Original Paper

Eur J Hum Genet 1995;3:56–60

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Mapping of the Spinal Muscular Atrophy (SMA) Gene to a 750-kb Interval Flanked by Two New Microsatellites

Abstract

The gene for autosomal recessive proximal spinal muscular atrophy (SMA) has recently been mapped between D5S629 and D5S557. We report here a new single-locus microsatellite A31 (D5S823) and two multicopy microsatellites 97T-CA and 95/23-CA. The marker A31 maps to the region of overlap between YACs y116, y55 and y122, distal to D5S629; 97T-CA originates from a cosmid corresponding to the STS 97T, localized distally to A31, while 95/23-CA derives from a cosmid corresponding to the STS 97U, localized proximally to D5S557. We tested all our key recombinant families with these markers. In one type I/II SMA family, a recombinant was found that placed the SMA locus distal to D5S823. Homozygosity mapping in a consanguineous type I SMA family indicates that the SMA gene lies proximal to 95/23-CA. Thus, the two new markers, A31 and 95/23-CA further refine the SMA gene to an approximately 750-kb interval.

Key Words
Spinal muscular atrophy
SMA
Microsatellites
Multicopy markers
Linkage analysis

Introduction

The autosomal recessive proximal spinal muscular atrophy (SMA) candidate region on 5q11.2-13.3 has been narrowed to a region of about 1.4 Mb between the loci D5S435 and D5S557 [1–6]. More recently, a new marker, D5S629, which is more distal to D5S435 has been defined as the closest proximal border of the SMA region [7]. This region containing the SMA gene is still large complicating the search for the gene. Apart from the size of the region, there are several unusual features of this part of the genome. It contains multiple copies of various loci [2, 9–12], chromosome-specific repeats [2–4, 13] and is highly unstable in YACs [2, 8, 9]. It also appears to encode a large number of cDNAs [authors' obs.]. Due to this complexity, it is imperative to narrow the area containing the SMA gene.

Received: September 5, 1994
Revision received: December 5, 1994
Accepted: December 13, 1994

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1018-4813/95/
0031-0056$8.00/0

Materials and Methods

Family Assessment

The 7 key recombinant families have been previously reported [4, 5]. All patients with proximal SMA were carefully investigated and diagnosis was performed according to the criteria defined by the International SMA Consortium [14].

Construction of Cosmid Libraries from YACs

High-molecular-weight YAC DNA (about 7.5 μg/plug) was prepared in 1.5% low-melting agarose by standard methods [15] and stored in 0.5 *M* EDTA. The plugs were washed 3 times in 1 × TE at 50 °C, then partially digested with *Sau*3AI (about 0.06 U/plug). For the analytical test, one plug was melted in a final volume of 250 μl containing enzyme reaction buffer. Aliqouts of 50 μl were directly transferred into tubes containing 5 μl of 0.5 *M* EDTA at 0, 5, 10, 15 and 20 min. These were separated on 0.3% agarose gel at 30 V overnight. The digestion times giving the best sizes (about 20–40 kb) were between 5 and 10 min. The preparative digest was performed with 3 plugs using digestion times of 5, 7.5 and 10 min. The enzyme was immediately inactivated with EDTA (15 m*M* final concentration) and the plugs were melted at 68 °C for 10 min. The dephosphorylation (5 U alkaline phosphatase/plug, Boehringer) and agarase treatment (2U agarase/plug, Calbiochem or Eurogentech) were performed simultaneously at 37 °C for 2 h. The alkaline phosphatase inactivation was achieved by adding 15 m*M* EDTA and heating the reaction at 68 °C for 10 min. NaCl was added to a final concentration of 100 m*M*, then the DNA was extracted twice with phenol, pH8 (no chloroform!) and twice with ether (saturated with distilled water). The DNA was precipitated with 1/10 vol 3 *M* sodium acetate, pH 6 and 2.5 vol ice-cold ethanol, placed on ice for 5 min, and then the DNA was pelleted at 5,000 rpm in an Eppendorf centrifuge for 20 min. The DNA was pooled and resuspended in 6 μl 1 × TE. The DNA concentration was tested by dropping 0.5 μl of the sample on an ethidium-bromide-stained agar plate. About 2–4 μg of YAC DNA were ligated to 1–2 μg cosmid DNA SuperCos1 (Stratagene) in a total volume of 12 μl overnight at 14 °C. The vector DNA was prepared as recommended by the supplier. 4 μl of the ligation mix were packaged with Gigapack Gold (Stratagene) and plated out on two large plates (222 × 222 mm). About 30,000 colonies/plate were obtained. Human clones were identified by hybridization with radioactively labelled human pool DNA mixed with an Alu probe.

