European Journal of Human Genetics (2004) 12, 483–488

www.nature.com/ejhg

ARTICLE

Refined genetic mapping of autosomal recessive chronic distal spinal muscular atrophy to chromosome 11q13.3 and evidence of linkage disequilibrium in European families

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Chronic distal spinal muscular atrophy (Chronic DSMA, MIM *607088) is a rare autosomal recessive disorder characterized by a progressive motor weakness and muscular atrophy, predominating in the distal parts of the limbs. A form of Chronic DSMA gene has been previously mapped to chromosome 11q13 in the 10.3 cM interval defined by loci D11S1889 and D11S1321. By linkage analysis in 12 European Chronic DSMA families, we showed that a disease gene maps to chromosome 11q13.3 ($Z_{max} = 6.66$ at $\theta = 0.00$ at the DSM4 locus) and suggested that this condition is genetically homogeneous. Recombination events allowed us to reduce the genetic interval to a 2.6 cM region, telomeric to the IGHMBP2 gene, excluding this gene as the disease causing gene in Chronic DSMA. Moreover, partial linkage disequilibrium was found between three rare alleles at loci D11S1369, DSM4 and D11S4184 and the mutant chromosome in European patients. Analysis of the markers at these loci strongly suggests that most Chronic DSMA

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Received 3 October 2003; revised 21 January 2004; accepted 6 February 2004

chromosomes are derived from a single ancestor. Refinement of the Chronic DSMA locus will hopefully allow to test candidate genes and lead to identification of the disease-causing mutations.

European Journal of Human Genetics (2004) **12,** 483–488. doi:10.1038/sj.ejhg.5201177
Published online 31 March 2004

Keywords: chronic DSMA; SMAR; dHMN; linkage disequilibrium; IGHMBP2

Introduction

Distal spinal muscular atrophy (DSMA) forms a heterogeneous group of rare neuromuscular disorders characterized by progressive anterior horn cell degeneration, leading to motor weakness and muscular atrophy predominating in the distal part of the limbs. Numerous DSMA phenotypes have been reported, differing by their mode of inheritance, age at onset and distribution of paralyses.[1–3]

We have previously reported the clinical profile of chronic distal spinal atrophy (Chronic DSMA) with autosomal recessive inheritance in a large inbred family from Lebanon.[4] The disease was characterized by (i) a broad range of age at onset (6 months–19 years), (ii) the distal predominance of lower limb paralyses, (iii) the slow progressive course and secondary involvement of proximal and trunk muscles, (iv) late diaphragmatic dysfunction. The disease gene has been mapped to chromosome 11q13, in the 10 cM interval defined by loci D11S1889 and D11S1321, which encompasses the IGHMBP2 gene, already accounting for early and severe spinal muscular atrophy (SMA) with distal predominance and diaphragmatic paralysis (SMARD1).[5,6] However, sequence analysis of the IGHMBP2 gene failed to detect any mutation in our Chronic DSMA patients.[4]

We have performed haplotype analysis at the Chronic DSMA locus in a series of 12 European families. Using a homozygosity mapping strategy, we mapped the Chronic DSMA locus to a short genetic interval (2.6 cM) and showed evidence of a founder effect in 8/12 families.

Patients and methods

Families

Inclusion criteria for Chronic DSMA were: (1) motor weakness predominating at the distal part of the limbs, (2) early and severe foot extensor muscle involvement with intrinsic foot muscle atrophy, (3) secondary hand muscle weakness, predominating at the wrist and finger extensor muscles with interosseous muscle atrophy, (4) trunk muscle weakness with marked hyperlordosis, (5) evidence of muscle denervation on neurophysiological assays and/or muscle biopsy, with normal sensory action potentials and normal motor and sensory nerve conduction velocity, and (6) absence of SMN1 gene deletion.

A total of 12 European families were studied and blood samples were collected from 15 patients and 35 relatives, with informed consent. In five families, at least one parent originated from Italia (families 1, 4, 9, 10 and 12). Parents of the other families originated from France except for family 2, originating from Portugal (Figure 1).

