**RET Mutation Screening in Familial Cutaneous Lichen Amyloidosis and in Skin Amyloidosis Associated With Multiple Endocrine Neoplasia**

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In several families, multiple endocrine neoplasia type 2A (MEN 2A) has been found in association with cutaneous lichen amyloidosis. It has been debated, however, whether the skin amyloidosis found in MEN 2A families, localized exclusively in the interscapular area, represents the same anomaly as that found in autosomal dominant familial cutaneous lichen amyloidosis, which is more generalized. We screened two MEN 2A families with associated skin amyloidosis for germline mutations in the RET gene responsible for the MEN 2A cancer syndrome, and found the same mutation characteristic of MEN 2A in both families.

We also screened probands from three pedigrees with familial cutaneous lichen amyloidosis for RET mutations. In none of the RET coding and flanking intronic sequences was a mutation detected. This most probably indicates that skin amyloidosis found in some MEN 2A families and familial cutaneous lichen amyloidosis are different conditions. Consequently, patients with apparent familial cutaneous lichen amyloidosis do not appear to be at risk for MEN 2A.

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Cutaneous lichen amyloidosis (CLA) is a rare disorder characterized by deposits of amyloid in the papillary dermis. Sporadic as well as autosomal dominant hereditary forms have been documented. Gagel et al (1989) reviewed 63 of these hereditary cases. Here we refer to the hereditary form as familial cutaneous lichen amyloidosis (familial CLA).

CLA-like skin lesions have also been found in patients with multiple endocrine neoplasia type 2A (MEN 2A) (Gagel et al, 1989; Nunziata et al, 1989; Ferrer et al, 1991; Kousseff et al, 1991; Chabre et al, 1992; Robinson et al, 1992; Pacini et al, 1993). MEN 2A is a neoplastic syndrome characterized by C-cell hyperplasia, medullary thyroid carcinoma, pheochromocytoma, and parathyroid hyperplasia. The disorder is caused by specific germline mutations in the RET proto-oncogene (Donis-Keller et al, 1993; Mulligan et al, 1993).

Because patients have been found with CLA lesions in several MEN 2A families, it has been suggested that patients having sporadic or familial CLA should be considered at risk for the MEN 2A syndrome and therefore should be tested for MEN 2A mutations (Nunziata et al, 1989; Ferrer et al, 1991; Chabre et al, 1992). Based on the association of both of these conditions, RET gene mutations have been thought responsible for the skin amyloidosis found in MEN 2A patients. We therefore screened two MEN 2A families with associated CLA and three families with familial CLA for RET germline mutations.

**MATERIALS AND METHODS**

**Familial CLA** The three families participating in this study had CLA in at least two generations (Fig 1). All affected family members were examined by a dermatologist. Light microscopic and electron microscopic evidence of amyloid was found in skin biopsy specimens from at least two individuals in each of the familial CLA families, FCLA-2 and FCLA-3. Although no electron microscopic analysis was performed on patient material from family FCLA-1, the diagnosis in this family was based on a characteristic clinical picture and on histopathologic and immunofluorescence examination of skin specimens. In all three families, the CLA lesions were found mainly on the arms and legs.

**MEN 2A/CLA Families** Families MEN 2A/CLA-1 and -2 have been described before. Figure 2 shows the relevant parts of the pedigrees. Family MEN 2A/CLA-1 has been reported by Kousseff et al (1991), who gave a detailed description of the CLA lesions. Family MEN 2A/CLA-2 has been described as family B by Lips et al (1994). Some of the patients in this family appeared with lesions in the interscapular region only and were clinically diagnosed as having CLA upon examination by a dermatologist. Light microscopic evaluation of biopsy specimens of the lesions failed, however, to detect amyloid.

