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Mapping of Papillon-Lefèvre Syndrome to the Chromosome 11q14 Region

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Abstract

Papillon-Lefèvre syndrome (PLS) is an autosomal recessive disease which belongs to the palmo-plantar keratoderma (PPK) group. It is characterized by a premature loss of primary and permanent teeth and early onset periodontitis. High consanguinity has been observed in over one-third of PLS families. No candidate genes or gene localizations have been described to date for this disorder. A primary genome-wide search by homozygosity mapping using samples from a large consanguineous family in which 4 siblings were affected by the disease showed homozygosity and linkage in the region of 11q14. Linkage was confirmed in 4 additional families with diverse ethnic and geographic backgrounds, 2 of which were consanguineous. A maximum two-point lod score of 8.19 was obtained for the marker AFM063yg1 (D11S901= for $\Theta = 0$. Analysis of recombination events places the gene within a 7-cM interval between AFM063yg1 and AFM269yg9 (D11S4175). No shared haplotype was found for the 5 families analysed.

Key Words

Papillon-Lefèvre syndrome
Homozygosity mapping
Palmo-plantar keratoderma (PPK)
Chromosome 11q

Introduction

Palmo-plantar keratoderma (PPK) is a general term for a heterogeneous group of skin diseases characterized by hyperkeratosis of palms and soles. The traditional classification, which is based on histopathological findings (the presence or absence of epidermolysis) and on the nature of the palmo-plantar lesions (diffuse, focal or linear) seems to be insufficient for understanding the pathological mechanisms. A new classification comprising 19 different forms has recently been proposed and seems to be very useful [1].

Papillon-Lefèvre syndrome (PLS) was first described in 1924 by Papillon and Lefèvre [2] and denotes an association of diffuse transgredient palmar-plantar hyperkeratosis, severe periodontopathy and premature loss of primary and permanent dentition. PLS is a rare disease transmitted in an autosomal recessive manner with a prevalence in the general population of 1–3 per million. At least one-third of the families are consanguineous and there is no sex or race preference [3]. Until 1995, only about 200 cases had been reported in the literature [4].

The periodontal lesions are the most constant features and are therefore considered as the most important for diagnosis. The skin lesions in PLS are in general diffuse, not marked and often erythematous. They are sharply outlined, often with a livid margin. An early onset of cutaneous lesions at birth has been noted but more commonly they appear between 6 months and 3 years of age [3, 5]. The most frequently observed facultative signs are ectopic intracranial calcifications, mental retardation and increased susceptibility to infections [3].

As a preliminary approach to identify the gene defect in PLS, we used the method of homozygosity mapping

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[6], which has already been used to map genes for autosomal recessive diseases in consanguineous families.

Methods

Blood samples for DNA extraction were collected from 28 members of 5 families. DNA was extracted from whole blood using standard procedures.

Fluorescent Genome Mapping and Determination of Haplotypes

We used the AFM/Genethon panel of fluorescent PCR primers. Microsatellite primers were labelled with either 6-Fam, Hex or Tet phosphoramides [7]. PCR reactions were performed in 192-well microtitre plates (Falcon) in a final volume of 15 µl containing 30 µg/µl DNA, 0.16 m*M* of dNTP, 1 × NBL buffer, 0.33 µ*M* of each primer and 0.03 IU of Taq polymerase.

After a hot-start procedure (enzyme is added at 94 °C after denaturation for 5 min at 96 °C) 26 cycles (40 s 94 °C, 30 s 55 °C) were performed. The rest of the procedure is similar to that described by Reed et al. [7].

Non-Fluorescent Genotyping

Genotypings for the other microsatellite markers, using unlabelled primers were carried out for the region of interest.

PCR were performed in 96-well microtitre plates (Falcon) in a final volume of 50 µl containing 40 ng of genomic DNA, 125 µ*M* dNTP, 1 µ*M* of each primer and 0.05 IU Taq polymerase. The amplification products from the same DNA sample generated with different primer sets were pooled and allowed to co-migrate in a single lane of a 6% polyacrylamide gel. After transfer to Hybond N+ (Amersham) membranes, hybridization was performed either with peroxidase-labelled PCR primers, or with a poly-(AC) probe, according to Vignal et al. [8].

Linkage Analysis

Linkage analysis was performed using the LINKAGE 5.1 program. Two-point lod scores were calculated with the MLINK program [9]. The disease was considered to be inherited in an autosomal recessive manner with a penetrance of 95% and with a frequency of 1 per 1,000,000. Consanguineous loops were incorporated in the pedigree files. Allele frequencies used were based on unrelated individuals from 8 CEPH families [10].