Genotyping

Genomic DNA was extracted according to the standard procedures. Linkage studies were performed using nine contiguous microsatellite DNA markers, mapping to the 11q13 region and spanning a 10.3 cM interval (sex average distance) in the following order: cen–D11S1889–D11S913–D11S4113–D11S4136–D11S1314–D11S1369–DSM4–D11S4184–D11S916–D11S1321–tel. Most of the markers were obtained from the Genethon map and ordered from the genome-integrated map of the UCSC genome database.[7] Marker DSM4 was found in the computerized sequence of BAC AP002381, from the Human Genome Project database, in the vicinity of loci D11S1369 and D11S4184. Polymorphic markers at the DSM4 locus were amplified using the forward primer: CATCTATGGAAACAGAAGCAC and the reverse primer: CAAGATTCTGATATGTAAGGAG. Allele frequencies were obtained by screening a panel of 50 unrelated healthy European individuals. Informativity of markers varied from 71.5 to 77.8%. PCR amplification of microsatellite markers was carried out with 100 ng genomic DNA in a 25 μl total reaction volume containing: 0.2 U of *Taq* polymerase (Invitrogen Life Technology), 1 μM of the 5′-fluorescent labeled forward primer and 1 μM of the reverse primer, 200 μM dNTPs, and 1 $\times$ reaction buffer (50 mM KCl, 20 mM Tris-HCl pH 8.4, 2 mM $MgCl_2$). Amplification was performed using the following conditions: 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final 5 min extension at 72°C. PCR products were diluted, denatured at 94°C during 5 min, and loaded on the ABI prism 3100 Genescan analyzer (Applied Biosystems). For the data analyses, the Genescan Analysis and Genotyper softwares were used.

Sequence analysis

For the IGHMBP2 gene sequence analysis, all 15 exons were amplified from family 1, 2 and 3 probant's genomic DNA, using the intronic primers and PCR conditions reported by Grohmann *et al*[6] Fragments were sequenced using the Big Dye Terminator Cycle Sequencing Kit v2 on a 3100 automated sequencer (ABI Prism, Applied Biosystems,

