, 14, 15, 16, 18) and the *MEN1* coding regions, including the exon–intron boundaries, were amplified by PCR and directly sequenced. We identified two germline mutations in the proband: the first one, K666M, located at the exon 11 of *RET* proto-oncogene and the second one, IVS4+1G>T, located in the *MEN1* gene. The functional characterization of IVS4+1G>T variation, located in the splicing donor site of exon 4 of *MEN1* gene, caused the in-frame junction of exon 3 to exon 5, thus obtaining a shorter protein. The same proband's germline mutations were found in 16 relatives out of 21 screened subjects: 8 carried IVS4+1G>T, 4 RET K666M, and 4 both the mutations. This is the second report in literature of coexistence in the same family of germline mutations of both *RET* proto-oncogene and *MEN1* gene. The simultaneous presence of the two mutations was not apparently associated with more aggressive diseases, since at last follow-up all patients appeared to be disease-free or well compensated by medical therapy; finally, no one exhibited metastatic diseases.

Keywords MEN1 · MEN2 · Primary hyperparathyroidism · Medullary thyroid cancer

Introduction

Multiple endocrine neoplasia type 1 (MEN1, OMIM 131100) is an autosomal dominant disorder characterized by the combined occurrence of parathyroid glands, pancreatic islet cells, and anterior pituitary tumors (PI) [1–3].

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Less frequently, tumors of the foregut, carcinoids, and lipomas can occur. Prevalence of disease is approximately 1/30,000. The *MEN1* gene is located on chromosome 11q13 and encodes a 610 aminoacid nuclear protein, menin, with no sequence homology to other known human proteins [4]. Mutations of *MEN1* gene were identified in about 80% of families with clinical manifestations of MEN1; the remaining patients may not harbor mutations within the coding region of the *MEN1* gene; these individuals may have mutations in the promoter or untranslated regions (UTRs), which remain to be investigated [5]. The most common *MEN1* mutations are inactivating and commonly result in truncated forms of menin that cannot interact properly with their interactors [6].

Multiple endocrine neoplasia type 2 (MEN2; MIMs 171400, 162300) is an inherited, autosomal dominant disorder that affects 1 in 30,000 individuals [7, 8]. MEN2 is caused by deleterious germline mutations found exclusively within the *RET* protooncogene (OMIM 164761), located on chromosome 10q11.2, which encodes the RET tyrosine kinase receptor, a transmembrane protein present on neuroendocrine cells of neural crest origin. Patients affected by MEN2 have a very high lifetime risk of developing medullary thyroid carcinoma (MTC) (from 95 up to 100%), and may be at increased risk for pheochromocytoma and primary hyperparathyroidism (PHPT), depending on the *RET* mutation [8]. *RET* mutations consist mainly of missense sequence changes found in hot spot exons 10, 11, and 13–16 [7].

By a clinical point of view, MEN1 is characterized by genetic and corresponding phenotypical heterogeneity, whereas MEN2 by a more stringent genotype/phenotype relationship: for example, subjects with RET mutations in codon 634 have a more than 90, 50, and 30% probability of developing MTC, pheocromocytoma, and PHPT, respectively [8]. However, even in MEN2 syndrome, the exact relationship between genotype and disease manifestations is not straightforward. Indeed, the effects of specific genetic changes sometimes do not translate into an expected feature or clinical behavior [9, 10].

In this context, here we report the coexistence of a germline intronic heterozygote variation at the *MEN1* gene (IVS4+1G>T) and a germline mutation of exon 11 of *RET* proto-oncogene (K666M) in a large Italian family and describe the corresponding clinical manifestations in the carriers, followed-up since 2004.

