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Lethal congenital contracture syndrome (LCCS), a fetal anterior horn cell disease, is not linked to the SMA 5q locus

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Abstract

The lethal congenital contracture syndrome (LCCS) is an autosomal recessive syndrome (McKusick 253310) leading to perinatal death owing to early onset degeneration of the anterior horn motor neurones of the spinal cord. The neuropathological findings in the LCCS closely resemble those of spinal muscular atrophy (SMA). Since all the three types of SMA have been localised to the same gene locus on the long arm of chromosome 5, we analysed samples from seven families with 10 LCCS fetuses with the microsatellite markers assigned to the SMA 5q region. Linkage analyses between the SMA linked DNA markers and the disease allele in the LCCS families excluded the critical chromosomal region around the SMA locus as the critical chromosomal region for the LCCS locus.

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The lethal congenital contracture syndrome (LCCS) is an autosomal recessive syndrome (McKusick 253310), so far reported mostly in Finland.[1] It leads to perinatal death and the fetuses typically have hypoplastic lungs, marked skeletal muscle hypoplasia, and contractures of the extremities.[2] The pathological mechanism of LCCS, the early onset degeneration of the anterior horn motor neurones of the spinal cord, has been well documented.[3]

The neuropathological findings in LCCS closely resemble those of spinal muscular atrophy (SMA). Three different forms of SMA can be distinguished by the age of onset and the course of the disease.[4] Since all three types share a gene locus at 5q13.3, between the polymorphic DNA markers D5S435 and D5S39,[5–8] the question arises whether LCCS might represent an extreme form of SMA. To study this hypothesis, we performed linkage analysis using samples from seven Finnish families with 10 LCCS fetuses and the markers closely linked to or flanking the SMA locus on 5q.

Methods and results

Fig 1 shows the LCCS pedigrees used in this study. Peripheral blood samples were stored at −20°C and were available from 14 parents and seven healthy children. Total DNA was isolated according to standard procedures.[9]

The polymorphic microsatellite markers (D5S407, D5S435, D5S351, D5S39, D5S424)[8 10 11] assigned to the SMA 5q region were amplified using polymerase chain reaction (PCR). PCR was performed in a microtitre well format as described previously.[12] All the primer sequences of the markers originated from the amplifiable marker collection of Généthon (The Généthon Microsatellite Map Catalogue 1993) or that of the Nordic Human Genome Organisation.

We carried out data simulation for the LCCS family material in the linkage analyses assuming a single marker locus and double heterozygosity

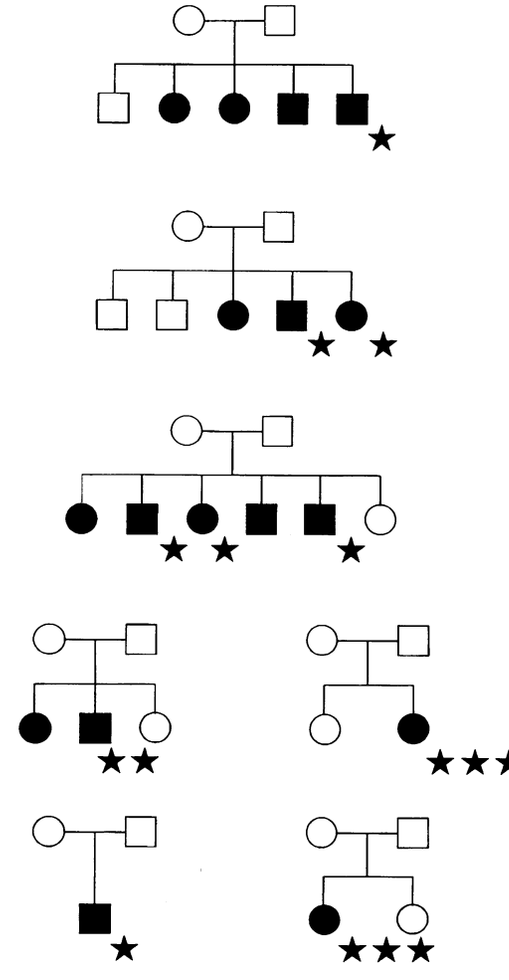

Figure 1 The pedigrees of the families studied. Filled symbols denote affected members, open symbols denote unaffected spouses. One star indicates subjects from whom fibroblasts were available for DNA isolation, two stars those from whom placenta was collected, and three stars those from whom liver biopsy was available for DNA isolation. Peripheral blood samples were available from all unaffected family members.

