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Localization of a Gene for Autosomal Dominant Larsen Syndrome to Chromosome Region 3p21.1-14.1 in the Proximity of, but Distinct from, the COL7A1 Locus

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Summary

Larsen syndrome (LS) is a skeletal dysplasia (osteochondrodysplasia) in which multiple dislocations of the large joints are the major feature. Nosology in this group of diseases, which constitutes 8% of Mendelian disorders in man, is primarily based on clinical and radiographic features. Hopes for more accurate classification grounds are currently being met by progress in elucidation of underlying genetic defects. We have performed linkage analysis in a large Swedish kindred with autosomal dominant LS and found the gene (LAR1) to be strongly linked to chromosome 3p markers (Z_{max} = 13.4 at (θ = .00). Recombination analysis indicates that the LAR1 locus is located in a region defined distally by D3S1581 and proximally by D3S1600, which cytogenetically maps to chromosome region 3p21.1-14.1. Linkage and recombination analysis of a COL7A1 *Pvu*II intragenic polymorphism versus LS and chromosome 3 markers indicate that COL7A1 is located close to, but distinct from, the LAR1 locus.

Introduction

Larsen syndrome (LS) is a congenital disorder involving the large joints, the hands, and the face (Larsen et al. 1950). The most severe symptoms are multiple dislocations of the large joints, including the knees, the hip joints, and the elbows. Club foot varus or valgus is often found. The thumbs are spatulate with broad nails, the fingers cylindrically formed, and the metacarpi are variably misshaped. The facial symptoms are flat nasal bridge, widely spaced eyes, and prominent forehead. Several additional but variable abnormalities have also been reported (Silverman 1972; Steel and Kohl 1972; Salmon et al. 1973; Robertson et al. 1975; Micheli et al. 1976; Henriksson et al. 1977; Marques 1980; Kiel et al. 1983; Eiferman et al. 1993; Petrella et al. 1993). The incidence in Western countries is estimated to be 1 per 100,000 births (Bonaventure et al. 1992) but is probably higher, since the syndrome is often unrecognized or misdiagnosed (Latta et al. 1971; Houston et al. 1981; Chen et al. 1982). The basic defect in LS is not yet known; however, earlier observers pointed out the possibility of generalized mesenchymal disorder resulting in early laxity of the supporting tissues of joints (Latta et al. 1971; Takashi et al. 1976; Marques 1980). Biochemical and structural studies of collagen from connective tissue in LS have shown variable and occasionally contradictory results (Cetta et al. 1979; Chen et al. 1982; Pierquin et al. 1991; Bonaventure et al. 1992). The condition may be inherited in either an autosomal dominant (MIM 150250) or recessive (MIM 245600) form (McKusick 1992). The two genetically different forms of LS are not clearly discernible at clinical examination; however, the recessive form is reported to be more debilitating and with increased lethality (Maroteaux 1975; Chen et al. 1982; Bonaventure et al. 1992). Germ-line mosaicism (Petrella et al. 1993; Rochelson et al. 1993) and sporadic new mutations (Latta et al. 1971) have also been considered. Apparently identical unbalanced chromosome translocations have been found in two unrelated children exhibiting LS, which indicated a possible locus for the defective gene to chromosome 1q or 6p (Pierquin et al. 1991). Genetic linkage studies of a well-defined form of recessive LS have failed to demonstrate linkage of the disorder to major collagen genes (Bonaventure et al. 1992). We have investigated a large Swedish multigeneration kindred with dominant LS (fig. 1), detected through the index case with typical symptoms (fig. 2). In the present article we report the localization of a gene liable to development of this disorder to chromosome 3p21.1-14.1.

Material and Methods

A large multigeneration family (fig. 1*A*) from western Sweden was detected through the index case (fig. 2) pre-

