Genomic rearrangements in childhood spinal muscular atrophy: linkage disequilibrium with a null allele

Rachael J Daniels, Louise Campbell, Nanda R Rodrigues, Michael J Francis, Karen E Morrison, Michael McLean, Alex MacKenzie, Jaakko Ignatius, Victor Dubowitz, Kay E Davies

Abstract

Autosomal recessive childhood onset spinal muscular atrophy has been mapped to chromosome 5q13. We report the analysis of a polymorphic microsatellite which shows linkage disequilibrium with the disease. The linkage disequilibrium is observed with a null allele which is seen as the non-inheritance of alleles from one or both parents. The inheritance of a null allele was observed in 26 out of 36 (72%) informative childhood onset spinal muscular atrophy (SMA) families tested, of all types of severity and from a variety of ethnic backgrounds. In seven families segregating for the severe Werdnig-Hoffmann or SMA type I, no alleles were inherited from either parent using this microsatellite. This null allele may represent a deletion which is either closely associated with, or causes, the disease.

(J Med Genet 1995;32:93-96)

Childhood onset proximal spinal muscular atrophy (SMA) has been well defined clinically and genetically.¹⁻⁸ It is a common autosomal recessive neurological disorder in which the initial biochemical defect is unknown, which results in abnormalities of the alpha motor neurones in the anterior horn of the spinal cord. Molecular genetic research has localised the mutation(s) to chromosome 5q11.2-q13.3.⁹⁻¹³ Genetic linkage analysis and the consideration of key recombinant family data has mapped the gene to the interval between the markers D5S435 and D5S557.^{9-11,13} Yeast artificial chromosome (YAC) contigs of this region have been constructed and new microsatellites reported.¹³⁻¹⁶ The candidate region contains chromosome 5 specific repeat sequences as evidenced by the detailed study of genomic and cDNA sequences that map within it.^{13,17} A microsatellite repeat, CATT-1, isolated by Burges *et al*,¹⁶ has multiple sites within the region, with as many as eight alleles in any one person. In an attempt to distinguish between the individual sites for CATT-1, alternative primers flanking the various copies of this dinucleotide repeat were obtained. Genotyping with one set of these alternative primers (defining the locus CATT/alt) shows linkage disequilibrium with SMA.

Melki *et al*¹⁵ recently published three microsatellites, C272, C212, and C161, which

also have multiple copies in the candidate region and show abnormal genetic changes within a small proportion of SMA families. We have analysed these markers in our data set and compare them with the results obtained with CATT/alt.

Materials and methods

FAMILIES

A total of 130 SMA families was used in this study, 101 of which have been previously tested

Figure 1(A) Example of the lack of inheritance of one parental allele at the CATT/alt locus in an SMA type I family (UK). (B) Example of lack of inheritance of alleles from each parent at the CATT/alt locus in an SMA type I family (UK). The father's DNA was unavailable for analysis.

Molecular Genetics Group, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK
R J Daniels
L Campbell
N R Rodrigues
M J Francis
K E Morrison
K E Davies

GenoSPHERE Project (ERATO, Japan), Faculty of Medicine, University of Ottawa, 451 Smyth Road, Ottawa, Ontario K1H 8M5, Canada
M McLean

Children's Hospital of Eastern Ontario, 401 Smyth Road, Ottawa, Ontario K1H 8L1, Canada
A MacKenzie

Department of Medical Genetics, The Family Federation of Finland, Kalevankatu 16, FIN 00100 Helsinki, Finland
J Ignatius

Department of Paediatrics and Neonatal Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK
V Dubowitz

Correspondence to: Professor Davies.

Received 12 October 1994
Accepted for publication 28 October 1994

Table 1 Analysis of CATT/alt in SMA families and controls

Families	Inheritance		
	No allele absence	Allele absence from one parent	Allele absence from both parents
Finnish I	2	3	4
Finnish II	3	6	0
Finnish III	0	4	0
Other I	5	6	3
Totals	10	19	7
Non-SMA	24	0	0

I, II, and III refer to the types of SMA.

"Other" includes families from the UK, elsewhere in Europe, and Asia.

