Survival Motor Neuron Gene Deletion in the Arthrogryposis Multiplex Congenita–Spinal Muscular Atrophy Association

Lydie Bürglen,* Jeanne Amiel*, Louis Viollet*, Suzie Lefebvre,* Philippe Burlet,* Olivier Clermont,* Valérie Raclin,* Pierre Landrieu,‡ Alain Verloes,§ Arnold Munnich,* and Judith Melki*

**Unité de Recherches sur les Handicaps Génétiques de l'Enfant. INSERM, Unité 393, I.F.R.E.M, Institut Necker. Hôpital des Enfants Malades, 75743. Paris Cedex 15, France; ‡Departement of Pédiatrie, Hôpital du Kremlin-Bicêtre, France; and §Centre de Génétique, Université de Liège, Belgium*

Abstract

The survival motor neuron (*SMN*) gene was lacking in 6/12 patients with arthrogryposis multiplex congenita (AMC) associated with spinal muscular atrophy (SMA). Neither point mutation in the *SMN* gene nor evidence for linkage to chromosome 5q13 were found in the other patients. Hitherto, arthrogryposis was regarded as an exclusion criterion in SMA. Our data strongly suggest that AMC of neurogenic origin is genetically heterogeneous, with a subgroup being allelic to SMA. Absence or interruption of the *SMN* gene in the AMC-SMA association will make the diagnosis easier and genetic counselling will now become feasible. (*J. Clin. Invest.* 1996. 98:1130–1132.) Key words: motor neurons • Werdnig-Hoffmann disease • inherited • congenital contractures • gene

Introduction

Arthrogryposis multiplex congenita (AMC)[1] is a frequent sequence of congenital joint fixation (incidence: 1/3000 live births) secondary to decreased fetal movements in utero (1, 2). AMC has been ascribed to either oligohydramnios or a variety of diseases involving the central nervous system, skeletal muscle, or spinal cord. Since neuronal degeneration and neuronophagia occur in the anterior horns, it has been hypothesized that the AMC of neurogenic origin could be related to acute spinal muscular atrophy (SMA type I, Werdnig-Hoffmann disease) (3). SMA type I is characterized by severe, generalized muscle weakness and hypotonia in the first six months. Death, from respiratory failure, usually occurs within the first two years. This disease may be distinguished from the intermediate (type II) and juvenile forms (type III, Kugelberg-Welander disease) (4). The underlying biochemical defect(s) remain(s) unknown. Recently, we have identified the survival motor neuron (*SMN*) gene as the SMA-determining gene, since it is either absent or interrupted in 90–100% of typical SMA patients (5–7) and patients retaining the gene carried intragenic *SMN* mutations (5, 7). Yet, variants of infantile SMA with cerebellar hypoplasia, pontocerebellar degeneration, multiple long bone fractures at birth or congenital heart defects (CHD) with or without joint contractures have been described (9). Recently, we have shown deletions of the *SMN* gene associated with SMA and CHD (10). Here, we describe SMN gene deletion in 6/12 patients with the AMC-SMA association.

Methods

Families. A total of 12 unrelated patients including eight males and four females of various geographic origins was selected in this study. Inclusion criteria were (*a*) congenital joint contractures of at least two regions of the body (1); (*b*) generalized muscle weakness with muscular atrophy and areflexia, without extraocular involvement; (*c*) electromyographic studies showing denervation and diminished motor action potential amplitude; and (*d*) muscle biopsy consistent with denervation with no evidence of storage material or other structural abnormalities (4).

DNA analyses. DNA was extracted from peripheral blood leukocytes, lymphoblastoid cell lines or muscle tissues.

Dinucleotide repeat polymorphism analysis. For genotyping using markers *C272* (*D5F150S1*,*-S2*) and *C212* (*D5F149S1*, *S2*), amplification conditions were as follows: denaturation 94°C, annealing 55°C, extension 72°C, for 1 min each, for 30 cycles (11).

SMN gene analysis. Only two discrepancies in exons 7 and 8 have been described between *SMN* gene and the centromeric copy (5). Single strand conformation polymorphism (SSCP) analysis of exons 7 and 8 PCR amplified products allowed the *SMN* and the centromeric genes to be distinguished. *SMN* exons 7 and 8 were studied by SSCP analysis after PCR amplification of genomic DNA (200 ng) using unlabeled primers R111- 541C770 (exon 7) and 541C960-541C1120 (exon 8) (5). The other exons were studied by SSCP analysis after PCR amplification of genomic DNA (200ng) using unlabeled primers flanking each exon (12).