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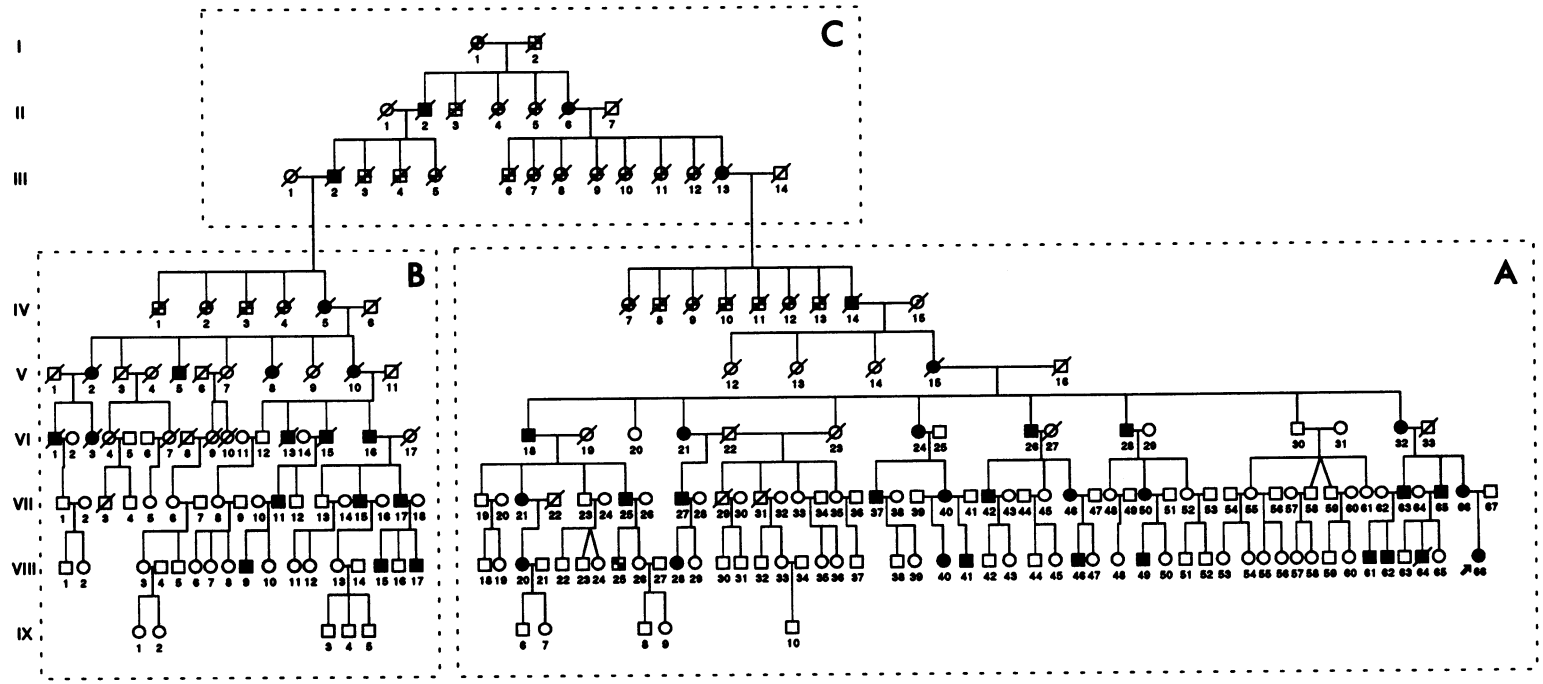

Figure 1 Pedigree of the investigated family. Rectangle A demarcates the first family branch detected, while rectangle B demarcates the second branch . Rectangle C demarcates common ancestral relatives to the two branches A and B. Symbols with plus signs indicate ancestral relatives with unknown disease status. Individual VIII-25, indicated with a checked pattern, had a cleft uvulae and cylindrical fingers but none of the other common signs of Larsen syndrome.

Table 1

Clinical Presentation of the 26 Affected Included in the DNA Study

	VI-16	VI-18	VI-21	VI-24	VI-26	VI-28	VI-32	VII-15	VII-17	VII-21	VII-25	VII-27
Face:												
Hypertelorism	−	+	+	+	+	+	+	−	+	+	+	+
Saddle nose	+	+	+	+	+	+	+	+	+	+	+	+
Prominent forehead	+	+	+	+	+	+	+	+	+	+	+	+
Hands:												
Cylindrical fingers	−	?	−	−	+	−	−	−	−	−	?	−
Short nails	−	?	+	+	−	−	+	−	−	+	+	+
Broad thumb(s)	−	?	+	+	−	−	+	−	−	+	+	−
Metacarpal symptoms	−	+	+	+	+	−	−	−	−	+	?	−
Clinodactyly-V	+	?	−	−	+	−	−	+	+	−	?	−
Large joints:												
Loose joints	−	?	−	+	−	+	−	+	−	−	+	+
Knee dislocation	−	−	+	−	−	−	−	+	−	−	−	−
Hip dislocation	−	−	?	−	−	−	−	+	−	+	−	−
Club foot	+	+	+	−	−	+	−	+	+	+	+	+
Surgically treated	−	+	+	+	+	+	+	+	−	+	?	−
Orthopedic device	+	+	+	+	+	+	+	+	+	+	+	+

NOTE.—A plus sign (+) denotes presence; and a minus sign (−) denotes absence of the symptom. A question mark (?) denotes uncertain or unknown phenotype.