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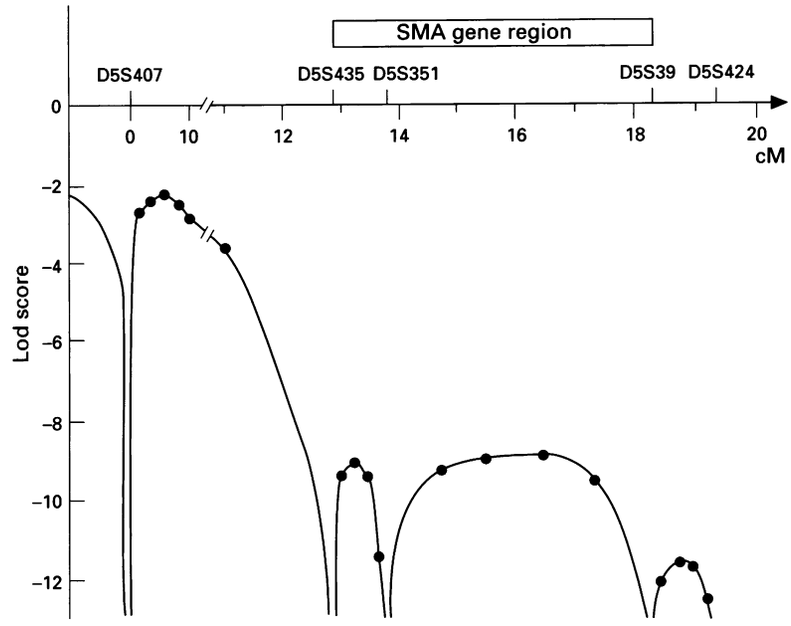

Figure 2 The result of multipoint linkage analyses of LCCS and SMA. The critical chromosomal region was considered excluded with lod score values below −1·5 of flanking or closely linked markers. The markers are shown above the horizontal axis and genetic distances between markers are expressed in centiMorgans (cM).

Pairwise linkage data between LCCS and markers flanking the SMA locus

Marker	*Recombination fraction*				
	0·00	*0·01*	*0·05*	*0·07*	*0·1*
D5S407	−∞	−1·7	−0·51	−0·30	−0·12
D5S435	−∞	−2·9	−1·5	−1·2	−0·93
D5S351	−∞	−6·1	−2·8	−2·2	−1·6
D5S39	−∞	−4·3	−2·2	−1·8	−1·4
D5S424	−∞	−5·0	−2·4	−1·9	−1·4

in the parents to analyse the informativeness of our family material. The MSIM option of the SLINK computer program[13] was used with 2000 replicates (one replicate equals one round of generating marker genotype for each subject) to obtain the elod values (expected logarithm of odds score). This simulation analysis showed an average elod of 1·4 at the recombination fraction (θ) 0·07, SD 0·5. The elod values remained over 1·0 at θ 0·13 with 85% of replicates.

Linkage analyses between the disease locus of LCCS and the polymorphic marker loci were carried by using the MLINK and LINKMAP options of the LINKAGE package computer program (version 5·1).[14] The frequency applied for the disease allele was 0·016[1] with complete penetrance. Locus homogeneity was assumed owing to the enrichment of the diseases in the genetically isolated Finnish population and the homogeneity of the clinical presentation.

The marker D5S39 is reported to be the closest marker to the SMA locus with D5S39 and D5S435 being distal and proximal flanking markers, respectively.[8 11] The results of the two point analyses are summarised in the table. No evidence of linkage between any of the markers and the LCCS locus was found, and only negative lod scores were obtained at the recombination fractions $\leq 0{\cdot}1$. Also the multipoint linkage analyses with the markers resulted in negative lod scores excluding a continuous area 13·7 cM proximal to D5S351, the closest marker to the SMA locus, and 11 cM distal to it (fig 2). These data show that the LCCS locus is not allelic with SMA.

Discussion

Diseases affecting anterior horn cells are a heterogeneous group of neurodegenerative disorders which may become manifest at any time of life.[15] The most common of these disorders is spinal muscular atrophy. The assignment of the SMA loci to chromosome 5q has made prenatal diagnosis possible for SMA families. However, it has led to confusion in the families with so-called variants of SMA, that is, diseases with anterior horn cell involvement and a phenotype atypical of SMA.[16] The group of "variants" of infantile SMA, or more precisely anterior horn cell disease (AHD), includes two subgroups that resemble LCCS: cases with AHD and multiple congenital fractures and cases with AHD and early respiratory insufficiency.[17]

It has been suggested that the SMA variants differ genetically from SMA 5q.[4] The reported pedigrees with AHD and arthrogryposis suggest autosomal recessive transmission,[18] but X linked inheritance cannot be excluded.[19 20] A linkage study in a consanguineous family with two affected males out of five sibs was performed resulting in exclusion of 5q.[21] Our results excluding the SMA 5q locus as the LCCS gene locus show that LCCS does not represent a subtype of SMA, but is a genetically distinct syndrome.

At present, the prenatal diagnosis of LCCS is based on sonographic findings of fetal akinesia and hydrops.[22] The localisation of the LCCS gene would make specific prenatal diagnosis available for LCCS families. In addition to the clinical advantage, the further characterisation of LCCS would provide data on the molecular pathomechanism of motor neurone disease.

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