Results

Clinical Data

Five families with PLS, 3 of which were consanguineous, were collected and the DNA stored in the Généthon DNA bank (fig. 1). All the members of these families were examined by a dermatologist and a dentist. There were 14 affected and 14 non-affected individuals. Detailed medical history, physical findings and pedigree data were obtained for each family member. Families were from France (4894, 6243), North Africa (1338), Martinique (3850) and the Netherlands (6460).

Linkage Analysis

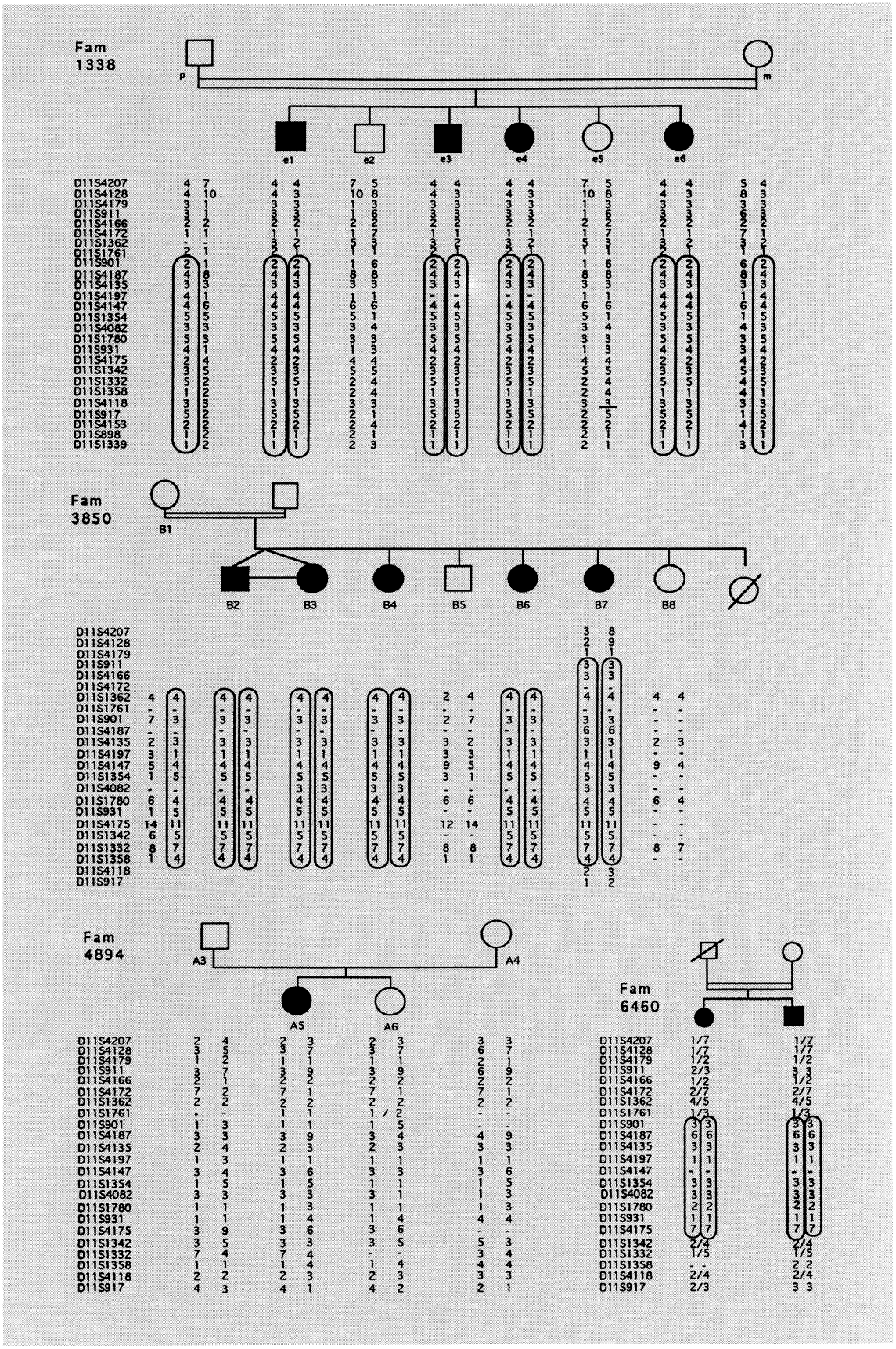
Since there was no candidate gene suspected to be responsible for PLS, we first undertook a genome-wide scan of a large consanguineous family (1338) with 8 members, including 4 affected sibs. The parents were first cousins and the offspring share 1/16 of the ancestral genome. According to Lander and Botstein [6] an average spacing of 15 cM between only modestly polymorphic markers and using about 15 inbred individuals is sufficient to find the linked homozygous region in consanguineous pedigrees in which the parents are first cousins. However, only 5–10 inbred family members should suffice using more highly polymorphic markers or more closely spaced restriction fragment length polymorphisms (RFLPs). The collection of 243 markers selected from the AFM/Généthon panel [10] and spaced over the autosomes at 15-cM intervals was thus considered as appropriate.