senting with anterior bilateral dislocation of the knees, bilateral hip joint dislocation, and bilateral club foot varus. The face exhibited hypertelorism, depressed nasal bridge, and long philtrum. The fingers were normally tapering. Incidentally, another index case (VII-17) was detected representing a new multigeneration family with dominantly inherited LS (fig. 1*B*). Grandparents to both index cases originated from the same parish. Suspicion arose concerning a common ancestral carrier, which was later confirmed by genealogical studies of county archives (fig. 1C). The common ancestor-couple was traced back to the year 1751. Later, haplotype analyses of individuals from the two branches from this pedigree further supported genetic relationship in the two families (see Recombination Analysis, below). One hundred thirty-seven descendants of a common ancestor-couple distributed in nine generations constitute the combined pedigree used for linkage studies. The evaluation of the affection status was done by one of us (M.V.). Criteria for positive diagnosis were as follows: typical facial symptoms combined with hand and/or foot anomalies described in LS. These symptoms are the most commonly found in patients described in the literature (De Smet et al. 1993). Evaluation included physical examination of the phenotype and medical history (73 individuals). In cases where the individuals were unavailable for examination, the evaluation consisted of medical history report given by near relatives and examination of photographs (15 individuals). Affection status of four ancestral relatives (II-2, II-6, III-2, and III-10) was ascertained by exclusion and by descent. Affection status of 26 additional ancestral relatives could not be determined. In total, 49 individuals were considered carriers of the defective gene. Altogether, 48 family members, of whom 26 were affected, were included in the DNA study. The most common symptoms described in LS and found in the 26 affected are presented in table 1.

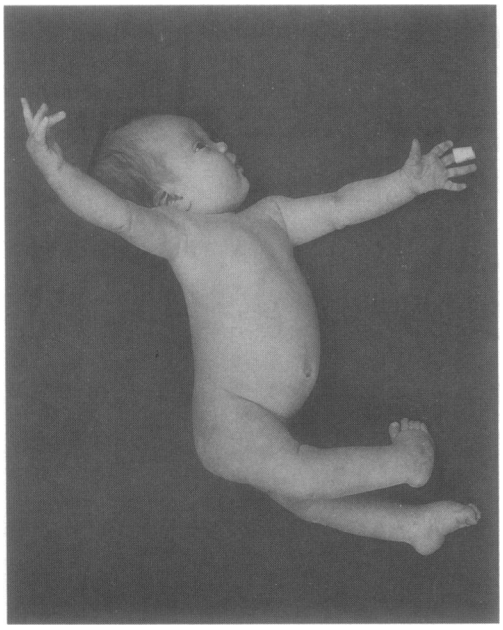

Figure 2 Photograph of the index case from pedigree A (indicated by an arrow in fig. 1) at 4 wk of age. Note the dislocated knees, club feet, and typical face.

DNA Isolation, Genotyping, and Linkage Analysis

DNA was isolated from venous blood samples anticoagulated with EDTA and extracted using standard pro-

VII-37	VII-40	VII-42	VII-46	VII-63	VI-65	VII-66	VIII-15	VIII-20	VIII-28	VIII-40	VIII-41	VIII-46	VIII-66
+	+	+	+	+	+	+	−	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	−	+	+	+	+
+	+	+	−	+	+	−	−	−	+	−	−	−	−
+	+	+	+	+	+	−	−	+	+	−	−	−	−
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+	+	+	−	−	−	−	−	+	+	+	+	+	+
+	+	+	+	−	−	−	+	+	+	−	+	+	+
+	+	+	+	+	+	+	−	+	+	−	+	+	+

cedures. PCR reactions were performed according to standard procedures. In brief, amplifications were carried out in a 20-μl volume containing 125 ng genomic DNA; 15 pmol of each primer; 10 mM Tris HCl (pH 8.3); 50 mM KCl; 1.5 mM $MgCl_2$; 0.001% (w/v) gelatin; 4.4 nmol of each dATP, dCTP, dGTP, dTTP; 0.66 μCi ^{32}P-dCTP; and 0.55 U *Taq* DNA polymerase (Perkin-Elmer). Amplifications were performed in a Hybaid Omnigene temperature cycler.

Simple-sequence-repeat polymorphisms (SSRPs) were determined by the length of amplified PCR products on a 6% polyacrylamide/7 M urea sequencing gel. After drying, the gel was exposed to a Fuji x-ray film overnight at room temperature.

Two-point linkage analyses were performed with the MLINK program from the LINKAGE 5.1 package (Lathrop et al. 1984). The model used for analyses was autosomal dominant inheritance with full penetrance (1.0) and a disease allele frequency of 0.0001.

A screen of the genome was performed using primer pairs from Généthon (Weissenbach et al. 1992; Gyapay et al. 1994) and the CHLC (Murray et al. 1994) linkage maps. When ~90% of the genome was excluded (data not shown), linkage was found for markers in chromosome region 3p. Additional markers from this region were subsequently used and analyzed. The chromosomal location of Généthon markers was derived from Généthon Quick-map utility via anonymous FTP from ceph-genethon-map.genethon.fr. In order to further map the LAR1 locus on the Généthon map, multipoint analysis was performed as a combination of several three-point analyses using the LINKMAP program from the LINKAGE 5.1 package (Lathrop et al. 1984).

Linkage Analysis of a Human Type VII Collagen (COL7A1) PvuII Intragenic Polymorphism

A *Pvu*II intragenic polymorphism in the COL7A1 locus with a PIC value of .36 (Christiano et al. 1992) was analyzed on all 48 family members. A PCR amplification was performed using primer pairs from Christiano et al. (1992), and PCR reactions were performed according to standard procedures. The amplified PCR products were digested with *Pvu*II and separated by electrophoresis on a 2% agarose gel.