Results

The diagnosis of AMC-SMA association was made at birth with an uniform phenotype characterized by a severe hypotonia, absence of movements except extraocular mobility and congenital joint contractures of at least two regions of the body (Fig. 1 and Table I). The contractures involved either distal joints only (cases 9 and 10) or distal and proximal joints (cases 1–8, 11–

Address correspondence to Judith Melki, INSERM Unité 393, Hôpital des Enfants Malades, 149 rue de Sèvres 75743 Paris Cedex 15, France. Phone: 1 44 49 51 63; FAX: 1 47 34 85 14; E-mail: melki@necker.fr

Received for publication 23 April 1996 and accepted in revised form 23 July 1996.

1. *Abbreviations used in this paper:* AMC, arthrogryposis multiplex congenita; CHD, congenital heart defect; SMA, spinal muscular atrophy; *SMN*, survival motor neuron; SSCP, single strand conformation polymorphism.

J. Clin. Invest.

0021-9738/96/09/1130/03 $2.00
Volume 98, Number 5, September 1996, 1130–1132

Table I. Clinical and Genetic Characteristics of AMC-SMA Patients

	Case											
	1	2	3	4	5	6	7	8	9	10	11	12*
Sex	m	f	m	m	m	m	m	m	m	f	f	f
Age of death	d 8	d 6	d 1	d 25	d 11	d 13	4 mo	> 3 yr	> 3 yr	d 20	> 9 yr	> 16 mo
Decreased fetal movements	+	+	−	+	−	−	+	−	+	−	+	+
Hypotonia at birth	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory involvement at birth	+	+	+	+	+	+	+	−	+	+	−	−
Neurogenic EMG	nd	+	+	+	+	+	nd	+	+	+	+	+
Muscle atrophy (MB)	+	+	+	+	+	+	+	+	+	+	+	+
Contractures												
Hips	−	+	−	−	−	+	−	+	−	−	+	+
Knees	+	+	+	+	+	+	−	+	−	−	+	+
Ankles	+	−	−	+	−	−	−	+	+	+	+	+
Elbows	−	+	+	+	−	−	+	+	−	−	−	−
Wrists	−	+	+	−	+	+	+	+	−	+	−	−
Fingers	−	+	−	−	+	+	−	−	−	−	−	−
Associated signs	facial micro	Ao.Co	−	−	−	−	fract.	−	facial micro	facial micro	facial micro	−
C212/C272 markers	+	+	del/+	del/+	+	del/+	+	+	unlink	+	+	+
SMN gene	del	del	del	del	del	del	+	+	+	+	+	+

+, present; −, absent; *Ao.Co*, aortic coarctation; *Fract.*, bone fracture; *Facial. micro*, facial involvement with micrognathia; *nd*, not done; *MB*, muscle biopsy; del, homozygous deletion; unlink, disease locus unlinked to chromosome 5q13. *Both the child and her father were affected.

12). Joint involvement ranged from two affected joints (case 9) to severe generalized postural defects (cases 2 and 8, Table I). Decreased fetal movements were noted in 7/12 patients and neonatal respiratory distress was observed in 9/12 patients requiring artificial ventilation. Four infants are still alive but most of them (8/12) died within the first month of life due to respiratory failure. Facial involvement associated with micrognathia was noted in 4/12 patients. No family history was noted except in family 12 in which both the child and her father were affected suggesting an autosomal dominant form of AMC.

Table I shows that *SMN* exons 7 and 8 were lacking on both mutant chromosomes in 6/12 patients (cases 1–6, Fig. 2). Analysis of loci detected by markers *C212* and *C272* mapping upstream from the *SMN* gene showed that 3/6 patients had a large inherited deletion involving both loci on one parental allele, the other parental allele carrying only one locus instead of the expected two (Fig. 2). Analysis of the other *SMN* exons and exon-intron boundaries did not reveal intragenic mutations in the patients retaining the *SMN* exons 7 and 8 (cases 7–12, not shown, available on request). In addition, genetic analysis of family 9 showed that both the affected and the healthy sibs carried the same 5q13 haplotype using markers flanking the *SMN* gene suggesting that the disease gene was not linked to chromosome 5q13 (not shown). Exclusion of chromosome 5q has also been shown in one family with two AMC-SMA patients (13).

