A novel RET gene mutation in a patient with apparently sporadic pheochromocytoma

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Abstract. Pheochromocytoma (Pheo) is a chromaffin tumor arising from the adrenal medulla. The recent discovery of new germline mutations in RET, SDHA, SDHB, SDHC, SDHD, VHL, NF1, TMEM127, MAX genes, increased the rate of genetic disease from 10% to 28% in patients with apparently sporadic tumor. RET germline mutations cause multiple endocrine neoplasia type 2 syndrome (MEN 2A) characterized by complete penetrance of medullary thyroid cancer (MTC), and lower prevalence of Pheo and hyperparathyroidism. We describe the genetic etiology of an apparently sporadic case of monolateral Pheo in a 42-year-old male patient. A new (not previously reported) MEN 2A-associated germline RET mutation located in exon 11 (Glu632Gly, caused by an A>G point mutation at position 1895 of the RET cDNA) was found in the patient but not in his living first-degree relatives. This observation increases the number of possible germline RET mutations. Genotype-phenotype correlation of this new genetic alteration is unknown, but this rare mutation is probably associated with a low risk for MTC (usually the first tumor diagnosed in MEN 2A syndrome) and with the development of Pheo before the onset of MTC. Since we expect MTC to occur in our patient, strict follow-up is mandatory. Our findings emphasize the relevance of genetic testing in patients with Pheo, especially when the clinical presentation (family history, young age at diagnosis, multiple locations, malignant lesions, and bilateralism) is suggestive.

Key words: Pheochromocytoma, MEN 2A, RET mutation

THE WHO tumor classification defines pheochromocytoma (Pheo) as a chromaffin tumor arising from the adrenal medulla, and paraganglioma (PGA) as an extra-adrenal tumor [1]. Adrenal and extra-adrenal Pheo have an incidence of 2-8 cases per million per year [2]. In contrast to the previous estimation that only 10 % of Pheo have a genetic component [3], recently germline mutations in RET, SDHA, SDHB, SDHC, SDHD, VHL, NF1, TMEM127, MAX genes have been reported up to 28 % of patients with Pheo or PGA. Young age at diagnosis, multiple tumors, personal and familiar history should be suspicious of genetic disease [4]. Hereditary Pheo may be a phenotype of multiple endocrine neoplasia type 2 (MEN 2A).

MEN2 is a rare autosomal dominant condition resulting from an activating germline mutation of RET proto-oncogene [5]. In MEN 2A syndrome, carriers of a RET mutation are at high risk of medullary thyroid carcinoma (MTC, 95% of cases), hyperparathyroidism (HPTH, 25% of cases) and Pheo in 50% of cases; in MEN2B, a more aggressive subtype, MTC is present in 100% of cases and it is associated with Pheo (50%), marfanoid habitus and mucosal neuromas, while HPTH has never been described; familial MTC (FMTC) is considered a variant of MEN2 in which only MTC is found [6].

About 98% of patients with MEN 2 have germline mutations in exons 5, 8, 10, 11, 13, 14, 15 or 16 of the RET gene. The most frequent RET mutation in MEN 2 syndrome is located in exon 11 at codon 634. Less common mutations of other cysteine residues can be found at codons 609, 611, 618, 620. Rare mutations are localized at codons 630, 768, 790, 791, 804, 883, 891 and extremely rare ones are at codons 631 and 632 [7].

The MEN 2 syndromes are characterized by a strong genotype-phenotype correlation: a specific RET muta-
tion may be responsible for a particular phenotype (average age of onset, penetrance, clinical features and a more or less aggressive clinical course) [8].

In a continuous series of 246 RET mutation carriers, Imai et al, described that the age-dependent penetrance of Pheo was 32% by age 50 and 50% by age 76 [9].

In MEN 2A, the onset of Pheo is usually concomitant or subsequent to MTC [10], but in 13-27% of cases it represents the first manifestation of the syndrome [9, 11]; moreover it has also been reported a codon specific age-related onset of Pheo [12, 13].

In this paper we describe a patient with an apparently sporadic Pheo with a novel germline RET mutation.