Genotypes were scored and two-point linkage analysis was performed with the MLINK program from the LINKAGE 5.1 package. To further map COL7A1 on the Généthon map, multipoint analysis was performed with the LINKMAP program from the LINKAGE 5.1 package. A comparison was made between the mapping data of the COL7A1 and LAR1 gene loci. The comparison is illustrated in figure 3.

Results

Linkage Analysis

After exclusion of ~90% of the genome (data not shown) linkage was detected with markers in chromosome region 3p. Subsequently, several markers in this region were employed, and it could be shown that markers in a region from D3S1613 to D3S1287 (fig. 4) on the Généthon map were strongly linked to the LS locus (LAR1). For seven of the markers the LOD score was

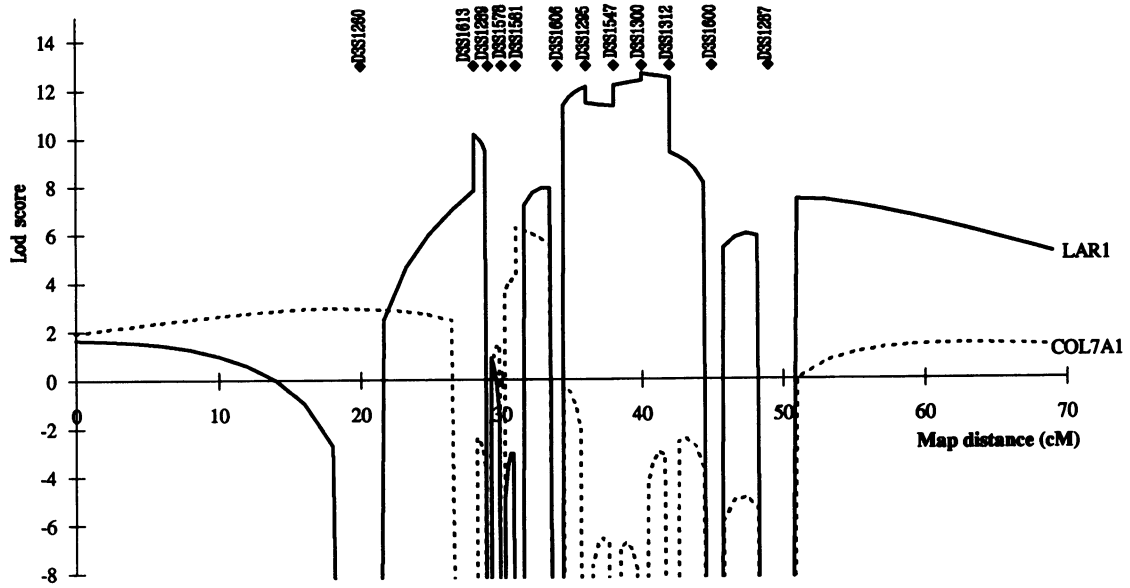

Figure 3 Multipoint linkage map generated with LINKMAP using chromosome 3 markers. The orders of markers are also shown in fig. 4. The solid lines correspond to multipoint analyses of the LAR1 locus, the dotted lines correspond to multipoint analyses of a *Pvu*II intragenic polymorphism in the human type VII collagen (COL7A1) gene locus. An 11-cM region defined by markers D3S1606 and D3S1600 gives the highest likelihood for location of the LAR1 locus. The highest likelihood for location of the human type VII collagen gene is between markers defined by markers D3S1578 and D3S1606.

at maximum at a recombination fraction of zero (θ = .00), and for marker D3S1300 the maximal LOD score was 13.4. This marker was informative at each meiosis. Pairwise LOD score values for all used markers in the region versus LAR1 are shown in table 2. A multipoint analysis of LAR1 versus markers in the region is shown in figure 3.

Recombination Analysis

To further narrow the region of the LAR1 locus, haplotype analyses were performed for the region D3S1260-D3S1287. Data from the haplotype analyses are shown in figure 5.

Recombination events between marker D3S1581 and marker D3S1295 in four individuals (VII-40, VII-46, VIII-38, and VIII-46) indicate that the LAR1 locus is most likely located in a region proximal of marker D3S1581. Individual VIII-63 displays recombination events both between markers D3S1606 and D3S1295 and between D3S1581 and D3S1606 (fig. 5). These recombination events do not allow for exclusion of the 3-cM region between markers D3S1581 and D3S1606. Marker D3S1613 has a maximal LOD score of 8.12 at a recombination fraction of zero (θ = .00), although it is mapped distal of marker D3S1606. Tested markers distal of D3S1613 show no linkage to LS. For a genetic and physical map of the markers see figure 4. A recombination event between marker D3S1312 and marker D3S1600 in individual IX-7 indicates that the LAR1 locus is located distal of marker D3S1600.