Discussion

The International SMA Consortium has established a number of inclusion and exclusion criteria for classical SMA (4). In-

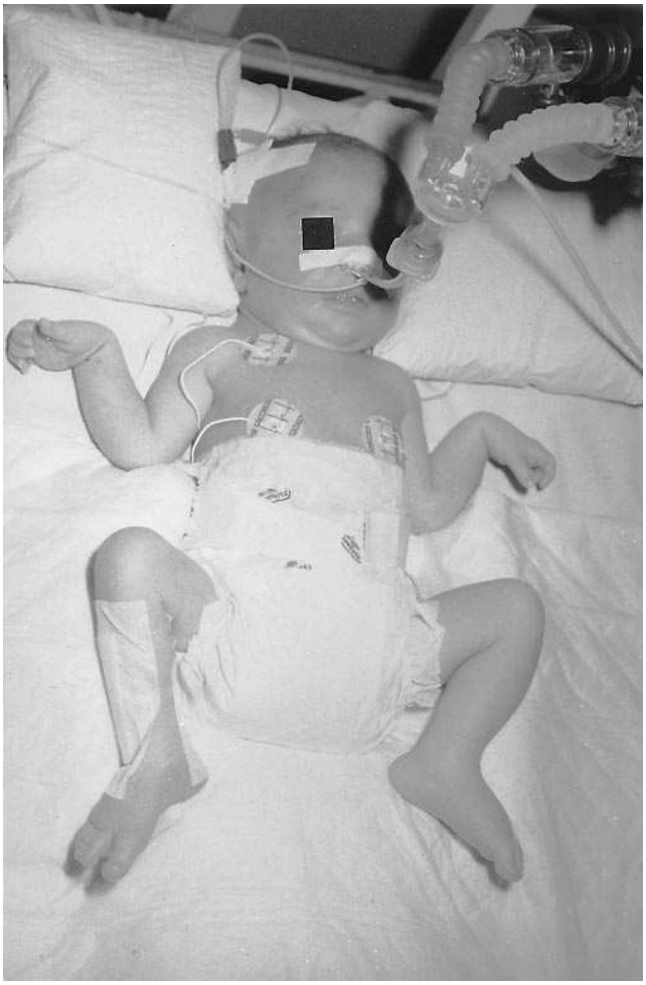

Figure 1. Clinical characteristics of patient 2. Note the flexion contractures of hips, knees, elbows, and wrists with ulnar deviation and fingers.

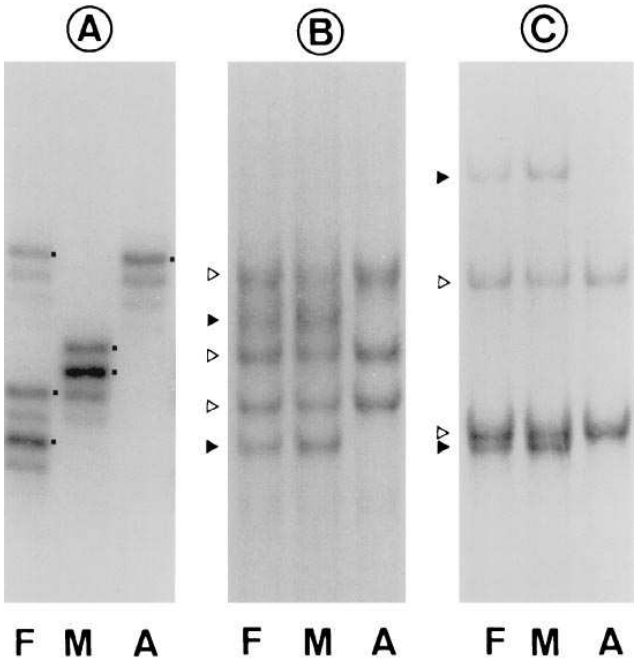

Figure 2. Genetic analysis of family 6. (*A*) Family study based on microsatellite marker *C272*. Note the non contribution of the mother to his affected child. Dots indicate allelic fragments. (*B–C*) SSCP analysis of PCR-amplified exons 7 (*B*) and 8 (*C*) of *SMN* (*closed arrowheads*) and its centromeric copy (*open arrowheads*). *SMN* exons 7 and 8 are present in parents but not in patient. *F*, father; *M*, mother; *A*, affected infant.

deed, atypical forms of infantile SMA with cerebellar hypoplasia, pontocerebellar degeneration, multiple long bone fractures at birth or congenital heart defects (CHD) have been described and could represent separate entities with a different genetic basis (4, 9). One of the exclusion criteria was in fact arthrogryposis with severe and generalized contractures.