Subjects and methods

Subjects

The proband was a 45-year-old male (subject II-5, Fig. 1; Table 1), affected by macroprolactinoma, PHPT, duodenal micro-gastrinomas, and gastric carcinoid ((laboratory values: serum prolactin 87 ng/ml (n.v. <15), calcium 11.7 mg/dl (n.v. <10 mg/dl), PTH 92 pg/ml (n.v. <60), gastrin 370 pg/ml (n.v. <100)). Moreover, because of the presence of a nodular goiter with a suspected cytology of papillary thyroid cancer, total thyroidectomy plus subtotal parathyroidectomy were simultaneously performed. The pathological diagnosis was parathyroid hyperplasia, papillary thyroid cancer and an unexpected MTC of 6 mm with C-cell hyperplasia of the surrounding thyroid tissue; moreover, because of the persistence of high serum gastrin levels (up to 2300 pg/ml, in the absence of proton pump inhibitory therapy), the patient underwent pancreatic echoendoscopy and a 111-In-octreoscan, that showed a 25 mm lesion located on the head of pancreas and a corresponding uptake of the radiotracer in the same area, respectively. In January 2007, the patient underwent surgical excision of head of pancreas, duodenum, and stomach. The pathologist confirmed the diagnosis of pancreatic gastrinoma, with two peri-pancreatic metastatic lymph nodes and multiple microscopic neuroendocrine tumors of the pancreas, duodenum, and stomach. Serum gastrin levels completely normalized after treatment and, at last follow-up, also serum calcitonin, prolactin (on medical therapy) and both serum calcium and PTH were found in the normal range. Due to the presence of clinical manifestations possibly related to both MEN1 and MEN2 syndromes, molecular analyses for *MEN1* and *RET* genes were performed and a germline heterozygote variation at the *MEN1* gene (IVS4+1G>T) plus a germline mutation of exon 11 of *RET* proto-oncogene (K666M) were, respectively, individuated. Genetic analysis was then extended to relatives and the same *MEN1* gene variation was found in two nieces (subjects III-2 and III-4, Fig. 1), both previously operated on for the removal of insulinomas (IN). To date, 21 relatives have been screened, and 16 of them exhibited one or both mutations (Fig. 1; Table 1).

Methods

After written informed consent, genomic DNA was isolated from peripheral blood leukocytes of all studied subjects by using the phenol/chloroform standard method. Polymerase chain reaction (PCR) amplifications for all the 9 *MEN1* coding regions, including the exon–intron boundaries and for exons 8, 10, 11, 13, 14, 15, 16, 18 of *RET* gene in all recruited subjects and functional characterization of *MEN1* splicing affecting genomic variants (SpaGVs) were performed as previously described [11, 12]. Screening for the MEN1 and MEN2 carriers comprised the following: serum ionized Ca++, glucose, PTH, PRL, GH, gastrin, chromogranin-A, neck ultrasound, abdomen CT and pituitary MRI, and serum calcitonin (basal and after stimulation by pentagastrin),

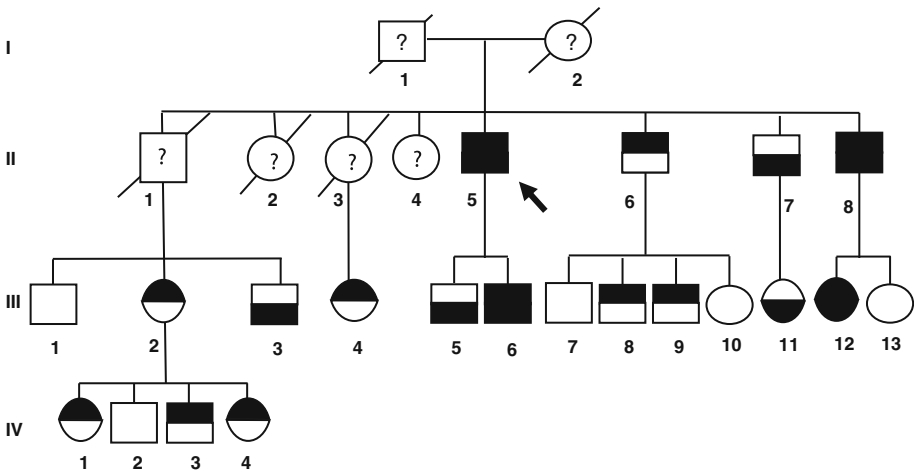

Fig. 1 Pedigree and genotypes of the family. *Upper black filled boxes MEN1* mutation carriers; *lower black RET* mutation carriers; *full black* carriers of both mutations; *open symbols*: unaffected subjects; ? unknown status. An *arrow* indicates the proband

