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Features of Multiple Endocrine Neoplasia Type 1 and 2A in a Patient with Both *RET* and *MEN1* Germline Mutations

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The coexistence of multiple endocrine neoplasia type 1 (MEN1) and type 2A (MEN2A) is a rare occurrence and has been reported only twice in the literature. We present a patient with primary hyperparathyroidism and medullary thyroid cancer with strong family history of both MEN1- and MEN2A-associated conditions. Genetic testing showed the patient had a novel *MEN1* loss-of-function mutation, c0.525_526insTT (p.Ala176Leufs*10), and an uncommon Cys630Tyr *RET* mutation. This case highlights the importance of obtaining a detailed family history when heritable endocrine disorders are suspected.

Key Words: MEN1, MEN2A, *RET*, *MEN1*, medullary thyroid cancer, hyperparathyroidism

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant inherited syndrome primarily caused by inactivating mutations of the *MEN1* tumor suppressor gene [1,2]. However, approximately 10% of patients have other mutations involving genes such as *CDKN1B*, *CaSR*, *AIP*, and *CDC73* that give rise to familial or sporadic MEN1 phenotypes [3,4]. MEN1 is characterized by benign or malignant tumors of parathyroid glands, gastro-entero-pancreatic cells, and/or the pituitary. Less commonly, adrenocortical and carcinoid tumors occur [5]. Multiple endocrine neoplasia type 2A (MEN2A) is also inherited in an autosomal dominant pattern and is characterized by mutations in the rearranged during transfection (*RET*) proto-oncogene [6]. Activating *RET* mutations give rise to clinical conditions most commonly seen in patients with MEN2A, including medullary thyroid carcinoma (MTC), pheochromocytoma, parathyroid hyperplasia, and, less commonly, cutaneous lichen amyloidosis, while inactivating *RET* mutations have been seen in MEN2A-associated Hirschsprung's disease [2]. While *RET*-associated MTC in families without other features of MEN2A has been called familial MTC (FMTC), recent expert consensus is that FMTC should be considered as a variant of MEN2A disease expression and not an independent hereditary form of MTC [7]. For this reason, we refer to our proband and proband's Spanish family members with MTC *RET* germline mutations as having MEN2A.

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Here we present a case where the proband was found to have MEN1 and MEN2A with genetic testing confirming a previously unreported *MEN1* germline mutation and a *RET* germline mutation. The coexistence of MEN1 and hereditary MTC syndromes in one family is an extremely rare and has only been reported twice in the medical literature [8,9].

Methods

A 7-gene hyperparathyroidism panel was ordered through the Invitae diagnostic laboratory (www.invitae.com) as a result of the clinical and family history following genetic counseling. Genomic deoxyribonucleic acid obtained from the proband's blood sample was enriched for targeted regions using a hybridization-based protocol and sequenced using Illumina technology. All targeted regions were sequenced with ≥50× depth or were supplemented with additional analysis. Sequencing with deletion/duplication testing was performed on each of the following genes: *AP2S1*, *CASR*, *CDC73*, *CDKN1B*, *GNA11*, *MEN1*, and *RET*. A pathogenic variant, c0.525_526insTT (p.Ala176Leufs*10), was identified in *MEN1*, and a pathogenic variant, c0.1889G > A (p.Cys630Tyr), was identified in *RET*.

Diff-Quik (StatLab Medical Products, Mckinney, TX, US) and Papanicolaou (Richard-Allan Scientific, San Diego, CA, US) stains were performed in the smears from the fine-needle aspiration (FNA) of the thyroid lesion. Hemoxylin and eosin (StatLab Medical Products, McKinney, TX, US) stain was used in the specimens of total parathyroidectomy and thyroidectomy. Images were captured on an upright Olympus BX51 microscope (Olympus, Tokyo, Japan) with the Leica MC170 camera (Leica, Wetzlar, Germany).

