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Confirmation and Refinement of the Genetic Localization of the Coffin-Lowry Syndrome Locus in Xp22.1-p22.2

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Summary

The Coffin-Lowry syndrome (CLS) is an X-linked inherited disease of unknown pathogenesis characterized by severe mental retardation, typical facial and digital anomalies, and progressive skeletal deformations. Our previous linkage analysis, based on four pedigrees with the disease, suggested a localization for the CLS locus in Xp22.1–p22.2, with the most likely position between the marker loci DXS41 and DXS43. We have now extended the study to 16 families by using seven RFLP marker loci spanning the Xp22.1–p22.2 region. Linkage has been established with five markers from this part of the X chromosome: DXS274 (lod score [Z] (θ) = 3.53 at θ = .08), DXS43 ($Z(\theta)$ = 3.16 at θ = .08), DXS197 ($Z(\theta)$ = 3.03 at θ = .05), DXS41 ($Z(\theta)$ = 2.89 at θ = .08), and DXS207 ($Z(\theta)$ = 2.73 at θ = .13). A multipoint linkage analysis further placed, with a maximum multipoint Z of 7.30, the mutation-causing CLS within a 7-cM interval defined by the cluster of tightly linked markers (DXS207-DXS43-DXS197) on the distal side and by DXS274 on the proximal side. Thus, these further linkage data confirm and refine the map location for the gene responsible for CLS in Xp22.1–p22.2. As no linkage heterogeneity was detected, this validates the use of the Xp22.1–p22.2 markers for carrier detection and prenatal diagnosis in CLS families.

Introduction

The Coffin-Lowry syndrome (CLS) (McKusick [1990, pp. 1576–1577] 303600) constitutes a well-defined clinical entity, showing an X-linked semidominant mode of inheritance. It was described independently by Coffin et al. (1966) and by Lowry et al. (1971) and was definitively distinguished by Temtamy et al. (1975). Affected males exhibit severe mental retardation, with marked delay in acquisition of speech, a characteristic coarse facies with epicanthic folds, hypertelorism, thick lips and anteverted nares, typical large soft hands with puffy tapering fingers, hypotonia with hyperlaxity of joints, and various progressive skeletal abnormalities. Manifestations in female carriers are highly variable, but generally the signs and symptoms are much less severe than in males, with often normal or near-normal intelligence. Heterozygote detection in most but not all carrier females is possible (Haspeslagh et al. 1984; Young 1988). Although the incidence of this disorder is unknown, it appears to be widely distributed and not uncommon: over 50 cases have been reported in families of European, Asian, and African origin (see references in Young 1988). Temtamy et al. (1975) have suggested that CLS is a progressive and primary connective-tissue disorder. Beck et al. (1983) found abnormalities in proteodermatan sulfate metabolism in cultured fibroblasts, and Vine et al. (1986) further suggested a lysosomal storage disorder. However, the underlying biochemical defect of CLS is still unknown, and linkage analysis provides a tool in the effort to identify the

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gene responsible for the defect and offers a potential means for prenatal diagnosis.

In our initial linkage study, based on four pedigrees segregating for the disorder, we found a positive lod score (Z) with the markers DXS28 ($Z(\theta) = 2.00$ at $\theta = .05$) and DXS41 ($Z(\theta) = 1.26$ at $\theta = .10$), suggesting a localization of the CLS locus on the distal part of the X chromosome short arm. A multipoint Z of 3.41 was obtained for a most probable position between DXS41 and DXS43, at Xp22.2-p22.1. Further support for assignment of the disease gene to the distal part of Xp has been reported by Partington et al. (1988), who obtained positive Z values for DXS43 ($Z(\theta) = 2.71$ at $\theta = .001$), DXS28 ($Z(\theta) = 0.90$ at $\theta = .00$), and DXS84 ($Z(\theta) = 1.56$ at $\theta = .09$) by analyzing a three-generation CLS family.

In an attempt to confirm this localization and to define the position of the CLS locus more precisely, we have now extended the linkage analysis to 16 CLS families and have used seven RFLP marker loci spanning the Xp22.2–p22.1 region. The data that we report here confirm and refine our previous conclusion.