Table 1 Clinical manifestations and age of onset

	Gender	Birth date	Age (years) at the time of first examination	Clinical manifestations and age of onset (years)
Members carrying *RET* proto-oncogene mutation				
II-7	M	1962	44	CCH (45)
III-3	M	1967	38	CCH (41)
III-5	M	2003	3	NH
III-11	F	1992	15	CCH (16)
Members carrying *MEN1* gene mutation				
II-6	M	1960	46	ZES (46), PHPT (46)
III-2	F	1965	40	IN (17), PHPT (39), AF (41)
III-4	F	1984	20	IN (17), PHPT (18)
III-8	M	1983	22	PHPT (22), IN (23), GL (23), LI (26)
III-9	M	1987	18	PHPT (18)
IV-1	F	1987	17	PHPT (20), PI (20)
IV-3	M	1996	8	NH
IV-4	F	1999	5	NH
Members carrying both *MEN1* gene and *RET* proto-oncogene mutations				
II-5	M	1958	45 (proband)	PI (38), PHPT (45), PTC (46), MTC (46), CA (47), GA (47)
II-8	M	1965	39	PHPT (40), CS (40), CA (40), LI (40), AF (40), PTC (40), MTC (40), GA(41)
III-6	M	2005	1	NH
III-12	F	1993	13	PHPT (13), PI (15)

NH no hormonal alteration, *PHPT* primary hyperparathyroidism, *PI* pituitary tumor, *GA* gastrinoma, *IN* insulinoma, *CA* carcinoid, *CS* Cushing syndrome, *AF* angiofibroma, *GL* glucagonoma, *LI* lipoma, *PTC* papillary thyroid cancer, *MTC* medullary thyroid cancer, *ZES* Zollinger–Ellison syndrome, *CCH* C-cell hyperplasia

serum ionized Ca++, PTH, 24-h urinary metanephrines, and neck ultrasound, respectively [3, 8].

Results

In the proband two germline mutations, the first (K666M) located at the exon 11 of *RET* proto-oncogene, and the second (IVS4+1G>T) in the *MEN1* gene were detected. The functional characterization of IVS4+1G>T heterozygote variation, located in the splicing donor site of exon 4 of *MEN1* gene, showed that it alters splicing of the *MEN1* mRNA leading to exon 4 skipping (Fig. 2) and causes the in-frame junction of exon 3 to exon 5, thus obtaining a lack of 43 amino acid residues in menin. Both proband's parents (subjects I-1 and I-2, Fig. 1; Table 1) had previously died

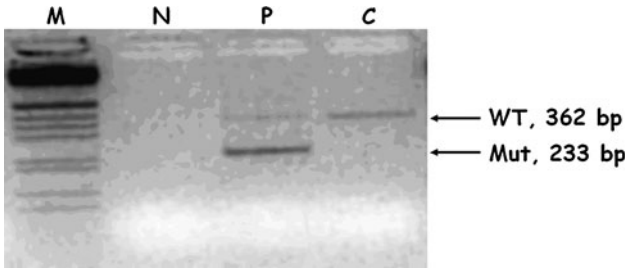

Fig. 2 PCR performed on the cDNA obtained from the proband and from a healthy control. *M* marker, *N* negative control, *P* proband, *C* positive control. In the proband an additional band is present

in old ages and a history of gastric ulcer was present in the father. Two sisters and one brother had previously died at 38, 55, and 29 years, respectively: the first one was affected by PHPT, pituitary tumor and Zollinger–Ellison syndrome, the second by gastrointestinal tumor (not better specified), respectively, whereas no clinical information about the brother is currently available. The same proband's germline mutations were found in 16 out of 21 screened relatives: 8 of them carried IVS4+1G>T, 4 *RET* K666M, and 4 both the mutations (Fig. 1; Table 1). The clinical manifestations of the syndromes were found only in subjects carrying the genetic variations, while they were not found, as expected, among the subjects without *MEN1* or *RET* mutations. In all cases, the patients evaluated and surgically or medically treated by us (not including those who died before, whose medical records are lacking), did not show aggressive diseases, because at last follow-up they appeared to be disease-free or well compensated by medical therapy; finally, no one exhibited metastatic diseases.