Ten SMA families were uninformative, 6 non-SMA control families were uninformative.

Table 2 Analysis of C272 in SMA families and controls

Families	Inheritance	
	No allele absence	Allele absence from one parent
Finnish I	2	0
Finnish II	7	0
Finnish III	4	0
Other I	4	1
Other II	12	0
Other III	2	1
Totals	31	2
Non-SMA	28	0

I, II, and III refer to the types of SMA.

"Other" includes families from the UK, elsewhere in Europe, and Asia.

Seventy-five SMA families were uninformative, 1 non-SMA control family was uninformative.

with other markers within the region which show genetic linkage with SMA. The remainder represent families who have a single affected child or families for whom no haplotyping has yet been performed. All cases conform to the international criteria for diagnosis of SMA.²

ISOLATION OF MICROSATELLITES

Cosmids constructed from the YAC 17F5¹³ were screened with radiolabelled poly (dC-dA)_n(dG-dT)_n. Eight positive clones were selected, subcloned into plasmid, and the microsatellite repeats sequenced. Most of the sequences flanking the (CA)_n repeat identified in these clones were identical to the sequence published in the original CATT-1 paper.¹⁶ A few of the clones, however, showed stretches of new sequence, and it was to these new sequences that primers were designed to amplify a subset of the CATT-1 loci, CATT/alt. These primer sequences have also been identified by McLean *et al*¹⁸ as amplifying a specific CATT-1 sublocus.

PCR ANALYSIS

The PCR analysis was carried out in a standard fashion incorporating radiolabelled nucleotides directly in the reaction as previously described.^{7,8} Loci C272, C212, C161, and CATT-1 were analysed as initially reported.^{15,16} CATT/alt PCR was performed in 1.5 mmol/l MgCl₂, using standard cycles with an annealing temperature of 55°C. Primers for CATT/alt are: 5'-tta cag gca tga gct act gca-3' and 5'-gag aag gct tcc tcc tga gta tgc-3'. The reaction products were electrophoresed on 6% denaturing acrylamide gels at 60 W. CATT/alt loci were analysed on gels run for four to five hours and required exposure to x ray film for three to 10 days.

LINKAGE DISEQUILIBRIUM

Linkage disequilibrium calculations were performed using standard χ^2 formulae with Yates's correction where appropriate.

Results

FAMILY STUDIES

We analysed 28 Finnish families segregating for all three types of SMA and 18 SMA type I families from Europe (predominantly the UK)

and Asia, with the microsatellite primers from the CATT/alt locus (see Methods). The results are summarised in table 1. An example of a family for whom the affected child fails to inherit an allele from one of its parents is given in fig 1A. In the left half of the pedigree, the father of the affected child has not inherited any alleles from his father. The next mating is uninformative because both parents have the same allele. Similarly, on the far right of the pedigree, the carrier grandmother does not give an allele to her carrier son, who in turn does not pass on any visible allele to his affected son.

An example of an affected child who fails to inherit a visible allele from either parent is given in fig 1B. This is not the result of technical failure because the upper constant band at 299 bp (marked) is present. This band appears in the majority of tracks, except the negative control. This child's DNA sample gave appropriate PCR products for all other closely linked markers from the region. For the families that are informative at this locus, 72% (26/36) show an absence of an allele indicating the segregation of a null allele or deletion. Thirty families not suffering from SMA were also studied, none of which showed any absence of alleles (24 were informative, see table 1).

We also analysed the microsatellites reported by Melki *et al*,¹⁵ C272, C212, and C161. The C272 microsatellite was analysed in the same families as those studied at the CATT/alt locus. None of these families showed allele absence, nor did the 29 control families. Since the frequency of allele absence at these loci is low,¹⁵ we extended the study in a further 62 SMA families. The results for C272 are summarised in table 2. In two SMA families (type I and type III), the affected sibs failed to inherit a product from the mother (fig 2A and B). Neither of these families showed the allele absence at the CATT/alt locus, where they were informative.