Only one homozygous region was found in all the affected children. One marker (AFM063yg1) on chromosome 11q14 at the locus D11S901 exhibits a pairwise lod score of 2.80 for $\Theta = 0$. To find the limits of this homozygous region, we tested 30 neighbouring markers, covering a distance of 25 cM. The genotypes of markers from the central part of this region are shown in figure 1.

We further confirmed the positive linkage of this region by analysing 4 other families of which 2 were consanguineous. All the 5 informative families showed a positive lod score for the region of interest. The maximum pairwise lod score for the 5 families for marker AFM063yg1 (D11S901) was 8.19 for $\Theta = 0$ (table 1). Most of the informative pedigrees are shown in figure 1.

The 5 families were also analysed to refine the linkage interval and to test for a possible common haplotype. We established the smallest co-segregating region in the 5 informative families using haplotype analysis (fig. 2). The haplotypes were constructed assuming the most parsimonious linkage phase. The telomeric limit is defined by marker AFM269yg9 (D11S4175) in family 6460 and the centromeric limit by the marker AFM063yg1 (D11S901) in the families 1338 and 6460. The interval between the two markers is approximately 7 cM [10]. In 3 consanguineous pedigrees, this region was easy to identify by homozygosity of the affected children (fig. 1). In the non-consanguineous pedigree 4894, a recombination event was observed between marker D11S1362 and D11S901, but the maternal haplotype phase is unknown. So it is not possible to determine which child has received the recombined chromosome.

No common haplotype was observed in the 5 pedigrees.


Fam 1338
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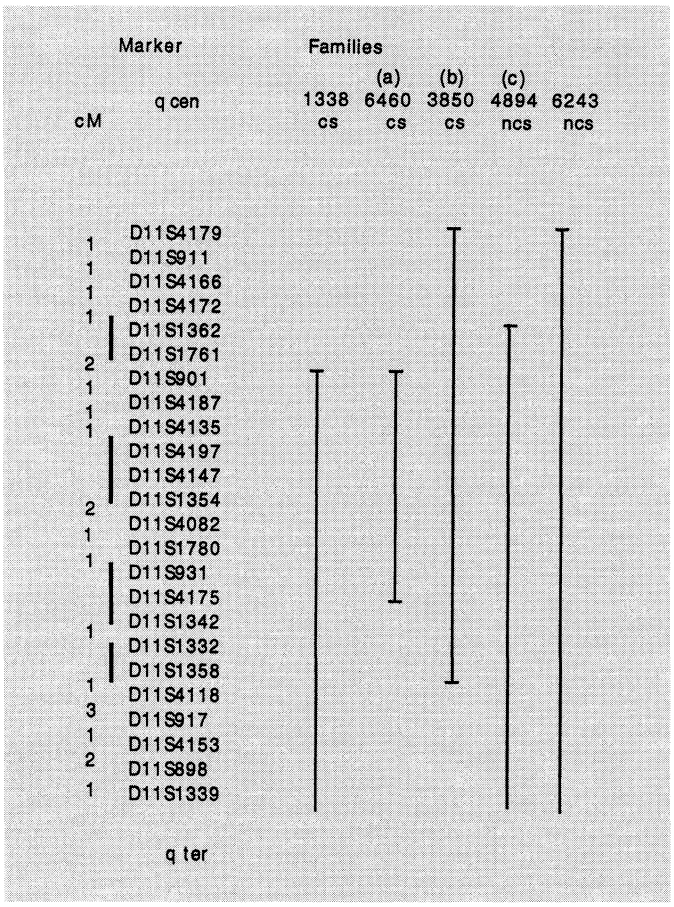

Fig. 2. This figure shows the limits of the linked interval in the 5 different families. The centromeric limit (qcen) is defined by the marker AFM063yg1 (D11S901) in the families 1338 and 6400 and the telomeric limit (qter) by marker AFM269yg9 (D11S4175) in family 6460. The distances between the markers are indicated in centimorgans on the left. Lane a: in the consanguineous family (cs) 6460 only two affected members have been genotyped. Thus the haplotype phase can only be determined for the region of homozygosity; lane b: based on individual B7 (fig. 1); lane c: a recombination was observed in the nonconsanguineous (ncs) family 4894 in the disease-bearing maternal chromosome between D11S4172 and D11S1761, but maternal phase could not be defined. It is thus not possible to determine which child has received the recombined chromosome.