Discussion

We have identified a large Italian family affected by both *MEN1* and *RET* mutations. The clinical manifestations of MEN1 in this family co-segregated with the presence of the *MEN1* gene variation (IVS4+1G>T), thus confirming a pathogenetic role of this mutation. Moreover, MEN1 affected individuals showed a variable phenotype, thus confirming the absence of a genotype-phenotype correlation in patients affected by this mutation, also within the same family. In our patients, PHPT was diagnosed only in patients with *MEN1* mutation or carrying both mutations, thus supporting the hypothesis that it was caused by *MEN1* and not *RET* mutation. Germline *MEN1* gene mutation IVS4+1G>T, previously described, leads to the loss of exon 4, causing the in-frame junction of the remaining part of the transcript [6]. To date, only 3 very young subjects out of 12 with this mutation, of age 6, 12, and 15 years, respectively, do not still show any clinical manifestation of MEN1 syndrome. The remaining 9 subjects resulted variably affected by at least one of the following diseases: Zollinger–Ellison syndrome, PHPT, insulinoma, pituitary tumor, gastrinoma, carcinoid, Cushing's syndrome, glucagonoma, angiofibromas, lipomas.

A germline mutation (K666M) of exon 11 of *RET* proto-oncogene was also found in the same family. Five out of the 8 affected family members underwent total thyroidectomy because of high calcitonin serum levels after pentagastrin stimulation test: micro-medullary thyroid cancer (MTC, <1 cm) without neck lymph node metastases (pT1N0M0) was found in 2 and C-cell hyperplasia in the remaining 3 subjects. The two patients with MTC (II-5 and II-8 in Table 1) had also unifocal micro-papillary thyroid cancer at histological examination. To date, both subjects are disease-free. The observations that (1) MTC was in both cases of small size and without metastases and (2) only C-cell hyperplasia, but not MTC, was present in older patients (age 41 and 45 years, respectively), although limited by the small numbers of studied subjects, may suggest that this mutation does not induce an early and aggressive neoplastic disease; moreover, mutation K666M seems to be associated only to MTC but not PHPT and phaeocromocytoma, since PHPT was found only in MEN1 affected family members and phaeocromocytoma, to date, has not been detected in any patient. Two slightly different mutations (K666E and K666N) have been previously described: the first one in three families and only in one subject phaeocromocytoma also occurred, the second one in a single patient affected only by MTC [13, 14]. Four subjects in our family (the proband and three relatives) carried both mutations (Table 1). At the best of our knowledge, the coexistence of MEN1 and MEN2 syndromes has been previously described only in 3 subjects from a German family by Frank-Raue et al. [15]. In that report, the authors observed that affected patients did not have an earlier onset of clinical manifestations of MEN1 or 2 syndromes, thus suggesting that the coexistence of the two mutations did not apparently change the natural history of the diseases. Similarly, the 4 subjects with both mutations in our family did not present an earlier development and/or a more aggressive behavior of the MEN1 and MEN2 related diseases. The pathogenesis of these syndromes is different. MEN1 is due to mutations in a tumor suppressor gene, while MEN2 is due to mutations that induce a constitutional activation of the proto-oncogene *RET*, that encodes a putative tyrosine kinase receptor. The *MEN1* gene mutations found in German and our family are located at the splice junction of different exons (IVS5+1G>A and IVS4+1G>T, respectively). At variance with the German study, the two subjects in our family with both mutations and with histological diagnosis

of MTC had also micro-papillary thyroid cancer (PTC). PTC has been reported to be associated with MEN1 but not MEN2 syndrome [16]. Actually, the incidence of PTC, and particularly micro-PTC, is progressively increased in the last years, so that this finding in our patients could be incidental and not correlated with the presence of MTC or MEN1 syndrome [17]. However, the germline mutation K666M of exon 11 of *RET* proto-oncogene is here described for the first time in only 8 patients, and, possibly, larger patients series are needed in the future to better individuate the corresponding phenotype. Consequently, since PHPT and phaeocromocytoma cannot be reliably excluded, follow-up of these last patients should include also yearly determination of serum ionized Ca++, PTH, and urinary metanephrines.

Conclusion

We present here a large Italian family, with coexistence in some members of both *MEN1* (IVS4+1G>T) and *RET* (K666M) sequence gene variations. The coincidence of both mutations in the same patients did not affect the typical phenotype of MEN1 and the clinical course of disease, while *RET* K666M mutation was only associated with MTC/CCH in our series.

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Conflict of interest The authors declare that they have no conflict of interest.

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