**Case Report**

A Caucasian 42 year-old male was referred to his physician because of abdominal pain, sweating, chills and hypertension. Abdomen ultrasound examination revealed a 3 cm right adrenal mass, hypoechoic, with regular shape, contiguous but not infiltrating renal vein. CT scan showed a heterogeneous adrenal mass with a mild enhancement after contrast media infusion (Fig. 1).

Hormonal measurements showed high urinary noradrenaline levels (100 μg/24h; normal values less than 85) with normal dopamine and epinephrine.

The patient underwent to laparoscopic adrenalectomy with an histopathological diagnosis of intra-adrenal Pheo of 3.7 cm in size.

**Post-operative evaluation**

After diagnosis the patient referred to our Medical Center. Negative post-operative urinary catecholamines confirmed the radical resection. Serum calcitonin (CT), CEA (carcinoembryonic antigen), calcium, PTH were in the normal range.

No evidence of thyroid nodules neither suspicious lymph-nodes were observed at neck ultrasound.

In consideration of patient's young age, in order to exclude a genetic form of disease, genetic tests were planned after patient’s informed consent starting with RET gene analysis.

Genomic DNA was purified from peripheral blood lymphocytes of the index case using the High Purification PCR Template Preparation Kit (Roche Diagnostic GmbH, Germany). The RET gene exons 10, 11, 13, 14, 15 and 16 were analyzed using PCR and DNA direct sequencing. Exon 11 mutation was confirmed on an independent sample of the index case and mutation analysis of his relatives was performed using specific amplification primers (5’-CCTCTGGCGGTGCCAAGCCTC-3’; 5’-CCTCGTCTGCCCAGCGTTG-3’) and exon 11 direct sequencing.

**Results**

We identified a new germline RET mutation in exon 11 which has not been previously reported. Glu632Gly substitution is caused by a A>G point mutation at position 1895 of the RET cDNA (Genebank n. NM_020975.4) (Fig. 2).

Our finding led to the diagnosis of MEN 2A of the index case and subsequently alive first-degree relatives (two siblings and two children) with no apparent history of thyroid cancer and endocrine disease underwent to genetic analysis but no RET gene mutation was found. After 3 months serum basal CT, urinary catecholamine levels and abdomen CT scan were normal; stimulated CT level was no checked because of pentagastrin unavailability.

After 46 months, the patient is alive with no evidence of recurrence and/or concomitant diseases.

Since the genotype-phenotype correlation of this new mutation is unknown and therefore the optimal timing for prophylactic surgery for MTC is not defined, we planned, with the consent of the patient, a strict follow-up based on periodic CT measurement and neck ultrasound.

**Discussion**

Pheo is defined as a tumor arising from catecholamine-producing chromaffin cells in the adrenal

**Fig. 1** Abdominal computed tomography scan shows right adrenal mass.
Pheochromocytoma and RET mutation

Machens et al. showed that, in relation to these three risk categories in patients affected by MEN2A, on average, Pheo occurred after MTC by 12.1 years in highest risk, and 4.8 years in high risk categories, respectively, but preceded MTC by 5.1 years in the least-high risk category [12, 13].

In our case, we found a new germline mutation located at codon 632 in exon 11 of RET proto-oncogene. It consists in a point mutation changing a GAG codon to GGG, determining the amino acid substitution from glutamate to glycine. Only one report in literature described Pheo in a patient harboring germline mutation located at codon 632, but authors reported a GAG>AAG mutation, resulting in glutamate to lysine substitution [20]; moreover a rare mutations at the same codon (E632K), but with different amino acidic substitution (c1894G>A), has been described in single individual report in a patient with multifocal MTC [21].

Limitation of our report is the lack of demonstration of transforming activity of mutant protein. However it’s known that codon 632 is localized in the cistein-rich extracellular domain, which is important for tertiary structure and protein dimerization. Substitution among this domain of the reactive, idrophilic side-chained glutammic acid with the small, apolar aminoacid glycine might lead to alteration in protein folding and subsequent modification of protein activity.