Subjects and Methods

Families

Sixteen CLS families were identified by contacting consultant clinical geneticists in several European countries. Thirteen families included at least two affected males (or one affected male and a manifesting female) per sibship, and three families included one affected and at least two healthy males. Four of the families were used in our initial linkage analysis (Hanauer et al. 1988). All patients were examined by experienced clinicians and scored as affected if they presented with the clinical signs noted above. All families except one consisted of two generations. Clinical data for some of these families have been reported elsewhere (Collacott et al. 1987; Gilgenkrantz et al. 1988). Altogether, 125 individuals were studied, including 33 affected males. At-risk females were scored as heterozygous for CLS either on genetic grounds (obligate carriers) or by the presence of unequivocal signs of the condition (reduced mental fitness, variable facial changes and/or tapered fingers). Otherwise they were entered into linkage calculations as "unknown."

DNA Analysis

The procedures for genomic DNA extraction, restriction-enzyme digestion, gel electrophoresis, Southern blotting, and hybridization to radioactively labeled probes have been described elsewhere (Oberlé et al. 1986).

The probes used in the present study and some of their relevant properties are listed in table 1. Results with the two probes at the DXS16 locus were combined.

Linkage Analysis

Pairwise and multilocus linkage analyses were performed using the LINKAGE program package (version 4.8) (Lathrop et al. 1985). The frequency of CLS was estimated to be 10^{-5}. Linkage analysis was conducted assuming 80% penetrance in carrier females and complete penetrance in males. Both the order of loci and genetic distances used in the multipoint linkage analysis were derived from data reported elsewhere (Thakker et al. 1990; Alitalo et al. 1991).

Table 1

Xp22.1–p22.2 DNA Markers Used in the Present Study

Locus	Probe	Location	Enzyme	Allele Length (kb)	Reported Allele Frequencies
DXS16	pXUT23	Xp22.2	*Bgl*II	17.5/12.5	.84/.16
	pSE3.2-L		*Msp*I	7.0/5.5	.53/.47
DXS207.....	pPA4B	Xp22.2	*Xba*I	12.0/9.5,2.5	.61/.39
DXS43	pD2	Xp22.2	*Pvu*II	6.0/6.6	.55/.45
DXS197.....	pTS247	Xp22.2	*Bgl*II	20.0/25.0	.74/.26
DXS274.....	CRI-L1391	Xp22.2–p22.1	*Msp*I	8.5,2.5/11.0	.53/.47
DXS41	p99-6	Xp22.1	*Pst*I	22.0/13.0	.56/.44
DXS92	pXG-16	Xp22.1	*Taq*I	3.8/7.1/3.5	.50/.40/.10

NOTE.—For references, see Mandel et al. (1989).

Table 2

Pairwise *Z* Values for Linkage between CLS and the Seven Xp22.1-p22.2 Marker Loci

Marker Locus	Z at θ of							
	.00	.01	.05	.10	.20	.30	θ_{max}	$Z_{(\theta max)}$
DXS16[a]	$-\infty$	−.78	.95	1.37	1.26	.77	.13	1.41
DXS43	$-\infty$	2.07	3.07	3.13	2.54	1.61	.08	3.16
DXS207	$-\infty$	−.18	2.12	2.68	2.47	1.65	.13	2.73
DXS197	$-\infty$	2.67	3.03	2.90	2.28	1.45	.05	3.03
DXS274	$-\infty$	2.46	3.45	3.50	2.86	1.82	.08	3.53
DXS41	$-\infty$	1.79	2.79	2.86	2.28	1.36	.08	2.89
DXS92	−3.05	−.33	.32	.53	.58	.42	.16	.59

[a] Results for the two probes have been combined.

