Genetic Heterogeneity of Familial Primary Cutaneous Amyloidosis: Lack of Evidence for Linkage with the Chromosome 10 Pericentromeric Region in Chinese Families

Ding-Dar Lee, Jium-Yu Huang, Chu-Kwan Wong, Robert F. Gagel,* and Shih-Feng Tsai†
Department of Dermatology, Veterans General Hospital-Taipei and School of Medicine, National Yang-Ming University, Republic of China; *Section of Endocrinology, Department of Medical Specialties, University of Texas, M.D. Anderson Cancer Center, U.S.A.; and †Institute of Genetics, National Yang-Ming University, Republic of China

Primary cutaneous amyloidosis is a relatively common skin disease in Southeast Asia, South America, and the Republic of China. Although most cases are sporadic, some patients have a family history, suggesting that genetic factors may play a role in its pathogenesis. Some patients with multiple endocrine neoplasia type 2A also have a clinical picture of primary cutaneous amyloidosis. It is thus suggested that the gene of familial primary cutaneous amyloidosis is linked to the pericentromeric region of chromosome 10, the location of the RET proto-oncogene. We have carried out linkage analysis in seven families with cutaneous amyloidosis using four dinucleotide repeat markers from the RET region. Negative lod scores at all recombination frequencies were obtained. We thus conclude that there is no evidence for linkage between Chinese families with primary cutaneous amyloidosis and the pericentromeric region of chromosome 10. The distinct genetic basis, plus their apparent phenotypic differences in sex ratio, age of onset, and sites of cutaneous lesions, suggests that familial primary cutaneous amyloidosis includes clinical subtypes attributable to genetic heterogeneity. J Invest Dermatol 107:30-33, 1996

Primary cutaneous amyloidosis, such as lichen amyloidosis or maculopapular amyloidosis, characterized by the deposition of amyloid in the dermal papilla, is a relatively common skin disease in Southeast Asia (Tan et al., 1987), South America (Ollague et al., 1980), and the Republic of China (Wong, 1974). Lichen amyloidosis, the most common variant, presents discrete, firm, hyperkeratotic, closely set, pinhead-to-matchhead-sized, dome-shaped or hemispheric, brownish papules. The prebintestinal area is the site most frequently affected (Wang, 1990). Another variant, macular amyloidosis, showing oval, closely aggregated, brownish macules in a rippling pattern, may co-exist with lichen amyloidosis (Wang, 1990). Cutaneous amyloidosis usually causes severe pruritus, and its appearance tends to cause embarrassment. The cutaneous amyloid is believed to originate from degenerated epidermal cells (Kumakiri and Hashimoto, 1979). To date, the etiology of primary cutaneous amyloidosis is still unknown, but it is believed to be multifactorial, including environmental and frictional epidermal damage, immunology, and other factors (Wong, 1990). Although most cases are sporadic, some patients have a family history (Rajagopal and Tay, 1972; Vasily et al., 1979; Newton et al., 1985). In particular, about one-third of the South American cases have a family history of this disorder (Ollague et al., 1990). Taken together, the familial aggregation and the racial susceptibility suggest that genetic factors play an important role in its pathogenesis. In the reported cases of familial primary cutaneous amyloidosis (FPCA), the disease is inherited as a Mendelian autosomal dominant trait with variable penetrance (Rajagopal and Tay, 1972).