Clinical Case

The proband is a 28-year-old male referred for evaluation of a possible MEN1 syndrome. Recent lab results showed elevated calcium and parathyroid hormone levels consistent with primary hyperparathyroidism (PHPT). Dual-energy X-ray absorptiometry scanning revealed low bone mass for age. He did not have a history of kidney stones. He was also found to have a 9 mm thyroid nodule 1-year earlier in Mexico. At that time, a FNA was performed, and cytology classified the nodule as "suspicious for follicular neoplasm." Extensive family history was obtained, and the proband's pedigree is shown in Fig. 1. His family history was significant for a sister (IV0.1) with Zollinger–Ellison syndrome (ZES) and PHPT; a father (III0.2) with ZES and thymic carcinoid; and a paternal uncle (III0.1) with ZES. His paternal great aunt (II0.6) in Spain was diagnosed with MTC and had a Cys630Tyr *RET* germline mutation. This great aunt had several children and grandchildren with MTC, some of whom had confirmed *RET* germline mutations as noted in Fig. 1. There was no family history of hyperparathyroidism, pheochromocytoma, or other adrenal tumors. Our proband had initial commercial genetic testing through Quest Diagnostics (www.questdiagnostics.com) for *MEN1* mutations that did not identify any clinically significant known variants. Following extensive laboratory and imaging evaluation for additional MEN1 conditions (Table 1), he was referred to Genetics and Endocrine surgery. A pituitary magnetic resonance image did not show any lesions, and computed tomography scans of chest, abdomen, and pelvis did not show any masses or lymphadenopathy. He did not have any signs or symptoms suggesting insulinoma, gastrinoma, carcinoid, or functional adrenal tumors. Repeat FNA of the thyroid nodule was read as consistent with medullary carcinoma, with immunohistochemistry showing calcitonin positive cells. His calcitonin and carcinoembryonic antigen levels were elevated to 50.8 pmol/L and 5.2 μg/L, respectively.

Genetic testing revealed a germline heterozygous loss-of-function *MEN1* mutation in exon 3 p.Ala176Leufs*10 as well as a Cys630Tyr *RET* germline mutation (identical to that in his paternal great aunt). Other family members have not yet been tested for the MEN1 mutation as of the writing of this manuscript. He ultimately underwent total parathyroidectomy with left forearm reimplantation, parathyroid cryopreservation, total

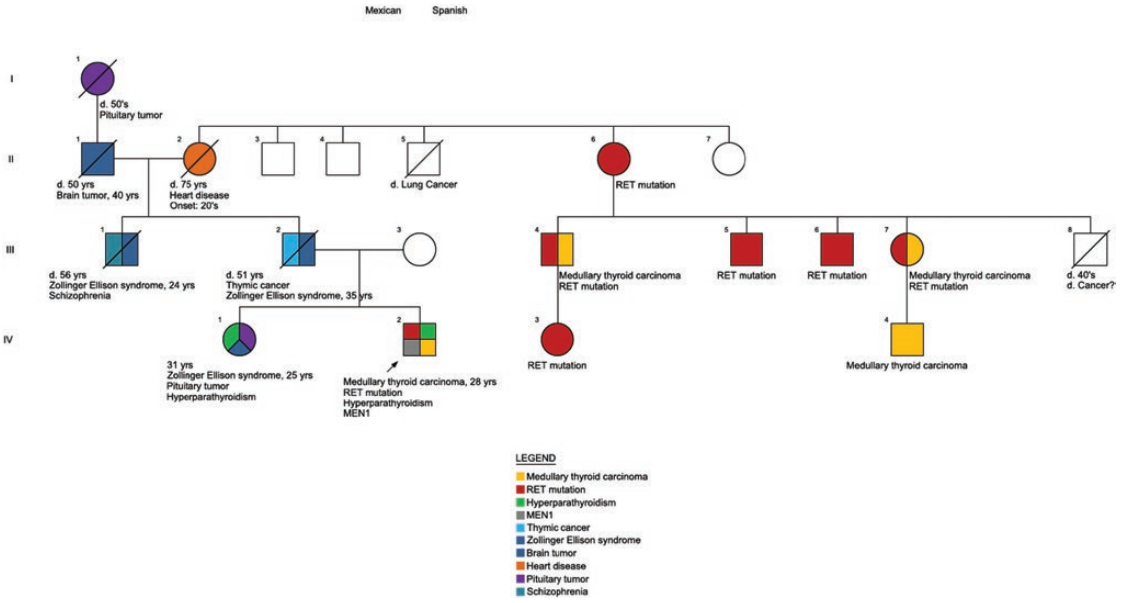

Figure 1. Multigenerational pedigree reflecting both MEN1 and MEN2A in the family history. Pertinent medical histories are listed under individual family members. The designation "d" represents age of death. RET mutation is noted for those members who had genetic testing confirming presence of Cys630Tyr RET germline mutation. MEN1 similarly indicates genetic testing confirming presence of MEN1 germline mutation, c.525_526insTT (p.Ala176Leufs*10).

