SD, Trush MA, et al (1998) Somatic mutations of the mitochondrial genome in human colorectal tumors. Nat Genet 20:291–293

Rando JC, Pinto F, Gonzales AM, Hernandez M, Larruga JM, Cabrera VM, Bandelt HJ (1998) Mitochondrial DNA analysis of northwest African populations reveals genetic exchanges with European, near-eastern, and sub-Saharan populations. Ann Hum Genet 62:531–550

Torroni A, Huoponen K, Francalacci P, Petrozzi M, Morelli L, Scozzari R, Obinu D, et al (1996) Classification of European mtDNAs from an analysis of three European populations. Genetics 144:1835–1850

Torroni A, Petrozzi M, D'Urbano L, Sellitto D, Zeviani M, Carrara F, Carducci C, et al (1997) Haplotype and phylogenetic analyses suggest that one European-specific mtDNA background plays a role in the expression of Leber hereditary optic neuropathy by increasing the penetrance of the primary mutations 11778 and 14484. Am J Hum Genet 60: 1107–1121

Vilarinho L, Chorão R, Cardoso ML, Rocha H, Nogueira C, Santorelli FM (1999) The ND1 T3308C mutation may be a mtDNA polymorphism: report of two Portuguese patients. J Inher Metab Dis 22:90–91

Watson E, Forster P, Richards M, Bandelt HJ (1997) Mitochondrial footprints of human expansions in Africa. Am J Hum Genet 61:691–704

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Diaphragmatic Spinal Muscular Atrophy with Respiratory Distress Is Heterogeneous, and One Form Is Linked to Chromosome 11q13-q21

To the Editor:

Diaphragmatic spinal muscular atrophy (SMA) has been delineated as a variant of infantile SMA (SMA1 [MIM 253300]) (Mellins et al. 1974; Bertini et al. 1989). The most prominent symptoms are severe respiratory distress resulting from diaphragmatic paralysis with eventration shown on chest x-ray and predominant involvement of the upper limbs and distal muscles. In contrast to classic SMA1, in diaphragmatic SMA the upper spinal cord is more severely affected than the lower section. The *pmn* mouse presents with progressive motor neuronopathy and a disease that closely resembles diaphragmatic SMA (Schmalbruch et al. 1991). The *pmn* locus has been mapped to murine chromosome 13 (Brunialti et al. 1995).

Here we report on nine patients from three families with diaphragmatic SMA following autosomal recessive inheritance. The diagnosis of diaphragmatic SMA was made on the basis of clinical criteria (Rudnik-Schöneborn et al. 1996). Family 1 is of Lebanese origin; family 2, German origin; and family 3, Italian origin. We obtained DNA samples from these families after receiving informed consent, in accordance with the Declaration of Helsinki.

In family 1 (fig. 1*A*), the parents are first cousins. The first affected son died, at the age of 10 wk, of suspected sudden infant death syndrome (SIDS). One daughter presented, at the age of 6 wk, with feeding difficulties and progressive respiratory distress. Chest x-ray showed eventration of the diaphragm. Mechanical ventilation was initiated at the age of 8 wk. She developed progressive muscular atrophy with complete paralysis of the upper and lower limbs and mild contractures of the knee and ankle joints. Three other children, nonidentical twin daughters and the youngest daughter, died of respiratory failure—the twins at the age of 8 and 9 wk and the youngest daughter at the age of 8 wk. Autopsy specimens were taken from gastrocnemius muscle in both twins and from the upper spinal cord in one twin. Skeletal-muscle histology revealed neurogenic atrophy without signs of reinnervation. Ultrastructurally, the motor end plates lacked nerve terminals and showed postsynaptic degenerative changes characterized by deep invaginations. The diameter of anterior spinal roots was reduced in the upper spinal cord. The remaining motor neurons showed chromatolysis. These findings offer two different pathophysiological concepts: (1) degeneration of the anterior horn cells of the spinal cord with neurogenic muscular atrophy suggests dying-forward atrophy, and (2) presynaptic and postsynaptic signs of motor end-plate degeneration suggest dying-back atrophy. In family 2 (fig. 1*B*), the first child had severe muscular hypotonia and died, at the age of 9 wk, of cardiorespiratory failure. The third child has been mechanically ventilated since the age of 3 mo. In family 3 (fig. 1*C*), which has been reported in detail elsewhere (Novelli et al. 1995), the gene locus for SMA1, on chromosome 5q, has been excluded. Both affected sibs presented with respiratory insufficiency right after birth and with the typical signs of diaphragmatic SMA.

