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Case report

A rare missense variant in *RET* exon 8 in a Portuguese family with atypical multiple endocrine neoplasia type 2A

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ABSTRACT

BACKGROUND AND OBJECTIVE: Multiple Endocrine Neoplasia type 2 (MEN2) is a rare genetic disorder characterized by medullary thyroid carcinoma (MTC), pheochromocytoma and primary hyperparathyroidism. MEN2 is an autosomal dominant syndrome caused by mutations in the *RET* proto-oncogene. In the vast majority of patients, the mutations are localized in exons 10, 11 and 13-15 of the *RET* gene. Rare variants located in exon 8 were recently identified but their clinical significance remains unclear. DESIGN AND METHODS: We studied two sisters presenting with pheochromocytoma as the first tumor. One of the sisters was diagnosed with a right pheochromocytoma at the age of 44 and at age 53 she developed an invasive left pheochromocytoma with no other endocrine neoplasia. The other sister was diagnosed with a left pheochromocytoma at age 50 and at age 64 she had a right phemochromocytoma and MTC. Neither of the two sisters presented evidence of primary hyperparathyroidism. Mutations of the *RET* proto-oncogene were investigated by DNA sequencing. RESULTS: We detected a germline missense variant in *RET* exon 8 (p.Cys531Arg) in both sisters. The p.Cys531Arg variant was not present in a third 50-year-old sister who has remained to date clinically unaffected. CONCLUSION: This is the first case showing the p.Cys531Arg variant in *RET* exon 8 co-segregating with family members affected by a syndrome reminiscent of MEN2A. Our results suggest that this variant has a specific genotype-phenotype correlation as it is associated with the development of pheochromocytoma before the onset of MTC.

Key words: MEN2A, Pheochromocytoma, RET mutation

INTRODUCTION

Multiple Endocrine Neoplasia type 2 (MEN2, OMIM 171400) is a rare (approximately one in 200,000 live births) autosomal dominant tumor syndrome. Distinct MEN2 subtypes have been recognized.[1-5] MEN2A is the most common form of MEN2 and its first manifestation is often MTC, usually occurring between the ages of 20 and 30 years.[6] MEN2A is characterized by MTC and pheochromocytoma plus primary hyperparathyroidism.[7]

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Since the discovery of the MEN2 causative gene, the *RET* proto-oncogene, direct genetic testing for at-risk individuals has been recommended.[8] Patients with germline *RET* mutations may undergo risk assessment and may be offered thyroidectomy prior to developing clinically evident MTC.[9,10] However, due to the varying clinical effects of *RET* mutations, establishing precise genotype-phenotype correlations remains challenging. The *RET* gene is located on chromosome 10 (10q11.2) and comprises 21 exons that encode for a transmembrane tyrosine kinase receptor involved in growth and differentiation of neural crest-derived tissues.[7,11,12] Most *RET* mutations are located in one of six conserved cysteines in the extracellular domain that induce a ligand-independent RET domerization.[13-16] Although most characterized *RET* mutations are located in exons 10, 11, 13, 14, 15 and 16,[17,18] additional mutations in exons 5 and 8 have been reported.[19]

Here we report a rare germline missense variant in *RET* exon 8 (p.Cys531Arg) that co-segregates with affected siblings in an atypical Portuguese MEN2A family. The p.Cys531Arg variant was recently identified in apparently sporadic cases of MTC,[19] but its clinical significance remains unclear. Our results show that this variant is associated with the development of pheochromocytoma before the onset of MTC, thus suggesting a novel genotype-phenotype correlation.

CASE REPORT

A 44-year-old Caucasian female (index case) was admitted to our endocrine outpatient department in 1995 because of paroxysmal hypertension and headaches lasting for several weeks. As biochemical and functional evaluation was indicative of left-sided pheochromocytoma, she was submitted to left adrenalectomy. There was no evidence of any other endocrine neoplasia. At age 53 a right pheochromocytoma was confirmed on ^{123}I-MIBG scanning. Right adrenalectomy was performed. Two years later liver and paravertebral metastasis were found and treated with ^{131}I-MIBG (total activity 800 mCi). At age 57 a slight calcitonin elevation was first detected [6pg/mL (Reference Value <5pg/mL)], as well as a 17×14mm hypoechogenic thyroid nodule that was found to be benign after histological analysis. When she was 63 (in 2013), hyperparathyroidism with hypercalcemia lacking surgical criteria were detected (Table 1). Both parathyroid function and calcitonin are under careful follow-up. The authors do not exclude the possibility that the moderate increase in calcitonin is related either to the hyperparathyroidism or to the malignant pheochromocytoma, as this hormone is also produced in chromaffin cells.[20] Indeed, both sporadic and familial pheochromocytomas have previously been associated with calcitonin production.[21,22] At the same time, normetanephrines were still high and did not suppress on the clonidine test (Table 2). ^{123}I-MIBG scanning revealed high uptake in lesions diagnosed in the mediastinum and left pulmonary apex, as well as in the lower right paramedian mediastin and left dorsal paramedian region. A new therapeutic session with ^{131}I-MIBG was offered.