Taken together the data of the recombination analysis indicate that the LAR1 locus is located in a region defined distally by marker D3S1581 and proximally by marker D3S1600, which cytogenetically correspond to chromosome region 3p21.1-14.1.

Linkage Analysis of a Human Type VII Collagen (COL7A1) PvuII Intragenic Polymorphism

A two-point analysis of a COL7A1 *Pvu*II intragenic polymorphism versus LS gave a maximum LOD score of 0.36 at a recombination fraction of .25 (θ = .25; table 2). Two-point analyses of COL7A1 versus markers in the region, from D3S1260 to D3S1287 on the Généthon map, resulted in a maximum LOD score of 3.55 at locus D3S1581 (θ = .00). In the region most likely to harbor the LAR1 locus (from marker D3S1581 to D3S1600), LOD scores were $-\infty$ at = .00. Recombination events in two different meioses (fig. 6) combined with the linkage data indicate that COL7A1 is most likely located distal of the LAR1 locus. Pairwise LOD scores for COL7A1 versus all markers in the region are shown in table 3.

Multipoint analyses indicated that the most likely location of COL7A1 is in the region between marker D3S1581 and D3S1606 with a maximum LOD score of 6.25. Multipoint analyses of LAR1 versus chromosome

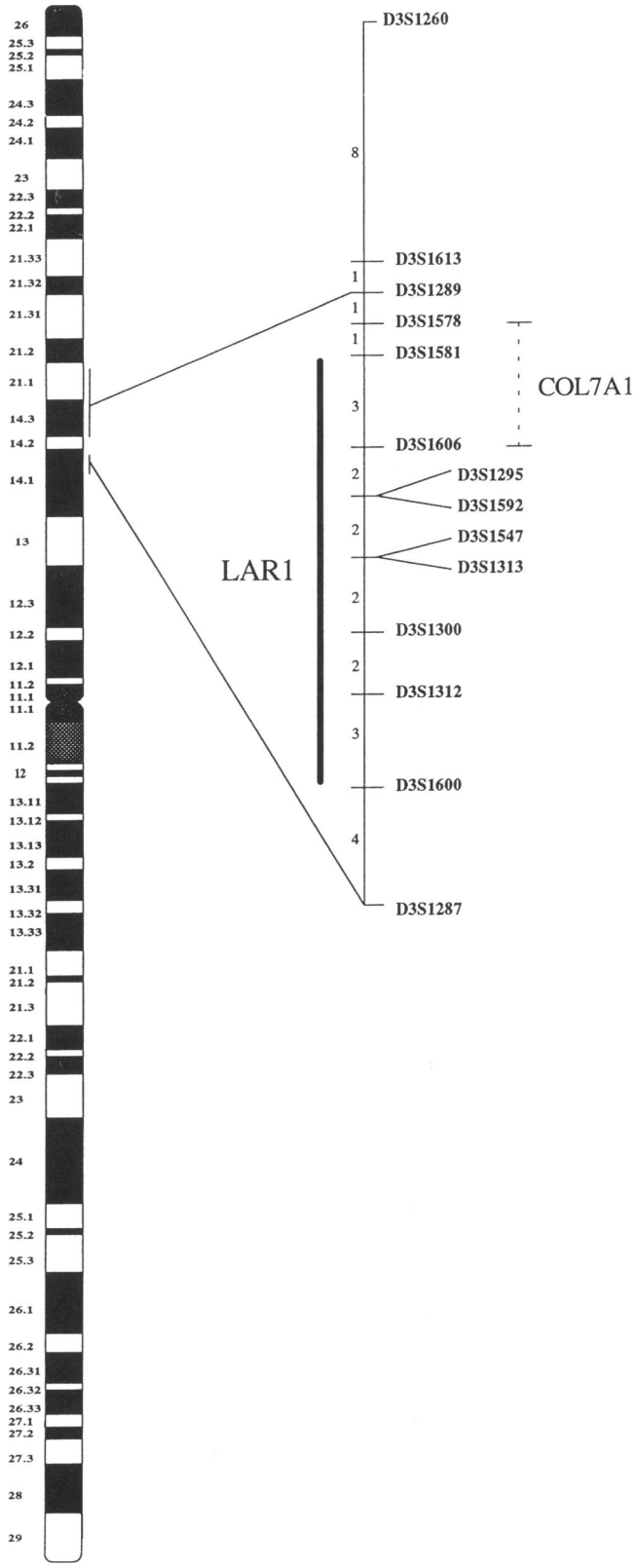

Figure 4 Ideogram of chromosome 3, illustrating the most likely location of the Larsen gene (LAR1) locus. All markers were from the Généthon map (Gyapay et al. 1994), and the sex-averaged recombination distances in cM are indicated between markers. The location of the LAR1 gene is indicated with a black bar. On the basis of the genetic and physical maps of the location of flanking markers, the LAR1 gene is cytogenetically located in region 3p21.1-14.1. The dotted line indicates the position of the COL7A1 locus according to our data (where multipoint LOD score exceeds 3.0). The chromosome 3 ideogram is according to the 850-band level of ISCN (1985).