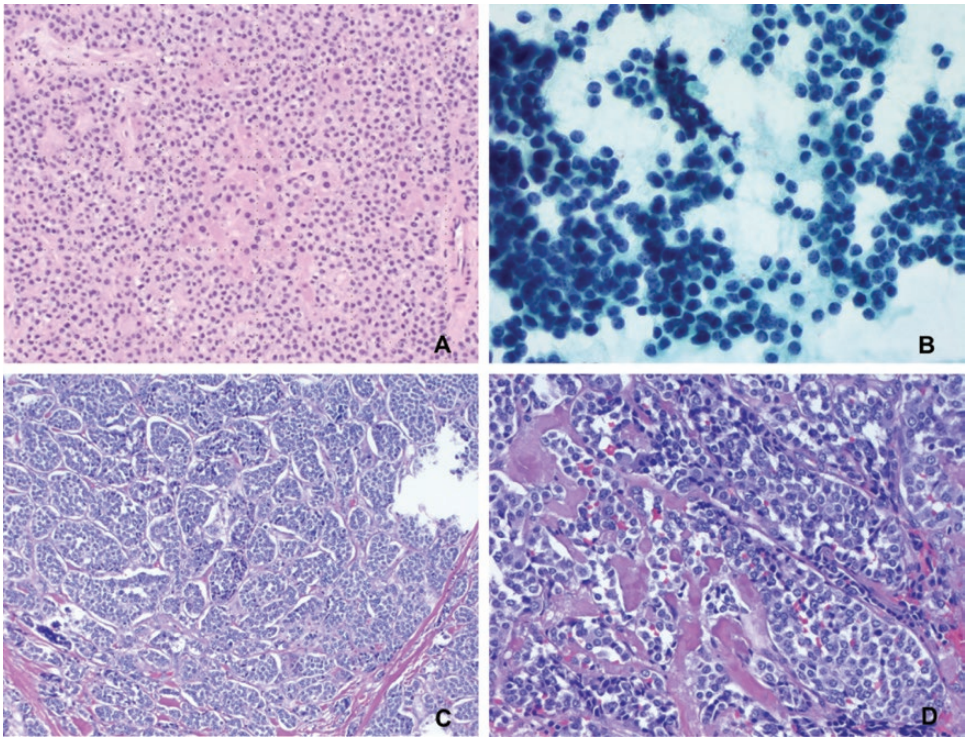

Figure 2. Surgical pathology.

thyroidectomy with regional lymph node dissection, and partial thymectomy. Final pathology (Fig. 2.) confirmed a 1.1 cm MTC in the left thyroid lobe with a 1 mm metastatic focus in one level VI lymph node (Stage III, T1bN1aM0). Pathology also showed normal

Table 1. Laboratory Testing

Laboratory test	Ref. range	Pre-op	Post-op
PTH (ng/L)	14.0–64.0	103.0	10.0
Calcium (mmol/L)	2.2–2.6	2.6	2.2
Gastrin (pmol/L)	<48.1	17.0	—
Pancreatic polypeptide (ng/L)	<274.0	221.0	—
Prolactin (pmol/L)	87.0–782.6	343.5	—
Insulin, fasting (pmol/L)	18.1–172.9	66.7	—
Glucose, fasting (mmol/L)	3.6–5.5	5.1	—
IGF-1(nmol/L)	8.3–48.9	25.2	—
CEA (μg/L)	0.0–3.8	5.2	1.8
Calcitonin (pmol/L	0.0–2.2	50.8	<0.6
Plasma-free metanephrines (pmol/L)	<289.0	248.4	—
Plasma-free normetanephrines (pmol/L)	<808.1	382.2	—

Abbreviations: CEA, carcinoembryonic antigen; IGF-1, insulin-like growth factor-1; PTH, parathyroid hormone.

thymic tissue and parathyroid hyperplasia with the largest gland weighing 80 mg. He developed postoperative hypoparathyroidism that was improving at 3 months postsurgery with minimal calcium supplementation (Table 1). Postsurgical labs also showed his serum calcitonin level was undetectable, and serum carcinoembryonic antigen normalized to 1.8 μg/L.