Another disease, multiple endocrine neoplasia type 2A (MEN 2A), is a rare, autosomal dominant genetic syndrome characterized by medullary thyroid carcinoma, pheochromocytoma, and parathyroid hyperplasia. Interestingly, some patients with MEN 2A or hereditary medullary thyroid carcinoma also have clinical pictures of cutaneous amyloidosis. The association of MEN 2A and hereditary localized pruritus was first reported in an Italian family (Nunziata et al., 1989a) and a further evaluation of this family confirmed the finding of amyloid (Nunziata et al., 1989b). At least nine such families have been reported so far (Ferrer et al., 1989a, b; Gagel et al., 1989; Nunziata et al., 1989a, b; Kousseff et al., 1991; Chabre et al., 1992; Robinson et al., 1992; Pacini et al., 1993). Gagel and colleagues postulated that MEN 2A and primary cutaneous amyloidosis may be associated because of one genetic defect that affects two contiguous or overlapping genes (Gagel et al., 1989). Efforts to map the predisposing gene have provided evidence that MEN 2A is related to the germline mutation of the RET proto-oncogene, which is located in the pericentromeric region of chromosome 10. Further studies revealed that mutation in the RET proto-oncogene could lead to disease of developmental defects or tumors of the neuroendocrine system (Heyningen, 1994). The RET mutations identified in MEN 2A have occurred at sites encoding the cysteine residues (Donis-Keller et al., 1993; Mulligan et al., 1993, 1994). In addition, a Cys→Tyr mutation of the RET proto-
oncogene in one of these pedigrees with MEN 2A and cutaneous amyloidosis was reported (Ceccherini et al., 1994). It is likely that the gene of FPCA is also linked to the pericentric region of chromosome 10. To verify this assertion, we have carried out linkage analysis in seven families of Chinese descent with primary cutaneous amyloidosis, using four polymorphic dinucleotide repeat markers in the pericentric region of chromosome 10. Our results demonstrate that there is no evidence for linkage between our FPCA patients and the genetic markers from this region.

MATERIALS AND METHODS

Families Family history was routinely traced for every patient with primary cutaneous amyloidosis in our outpatient clinics, and seven families with at least two affected members were enrolled in this study (pedigrees not shown). The diagnosis was made on the basis of clinical pictures, because most patients showed typical lesions of macular or lichenoid amyloidosis. Skin biopsies were performed for all affected patients. Biopsy specimens were fixed in 10% formalin, embedded in paraffin, and 4-μm sections were stained with hematoxyline and eosin, and Congo red. These patients were examined for physical signs of MEN 2A phenotype. None of the family members had histories of medullary thyroid carcinoma, phaeochromocytoma, or parathyroid hyperplasia.

Genotyping Blood samples were collected from sixty-two members of the seven families, including nineteen affected individuals with cutaneous amyloidosis. DNA from peripheral blood leukocytes was extracted manually with phenol/chloroform as previously described (John et al., 1991). Four markers with dinucleotide repeat polymorphism, sTCL-1 (D10S176) (Howe et al., 1992), D10S141 (Love et al., 1993), sTCL-2 (RET) (Lairmore et al., 1993), and sJRH-1 (RBP3) (Howe et al., 1992), were used in this study. The most likely genetic map is pter-D10S176-cen-D10S141-RET-RBP3-pter (Tuntachille et al., 1994), covering 3.1 cM of the pericentric region of chromosome 10 (sex-average distance) (Howe et al., 1992) (Fig. 1).

For each marker, one of the two primers flanking the repeat was end-labeled by T4 polynucleotide kinase with [γ-32P]ATP. The dinucleotide repeat element was PCR amplified from 500 ng of genomic DNA for 20 cycles in a reaction volume of 40 μl, which included 1.5 mM MgCl2, 200 μM dNTP, 2 units of Tag DNA polymerase, and 40 pmol of each primer. Denaturation was set for 1 min at 94°C, annealing for 2 min (at 57°C for sTCL-1, 55°C for D10S141, 55°C for sTCL-2, and 63°C for sJRH-1), and polymerization for 2 min at 72°C. The reaction products were electrophoresed on 6% denaturing polyacrylamide sequencing gels using radiolabeled MspI-digested Bluescript KS DNA as size standards. Gels were exposed to the Kodak XAR film for autoradiography using one intensifying screen (Dupont, Lighting Plus) for 24–48 h at -70°C.

Linkage Analysis Two-point genetic linkage analyses involving the disease locus and each marker were performed using the LINKAGE program (Turbo Pascal version 5.2) (Lathrop et al., 1985). The MLINK option was used to calculate the lod scores. Penetrance was assumed to be 90% and a gene frequency of 1 in 10,000 was used based upon the estimated prevalence of primary cutaneous amyloidosis in Taiwan.