3 markers and COL7A1 versus the same markers are shown in figure 3.

Discussion

We have shown that a locus for an autosomal dominant form of LS (designated LAR1) resides in chromosome region 3p where two-point linkage and recombination analyses localize the causative genetic defect to the 14-cM interval between markers D3S1581 and D3S1600. Only one individual (VIII-63) in the recombination analysis indicated that the most likely location of the LAR1 locus is proximal of marker D3S1606, this very same individual had another recombination event within 3 cM distal of marker D3S1606. This is a rather unlikely event and should be looked upon with some caution. There is a possibility that it reflects a rare case of mutation within marker D3S1606 for this individual. Nevertheless there are four other independent events of recombination between marker D3S1581 and D3S1295 in individuals VII-40, VII-46, VIII-38, and VIII-46, which strongly indicates that the LAR1 locus maps proximal of marker D3S1581. For recombination analysis see figure 3.

The LOD score (at $\theta = .00$) for marker D3S1613 reaches 8.12, even though recombination analysis indicates that the most likely location of the LAR1 locus is proximal of this marker. This indication is probably due to uninformative meioses in several individuals in generation VI of the pedigree for marker D3S1613. Accordingly, recombination events in some individuals (e.g., VII-40 and VII-46) between marker D3S1613 and LAR1 are undetected.

Taken together, the multipoint analysis and the recombination analysis identified the 14-cM region between marker D3S1581 and D3S1600 as the most likely location for the LAR1 locus. On the basis of the genetic and physical maps of flanking markers (Gyapay et al. 1994), the LAR1 locus could be cytogenetically determined to chromosome region 3p21.1-14.1 (fig. 4).

A striking observation in our data was that individuals with a common ancestor seven generations back (e.g., pedigree members VIII-15 and VIII-28) have the same haplotype in the LAR1 region over at least a 21-cM region from marker D3S1613 to D3S1287 (fig. 1; fig.

Table 2

Two-Point LOD Score Values for Linkage between Larsen Syndrome and Chromosome 3 Markers

Locus (Marker)	LOD Score at Recombination Fraction								
	0	.01	.05	.1	.2	.3	.4	Z_{max}	θ at Z_{max}
D3S1260 (AFM038xc1)	−∞	−6.78	−2.60	−1.07	−.05	.16	.13	.17	.33
D3S1613 (AFM340xf1)	8.12	7.96	7.33	6.53	4.91	3.24	1.51	8.12	.00
D3S1289 (AFM198yf2)	−∞	7.14	7.16	6.59	5.12	3.43	1.56	7.29	.03
D3S1578 (AFM268wg9)	−∞	.75	3.54	4.13	3.75	2.67	1.23	4.16	.12
D3S1581 (AFM273ve9)	−∞	4.57	5.42	5.27	4.23	2.84	1.26	5.43	.06
D3S1606 (AFM318wc5)	−∞	5.40	5.49	5.02	3.77	2.39	1.02	5.58	.03
D3S1295 (AFM210yc5)	10.25	10.06	9.32	8.37	6.40	4.31	2.02	10.25	.00
D3S1592 (AFM292xh1)	6.10	5.96	5.41	4.72	3.39	2.13	.97	6.10	.00
D3S1547 (AFM162xc9)	6.32	6.20	5.73	5.13	3.91	2.61	1.23	6.32	.00
D3S1313 (AFM259zg5)	6.58	6.42	5.81	5.04	3.50	1.99	.71	6.58	.00
D3S1300 (AFM220yh4)	13.38	13.16	12.25	11.07	8.53	5.74	2.72	13.38	.00
D3S1312 (AFM256ya9)	5.74	5.62	5.13	4.52	3.31	2.08	.86	5.74	.00
D3S1600 (AFM308xc9)	−∞	6.74	6.78	6.24	4.82	3.19	1.40	6.89	.03
D3S1287 (AFM198te7)	−∞	5.48	5.67	5.30	4.18	2.82	1.26	5.71	.03
COL7A1 *Pvu*II	−∞	−2.51	−.65	−.03	.33	.33	.18	.36	.25

5). This resemblance may indicate that the LS haplotype, in this pedigree, is associated with an inversion or some other chromosomal aberration that lowers recombination in the region. Cytogenetic analysis using high-resolution G-banding on prometaphase chromosomes has not shown any detectable aberration in the chromosomes of two affected members of the family (data not shown).

LS is a disorder of connective tissue, and likely candidates for such a disease include the collagen family of genes. Human type VII collagen gene is mapped in chromosome region 3p21.1 (Parente et al. 1991) and is therefore a candidate gene for LS. Human type VII collagen is, however, known to be expressed primarily in stratified squamous epithelia, such as the skin and the mucous membranes, and is known as causing the dystrophic forms of epidermolysis bullosa (Christiano et al. 1994). It has no obvious similarity to the phenotypic manifestations of LS, but several examples are known of single genes causing different clinical conditions when mutations involve separate parts of the gene. For the collagen family of genes this seems to be more a rule than an exception (for recent review see Francomono 1995).