Screening of a Chromosome-5-Gridded Cosmid Library with STSs Derived from the SMA Region

The PCR product of different STSs from the SMA region [2] were radioactively labelled by random priming, competed with 200 μg human pool DNA/5 μg Alu probe/5 μg SuperCos1 vector DNA and hybridized against gridded filters of the chromosome-5-specific cosmid library (Los Alamos). Filters were prehybridized for 4 h and hybridized overnight in 0.5 *M* sodium phosphate buffer pH 7.2/7% SDS/1 m*M* EDTA at 65 °C and washed in 2 × SSC/0.1% SDS, 1 × SSC/0.1% SDS and 0.1% SSC/0.1% SDS at 65 °C for 20 min each, then exposed on Kodak X-OMAT autoradiographies at −70 °C for 2–5 days.

Identification and PCR Analysis of Simple-Sequence Repeats (SSRs)

This was carried out as previously described [4, 16]. The sequences have been deposited within the EMBL Data Library under the Accession No.: X76698 (A31), X76699 (97T-CA) X76700 (95/23-CA), X76701 (97U-CA).

Results and Discussion

Isolation and Characterization of New SSRs Derived from the SMA Region between the Loci D5S629 and D5S557

We constructed a cosmid library from the YAC y116 [2], and isolated 96 human clones which were hybridized with radioactively labelled CA/GT-ds DNA. Seven positive clones were obtained. From the cosmid C31, the new polymorphic SSR A31 (D5S823) was isolated. The cosmid was physically mapped to the overlapping region between YACs y116, y55 and y122 by probing a Southern blot containing *Eco*-RI-digested YACs covering the SMA region, as well as genomic DNA from the somatic cell line PN/TS (which contains human chromosome 5) and HHW1064 (which contains a human chromosome 5 with a deletion of 5q11.2-q13.3). Thus, D5S823 lies distal to D5S629 (which was positive only for YACs y116 and y55, but not y122) and approximately 550 kb distal to D5S435.

Table 1. Characterization of the new SSRs

Marker	Location	Origin	Address	Primers	Repeat motif	Size or PCR product, bp	Annealing temp, °C
A31	overlap of y116/y55/ y122	cosmid C31	–	5′-GGCTACAGGC-CAGATGAGG-3′ 5′-CCAGGGGAAA-ATAGGCTTGT-3′	$(TG)_5$TATA-TTAA$(TG)_{15}$	128–150 (138[a])	60
97U-CA	y97U	cosmid C97U.1	253/H12	5′-ACCATTATGAA-AATGTTTTGC-3′ 5′-TATGTTCCTTC-CCTAGAGCAAG-3′	$(CA)_9C(CA)_6$-$AG(CA)_3A(CA)_5$	179[a] (not polymorphic)	57
95/23-CA	y97U	cosmid C97U.2	134/C1	5′-TGGAATATGC-TGCCAGAGT-3′ 5′-GGTAATTTCCT-TTAGCTGGG-3′	$(CA)_{17}$	96[a] (multicopy locus)	60
97T-CA	y97T	cosmid C97T	122/D7	5′-GACCCCAAGG-AAATACTTG-3′ 5′-GAGCAAGACT-CTGTCTCGAA-3′	$(CA)_{22}$	237[a] (multicopy locus)	55

[a] Size of the PCR product which was sequenced.