Discussion

To date, this is the third report in the literature of an individual with coexistent MEN type 1 and hereditary MTC with confirmed *MEN1* and *RET* mutations [8,9]. Notably, the *MEN1* mutation identified in our proband is the first report of this specific mutation. The mutation in exon 3, p.Ala176Leufs*10 is a loss-of-function change creating a premature translational stop signal, leading to absent or disrupted menin protein. Similar loss-of-function mutations in the *MEN1* gene are known to be pathogenic [10]. Importantly, our proband and several family members also manifested several MEN1-related endocrine tumors including ZES, PHPT, and carcinoid tumor, further supporting the pathogenic significance of this mutation. Our patient had undergone commercial testing for *MEN1* mutations that did not show any clinically significant known variants. Even in the face of "negative" genetic test results, review of family and clinical history may warrant consideration of further genetic testing that utilizes alternative deoxyribonucleic acid sequencing approaches such as the hybridization-based protocol used in our proband.

The Cys630Tyr *RET* germline mutation identified in our proband and documented in multiple family members is an uncommon mutation. It is not present in the gnomAD database (gnomad.broadinstitute.org) of 278 002 alleles or 139 001 individuals. In 95% of MEN2A patients, the most common *RET* mutations occur in codons 634, 609, 611, 618, and 620. Cys630Tyr *RET* mutations have been documented in a handful of cases in both MEN2A and FMTC [11,12]. Much is still unknown about this mutation, including penetrance, associated onset of disease, and prognosis. Certain *RET* mutations are associated with more aggressive MTC in MEN2A such as *RET* codon M918T, C634, and A883F mutations [7]. However, based on a few case reports, our patient's mutation is thought to be associated with a less aggressive course of MTC and lower incidence of associated pheochromocytomas [11,12]. Codon 630 mutations are currently categorized as moderate risk for MTC [7]. In our patient's family, members with MTC have not had aggressive disease courses, and all known relatives with MTC are still alive and are either cured or have undergone prophylactic thyroidectomies based on genetic testing, as of the preparation of this manuscript.

Our patient's germline mutations thus far have manifested typical phenotypes of MEN1 and MEN2A syndromes that have important implications in his treatment and surveillance.

Given his personal history of PHPT and MTC, along his with family history of MEN1- and MEN2A-related endocrine disorders, close surveillance will be needed for both MEN1 and MEN2A. In addition, follow-up with genetic counseling to assist in family planning was of particular interest to this patient. Referral of other at-risk family members, including his sister, for consideration of cascade testing and screening was also discussed extensively with our patient [13]. Additional next steps include analysis of our proband's tumor tissue for loss of heterozygosity at the *MEN1* locus, which would further support his mutation as pathogenic driver [14]. The interaction of these 2 germline mutations and whether they predict a worse prognosis remains to be seen given the limited literature. In the family with coexistent MEN1 and MEN2A identified by Mastroianno et al [9], affected family members showed variable phenotypes with 2 family members diagnosed with MTC. Similar to our case, the members affected in this family did not show aggressive disease course, and there was no history of pheochromocytomas. In the report by Frank-Raue et al [8], again, there was variable MEN1 phenotype, but, unlike our case aside, from the c-cell hyperplasia noted in the proband, no MEN2A phenotype was identified in any family members. Further monitoring of our patient and his family over time along with additional genetic analysis as previously noted may shed light to this question.

Conclusion

In this patient with coincident MEN1 and MEN2A syndromes, genetic testing confirmed a novel germline mutation in *MEN1* that was not identified on routine genetic testing and a Cys630Tyr mutation in *RET*. The presence of both germline mutations manifested with typical phenotypes of MEN1 and MEN2, including PHPT and MTC. Our case contributes to the existing limited literature document coexisting MEN1 and MEN2A. It also highlights the utilization of appropriate genetic testing in identifying mutations that may not be picked up by commercial genetic laboratories. In families where a pathogenic variant is identified, screening of additional family members who would benefit from genetic counseling and consideration of testing is valuable for disease monitoring and intervention.

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Additional Information

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Data Availability: Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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