Results

Results of the two-point linkage analysis are summarized in table 2. Based on documented clinical data, in these and in other families (Haspeslagh et al. 1984; Gilgenkrantz et al. 1988; Young 1988), linkage analysis was conducted assuming that 80% of carrier females would manifest some characteristics of the syndrome. When the estimate of penetrance was increased to 90%, the *Z* values were not altered substantially (results not shown). Of the seven Xp22.1-22.2 markers analyzed, five showed significant evidence of linkage to the CLS gene: DXS274 ($Z(\theta) = 3.53$ at $\theta = .08$), DXS43 ($Z(\theta) = 3.16$ at $\theta = .08$), DXS197 ($Z(\theta) = 3.03$ at $\theta = .05$), DXS41 ($Z(\theta) = 2.89$ at $\theta = .08$), and DXS207 ($Z(\theta) = 2.73$ at $\theta = .13$). The

Table 3

Lod Scores for Linkage between Xp22.1-p22.2 Markers, $Z_{max} > 1$

Markers	Z at θ of							
	.00	.01	.05	.10	.20	.30	θ_{max}	$Z_{(\theta max)}$
DXS16 and DXS43[a]	3.76	3.69	3.40	3.02	2.22	1.36	.00	3.76
	$-\infty$	12.94	12.53	11.38	8.61	5.48	.02	12.96
DXS16 and DXS207[a]	$-\infty$	2.75	3.07	2.88	2.17	1.31	.04	3.07
	$-\infty$	9.28	9.71	9.06	6.98	4.43	.03	9.76
DXS16 and DXS197[a]	1.02	1.00	.91	.80	.57	.34	.00	1.02
	3.41	3.36	3.12	2.79	2.06	1.26	.00	3.41
DXS16 and DXS41[a]	$-\infty$	1.13	1.53	1.47	1.04	.55	.06	1.54
	$-\infty$	3.17	5.68	5.91	4.72	2.86	.09	5.95
DXS43 and DXS207	5.50	5.40	5.00	4.47	3.34	2.10	.00	5.50
	27.87	27.42	25.56	23.14	17.92	12.17	.00	27.87
DXS43 and DXS197	6.02	5.92	5.51	4.97	3.80	2.50	.00	6.02
DXS43 and DXS274	$-\infty$	3.12	3.49	3.37	2.75	1.91	.05	3.49
	$-\infty$	13.09	16.30	16.21	13.57	9.53	.07	16.50
DXS207 and DXS274	$-\infty$	−.93	.85	1.33	1.37	.99	.15	1.43
	$-\infty$	4.05	9.64	10.80	9.85	7.26	.10	10.82
DXS197 and DXS274	$-\infty$	4.03	4.32	4.10	3.27	2.21	.04	4.33
	$-\infty$	7.58	7.60	7.03	5.44	3.55	.05	7.60
DXS197 and DXS41	$-\infty$	.87	1.34	1.35	1.06	.63	.07	1.38
DXS92 and DXS41	3.43	3.36	3.10	2.76	2.02	1.22	.00	3.43
	11.80	11.57	10.66	9.49	6.99	4.32	.00	11.80

NOTE.—Where two rows of data are given for a marker pair, the first row is data from the present study and the second row is data from the present study that have been combined with data from Alitalo et al. (1991).

[a] Results for the two probes at DXS16 have been combined.