**Single-Strand Conformation Polymorphism (SSCP) Analysis** High-molecular-weight DNA from patients from families MEN 2A/CLA-1, FCLA-1, FCLA-2, and FCLA-3 was used for SSCP analysis of all 20 RET exons. DNA amplification was carried out on 150 ng of DNA in 1 X Super Taq reaction buffer using 0.125 U of Super Taq (HT Biotechnology Ltd., Cambridge, UK) in a total volume of 30 μl containing 20 μM...
The polymerase chain reaction (PCR) consisted of 30 cycles of 92°C for 40 s, 72°C for 60 s, and another 60 s for annealing at the appropriate temperature. Table I lists the primers (100 ng of each primer) used for each exon of the RET gene, the annealing temperature, and the restriction enzymes used when relatively long PCR products were obtained. Electrophoresis was carried out in a 6% polyacrylamide gel under at least two different conditions. Glycerol concentrations used were 0%, 5%, or 10%, at 4°C, 20°C, or 30°C, respectively. We also used Mutation Detection Enhancer gel solution (At-Biochem, Malvern, PA) as a replacement for acrylamide and glycerol and ran the gels at 30°C in 0.5 X Tris-borate buffer at a maximum of 1750 V and 60 W in a temperature-regulated LKB 2010 Macrophore electrophoresis unit (Uppsala, Sweden).

**Polymorphisms in the RET Gene** Intrageneic RET polymorphisms (see Table II) were analyzed by carrying out digestions with the restriction enzymes listed or, in the case of exon 18, by SSCP analysis.

**Sequence Analysis** For all families, sequence analysis was carried out on exons 10 and 11, which are known to account for more than 95% of the mutations found in MEN 2A (Mulligan et al, 1994; Schuffenecker et al, 1994). In addition, all SSCP variants observed were sequenced. For SSCP and sequence analysis, the same primer sets were used. For sequence analysis, however, one of the primers of each set was biotinylated. DNA amplification was carried out as described earlier. PCR products were separated in a 2% low melting point agarose gel. After ethidium bromide staining, bands were cut out and isolated with the Sephaglas BandPrep kit (Pharmacia Biotech, Uppsala, Sweden). The two single strands were separated using Dynal beads (DYNAL AS, Oslo, Norway). They were sequenced with the T7 sequencing kit (Pharmacia Biotech, Uppsala, Sweden). The two single strands were separated using Dynal beads (DYNAL AS, Oslo, Norway). They were sequenced with the T7 sequencing kit (Pharmacia Biotech, Uppsala, Sweden). The two single strands were separated using Dynal beads (DYNAL AS, Oslo, Norway). They were sequenced with the T7 sequencing kit (Pharmacia Biotech, Uppsala, Sweden). The two single strands were separated using Dynal beads (DYNAL AS, Oslo, Norway). They were sequenced with the T7 sequencing kit (Pharmacia Biotech, Uppsala, Sweden).

**RESULTS**

**RET Mutation Screening in MEN 2A/CLA Families** A search for mutations throughout the entire RET gene by means of SSCP revealed in one family (MEN 2A/CLA-1) a conformation variant in exon 11 in all affected family members (MEN 2A and MEN 2A/CLA patients). Upon sequence analysis, this appeared to be caused by transition T1900—>C, resulting in the substitution of glutamic acid for a cysteine at codon 634 (Cys634→Arg) (Fig 3).

**DISCUSSION**

Phenotypic diversity due to mutations affecting different domains of a gene product is a frequent phenomenon known as allelic heterogeneity. The RET gene is a well-known example. Base-pair substitutions affecting one of five highly conserved cysteine residues in the extracellular part of the protein are associated with MEN 2A and familial medullary thyroid carcinoma (Mulligan et al, 1994; Schuffenecker et al, 1994). Furthermore, a missense mutation substituting threonine for methionine at codon 918 in the tyrosine kinase domain of the protein has been found responsible for a proportion of patients suffering from Hirschsprung disease (Edery et al, 1994; Romeo et al, 1994). The combined occurrence of both MEN 2A and CLA in some families of patients with familial CLA or HLA-2A/CLA phenotypes (data not shown) may indicate that the RET gene is a candidate gene for these syndromes.

