De novo deletions in spinal muscular atrophy: implications for genetic counselling

Proximal spinal muscular atrophies (SMA) are characterised by degeneration of anterior horn cells of the spinal cord, leading to progressive symmetrical limb and trunk paralysis associated with muscular atrophy. SMA represents the second most common fatal autosomal recessive disorder after cystic fibrosis (1 in 6000 newborns). The genomic region encompassing the disease gene is characterised by the presence of low copy repeats and a tendency to inherited or de novo deletions. Recently, we have identified a gene duplicated in the 5q13 region, one of which, the survival motor neurone gene (SMN), is missing in the majority of patients. Those retaining this gene carried intragenic mutations, providing strong evidence that this gene is the SMA determining gene. From a practical point of view, direct SMN gene analysis makes genetic counselling much easier by eliminating the need for complex polymorphism studies. Here, we report the relevance of these recent advances for genetic counselling and prenatal diagnosis in a non-consanguineous SMA type II family with an affected boy and a healthy brother.

PCR amplification conditions of microsatellite DNA markers at loci DSS465 (AFM114ye7), DSS435 (cVS19), D5F149S1/S2 (C212), D5F150S1/S2 (C272), and DSS351 (I105) were as follows: denaturation at 94°C for one minute, annealing at 55°C for one minute, and extension at 72°C for one minute, for 30 cycles. The procedure used for detection of dinucleotide repeat polymorphisms has been described elsewhere. Gels were transferred onto a nylon membrane, then hybridised overnight at 42°C with a poly-AC probe, labelled by chemiluminescence according to the manufacturer's instructions (ECL, direct nucleic acid labelling and detection system; Amersham Life Science), and exposed to x ray films for 10 minutes. For SSCP analysis of SMN exons 7 and 8, DNA from peripheral leucocytes (200 ng) was amplified by PCR using unlabelled primers (20 μmol/1) in an amplification mixture (25 μl) containing 200 μmol/1 dNTPs and 1 U Taq polymerase (Gibco BRL). Amplified DNA was mixed with an equal volume of formamide loading dye (95% formamide, 20 mmol/1 EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol). The samples (5 μl) were denatured for 10 minutes at 95°C, loaded onto a polyacrylamide gel (Hydrolink MDE, Bioprobe), and electrophoresed at 4°C for 18 to 24 hours at 4 W. To amplify genomic DNA containing divergent exon 7 sequences, we used oligonucleotides R111 and 54IC770. To amplify genomic DNA containing divergent exon 8 sequences, we used oligonucleotides 54IC660 and 54IC1120.

Both children carried identical haplotypes using polymorphic markers flanking the SMA locus (fig 1A). Analysis of the SMN gene showed that SMN exons 7 and 8 were missing in the affected child but not the healthy child. Moreover, allele segregation at the closest genetic loci detected by markers C212 and C272 showed a de novo deletion of one maternal locus detected by these markers in the affected but not the healthy brother (fig 1A, B), giving additional support to a de novo event involving the SMN gene and the closely flanking markers C212 and C272 in our patient (fig 1A, B).

A priori, the analysis of polymorphic markers flanking the SMA locus favoured the hypothesis of either genetic heterogeneity or misdiagnosis, as both the affected and unaffected sibs carried identical haplotypes. Yet, the use of SMN as probe had important implications for genetic counselling in this

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**Figure 1** (A) Pedigree of the SMA family. Haplotypes at loci DSS465 (AFM114ye7), DSS435 (cVS19), D5F149S1/S2 (C212), D5F150S1/S2 (C272), and DSS351 (I105) are presented (from centromere to telomere). Results of the SSCP analysis of SMN exons 7 and 8 are noted (−) for homozygous deletion and (+) for the detection of at least one copy of these exons. (B) Allele segregation analysis at loci detected by markers C212 and C272 and SSCP analysis of amplified exons 7 and 8 of the SMN gene in a SMA type II patient and his healthy brother. Note the incomplete contribution of the mother to her affected child using markers C212 and C272 compared to her healthy child who is haploidentical with flanking markers. Closed arrowheads correspond to the SMN exons. Open arrowheads correspond to the centromeric copy.
family. First, it allowed the diagnosis of SMA to be confirmed, since the affected child carried a homozygous deletion of SMN exons 7 and 8, as previously reported in more than 90% of SMA patients.12 Second, the direct analysis of the SMN gene and its flanking markers C212–C227 provided strong evidence for a de novo deletion of maternal origin and rejected the hypothesis of genetic heterogeneity since the affected and unaffected children carried different genotypes at the SMN locus. The risk for the mother of bearing another affected child should therefore be considered to be very low. Indeed, analysis of 41 families with two affected children did not provide any evidence for germlinal mosaicism using microsatellite DNA markers, suggesting that this situation is very rare (data not shown, available on request). For this reason, the combined analysis of SMN and its flanking markers C212 and C227 are still of interest for detecting de novo deletions, estimating their actual frequency, and delivering accurate prenatal prediction in SMA.

In conclusion, the use of SMN gene testing has important implications for genetic counselling in SMA families. First, it allows the diagnosis of SMA to be confirmed when a proband harbours a homozygous SMN gene deletion. In contrast, the absence of a homozygous deletion may suggest either a mis-diagnosis or the presence of intragenic mutations in the SMN gene, as previously reported.13 A clinical re-evaluation of the proband and the search for intragenic mutations by screening of each SMN exon should be undertaken before making prenatal diagnosis feasible.12 Second, the use of SMN and the closest genetic markers as probes enables detection of de novo deletions in the probands. Establishing the status of the parents at the SMN locus should be helpful in estimating the actual frequency of de novo deletions at this locus. Finally, several reports have estimated the frequency of homozygous SMN deletions in asymptomatic carriers to be below 1% (0.8%).11–13 Moreover, these observations have been reported in the mildest form of the disease only. Consequently, fetuses harbouring homozygous SMN gene deletions should be predicted to be affected in families with severely affected probands.

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NOTES

Standing Committee on Human Cytogenetic Nomenclature 1996–2001

Elections for the Standing Committee on Human Cytogenetic Nomenclature were held at the 9th International Congress of Human Genetics in Rio de Janeiro, Brazil, on 21 August 1996. The following members were elected for the period 1996–2001: Patricia A Jacobs, (UK) (Chairman), Uta Francke (USA), David H Ledbetter (USA), Norio Niikawa (Japan), Aviranchan T Tharapel (USA), Niels Tommerup (Denmark), Angela M Vianna-Morgante (Brazil). Issues regarding human cytogenetic nomenclature can be addressed to any member of the committee.

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The Second European Forum on Quality Improvement in Health Care will be held in Paris, France, on 24–26 April 1997. The forum will consist of one day teaching courses, invited presentations, posters, and presentations selected from submissions, and a scientific session. For more information contact: BMA, Conference Unit, PO Box 295, London WC1H YTE, UK. Tel: +44 (0) 171 383 6478. Fax: +44 (0) 171 383 6869.

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Correction

In the November 1996 issue of the journal, on page 940, the current affiliation of Dr F M Pope as MRC Connective Tissue Genetics Group, Strangeways Research Laboratory, Worts Causeway, Cambridge CB1 4RN, was inadvertently omitted.