The C212 microsatellite was also tested in 82 SMA families and 25 non-SMA families and showed no abnormalities in inheritance. The sequence of the C161 primers was compared to the sequence published for the CATT-1 loci and found to be very similar. When C161 was genotyped in the same families previously analysed with the CATT-1 primers, the PCR pattern was found to be the same (data not

Figure 2(A) Example of the allele loss at the C272 locus in an SMA type I family (UK). (B) Example of allele loss at the C272 locus in an SMA type III family (UK).

Figure 3 Linkage disequilibrium between SMA and the CATT/alt locus in the Finnish population.

shown). C161 was therefore not analysed further.

LINKAGE DISEQUILIBRIUM

Thirty Finnish SMA patients and 20 normal Finnish persons were genotyped with CATT/alt. The resulting products were difficult to score directly, but it was possible to score subjects on the basis of having one or both of the two bottom alleles (A: 248 bp, B: 246 bp), of having some form of upper allele (C), or of having both an upper and lower allele (A or B and C). The data are presented in fig 3. The results were corrected for the total number of persons and, with 2 degrees of freedom, a χ^2 value of 12.22 is obtained ($p < 0.001$), providing evidence of linkage disequilibrium between the CATT/alt genotypes and SMA in this Finnish population. There was no linkage disequilibrium apparent with either C272 or C212.

Discussion

Seventy-two percent (26/36) of all informative SMA families showed abnormal inheritance patterns at the CATT/alt locus. This phenomenon was seen in families with all three forms of SMA, from the UK, Finland, Germany, and Asia. Similar results have recently also been described in Canadian and North American SMA type I patients.¹⁸ It is interesting to note that all the families which showed a "double null" inheritance pattern were of the severe SMA type I subgroup; this may in part explain the varying severity of the disease, if this "null allele" is in some way pathogenic. Furthermore, the three generation families available for study showed that the null allele was inherited from previous generations

(fig 1A). The null allele was seen to be inherited from previous generations through both the maternal and paternal lines. When the genotypes from the other polymorphic loci in this region are studied, it is clear that the abnormality is exclusively inherited from carrier grandparents. None of the 30 non-SMA families studied showed an abnormality at the CATT/alt locus. The high numbers of SMA families showing allele absence suggests that this genetic change may have a direct link with the mutation involved in SMA.

Melki *et al*¹⁵ reported three families out of 201 (1.9%) showing abnormalities at the C272 locus and three families (1.9%) showing changes at the C212 locus. Dosage studies indicated that two of the families abnormal at the C212 locus represented a de novo change and one an inherited change, respectively. One further family showed a de novo rearrangement with both of these markers. In our studies, two out of 106 families (1.8%) showed a similar genotypic change at the C272 locus, a result comparable to that found in the French cohort. Although we found no changes at the C212 locus, our data set may not be large enough to detect changes present at a frequency of 1 to 2%. We found no evidence for de novo loss of alleles at the C272 locus as these changes were observed in more than one sib. Melki *et al*¹⁵ presented evidence for such de novo cases. If such de novo changes have occurred in our population, the parents must be germline mosaics.

In summary, we present in this paper the results of genotyping at a new locus, CATT/alt. This locus not only shows significant linkage disequilibrium with SMA in the Finnish population, implying that it may be very close to the gene, but the deletion/null allele present in at least half of the SMA families studied may relate to the mutation itself. As the families with null allele/deletions at the C272 locus are not the same as those which also show allele absence at the CATT/alt locus, the region over which rearrangements associated with the disease can occur may be quite large (several hundred kilobases). The seven SMA type I families showing no inheritance of visible alleles at the CATT/alt locus from both parents are worthy of further study as these might represent the extreme DNA rearrangement which results in the most severe phenotype.

We are very grateful to Dr Peter Harris for the DNA samples used as controls. We thank Helen Blaber for assistance with the preparation of the manuscript. We are indebted to the SMA families and the Finnish Red Cross for blood samples. We are grateful to the Medical Research Council, the Muscular Dystrophy Group of Great Britain and Northern Ireland, the Muscular Dystrophy Association in the USA, and the Finnish Muscular Dystrophy Association for generous support.

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