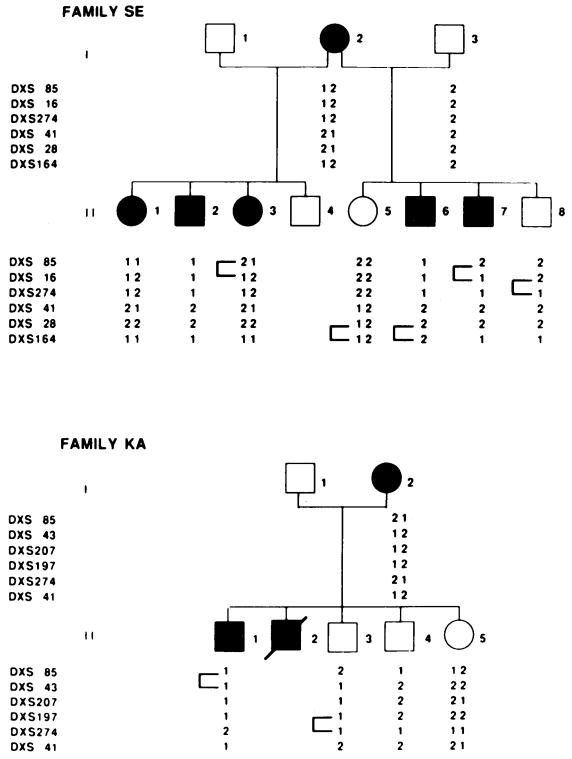

Figure 1 Segregation of informative Xp22.1-p22.2 markers in two CLS families in which obligatory recombination has occurred. Genotypes for Xp22.1-p22.2 flanking markers (DXS85 on the distal side and DXS28 and DXS164 on the proximal side), reported by Hanauer et al. (1988), have also been included. Allele 1 and allele 2 denote the largest and smallest fragments, respectively. The most probable DNA marker haplotypes are indicated below each individual. The location of each crossover is indicated by a square bracket linking the two informative markers that flank the crossover. Circles represent females and squares represent males. A blackened symbol indicates that the individual is affected with CLS.

1-lod-unit confidence intervals were .01–.23, .01–.25, .005–.23, .01–.25, and .03–.30, respectively. Pairwise Z values for the remaining markers, DXS16 and DXS92, although lower, were still positive: $Z(\theta) = 1.41$ at $\theta = .13$ and $Z(\theta) = 0.59$ at $\theta = .16$, respectively. At least one obligatory recombination with CLS (phase unknown) was observed for the markers DXS274, DXS197, DXS41, and DXS16, and two each were observed for both DXS43 and DXS207. Table 3 summarizes the analyses of the DNA markers' two-point linkage to each other in the CLS families. When combined with our data, the estimates established in 31 families segregating retinoschisis and in 40 families from the Centre d'Etude du Polymorphisme Humain reference panel, reported by Alitalo et al. (1991), are for most of the values confirmed (table 3).

Observation of key recombinants and multipoint analysis have been used to investigate the most likely position of the CLS locus relative to the Xp22.1-p22.2 map of markers. According to Alitalo et al. (1991), the most probable order of the markers, from telomere to centromere, is: DXS16–(DXS207-DXS43-DXS197)–DXS274–(DXS41-DXS92). The relative order for DXS43, DXS207, and DXS197, which are tightly linked, could not be determined in their study. Since the three latter markers also showed complete cosegregation between themselves in the CLS families (table 3), our data were not instructive with regards to the order of these loci either. The situation is similar for the more proximal cluster (DXS41-DXS92).

Tentative information regarding the location of the CLS locus was obtained by examining those recombination events involving CLS and several linked informative markers. Two pedigrees, illustrated in figure 1, were particularly informative in this study. Mothers in both families exhibited clinical signs of the condition, which eliminated the possibility of germinal mosaicism. Although the phase was unknown, the alleles have been arranged to accommodate the fewest crossovers. In family SE, the mother was only heterozygous for the markers DXS16, DXS274, and DXS41, and we observed cosegregation between the disease locus and the marker loci DXS16. The healthy son, II-8, was recombinant for DXS274 and DXS41, both proximal to DXS16, suggesting that the gene mutation most likely lies distal to DXS274. Furthermore, in our preliminary linkage study in four CLS families including the SE kindred (Hanauer et al. 1988), crossovers were noted, in individuals II-3 and II-7, between CLS and the DXS85 marker locus, which is located 10 cM distal to DXS16, at Xp22.2-p22.3 (Alitalo et al. 1991). On the proximal side of the Xp22.1-p22.2 region, crossovers were also observed between CLS and both DXS28 (Xp21.3) and DXS164 (Xp21.2) (Hanauer et al. 1988). Thus, the combined data from family SE suggested a location of the CLS locus between DXS274 and DXS85 and, in addition, excluded its placement proximal or distal to the Xp22.1-p22.2 region. In family KA, the healthy male II-3 was recombinant for DXS43, DXS207, and DXS197, but was not recombinant for DXS274 and DXS41, which provided support for placing the CLS locus proximal to the cluster (DXS207-DXS43-DXS197). One obligatory recombinant was also previously noted in this sibship with the more proximal marker DXS85 (Hanauer et al. 1988). Thus, taken together, the events observed in these two pedigrees suggest that DXS274

