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Correspondence

Rare manifestation of multiple endocrine neoplasia type 2A & cutaneous lichen amyloidosis in a family with *RET* gene mutation

Sir,

Multiple endocrine neoplasia type 2 (MEN 2) is an autosomal dominant condition, characterized by the presence of medullary thyroid carcinoma (MTC), pheochromocytoma (PHEO), and hyperparathyroidism (HPT). It is subtyped into MEN 2A, MEN 2B, and familial medullary thyroid carcinoma (FMTC) based on the different tissues involved[1]. MEN2A (OMIM # 171400) accounts for more than 80 per cent of MEN2 cases[2] and some of its rare variants are associated with Hirschsprung disease (HSCR), cutaneous lichen amyloidosis (CLA), nephrolithiasis and other neurological and mental disorders[3,4]. The gene implicated in MEN2 aetiology is *RET* (REarranged during Transfection) (OMIM# 164761) proto-oncogene located on chromosome 10q11.2 which encodes a putative receptor tyrosine kinase (TK). Various studies have identified germ line mutations in *RET* gene causing MEN 2A[5,6]. We report on a rare condition of MEN2A with CLA and identification of a *RET* mutation Cys634Arg in the patient and family members.

A 37 yr old female patient developed neck swelling 13 years back, consulted her family physician and was advised fine needle aspiration cytology (FNAC) based on which colloid goiter was diagnosed. The swelling increased gradually in size over the next 10 yr and at the age of 34 yr she had two thyroid nodules 4 x 3 cm and 3 x 2 cm, respectively. She was referred to the department of Endocrinology and Metabolism, All India Institute of Medical Sciences (AIIMS), New Delhi, India, in September 2011 for further assessment of these nodules. Histopathology confirmed the diagnosis of MTC but the investigations were negative for the presence of pheochromocytoma. She underwent total thyroidectomy and was on six monthly follow up. After one year, positron emission tomography (PET) scan revealed bilateral lesions in the adrenal glands with intense meta-iodobenzylguanidine (MIBG) uptake seen only in the left adrenal nodule indicating presence of PHEO. She underwent left adrenalectomy at the age of 35 yr and was routinely monitored every six months. Eighteen months later, at the age of 37 yr, MIBG uptake was seen in the right adrenal gland for which right adrenalectomy was performed and was put on physiological steroid replacement post-surgery. She had no history suggestive of hyperparathyroidism, pituitary mass lesion, hypoglycaemic episodes, carcinoid syndrome, vitiligo or gastrointestinal (GI) bleed, but she complained of itchy, non-raised skin lesions on the upper back and shoulders for the last 12 yr. On examination she was found to have skin thickening with hyper as well as hypopigmented macular lesions in dorsocervical area, consistent with diagnosis of cutaneous lichen amyloidosis (Fig.). She had a significant family history with her mother dying of MTC at the age of 44 yr. Her maternal aunt has been operated for MTC and PHEO.

Detailed family history and pedigree information was collected and peripheral blood sample (5 ml) was drawn in EDTA for molecular investigations after taking written informed consent from the patient and her family members. Genomic DNA was extracted using standard salting out protocol and subjected to PCR amplification of the *RET* gene with primers described previously[7]. All amplified products were purified and sequenced and the nucleotide sequences were compared with the published cDNA sequences of *RET* (GenBank accession number ENSG00000165731) gene.

Direct sequencing of the *RET* gene in the patient revealed a T to C transition in exon 11 (c.1900 T>C) leading to replacement of cysteine by arginine at codon 634, thereby resulting in a missense mutation.