First, we confirmed that, in families 1 and 2, there is no linkage of the trait to markers of the SMA locus on 5q11.2-q13.3, as there is in family 3. Second, the orthologous regions corresponding to the murine *pmn* gene region on human chromosomes 1q and 7p were excluded as gene loci responsible for the disease (Grohmann et al. 1998).

To locate the gene locus for diaphragmatic SMA, a whole-genome scan was undertaken in family 1. Microsatellite analysis was performed, by standard semiau-

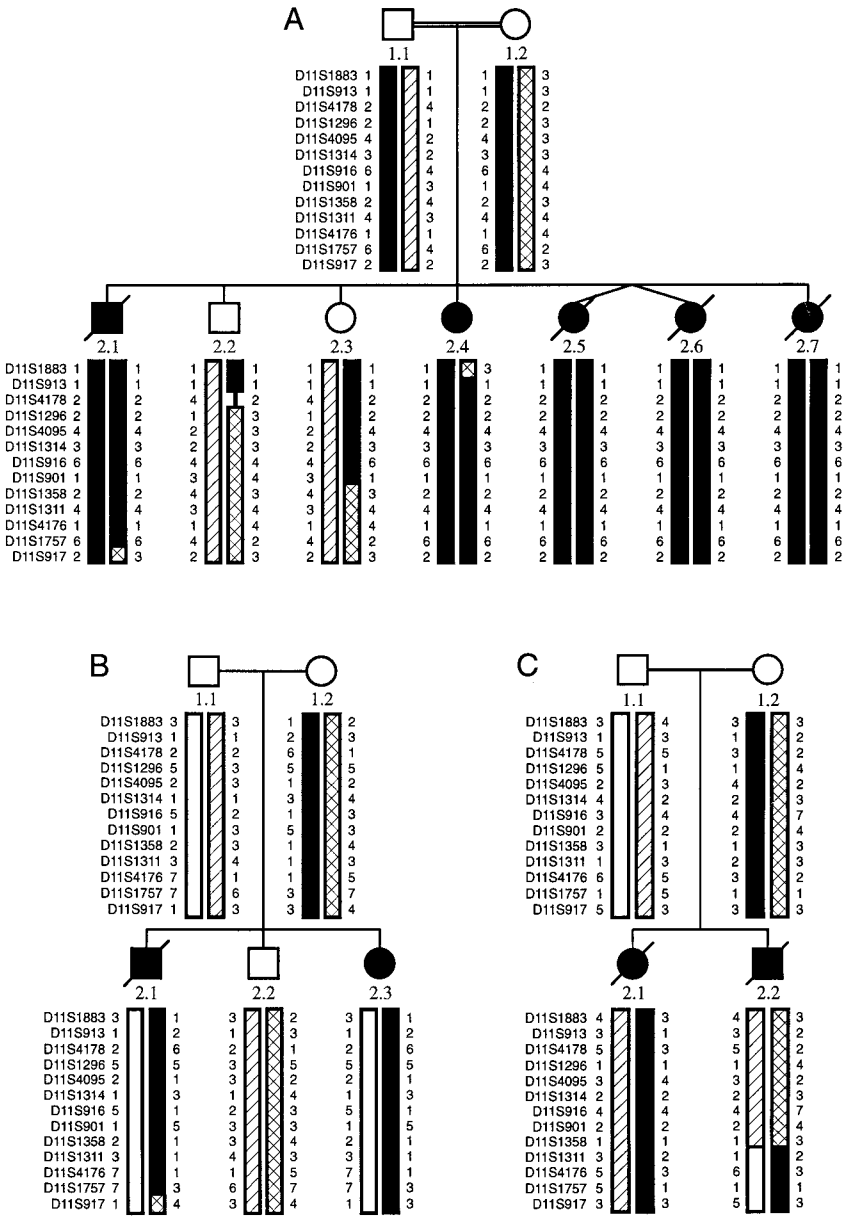

Figure 1 Haplotypes in families with diaphragmatic SMA subtypes. *A,* Family 1 (Lebanese origin): age at onset, 6–10 wk. *B,* Family 2 (German origin): age at onset, 9–12 wk. *C,* Family 3 (Italian origin): onset at birth. Haplotype analysis indicated that the cosegregating segment of the *SMARD* locus is flanked proximally by marker D11S1883 and distally by marker D11S917. Family 3 has no linkage to the *SMARD* locus. Blackened squares represent affected males; unblackened squares, unaffected males; blackened circles, affected females; unblackened circles, unaffected females; double line (in *A*), consanguinity.