The 50-year-old female sister of the index case presented in 1993 with clinical, analytical and imagiologic evidence of a left pheochromocytoma with no evidence of any other endocrine neoplasia. Left adrenalectomy was performed. Two years later, in 1995, when her sister was first diagnosed with pheochromocytoma, both sisters were submitted to genetic testing for the most common *RET* and *SHD* mutations but none were

Table 1. Analytic evaluation of Case 1 in 2013, when she was 63 years old. Mild hyyperparathyroidism with hypercalcemia, as well as mild calcitonin elevation were diagnosed

Parameter (Reference Value)	Value
PTH (14-72pg/mL)	128
Vitamin D (30-80ng/mL)	22.2
Serum Calcium (8.6-10.2mg/dL)	10.7
Serum Phosphorus 2.4-5.1mg/dL	3.4
Calcitonin (<5pg/mL)	6.4
NSE (0-16.3ug/L)	20

Table 2. Clonidine Suppression Test performed in Case 1 after bilateral adrenalectomy: normetanephrine values were initially high and did not suppress after clonidine administration

Clonidine Suppression Test (300mg po)		
	0 min	180 min
Metanephrines (<90ug/24h)	85	50
Normetanephines (<180ug/24h)	464	231

detected. At age 64 a right phemochromocytoma was found and surgically resected. At age 65 the patient presented increased serum calcitonin levels and was diagnosed with MTC following total thyroidectomy.

In 2013, genomic DNA was extracted from both patients and their unaffected sister. For the index case, exons 5, 8, 10, 11 13, 14, 15 and 16 of the *RET* gene (10q11.21, OMIM 164761, NM_020630.4) were analyzed. In addition, 100-200 intronic nucleotides flanking each splice site were sequenced. The nomenclature used to describe the genetic variants follows the Human Genome Variation Society (HGVS) guidelines. Two heterozygous variants were identified (Figure 1). One is located in exon 8 and