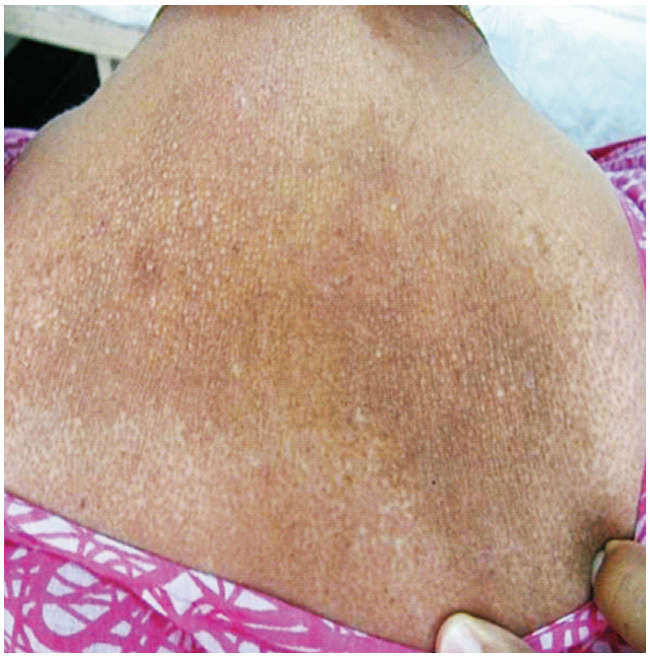

Fig. Photograph showing macular lesions in dorsocervical area indicating CLA.

Screening the asymptomatic children in her family also revealed presence of the same mutant allele following which they were screened for possible presence of MEN2A tumours. The older child, aged 14 yr showed MIBG uptake in the right adrenal gland. So it is planned to carry out adrenalectomy first and then prophylactic thyriodectomy as in the other sibling (4 yr old). The absence of the mutation in the other family members ruled out the risk of developing MEN2A tumours in them in later stages.

The majority of cases in MEN 2A harbour mutation at codon 634 and Cys to Arg replacement is the most common amino acid change found at this codon[8,9]. Normal signaling of the RET receptor requires a cell-surface complex consisting of RET tyrosine kinase domain (RTK), glial cell line-derived neurotrophic factor (GDNF) ligand family and ligand-specific co-receptor which together form the components of a tripartite complex. Two of such tripartite complexes ultimately lead to RET dimerization and promote normal downstream signaling.

RET receptor has an extracellular domain, a transmembrane and an intracellular tyrosine kinase domain which play an important role in the development and differentiation of tissues originating from neural crest by transducing signals for cell growth[10,12]. The extracellular domain of RET is enriched with cysteine residues which are involved in the formation of intra-chain disulphde bonds contributing to the specific orientation of tertiary structure of the RET protein.

The mutant cysteine in the cysteine-rich domain is unable to participate in intramolecular disulphide bonding to form the tripartite complex and instead allows formation of an intermolecular disulphide bond with a second normal tripartite complex molecule[11,13]. This abnormal intermolecular cysteine bridge between a tripartite complex and an independent RET receptor leads to constitutive dimerization. The aberrant signaling, therefore, causes formation of tumours.

Mutations at codon 634 have been associated with the presence of pheochromocytoma, hyperparathyroidism and rarely with CLA. CLA observed as local amyloids can exist as an independent entity (without MEN 2A) or in association with MEN 2A[14]. Not all the families with *RET* mutations develop CLA and, therefore, it is a rare entity. CLA may appear during early life and may precede the biochemical presentation of MTC[15] as seen in our patient where the cutaneous lesions had manifested about two years before the initial symptoms of MTC with characteristic presentation at the inter scapular region.

Accurate diagnosis followed by surgery at an early stage is a useful intervention for management of MTC in MEN 2A mutation carriers. This early prophylactic surgery remains the only curative option as RET tumours are prone to metastasize early and are resistant to chemo as well as radiotherapy. Therefore, early diagnosis and timely treatment are essential for their survival. Molecular study made a significant contribution in the early diagnosis especially in the case of children which is the most important outcome of the study.

In conclusion, we identified a rare variant of CLA with MEN 2A in a patient resulting due to Cys634Arg *RET* gene mutation. The study gives an insight on the important clinical parameters like age at onset of MTC and CLA and emphasizes the role of molecular genetic studies for early mutation detection which would aid in commencement of appropriate biochemical tests, their duration and frequency for tracking the progression of the disease. It also reaffirmed that CLA localized to the inter scapular region may present as one of the early manifestations of MEN2A and should be considered as an indicator of the potential risk for MEN2A. The report reiterates the importance of conducting

a comprehensive clinical, radiological and genetic evaluation of MEN2A patients and their relatives as *RET* mutation analysis is the fundamental test to confirm its diagnosis. It also highlights the need, awareness and availability of a simple genetic test which can be used as a diagnostic tool in MEN2A cases as early cancer diagnosis allows for timely preventive measures and suitable therapeutic and surgical management options.

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