Obviously genotype-phenotype correlation of this genetic alteration is unknown, but it is likely that this rare mutation may belong at low risk category for MTC, and that Pheo has therefore preceded onset of MTC. We can expect however occurrence of MTC in our patient. Neumann et al. examining a series of 271 apparently sporadic Pheos, found 13 patients (4.8%) with germline RET mutation, none of them had clinical evidence of MTC at presentation, but 12/13 developed MTC during the follow-up period [15]. In our patient a strictly periodic CT measurement (basal and/or stimulated) to detect early MTC is then mandatory.

Moreover this patient is at risk to develop a bilateral medulla [1]. It was a widespread assumption that only 10% of Pheos were hereditary [3], but more recent studies showed that frequency of germline mutations associated with Pheo is higher than previously estimated (hereditary predisposition for Pheo is approximately 30%). Moreover also in apparently sporadic Pheos, up to 25% of them harbor a germline mutation [14, 15]. Several genetic syndromes are known to be associated with an increased risk for Pheo including von Hippel-Lindau (VHL) syndrome, MEN2 and neurofibromatosis type 1 (NF1). Nine genes have been described as to be related with a predisposition to the development of Pheos and extra-adrenal paragangliomas (VHL, RET, NF1, SDHA, SDHB, SDHC, SDHD, TMEM127, MAX) [2, 4, 14].

Germline mutation of RET proto-oncogene (resulting on constitutive activation of the encoding receptor tyrosine kinase) causes MEN2, an autosomal dominant disease that shows three clinical subtype (MEN 2A, MEN 2B and FMTC) [6].

In MEN2A, Pheo is present in about 50% of cases and often diagnosed at younger age than patient with sporadic disease; it always produces epinephrine and, in 13-27% of cases, is the first manifestation of MEN2A; it is usually bilateral, diagnosed synchronously or metachronously and rarely many years later [16]; it is unlikely metastatic, and it is not associated with a more advanced stage of MTC at diagnosis or a shorter survival [17].

Clear association has been documented between specific RET mutations and development of Pheo. The Seventh International Workshop on MEN created a classification system for RET mutations - subsequently modified by the American Thyroid Association, ATA [18, 19] - based on the aggressiveness and onset of MTC; also mutations predisposing Pheo has been classified similarly: highest-risk category includes RET (codon 918), SDHD and SDHB mutations, high-risk category includes RET (codons 609, 611, 618, 620, 630, 634) and VHL missense mutations and least-high risk category includes RET (codons 768, 790, 791, 804, 891) and VHL truncating mutations [6, 13]. Moreover Machens et al. showed that, in relation to these three risk categories in patients affected by MEN2A, on average, Pheo occurred after MTC by 12.1 years in highest risk, and 4.8 years in high risk categories, respectively, but preceded MTC by 5.1 years in the least-high risk category [12, 13].

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Pheo and HPT; a surveillance of contralateral adrenal gland and serum calcium levels are advisable.

In addition, our patient was previously submitted to surgical excision of a skin melanoma. A Swedish study showed an increased risk for additional tumors and an increased tumor-related mortality in patients with Pheos. The most frequent cancers were liver, biliary tract and central nervous system tumors, melanoma and cervix carcinoma [22]. Moreover desmoplastic melanoma (an uncommon variant of melanoma) is associated with G691S polymorphism at the RET coding region [23]. More studies are necessary to improve knowledge about relationship between these two tumors that arise from cell types that share the same embryological origin.

In conclusion in our report, the discovery of this new mutation at codon 632 of RET gene emphasizes the relevance of a more widespread genetic testing of patients with Pheo; to date it is not cost effective to test every gene in every patient, so we all agree on a judiciously decision to test, and which genes to test, based on presence of significant clinical characteristics (family history, age at diagnosis, multiple locations, malignant lesions and bilateralism). In particular age less than 50 years, bilateral Pheo and significant increase in urinary or plasma metanephrines concentration are currently considered strong indications for screening RET mutations in apparently sporadic Pheo. Benefits of detection of hereditary Pheo include the identification of patients that may develop additional cancers, additional adrenal or extra-adrenal Pheos and additional diseases as well as early diagnosis in affected family members.

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