RESULTS

The total number of affected patients in the seven pedigrees is 19 with three males and sixteen females. The mode of inheritance is apparently autosomal dominant and the median age of onset in these patients is 21 years (range: 14–46 yr). The most frequently involved sites are pretibial areas (19 of 19), whereas the interscapular area is affected in only two patients. All the biopsy specimens demonstrate eosinophilic deposits of amorphous material in the papillary dermis and positive Congo red staining with greenish birefringence under polarized light, which is characteristic of cutaneous amyloidosis.

For linkage analysis, two alleles were assigned to each individual studied for each marker according to the gel images obtained. Cosegregation of the disease and the marker or recombination events can be deduced from the assigned genotypes (Fig. 2). The most likely haplotypes for each family member were constructed for the typed markers to minimize the number of double recombination events between close markers. By simple inspection and genetic analysis, recombination events could be detected in most of the families with the markers used. For example, individuals II2, II4, and II6 of family 3 (Fig. 3) have inherited the same haplotype (CDAC) from their affected mother, I2, but II6 was diagnosed as normal. Similarly, the same haplotype has been passed on by individual II4 to her daughter, III3 and III4, but only III4 had lesions of cutaneous amyloidosis. Furthermore, the affected individual III2 obtained the presumably grandpaternal (FDCC) rather than the grandmaternal haplotype (CDAC). Therefore, three recombinations would have had to occur to explain the disease-marker data among the affected siblings. The lod scores of two-point linkage analysis at different recombination fractions for the four markers are shown in Table I. As a whole, there is no significant evidence for linkage with any of the test markers. At θ = 0, lod scores of < -3.0 were observed at all loci. Of the four markers, the RET locus has negative lod scores even at higher recombination fractions, indicating that symptoms of our FPCA patients could not be caused by a mutation in the RET gene. In addition, using the exclusion criterion of a lod score less than -2,

![Figure 1. Map of the four loci covering 3.1 cM of the pericentric region of chromosome 10. cen, centromere.](image1)

![Figure 2. Genotypes of family 3 for the marker sTCL-1 (D10S176 locus). Two crossovers (*) were detected, assuming C allele is linked to the FPCA disease gene.](image2)
we were able to exclude a 17-cM region on either side of the RET locus as a location for the gene of FPCA.

**DISCUSSION**

In most reported cases of FPCA, it is transmitted as a Mendelian autosomal dominant trait with variable penetrance. For sporadic cases, the disease is more common in females, with a female-to-male ratio as high as 4.5:1 (Tan, 1990). In our collection of familial cases, the female patients also outnumber the male patients (female: male = 5.3:1). In contrast, equal gender distribution was observed in the pedigrees of combined MEN 2A and cutaneous amyloidosis (Robinson et al, 1992). Lichen amyloidosus usually occurs during the third to fifth decade of life, but there are no data available regarding the familial cases. The median age of onset in this study is 21 y, suggesting familial cases develop lesions earlier than sporadic ones. The median age of onset of cutaneous amyloidosis in five families with MEN 2A was even younger, 13 y (Robinson et al, 1992). The extensor surfaces of the pretibial regions are the most commonly involved sites for sporadic cases. This is also true for our familial cases; all patients in our study had lesions on their pretibial areas. The distribution of skin lesions in all reported cases of MEN 2A/cutaneous amyloidosis syndrome, however, was restricted to the interscapular area; no lesions on the pretibial areas were ever mentioned. Therefore, the apparent differences in sex ratio, age of onset, and distribution pattern of cutaneous lesions indicate the existence of distinct phenotypes for the combined and the isolated cases of primary cutaneous amyloidosis.