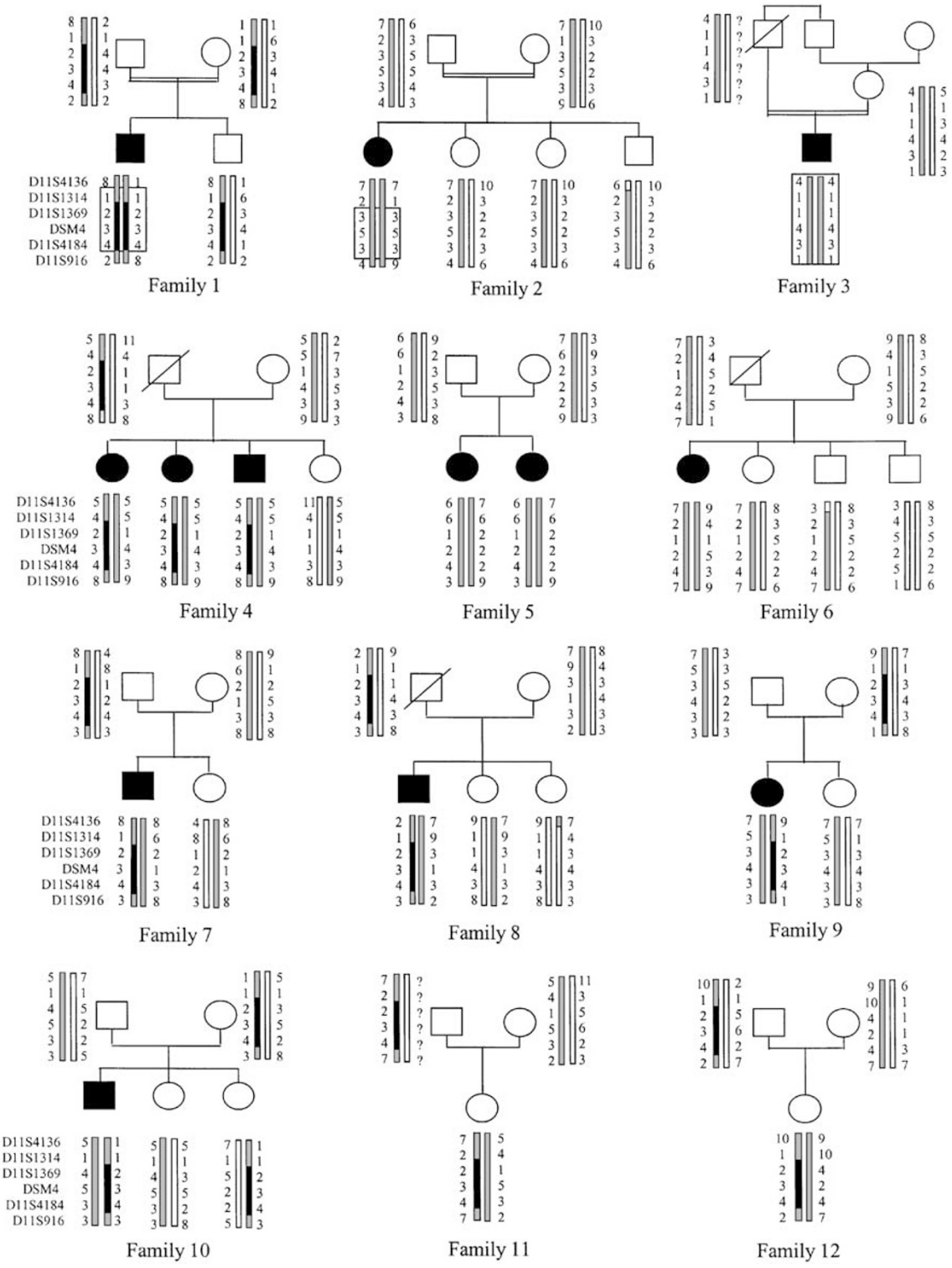

Figure 1 Chronic DSMA pedigrees. The polymorphic DNA haplotypes are presented with mutant (grey columns) and normal chromosomes (open columns). Black columns indicate the common ancestral haplotype.

Foster City, USA). Data were collected and analysed with an ABI DNA sequencing analysis software, version 3.4.1. DNA variants were tested in the parents and in a panel of unrelated European controls by genomic sequence analysis.

Statistical analyses

For linkage studies, pairwise linkage analyses were performed using the M-LINK and LINKMAP options of the 5.1 version of the LINKAGE program 5l.[8] We also compared the allele frequencies of mutant and control chromosomes at the D11S1369, DSM4 and D11S4184 loci. Significance of linkage disequilibrium was tested using the standard χ^2-test.

Results

Haplotype analyses in inbred families 1–3 revealed that all affected siblings were homozygotes for a common haplotype defined by loci D11S1369, DSM4 and D11S4184 in the 11q13.3 region (Figure 1). Recombination events in affected individuals placed the disease locus proximal to marker D11S916 in families 1 and 2 and distal to marker D11S1314 in family 2. The maximum lod score value was obtained at the DSM4 locus ($Z_{max} = 6.66$ at recombination fraction $\theta = 0.00$) (Table 1). These data support the view that the disease locus is located in the 2.6 cM interval defined by loci D11S1314 and D11S916.

IGHMBP2 sequence analysis in the probants of families 1, 2 and 3 revealed a small number of DNA variants. All these variants were detected in the parent's DNA and in the control population, suggesting polymorphisms (data not shown). These DNA polymorphisms were homozygous in family 3 probant but were heterozygous in the probants of families 1 and 2 (Het 1178G>A and Het 1284+5G>A, respectively).

Comparison of mutant chromosomes in the 12 European families suggested a common ancestral mutant haplotype. Indeed, Figure 1 shows that nine patients in eight families (pedigrees 1, 4, 7, 8, 9, 10 , 11 and 12) harboured a common mutant haplotype at loci D11S1369, DSM4 and D11S4184 (allele 2 of marker *CHLC.GATA5C04,* allele 3 of marker *DSM4* and allele 4 of marker *AFMc020yd5*). To test the hypothesis of a founder effect in Chronic DSMA, we compared the frequency of polymorphic alleles at these loci in affected individuals and in normal chromosomes of the European population ($n = 22$). The distribution of alleles 2, 3 and 4 was significantly different in affected individuals compared to European controls (Table 2). In particular, allele 2 at the D11S1369 locus displayed strong linkage disequilibrium with the Chronic DSMA allele (47% in mutant chromosomes *vs* 10% in control European chromosomes, $P < 0.001$). The same figure was observed for allele 3 at the DSM4 locus (38% in mutant chromosomes *vs* 5% in control European chromosomes, $P < 0.001$).

Table 1 Lod score values at standard recombination fractions for markers *AFMb032zg5*, *AFM212xe3*, *CHLC.GATA5C04*, *DSM4*, *AFMc020yd5 and AFM185ya1* at loci D11S4136, D11S1314, D11S1369, DSM4, D11S4184 and D11S916, respectively on chromosome 11q13.3

	θ			
Z	0.00	0.05	0.10	0.20
D11S4136	0.53	0.65	0.59	0.30
D11S1314	2.64	2.49	2.14	1.33
D11S1369	6.31	5.23	4.20	2.44
DSM4	6.66	5.62	6.61	2.78
D11S4184	3.41	2.79	2.24	1.33
D11S916	1.90	2.15	1.93	1.27

Discussion

Autosomal recessive Chronic DSMA is a rare clinical variant of SMA, characterized by progressive motor weakness and muscular atrophy, predominating in the distal part of the limbs. Familial and sporadic forms have been reported, but the clinical delineation of the syndrome has been described by Gardner-Medwin *et al*[9] in 1967, as a 'Benign spinal muscular atrophy arising in childhood and adolescence' and by Harding and Thomas[10] in a large series published in 1980. Pearn and Hudgson[11] reported other cases in a population survey of North Eastern England and estimated that distal SMAs account for less than 10% of all SMA cases. The remarkably variable clinical severity of Chronic DSMA has been emphasized by Anita Harding who recognized two subtypes of the disease, namely the 'mild juvenile distal hereditary motor neuronopathy type III' (distal-HMN III) and the 'severe juvenile form' (distal-HMN IV).[3]