**SSCP Analysis of Exons 10 and 11** For all families, sequence analysis was carried out on exons 10 and 11, which are known to account for more than 95% of the mutations found in MEN 2A (Mulligan et al, 1994; Schuffenecker et al, 1994). In addition, all SSCP variants observed were sequenced. For SSCP and sequence analysis, the same primer sets were used.
and patients might be associated with specific RET mutations. We therefore analyzed two families. In patients of one of these, MEN 2A/CLA-1, the presence of amyloid could be clearly demonstrated. Amyloid could not be demonstrated in specimens from the lesions of the patients from the other family. Because this is not a consistent feature of presumed CLA patients in previously reported MEN 2A/CLA families, however, and because all lesions were limited to the interscapular region, which is generally considered characteristic for the association of MEN 2A and CLA, family MEN 2A/CLA-2 was also included in this study (Gagel et al., 1989; Nunziata et al., 1989; Chabre et al., 1991; Chabre et al., 1992; Robinson et al., 1992; Pacini et al., 1993). In the two families, we found the same RET mutation in codon 634 (Cys634→Arg). The mutation was present in all MEN 2A patients, some of whom also had CLA. A Cys634→Tyr mutation (G1901A) has been reported previously in another family with MEN 2A and CLA (Ceccherini et al., 1994b). Although all the mutations affect codon 634, different amino acid substitutions result. The mutations found, notably Cys634→Arg, also occur frequently in MEN 2A families without CLA lesions. Although an association between MEN 2A/CLA and mutations in codon 634 may be postulated, the above-mentioned arguments make it hard to suggest a correlation between a specific RET mutation and the MEN 2A/CLA phenotype.

It might be suggested that the joint occurrence of MEN 2A and CLA would be due to the interaction of an apparently noncausative polymorphism and a disease-causing mutation, as has been described for the prion gene (Goldfarb et al., 1992). In the RET gene, several common noncausative polymorphisms have been found (Table II; Ceccherini et al., 1994a). None of the polymorphisms, however, seemed to co-segregate with the MEN 2A/CLA phenotype. Thus, for an explanation of the intrafamilial phenotypic variability, it may be necessary to look beyond the mutational-polymorphic genotype. Differential handling of the gene product by the paracrine growth mechanism of a particular individual may alter the pathogenesis of the condition and cause pleiotropy of the phenotype (Kousseff et al., 1991; Kousseff, 1992).

A search for RET mutations in patients from three “CLA only” families did not reveal a mutation other than already known noncausative polymorphisms. We also looked for possible cosegregation of these intragenic polymorphisms with the cutaneous phenotype in these families, but were unsuccessful. We therefore conclude that RET is not involved in these cases of familial CLA.

Our findings raise the question of whether the CLA found in MEN 2A and familial CLA are etiologically similar conditions. Clinically, there is a distinction in the affected sites. In MEN 2A patients, skin lesions are always found in the interscapular region, whereas in familial CLA patients, skin lesions are more generalized (Shuchardt et al., 1994). Dysfunction of the RET gene, which in developing mice is expressed in the peripheral nervous system (Kousseff et al., 1991), might lead to puritus and subsequently to scratching and degeneration of keratinocytes. It has been suggested that prolonged mechanical friction may produce a macular amyloidosis, or “friction amyloidosis” (Wong and Lin, 1988; Robinson et al., 1992). Because many chronic pruritic skin conditions do not show skin amyloidosis, however, this etiologic model might be an oversimplification.

The current results lend support to the idea that skin lesions in
familial CLA and MEN 2A/CLA patients are different from genetic, clinical, and etiologic points of view. Consequently, familial CLA patients do not appear to be at risk for MEN 2A. To settle this issue definitively, however, more data are needed. Mainly for this reason, it may still be reasonable for physicians of (apparent) familial CLA patients to have their patients screened for RET mutations.

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