tomated methods, by an ABI 377-Sequencer, and the results were processed by GENESCAN software, as described elsewhere (Saar et al. 1997). The whole-genome linkage scan was performed with the use of 340 polymorphic fluorescence–labeled markers spaced at ~10-cM intervals throughout the autosomal part of the genome. Subsequent fine mapping was performed with eight additional microsatellite markers. Markers were chosen from the Généthon final linkage map. Two-point parametric linkage analyses were performed with the LINKAGE package, version 5.2 (Lathrop and Lalouel 1984), under the following assumptions: a regular, fully penetrant autosomal recessive trait locus with a disease-allele frequency of .002 and no phenocopy rate, codominant marker loci with uniformly distributed allele frequencies, and standard recombination rates. Multipoint

Table 1

LOD-Score Values at Standard Recombination Rates for Markers on Chromosome 11q in Lebanese Family 1

			LOD Score at θ =						
Marker	Position[a]	Heterozygosity[b]	.00	.01	.05	.10	.15	.20	.30
D11S1883	68.5	.73	$-\infty$	−.87	−.24	−.03	.06	.09	.09
D11S913	70.9	.57	1.15	1.13	1.04	.93	.81	.69	.43
D11S1296	71.0[c]	.50	3.16	3.10	2.86	2.55	2.23	1.91	1.22
D11S4095	71.0	.64	3.16	3.10	2.86	2.55	2.23	1.90	1.22
D11S4178	71.5	.67	1.75	1.72	1.58	1.40	1.22	1.04	.66
D11S1314	77.5	.77	1.75	1.72	1.58	1.40	1.22	1.04	.66
D11S916	80.1	.72	2.96	2.90	2.67	2.38	2.07	1.76	1.09
D11S901	89.8	.82	3.16	3.10	2.86	2.55	2.23	1.90	1.22
D11S1358	96.3	.75	3.16	3.11	2.88	2.59	2.28	1.96	1.28
D11S1311	97.5	.75	1.75	1.72	1.58	1.40	1.22	1.04	.66
D11S4176	97.5	.82	1.75	1.72	1.58	1.40	1.22	1.04	.66
D11S1757	98.1	.65	3.16	3.11	2.88	2.59	2.28	1.96	1.28
D11S917	100.9	.80	$-\infty$	−.28	.30	.45	.47	.44	.30

[a] Sex-averaged genetic coordinates on chromosome 11 (cM), according to the Généthon map.
[b] Estimated value.
[c] Estimated from the genetic maps of the Marshfield Medical Research Foundation Center for Medical Genetics.

analysis was performed with the GENEHUNTER program, version 1.3 (Kruglyak et al. 1996).

Genomewide linkage scanning of family 1 revealed linkage of diaphragmatic SMA only to markers on chromosome 11q13-q21. In the following, we name this subtype of diaphragmatic SMA "spinal muscular atrophy with respiratory distress" (SMARD). For the markers D11S1296, D11S4095, D11S901, D11S1358, and D11S1757, a maximum two-point LOD score of 3.16 at recombination fraction (θ) 0 was obtained. The two-point LOD scores for 13 markers on chromosome 11q are summarized in table 1. Haplotype analysis revealed a recombination event in individual 2.4 that placed the disease locus distal to marker D11S1883 (fig. 1*A*). The crossing-over in individual 2.1 placed the disease locus proximal to marker D11S917. Consistent with parental consanguinity, all affected siblings from family 1 were autozygous for all markers within the cosegregating segment. Multipoint linkage analysis with the use of 13 markers yielded a maximum LOD score of 3.86, which clearly places the disease locus between D11S1883 and D11S917 (Généthon map positions 68.5 cM and 100.9 cM).

In family 2, the two affected sibs shared two identical parental haplotypes in the SMARD cosegregating segment on 11q13-q21, a finding that supports the assignment of the *SMARD* locus to this region (fig. 1*B*). In family 3, haplotype analysis was inconsistent with linkage to the markers tested (fig. 1*C*). Thus, this locus was excluded as being responsible for the disease in this family. Our finding that diaphragmatic SMA with onset at age 6–12 wk is linked to chromosome 11q markers in two apparently unrelated families from different countries (families 1 and 2) but that diaphragmatic SMA with onset at birth does not show such linkage (family 3) suggests that diaphragmatic SMA is both clinically and genetically heterogeneous.