We therefore analyzed a COL7A1 *Pvu*II intragenic polymorphism (Christiano et al. 1992) for genetic linkage, first versus LS in the affected family and second versus polymorphic markers in the 3p region. Multipoint analysis indicated that the most likely location of COL7A1 is in the interval between markers D3S1581 and D3S1606 with a maximal LOD score at marker D3S1581. Comparative multipoint analyses are displayed in figure 3. Consequently, the most likely region of the COL7A1 overlaps that of LAR1 (fig. 4). However, recombination events in two individuals excluded COL7A1 from the LAR1 region (fig. 6). The most likely explanation of the combined data is therefore that COL7A1 maps distal of LAR1 and that it is unlikely to play a causative role in the development of LS.

It will be interesting to see whether, in other families with the dominant form of LS, the disease locus will also map to chromosome region 3p21.1-14.1 or whether the disorder is heterogeneous. It also will be interesting to see whether the more clinically severe recessive form of LS (MIM 245600; McKusick 1992) maps to this region or is linked to a different locus.

The clinician unaware of LS could misdiagnose LS as arthrogryposis, diastrophic dysplasia, Ehlers-Danlos syndrome, or arthrochalasis multiplex congenita (Latta et al. 1971; Silverman 1972; Robertson et al. 1975; Marques 1980; Houston et al. 1981; De Smet et al. 1993). The view that "there may be hundreds of patients with LS misdiagnosed" (Houston et al. 1981, p. 211) is well supported by our study. The diagnosis was omitted previously in all affected from our pedigree before the index case, although 26 of 43 affected from generations V to VIII had been treated for joint conditions and some of them operated on 5–10 times. Variability of expression in LS is known (Harris and Cullen 1971; Latta et al. 1971; Samuel and Davies 1981; Petrella et al. 1993; De Smet et al. 1993) and is probably the reason why definite criteria for diagnosis are missing.

The localization of a gene for LS through positional cloning of LAR1 is a step toward elucidation of the underlying genetic defect. It will likely lead to a more

