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CASE REPORT

A germline *RET* proto-oncogene mutation in multiple members of an Arab family with variable onset of MEN type 2A-associated clinical manifestations

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KEYWORDS

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Abstract *Background:* Multiple endocrine neoplasia type 2A (MEN2A) is a rare cancer associated-syndrome, inherited in an autosomal dominant fashion and caused by germline mutation in *RET* proto-oncogene. Clinical diagnosis depends on the manifestation of two or more certain endocrine tumors in an individual, such as medullary thyroid carcinoma, pheochromocytoma, and parathyroid adenoma or hyperplasia. Prophylactic total thyroidectomy with central neck lymph node dissection is mandatory for mutation carriers, with periodic monitoring of the other concerned organs.

Subjects and methods: We have screened 27 individuals from a large Arab family with multiple affected members. Mutational screening involved the hotspot regions in the most commonly implicated exons 10 and 11 of *RET* proto-oncogene using PCR amplification of the coding and the flanking intronic regions followed by the Sanger sequencing. We aimed for confirmation of the clinical diagnosis and identification of at-risk asymptomatic mutation carriers.

Results: A pathogenic variant c.1901G > T (p.Cys634Phe), in exon 11 of *RET* proto-oncogene was identified in 15 members of different ages.

Conclusion: Genetic counseling plays a key role in the management of such high-risk families and hence helps in avoiding or reducing disease recurrence in their future generations.

1. Introduction

Multiple endocrine neoplasia type 2 (MEN2) is a rare cancer associated-syndrome, inherited in an autosomal dominant fashion and caused by germline mutation in *RET* proto-oncogene. The main manifestation is medullary thyroid

Abbreviations: ATA, The American Thyroid Association; FMTC, familial medullary thyroid carcinoma; MTC, medullary thyroid carcinoma; MEN2A, multiple endocrine neoplasia type 2

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carcinoma (MTC), which is a cancer of the parafollicular C/or calcitonin secreting cells. It is classified into three subtypes: MEN2A, familial medullary thyroid cancer (FMTC) and MEN2B. In FMTC, MTC is the only presented phenotype and it occurs at a later age of onset with a relatively better prognosis. MEN2B syndrome is characterized by MTC that occurs in childhood, with an increased risk for pheochromocytoma, mucosal neuroma of lips and tongue, ganglioneuroma of the gastrointestinal tract and Marfanoid habitus [1,2]. In MEN2A associated-families, individuals with pathogenic mutations are at increased risk (95%) for development of early adult onset MTC (multifocal or bilateral), which is often associated with C-cell hyperplasia; also risk is increased for pheochromocytoma (50%) and parathyroid adenoma or hyperplasia (20–30%) [2,3]. The prevalence of MEN 2A is estimated to be 1 per 50,000, with usual diagnosis age of 20–30 years. *De novo* mutations may to be responsible for around 5% of MEN2A patients [4].

RET proto-oncogene is located on chromosome 10 (10. q11.2); it comprises 21 exons and encodes a transmembrane tyrosine kinase receptor protein which plays an important role in transferring cell growth and differentiation signals. It is expressed in parafollicular C cells of the thyroid gland, parathyroid glandes, adrenal medulla and in the urogenital system [5].

The clinical phenotype depends on type and position of gene mutation [1,6,7]. All reported mutations; their associated phenotypes and the pertinent literature references are shown in the public mutation repository MEN2-RET databases: (http://www.arup.utah.edu/database/MEN2/MEN2_welcome.php); 2016 [accessed 1.8.16]. To date; 166 variants are stored in this database. The most frequently reported mutations are at codons 634 in exon 11, and 620, 618, 611, 609, which are located in exon 10. However, mutations at codon 634 have been reported in 85% of tested individuals, nearly 50% of them are amino acid cysteine to arginine substitution (Cys634Arg). Pathogenic variants at this codon reported to result in a higher incidence of pheochromocytoma, hyperparathyroidism and lichen amyloidosis [3,4,8]. Additionally, the risk of development of the Hirschsprung disease in carriers could reach 7% [9].

Many guidelines have been established for improvement of the diagnosis and for better management of patients with MTC or neuroendocrine tumors [10–13]. Based on the aggressiveness of thyroid tumors and the age of clinical detection, the revised guidelines by American Thyroid Association (ATA) Task Force on Medullary Thyroid Carcinoma has classified *RET* variants at codon C634 (C634F/G/R/S/W/Y) as high risk mutations (category H) [12]. In general, the recommendations included periodic assessment of the clinical status by thyroid ultrasound and biochemical screening for MTC, CT and MRI for pheochromocytoma, with serum calcium and parathyroid hormone level assessment [10–13].

MTC is the most common cause of death in MEN2-associated families. Therefore prophylactic total thyroidectomy with central neck lymph node dissection is mandatory for *RET* mutation carriers, with periodic monitoring for residual or recurrent MTC and annual calcium calcitonin stimulation test. Prophylactic surgery is recommended for young individuals of < 5 years of age, who are carriers for certain high-risk pathogenic variants in codon 634 [12,13], while biochemical screening for pheochromocytoma and hyperparathyroidism should start at 8 years of age [12].

We have screened the first Kuwaiti family with multiple affected members, for confirmation of the clinical diagnosis and as a measure for identification of at-risk asymptomatic mutation carriers.

2. Subjects and methods

2.1. Family data

The proband (P9) was 35 year old healthy male, approached the cancer genetics clinic seeking genetics counseling and predictive gene testing. Pedigree analysis and medical reports revealed many relatives manifesting thyroid/parathyroid/adrenal associated-diseases consistent with the diagnosis of MEN2A syndrome (Fig. 1, Table 1). Subsequently his high-risk family members (P1–P27) were seen in groups or in separate individualized sessions according to their request. Genetic counseling was provided, the importance of predictive gene test was discussed, and informed consent was obtained for blood collection and testing. The work has been carried out in accordance with The International Code of Medical Ethics of the World Medical Association (Declaration of Helsinki) and Ethical approval of the Kuwait Medical Genetics Center.

2.2. Molecular screening

Blood samples were obtained from 27 individuals in the family. Genomic DNA was extracted from peripheral blood leukocytes using the automatic Maxwell® 16 System DNA purification Kits (Promega, USA) according to manufacturer's protocol. We initially performed mutational screening for the hotspot regions in the most commonly implicated exons 10 and 11 of *RET* proto-oncogene using PCR amplification of the coding and the flanking intronic regions, followed by the Sanger sequencing of the PCR product using ABI PRISM® 3100 Genetic Analyzer (Applied Biosystem, USA). We used the previously designed primers and conditions for amplification of exons 10 and 11 [14]. We did not proceed to test other exons; soon the pathogenic variant was identified.

3. Result and discussion

We have identified a heterozygous variant in exon 11 of *RET* proto-oncogene; c.1901G > T; p.Cys634Phe (or C634F as commonly used in the literature) (Fig. 2); in the proband and 14 members of his family. Mulligan et al. had previously described this variant in 1993 as a disease causing mutation [15]. It was later published as pathogenic mutation by Gene Review, PMID:20301434 (http://www.ncbi.nlm.nih.gov/books/NBK1257), and was reported in ClinVar database, as NM_020975.4 at (http://www.ncbi.nlm.nih.gov/clinvar); 2016 [accessed 1.8.2016]. This is a missense mutation that occurred at the cysteine residue within the extracellular cysteine-rich domain. It substitutes cysteine for phenylalanine. This is a hotspot gene position for pathogenic mutations, which are commonly found in various populations and ethnic groups including North Africans, but with different frequencies due to different genetic background [3,4,16–20]. Codon 634