At least nine families of MEN 2A were reported to exhibit features of primary cutaneous amyloidosis. In five families reviewed by Robinson and colleagues, most patients (31 of 44) manifested both MEN 2A and cutaneous amyloidosis, and all but one patient showed skin problems prior to the diagnosis of MEN 2A (Robinson et al, 1992). Consequently, some authors considered the characteristic skin lesions to be a phenotypic marker heralding the full-blown medullary thyroid carcinoma in the MEN 2A kindreds (Ferrer et al, 1989, 1991; Chabre et al, 1992). Several possible explanations regarding the association of MEN 2A with primary cutaneous amyloidosis were proposed. Kousseff et al advocated that they represent the phenotypic variability of the expression of a pleiotropic gene (Kousseff et al, 1991). Other authors suggested that the primary cause is neurogenic and the skin lesion is a secondary phenomenon due to chronic pruritus and repeated scratching (Robinson et al, 1992; Pacini et al, 1993). Gagel et al (1989) proposed that the MEN 2A/cutaneous amyloidosis syndrome represents an overlap or contiguous gene syndrome in which a microdeletion or point mutation had caused two diseases. The predisposing gene of MEN 2A has been localized to the pericentromeric region of chromosome 10, and germline mutations of the RET proto-oncogene have recently been reported in association with MEN 2A (Donis-Keller et al, 1993; Mulligan et al, 1993, 1994). Furthermore, a Cys\(^{34}\)→Tyr mutation of the RET proto-oncogene was identified in all 11 families with MEN 2A and cutaneous

---

**Table 1. Pairwise Lod Scores of Four Chromosome 10 Markers in the Pericentromeric Region**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Recombination Fraction (θ)</th>
<th>Exclusion, cM*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.00</td>
<td>0.05</td>
</tr>
<tr>
<td>D10S176</td>
<td>−3.41</td>
<td>−2.76</td>
</tr>
<tr>
<td>D10S141</td>
<td>−5.64</td>
<td>−4.31</td>
</tr>
<tr>
<td>RET</td>
<td>−11.25</td>
<td>−6.78</td>
</tr>
<tr>
<td>RBP3</td>
<td>−7.31</td>
<td>−4.20</td>
</tr>
</tbody>
</table>

* cM, centimorgans excluded on either side of the tested markers, taking a lod score of −2.0 as the limit of exclusion.
amyloidosis (R. F. Gagel, manuscript in preparation). Because not all the MEN 2A patients have cutaneous amyloidosis, it is reasonable to postulate that the gene of familial primary cutaneous amyloidosis is separate but linked to the MEN 2A locus in the pericentromeric region of chromosome 10. As the previous reports were based on families of Caucasian origin, it would be of interest to determine whether the association of FPCA with chromosome 10 dinucleotide repeat markers also holds true for Chinese patients. We have performed linkage analysis in seven families with cutaneous amyloidosis and without MEN 2A using four polymorphic markers, including STCL-2 at the RET locus. These markers cover 3.1 cM of the pericentromeric region of chromosome 10 from 10p11.2 to 10q11.2. As shown in Table I, negative lod scores at most recombination fractions were obtained in our study for all markers. We thus conclude that, in Chinese patients affected with FPCA, there is no evidence indicating linkage between familial primary cutaneous amyloidosis and the pericentromeric region of chromosome 10. The genetic basis of "pure" familial cutaneous amyloidosis is probably different from that of cutaneous amyloidosis combining with the features of MEN 2A. It is notable that of the more than 100 families worldwide reported to have MEN 2A, only nine pedigrees are known to have cutaneous amyloidosis. The relationship between these two co-existing disorders and the genetic basis for their association awaits further study.

Finally, the current study, through genotyping chromosome 10 pericentromeric markers, formally excludes this region as the chromosomal location of the susceptibility gene of our FPCA patients without MEN 2A. Moreover, 17 cM on either side of the RET locus, for a total of 34 cM of the genome, has been excluded. Since the human genome was estimated to include 3300 cM (Renwick, 1971), this study has allowed exclusion of 1.0% of the genome. To identify the genomic position of the disease gene, we are currently conducting a genome-wide search to map the disease-susceptibility locus. The localization of the FPCA-linked region should establish a starting point for regional fine mapping and eventual isolation of the FPCA candidate gene employing the positional cloning approach.

We thank Shen-Jang Fan (Department of Statistical Genetics, New York State Psychiatric Institute, Columbia University) for critically reviewing the manuscript, and Ten-Yi Tsen and Gilbert J. Cote for excellent technical assistance. This work was supported in part by grants NSC84-2331-B-075-083 (C.-K. W.) and NSC85-2331-B-010-087 M02 (S.-F. T.), and the Clinical Research Center, VGH, Institute of Biomedical Science, Academia Sinica, Republic of China.

REFERENCES