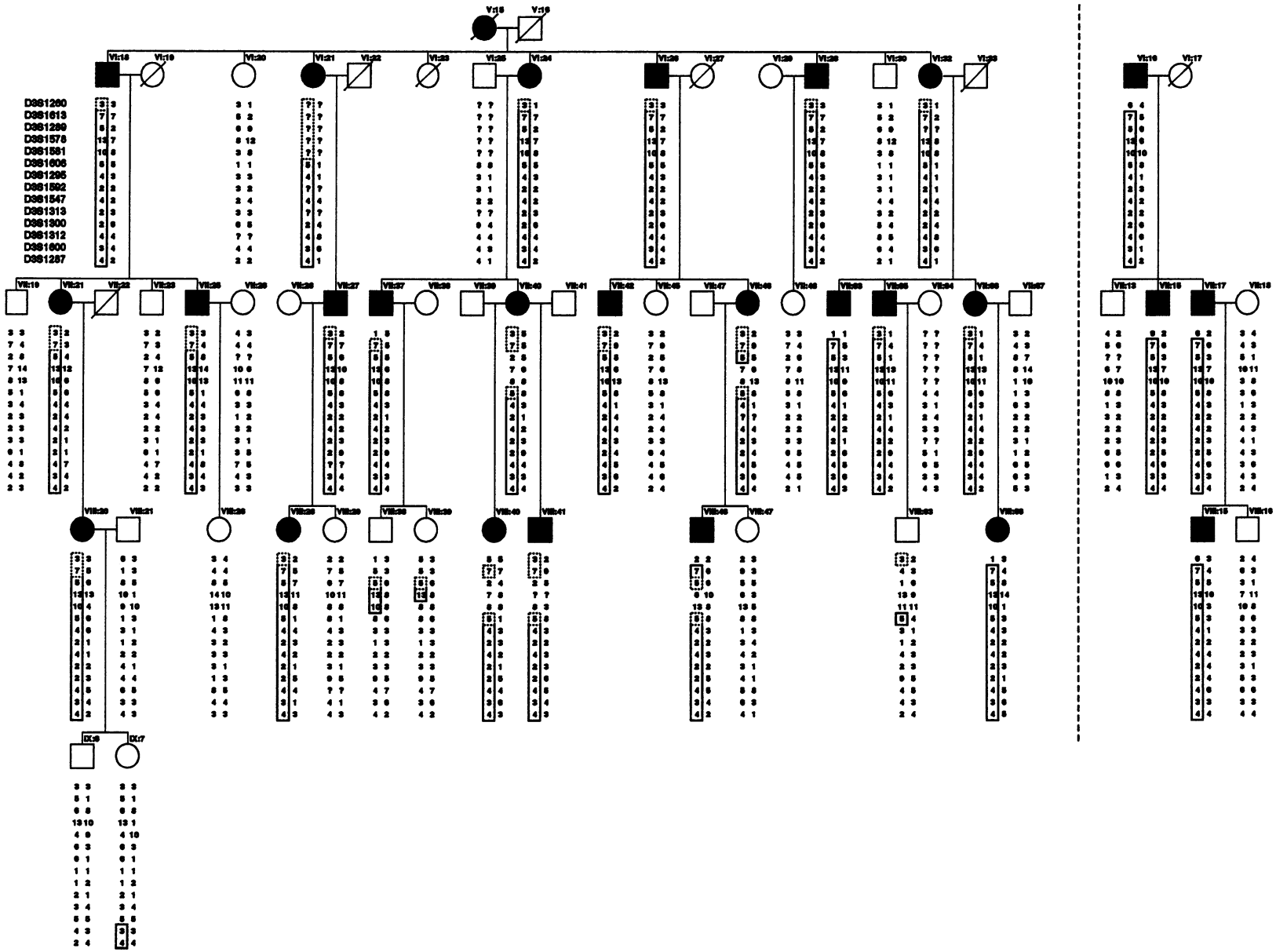

Figure 5 Recombination analysis of a Larsen syndrome family, indicating a distal and a proximal border for the LAR1 locus. The two pedigrees represent two different branches of the larger pedigree (fig. 1). The numbering of individuals is according to that of fig. 1. PCR typing results are grouped in the most likely paternal and maternal haplotypes for the region D3S1260-D3S1287 on the Généthon map (Gyapay et al. 1994). Affected individuals are indicated by blackened symbols, unaffected by unblackened symbols. The haplotype segregating with Larsen syndrome is boxed; and uninformative haplotypes are boxed with dotted lines. The allele from the affected parent is to the left under each symbol. Individuals VII-40, VII-46, VIII-38, and VIII-46 display recombination events giving a distal border for the LAR1 locus, and individual IX-7 indicates a proximal border.

Table 3

Two-Point LOD Score Values for Linkage between a COL7A1 *Pvu*II Intragenic Polymorphism and Chromosome 3 Markers

Locus (Marker)	LOD Score at Recombination Fraction							Z_{max}	θ at Z_{max}
	0	.01	.05	.1	.2	.3	.4		
D3S1260 (AFM038xc1)	1.58	1.54	1.37	1.17	.81	.51	.24	1.58	.00
D3S1613 (AFM340xf1)	−∞	−.58	.59	.88	.85	.56	.20	.93	.14
D3S1289 (AFM198yf2)	−∞	.20	1.79	2.08	1.79	1.16	.40	2.08	.11
D3S1578 (AFM268wg9)	−∞	3.83	4.06	3.77	2.87	1.82	.69	4.08	.04
D3S1581 (AFM273ve9)	3.55	3.47	3.14	2.74	1.94	1.16	.44	3.55	.00
D3S1606 (AFM318wc5)	−∞	1.00	1.48	1.49	1.20	.78	.32	1.51	.08
D3S1295 (AFM210yc5)	−∞	−1.04	1.24	1.79	1.71	1.15	.44	1.86	.14
D3S1592 (AFM292xh1)	−∞	−3.40	−1.38	−.60	−.01	.12	.06	.12	.30
D3S1547 (AFM162xc9)	−∞	.00	1.55	1.90	1.72	1.15	.45	1.92	.12
D3S1313 (AFM259zg5)	−∞	.23	.78	.85	.63	.32	.09	.85	.09
D3S1300 (AFM220yh4)	−∞	−1.37	.95	1.56	1.56	1.06	.40	1.66	.14
D3S1312 (AFM256ya9)	−∞	−.08	1.07	1.34	1.20	.78	.29	1.35	.12
D3S1600 (AFM308xc9)	−∞	−.87	.88	1.34	1.32	.88	.33	1.41	.14
D3S1287 (AFM198te7)	−∞	−1.72	.13	.69	.85	.59	.20	.87	.17

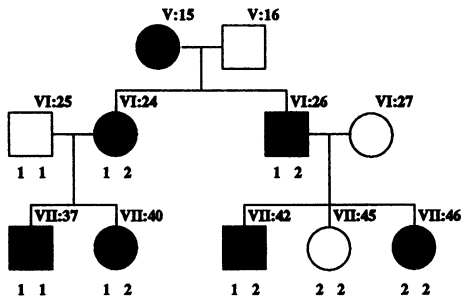

Figure 6 Family analysis of a *Pvu*II intragenic polymorphism in the human type VII collagen (COL7A1) showing recombinations between COL7A1 and LAR1 in two meioses. This analysis indicates that COL7A1 does not play a causative role in Larsen syndrome. Only family members with recombination information are displayed. The numbering of individuals is according to that of fig. 1.

accurate diagnosis, classification of different subtypes, genetic counseling, and, eventually, to prevention and/or treatment.

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