Our observation of *SMN* gene deletion in 6/12 patients (50%) showing either mild or severe generalized contractures strongly supports the view that this subgroup and classical SMA are allelic disorders as recently shown in the CHD-SMA association (10). Interestingly, all the patients harboring *SMN* gene deletion had a severe progressive course with a fatal outcome due to respiratory failure within the first month of life whereas 4/6 patients without SMN gene deletion had severe hypotonia at birth without progressive course such as described in Werdnig-Hoffmann disease, with death usually occurring within the first two years (4).

Thus, the *SMN* gene should be carefully investigated in AMC patients with evidence for spinal cord involvement. Yet, AMC of neurogenic origin remains a genetically heterogeneous condition as the SMN gene was not mutated in 6/12 patients. *SMN* gene analysis will make the diagnosis easier in the AMC-SMA association, thus contributing to clarify the nosology of AMC. *SMN* gene deletion should be of help in the genetic counselling of this association, and prenatal diagnosis will now become possible.

Acknowledgments

We thank Professor N. Philip, J.P. Harpey, R. Gilly, L. Vallee, Ch. Puissan, J. Costil, Dr. M. Mathieu, and P. Masson for their contributions to this study.

This work was supported by the Association Française contre les Myopathies (AFM), the Ministère de la Recherche et de la Technologie, and the Assistance Publique, Hôpitaux de Paris.

References

1. Stern, W.G. 1923. Arthrogryposis multiplex congenita. *JAMA.* 81:1507–1510.

2. Hall, J.G. 1985. Genetic aspects of arthrogryposis. *Clin Orthop. Relat. Res.*194:44–53.

3. Banker, B.Q. 1986. Arthrogryposis multiplex congenita: spectrum of pathological changes. *Hum Pathol.* 117:656–672.

4. Munsat, T.L. 1991. Workshop report: international SMA collaboration. *Neuromuscular Disorders.* 1:81.

5. Lefebvre, S., L. Bürglen, S. Reboullet, O. Clermont, P. Burlet, L. Viollet, B. Benichou, C. Cruaud, P. Millasseau, M. Zeviani et al. 1995. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell.* 80: 155–165.

6. Rodrigues, N.R., N. Owen, K. Talbot, J. Ignatius, V. Dubowitz, and K.E. Davies. 1995. Deletions in the survival motor neuron gene on 5q13 in autosomal recessive spinal muscular atrophy. *Hum. Mol. Genet.* 4:631–634

7. Bussaglia, E., O. Clermont, E. Tizzano, S. Lefebvre, L. Bürglen, C. Cruaud, J.A. Urtizberea, J. Colomer, A. Munnich, M. Baiget, and J. Melki. 1995. A frame-shift deletion in the survival motor neuron gene in Spanish spinal muscular atrophy patients. *Nature Genetics*. 11:335–337.

8. Chang, J.G., Y.J. Jong, J.M. Huang, W.S. Wang, T.Y. Yang, C.P. Chang, Y.J. Chen, and S.P. Lin. 1995. Molecular basis of spinal muscular atrophy in Chinese. *Am. J. Hum. Genet.* 57:1503–1505.

9. Munsat T.L. and K.E. Davies. 1994. Spinal muscular atrophy. *In* Diagnostic Criteria for Neuromuscular Disorders. A.E.H. Emery, Editor. European Neuromuscular Center, Baarn, The Netherlands. 48–54.

10. Bürglen, L., R. Spiegel, J. Ignatius, J.M. Cobben, P. Landrieu, S. Lefebvre, A. Munnich, and J. Melki. 1995. *SMN* gene deletion in variant of infantile spinal muscular atrophy. *Lancet.* 346:316–317.

11. Melki, J., S. Lefebvre, L. Bürglen, P. Burlet, O. Clermont, P. Millasseau, S. Reboullet, B. Bénichou, M. Zeviani, D. Le Paslier, D. Cohen, J. Weissenbach, and A. Munnich. 1994. De novo and inherited deletions of the 5q13 region in spinal muscular atrophies. *Science (Wash. DC).* 264:1474–1477.

12. Bürglen, L., S. Lefebvre, O. Clermont, P. Burlet, L. Viollet, C. Cruaud, A. Munnich, and J. Melki. 1996. Structure and Organization of the Human Survival Motor Neurone Gene. *Genomics.* 32:479–482.

13. Lunt, P.W., C. Mathew, S. Clark, N. Marlow, T. Moss, S. Love, L. Tyfield, T. Davies, and B. Spiedel. 1992. Can prenatal diagnosis be offered in neonatally lethal spinal muscular atrophy (SMA) with arthrogryposis and fractures? *J. Med. Genet.* 29:282.