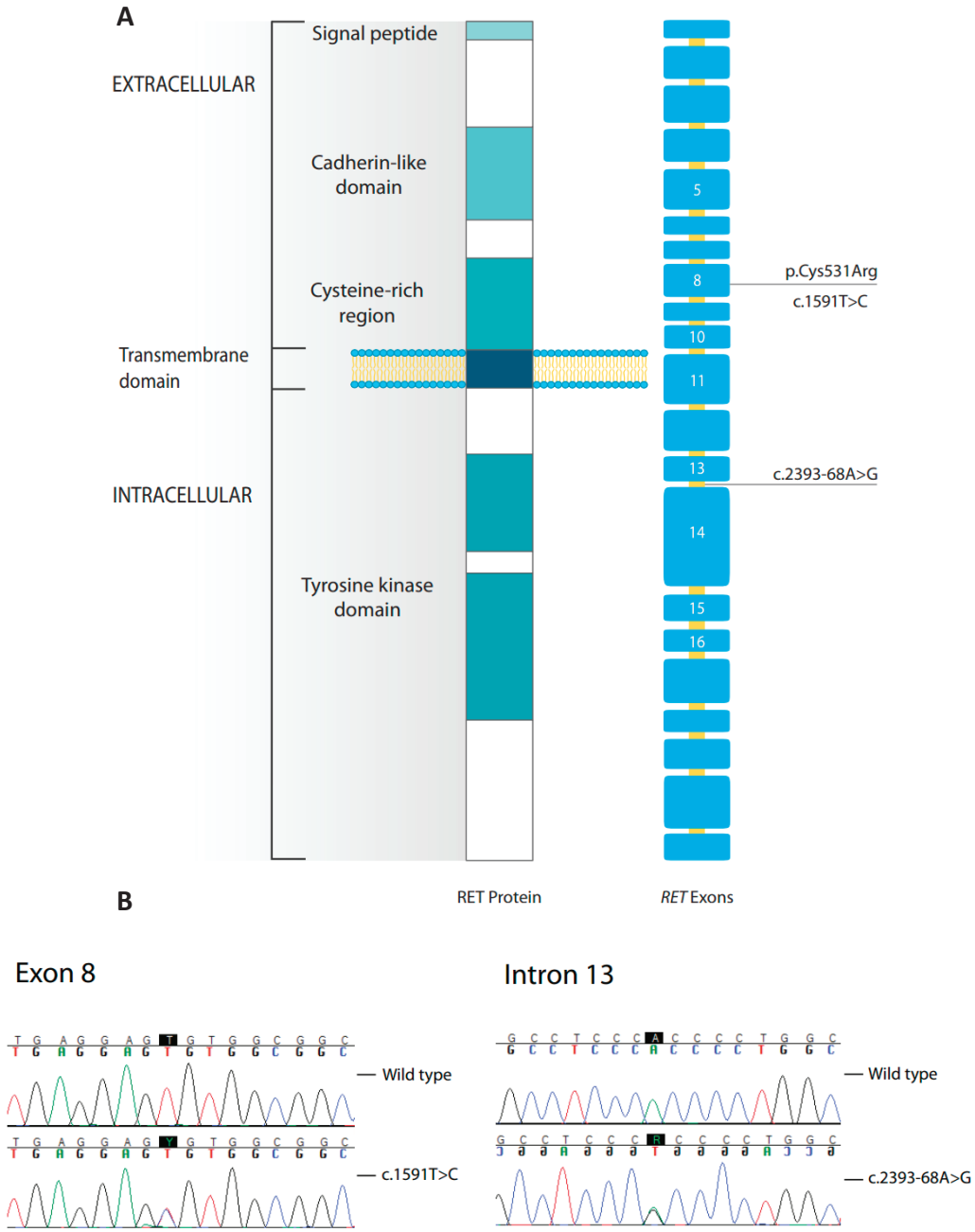

Figure 1. A) Schematic illustration of RET protein domains and exonic structure of *RET* mRNA. The locations of the variants are indicated. B) Identification of variants by sequence analysis of the *RET* gene.

consists of a T to C substitution at position c.1591, leading to a cysteine to arginine amino acid change in codon 531 (c.1591T>C, p.Cys531Arg). The second variant is located in intron 13 and consists of A to G substitution at position c.2393-68 (c.2393-68A>G). In light of these results, we next screened *RET* exons 8 and 14 in the two sisters. The affected sister was also heterozygous for both variants. The asymptomatic sister carried the intron 13 variant but the sequence of exon 8 is normal. The parents of the three sisters were unavailable for DNA testing. The offspring of the two affected sisters were not genetically analyzed; they are clinically and biochemically asymptomatic.

DISCUSSION

Most MEN2 families studied so far have germline-activating mutations of the *RET* proto-oncogene in exons 10, 11 and 13-15. Although genetic variants in other locations along the gene have been identified, their clinical significance remains unclear.[19,23-31] In this report we describe a family with a syndrome reminiscent of MEN2A that carries two distinct *RET* variants. The c.2393-68A>G intronic variant has been previously described[32] and is included in the NCBI database of single nucleotide polymorphisms (http://www.ncbi.nlm.nih.gov/projects/SNP/sn_pref.cgi?rs=57622093 6). An *in silico* analysis performed with MutationTaster (www.mutationtaster.org) indicates that this variant does not affect splicing. Consistent with the view that this variant is likely benign, it is present in the asymptomatic 50-year-old sibling of the two patients. In contrast, the c.1591T>C variant in exon 8 is present in the two affected sisters but not in the asymptomatic sibling.

The c.1591T>C variant is a missense genomic alteration in codon 531 causing substitution of a cysteine by an arginine in the extracellular cysteine-rich domain of the RET receptor. Although it is still described in the ARUP® MEN2 database (http://www.arup.utah.edu/database/MEN2/MEN2_welcome.php) as a variant of unknown functional significance, *in silico* analysis performed with MutPred (http://mutpred.mutdb.org/), PolyPhen (http://genetics.bwh.harvard.edu/pph2/) and UMD (http://umd-predictor.eu) indicates that this variant is probably damaging. When HEK 293T cells were transiently transfected with an expression vector for the C531R variant, the mutant protein was more tyrosine phosphorylated than wild-type RET, suggesting that receptors with this mutation can be activated even in the absence of the ligand.[19,33] However, the amount of phosphorylation found for p.C531R was significantly lower than that obtained with the most common MEN2A-causing mutation p.C634R.[9] Moreover, in contrast to cysteines encoded in exons 10 and 11, which are commonly mutated in hereditary MEN2, cysteine 531 is less conserved throughout evolution.[34] Taken together, these observations suggest that mutation of cysteine 531 can activate the RET receptor but has mild oncogenic potential. In agreement with this interpretation, the two patients studied developed tumours much later than typical MEN2A cases.

The two sibling patients reported in this study presented additional features that are unusual in typical MEN2A cases. First, the index case had malignant pheochromocytoma, which is very rare in MEN2A.[35] Second, pheochromocytoma was the only neoplasia identified in the index case and in her affected sister pheochromocytoma preceded MTC. This raises the hypothesis that the p.C531R mutation is associated with higher incidence of pheochromocytoma. In agreement with this view, patients with another mutation in exon 8 (G533C) had pheochromocytoma preceding MTC,[28,29] while an American family with 47 affected relatives also showed a strong association of this mutation with pheochromocytoma.[31]

In conclusion, we report a family carrying a rare germline missense variant in *RET* exon 8 that is associated with late-onset pheochromocytoma before development of MTC. Given that direct genetic testing is crucial for accurate diagnosis of MEN2, molecular risk assessment, informed family counseling and therapeutic profiling, our report provides new insight into genotype-phenotype associations involving mutations in *RET* exon 8.

DECLARATION OF INTEREST

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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