The repeat motif, size of PCR product and PCR conditions are given in table 1. The allele distribution on the SMA chromosomes, which was determined in 310 chromosomes, is as follows: 128, (0.12); 130, (0.00); 132 (0.01); 134 (0.02); 136 (0.64); 138 (0.08); 140 (0.04); 142 (0.01); 144 (0.01); 146 (0.07); 148 (0.01). Similar frequencies were found on the normal chromosomes. The heterozygosity frequency is 54%.

To obtain additional clones from the region, we isolated cosmids from the chromosome-5-specific Los Alamos library by hybridizing different STS-PCR products derived from the SMA region [2] against gridded cosmid filters.

From three different cosmids we isolated the following SSRs: 97T-CA, 95/23-CA and 97U-CA (table 1). The SSR 97T-CA ended at one side adjacent to an Alu repeat which is orientated with the 5′ end towards the $(CA)_n$ repeat. Therefore, the reverse primer is a 5′ Alu repeat sequence. Nevertheless, we obtained specific PCR products and the alleles showed Mendelian inheritance. Both markers, 97T-CA and 95/23-CA revealed a polymorphic multilocus pattern with up to 6 alleles. Since the evaluation of these complex patterns and the segregation analysis was often extremly difficult, no detailed analysis with respect to the distribution of alleles on normal and SMA chromosomes was performed. The marker 97U-CA was not polymorphic in 20 unrelated people.

Linkage Analysis in SMA Families

The new polymorphic marker A31 (D5S823) was tested against 7 multiplex recombinant families from Bonn and Ohio, who were recombinant with the closest flanking markers, D5S629 or D5S557. One of these families, OSU1, was recombinant for the marker A31 (D5S823). The two affected children inherited different alleles from their mother. All other proximal markers recombined as well, while the distal markers coseg-

regated with the SMA gene. These data were confirmed independently in Bonn and Ohio laboratories and indicate that the SMA gene lies within an approximately 850- to 1,000-kb interval flanked by the markers D5S823 and D5S557. The distance given is based on the YAC contigs reported by Kleyn et al. [2] and Carpten et al. [8]. However, because the YACs are unstable and possess frequent deletions, it is possible that the region is larger [8, 9].

Further markers, CATT-1 [10], C212 [9], Ag1-CA [11] and CMS1 [2] which are localized within the SMA candidate region did not recombine in any of these families.

The two other markers (97T-CA and 95/23-CA) revealed multiple copies within the SMA region. Due to the difficulty analyzing these complex markers, we only examined recombinant and consanguineous families. No clear recombinants were detected. However, in a type I SMA family in which the parents are first cousins, the marker 95/23-CA together with additional distal markers was heterozygous in the patient, whereas all markers proximal to 95/23-CA were homozygous. The most likely interpretation of these data is that a recombination occurred in the related grandfathers or the parents and that the SMA gene lies proximal to 95/23-CA. It is very unlikely that 6 higly polymorphic markers, Ag1-CA, C212, D5S435, LAS96, D5S680 and D5S76, that were all full informative in the parents, are homozygous by chance, while all distal markers are heterozygous.

This finding indicates that the SMA gene is flanked by 95/23-CA and D5S823 and further refines the SMA candidate gene interval to a 750- to 900-kb interval. However the identification of further recombinants, especially on the distal side, would be useful to confirm the new SMA candidate region.

Acknowledgments

We would like to thank Dr. Anne-Marie Frischauf for the chromosome-5-gridded cosmid filters and Dr. Larry Deavon for making the library available. We are grateful to the Deutsche Forschungsgemeinschaft and the MDA for funding this work.

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