Fig. 1. Three consanguineous pedigrees, in which the parents are first cousins, and one non-consanguineous pedigree are shown, including the disease-associated haplotypes (surrounded). Affected individuals are represented by black symbols and non-affected family members by open symbols.

Table 1. Combined pairwise lod scores table for PLS for 5 families (1338, 6460, 3850, 4894, 6243)

	Lod scores (Z) at recombination fraction (Θ)						
Θ	0.00	0.01	0.05	0.10	0.20	0.30	0.40
D11S4179	−2.14	0.37	1.07	1.18	0.97	0.61	0.25
D11S911	−0.34	2.92	3.41	3.25	2.47	1.55	0.67
D11S4166	−1.95	0.45	0.91	0.91	0.63	0.32	0.10
D11S4172	−2.48	0.05	0.81	1.00	0.90	0.61	0.27
D11S1362	−2.66	2.17	3.04	2.99	2.26	1.33	0.49
D11S1761	−8.52	−3.42	−1.39	−0.65	−0.16	−0.07	−0.05
D11S901	8.19	8.02	7.33	6.45	4.68	2.92	1.26
D11S4187	5.68	5.55	5.01	4.33	2.98	1.82	0.64
D11S4135	3.67	3.58	3.21	2.75	1.87	1.09	0.43
D11S4197	5.52	5.40	4.93	4.32	3.06	1.81	0.65
D11S4147	5.92	5.84	5.47	4.90	3.64	2.24	0.83
D11S1354	6.27	6.13	5.59	4.90	3.54	2.20	0.94
D11S4082	3.44	3.35	2.99	2.54	1.74	0.98	0.38
D11S1780	4.46	4.36	3.93	3.40	2.36	1.37	0.49
D11S931	7.05	6.90	6.28	5.49	3.90	2.37	0.98
D11S4175	3.38	3.37	3.25	2.98	2.24	1.39	0.54
D11S1342	2.69	4.99	5.08	4.60	3.35	2.02	0.77

Discussion

According to Stevens et al. [1], there are 19 different types of PPK with ectodermal dysplasia. The genetic defect has been identified for 4 of them and localized for 2 others. PLS is classified as type IV of the palmoplantar ectodermal dysplasia group but its pathogenic mechanism is unknown.

PPK disorders represent a clinically and genetically heterogenous group of diseases. Type I (Jadassohn-Lewandowsky syndrome) is due to mutations in keratin 16 and 6a genes on chromosome 17q12-q21 [11, 12]. Type II (Jackson-Sertoli syndrome) is due to a keratin 17 mutation on chromosome 17q12-q21 [13], type V (tyrosinaemia type II) is caused by a tyrosine aminotransferase deficiency on chromosome 16q22.1-q22.3 [14] and finally there is a loricrin mutation on chromosome 1q21 [15] in type VII (Vohwinkel syndrome). A localization has been found for type III (tylosis) on chromosome 17q, distal to the keratin gene cluster [16] and for type X (Fischer-Jacobsen-Clouston syndrome) on chromosome 13q [17].

However, the most characteristic feature of this disorder is periodontitis and premature loss of teeth. There are other examples of diseases associated with periodontitis. Juvenile periodontitis [18] and the statherin gene [19], a regulator of calcium in saliva, have been localized on chromosome 4q11-13 and several collagenase defects

have been described for the Ehlers-Danlos syndrome [20].

We showed here that PLS is linked to locus 11q14 in 5 families and that no common haplotype was observed. The smallest co-segregating region of 7 cM between marker D11S4175 and D11S901 was defined by recombination events in these families. The 5 families are from different geographical areas, but all of them showed positive linkage to the interval. We concluded therefore that in our PLS population a single gene is responsible for the disease. No common haplotype was observed suggesting that there is no founder effect. However, the existence of a smaller region with shared haplotypes cannot been excluded.

In the region of PLS localization on chromosome 11q14, there are some known genes, which could be tested as candidate genes: keratin 1 and a collagen binding protein 2 (colligen2) are on 11q13.5 and there is a tyrosinase gene on 11q14-q21 which is responsible for oculocutaneous albinism type IA.

We found 53 cDNAs in the interval between markers AFMa132xh9 and AFM269yg9 representing nearly 250 ESTs. One EST representing human alpha tubulin mRNA (SHGC 11690), which is localized near the locus D11S931, is expressed in keratinocytes, and two others from the same gene are expressed in fibroblasts (937212).

Since PLS is classified as a palmo-plantar hyperkeratosis, in which keratin genes have frequently been implicated, it is possible that a mutation in a keratin gene is also responsible for this disease.

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