The prevalence of diaphragmatic SMA is unknown. However, in a series of >200 patients with early-onset SMA, ~1% presented with diaphragmatic SMA and did not have a deletion of the survival motor-neuron gene (*SMN*) on chromosome 5q (Rudnik-Schöneborn et al. 1996). Considering the case history of the affected son from family 1 who had suspected SIDS, we presume that some of those infants with SIDS may possibly have been misdiagnosed. We are currently looking for further patients with SMARD, to refine the large cosegregating region on chromosome 11q.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Généthon, ftp://ftp.genethon.fr/pub/Gmap/Nature-1995/data/ (for genetic markers)

Marshfield Medical Research Foundation Center for Medical Genetics, http://www.marshmed.org/genetics/ (for genetic markers)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for SMA1 [MIM 253300])

References

Bertini E, Gadisseux JL, Palmieri G, Ricci E, Di Capua M, Ferriere G, Lyon G (1989) Distal infantile spinal muscular atrophy associated with paralysis of the diaphragm: a variant of infantile spinal muscular atrophy. Am J Med Genet 33:328–335

Brunialti AL, Poirier C, Schmalbruch H, Guénet J-L (1995) The mouse mutation progressive motor neuronopathy (*pmn*) maps to chromosome 13. Genomics 29:131–135

Grohmann K, Hübner C, Saar K, Stoltenburg-Didinger G, Wienker T (1998) Diaphragmatic spinal muscular atrophy (SMAD) is not the homologue counterpart to murine progressive motoneuron disease (*pmn*). Paper presented at the 5th Workshop Neurogenetics in Germany, Freiburg, Germany, October 22–24

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347–1363

Lathrop GM, Lalouel JM (1984) Easy calculations of LOD scores and genetic risks on small computers. Am J Hum Genet 36:460–465

Mellins RB, Hays AP, Gold AP, Berdon WE, Bowdler JD (1974) Respiratory distress as the initial manifestation of Werdnig-Hoffmann disease. Pediatrics 53:33–40

Novelli G, Capon F, Tamisari L, Grandi E, Angelini C, Guerrini P, Dallapiccola B (1995) Neonatal spinal muscular atrophy with diaphragmatic paralysis is unlinked to 5q11.2-q13. J Med Genet 32:216–219

Rudnik-Schöneborn S, Forkert R, Hahnen E, Wirth B, Zerres K (1996) Clinical spectrum and diagnostic criteria of infantile spinal muscular atrophy: further delineation on the basis of SMN gene deletion findings. Neuropediatrics 27:8–15

Saar K, Chrzanowska KH, Stumm M, Jung M, Nürnberg G, Wienker TF, Seemanová E, et al (1997) The gene for the ataxia-telangiectasia variant, Nijmegen breakage syndrome, maps to a 1-cM interval on chromosome 8q21. Am J Hum Genet 60:605–610

Schmalbruch H, Jensen H-J, Bjærg M, Kamieniecka Z, Kurland L (1991) A new mouse mutant with progressive motor neuronopathy. J Neuropathol Exp Neurol 50:192–204

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Further Evidence for a Susceptibility Locus on Chromosome 20q13.11 in Families with Dominant Transmission of Graves Disease

To the Editor:

The susceptibility loci for Graves disease (GD [MIM 275000]), which is a common complex trait (Brix et al. 1998), have been difficult to define (Roman et al. 1992; McLachlan 1993; Davies 1998; Farid 1998; Vaidya et al. 1999). Tomer et al. (1998) recently found evidence for linkage of GD to markers on the long arm of chromosome 20 (MIM 603388), with a peak multipoint LOD score of 3.5 at the marker D20S195. Their linkage analysis was performed by both parametric and nonparametric methods, and their cohort of 53 families with at least two first-degree relatives affected with autoimmune thyroid disease (AITD) was derived from the

Table 1

Phenotypes of Affected Sib Pairs with AITD

Sib-Pair Type	No. with Phenotype		
	GD-GD[a]	GD-AH[b]	All AITD
Full	66	6	72
Half	5	0	5
Total	71	6	77

[a] Sib pairs with GD only.

[b] Sib pairs with mixed GD and autoimmune hypothyroid. Families were selected on the basis of two affected sibs with GD. GD-AH sib pairs make up additional members of the same families.