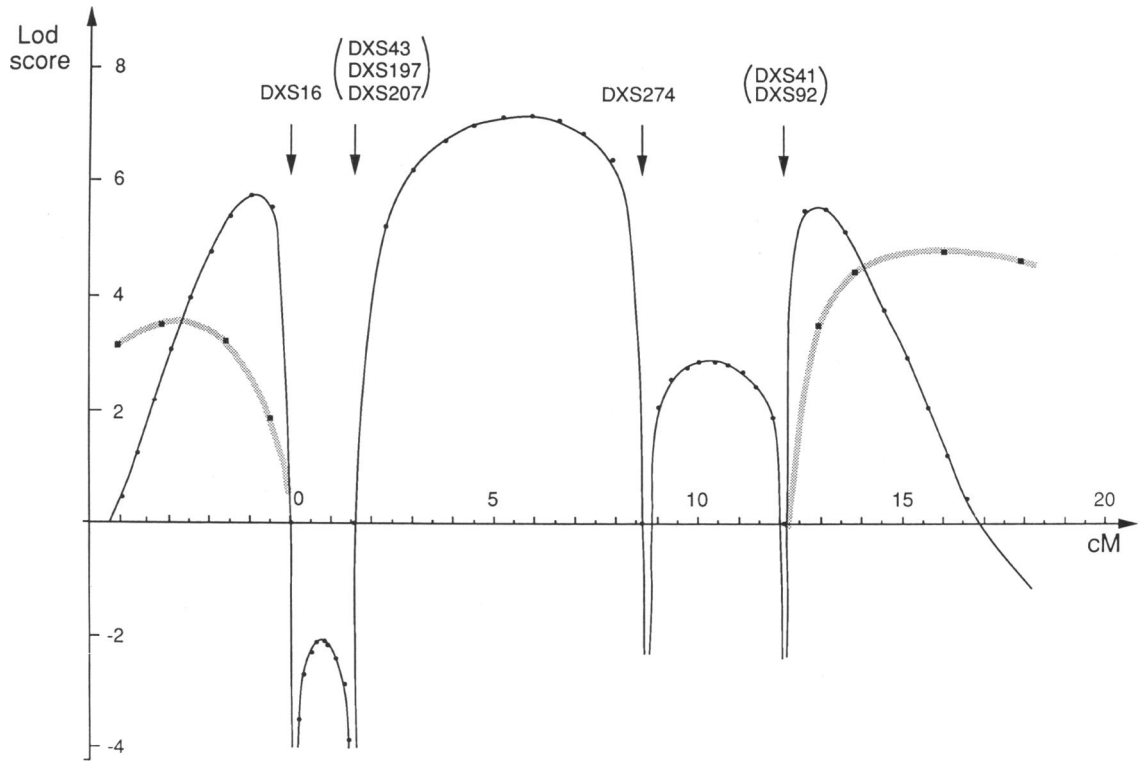

Figure 2 Multilocus *Z* analysis for the placement of the CLS locus with respect to the Xp22.1-p22.2 marker loci. The calculations were made either with only the results of the seven Xp22.1-p22.2 markers from the present study (thin line) or by adding the results obtained, in our preliminary study of four pedigrees (Hanauer et al. 1988), with DXS85 and DXS28 (thick line, represented only when different from the thin-line curve). Map distances and gene order for the marker loci were taken from Alitalo et al. (1991). DXS16 was arbitrarily placed at 0.0.

and DXS41 are proximal to the CLS locus while DXS207, DXS43, and DXS197 flank the disorder locus on the distal side.

In order to substantiate the above findings, we performed multipoint-linkage analysis, using the LINKMAP program (Lathrop et al. 1985), to vary the position of the CLS locus relative to fixed positions of the marker loci. The fixed genetic distances used for the test were as determined by Alitalo et al. (1991). As no probe-to-probe recombination between DXS43, DXS207, and DXS197 was observed, haplotypes for three markers were constructed and used in the location-score calculation. Similarly, DXS41 and DXS92 were also combined into a single haplotype system. Thus, a total of four distinct loci, in addition to the disease locus, were considered in the LINKMAP analysis. The analysis gave a peak multipoint *Z* of 7.30 for locating the CLS locus between DXS274 and the cluster (DXS207-DXS43-DXS197) (fig. 2).

Discussion

A first indication concerning the regional assignment of the CLS locus to distal Xp, between DXS41 and DXS43 in the Xp22.1-p22.2 region, came both from our previous linkage study based on four pedigrees segregating for the disease (Hanauer et al. 1988), and the Partington et al. (1988) study based on a single family. In the present investigation, we have extended the linkage analysis to 16 families with CLS and have focused on Xp22.1-p22.2 marker loci. Linkage has now been demonstrated between CLS and at least five loci from the region Xp22.1-p22.2: DXS274, DXS43, DXS41, DXS197, and DXS207, at 8% recombination for the first three markers and at 5% recombination for the fourth. Addition of our peak *Z* between CLS and DXS43 to that previously reported by Partington et al. (1988) yielded a maximum combined *Z* of 5.56 with $\theta = .05$, which further narrowed the confidence

interval for the CLS-DXS43 linkage, to .005–.16. Thus these additional two-point linkage data support strongly the previous tentative evidence that the CLS locus is on distal Xp, in the p22.1-p22.2 region (Hanauer et al. 1988; Partington et al. 1988).

The location of the gene responsible for CLS, relative to the seven marker loci, was further estimated by a multipoint linkage analysis. The maximum-likelihood estimate of the location of the CLS gene placed it between DXS274 and a group of tightly linked markers: DXS207, DXS43, and DXS197. The CLS locus could be excluded, with odds of 10^5:1, from the more proximal DXS274–(DXS41-DXS92) interval and, with odds of greater than 10^7:1, from the more distal DXS16–(DXS207-DXS43-DXS197) interval. CLS could only be excluded from lying distal to DXS16 or proximal to the cluster DXS41-DXS92 with odds of 25:1 and 50:1, respectively. However, if we take into account the results obtained in our preliminary study in four CLS pedigrees (Hanauer et al. 1988; for families SE and KA, see preceding discussion), with DXS28 (proximal to DXS41-DXS92) and with DXS85 (distal to DXS16), locations for CLS proximal to the cluster DXS41–DXS92 or distal to DXS16 become, respectively, 250 less likely and 5×10^3 less likely than the most probable location (fig. 2).

The 1-lod difference from the maximum-likelihood estimate, which was used to construct a support (confidence) interval, placed CLS 0.5–6 cM distal to DXS274. Thus, based on the present data, possible locations for the CLS gene were entirely confined between DXS274 and the cluster (DXS207-DXS43-DXS197), and the most likely gene order is Xtel–DXS16–(DXS207-DXS43-DXS197)–CLS–DXS274–(DXS41-DXS92)–Xcen.

The area bracketed by the CLS flanking markers, DXS274 and the cluster DXS207-DXS43-DXS197, comprises approximately 7 cM (Alitalo et al. 1991), which may mean than CLS is probably no more than 2–4 Mb away from the closest marker. As stated above, there is at least one phase-unknown recombination between CLS and the distal flanking cluster DXS207-DXS43-DXS197 and one between CLS and the proximal flanking marker DXS274. It should thus be possible to map genetically closer markers. New highly informative microsatellite markers are currently being generated between (DXS207-DXS43-DXS197) and DXS274, to localize the defective gene more accurately and to allow for the development of experimental strategies for isolating it. The linkage data presented in the present report are also important in view of the possibilities for carrier detection and prenatal diagnosis. Up to now, no evidence has been found for more than one CLS locus, although our data are not yet strong enough to rule out entirely the possibility of genetic heterogeneity. Linkage analysis of additional CLS families is required not only for a more accurate estimate of genetic distances but also to ascertain the genetic homogeneity of the disease.

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