Table 2 Allele frequencies at polymorphic loci of chromosome 11q13.3 in the European population

	D11S1369 *CHLC.GATA5C04*	*DSM4* *DSM4*	*D11S4184* *AFMc020yd5*
Alleles	2	3	4
Mutant chromosomes ($n = 24$)	47% (10/21)	38% (8/21)	52% (11/21)
Normal chromosomes ($n = 22$)	9% (2/22)	0% (0/22)	9% (2/22)
Control chromosomes	10% (8/80 tested)	5% (5/100 tested)	12% (12/100 tested)

Frequencies are indicated for mutant chromosomes and normal chromosomes originating from the parents of affected individuals and from unrelated European individuals

Table 3 Genes located in the 11q13.3 interval, according to the Human Genome database

Genes	*Protein homology domains*	*Predicted function*
PDE2A (Phosphodiesterase 2A, cGMP-stimulated)	2 GAF domains 1 PDEases domain	Would be involved in signal transduction
CENTD2 (Centaurin delta 2 ARAP1)	1 ARFGAP domain 1 RHO-GAP (or hRIP) domain 1 Ankyrin repeat 1 RA domain 1 PH domain	Probable DNA binding activity Would be involved in neuropeptide signaling pathway
STARD10 (Serologically defined colon cancer antigen 28)	1 Lipid-binding START domain	May be required in the acute regulation of steroidogenesis.
FLJ00012 (G-protein beta WD-40 repeat family member)	14 G-protein betaWD-40 repeats 2 Coiled-coil stretches 1 Leucine zipper motif	Could be involved in the transduction of signals generated by transmembrane receptors
KIAA0769 (SH3.20)	1 Cdc15/Fes/CIP4 motif 1 Lactate/malate dehydrogenase domain 4 SH3 domains 2 Coiled coil stretches 1 Leucine zipper motif	Would have oxido-reductase activity May play a role in protein transport and mediating the assembly of large multiprotein complexes
P2RY2 (Purinergic receptor P2Y)	1 Rhodopsin-like GPCR superfamily motif	Would be involved in G-protein coupled receptor protein signaling pathway
P2RY6 (Pyrimidinergic receptor P2Y)	1 Rhodopsin-like GPCR superfamily motif	Would localize in integral membrane protein
P164RHOGEF (Rho-specific guanine-nucleotide exchange factor 164 kDa)	2 DH domains 1 Coiled-coil stretch	Could modulate the activity of the Rho family GTPases, involved in a large number of cellular functions
TNFRSF19L (Tumour necrosis factor receptor superfamily, member 19-like)	6 Dileucine domains 1 Coiled-coil stretch	A member of the TNF-receptor superfamily, suggesting a regulatory role in immune response
D87470 (PT.b)	No protein homology	No predicted function

Putative protein domains are available at the Pfam protein family database and protein function from the Acembly/Aceview database

We have recently reported a large family originating from Lebanon, with childhood and adult onset in the same pedigree. We mapped the disease gene to a 10.3 cM region on chromosome 11q13 in this family and showed that the same gene accounts for different clinical forms differing by their severity and age at the onset of the disease.[4]

Here, studying a series of 12 additional patients with Chronic DSMA with severe foot and hand interosseous muscle atrophy, predominating to extensor muscles and marked hyperlordosis, we give support to the view that Chronic DSMA gene maps to chromosome 11q13.3 and that Chronic DSMA is probably a genetically homogeneous condition in patients of European ancestry. Using the homozygosity mapping strategy, we were able to narrow the Chronic DSMA region to a 2.6 cM interval, between loci D11S1314 and D11S916. The IGHMBP2 gene, which accounts for spinal muscular atrophy with respiratory distress (SMARD1), is situated in the genetic interval defined by loci D11S913 and D11S4113, according to the Genome database. This chromosomal region is located centromeric to the new refined Chronic DSMA region, suggesting the exclusion of this gene as the disease gene for Chronic DSMA.[4,6] In addition, evidence of two heterozygous IGHMBP2 DNA polymorphisms in the consanguinous individuals (families 1 and 2) strongly supports the absence of linkage to this gene.

Our study shows that 38% of Chronic DSMA chromosomes (8/21) derived from a single founder chromosome. Statistical analyses confirmed partial linkage disequilibrium between Chronic DSMA and loci D11S1369 and DSM4 in the European population. The size of the common ancestral haplotype indicates that Chronic DSMA is probably an ancient trait in the European population.

The Chronic DSMA region corresponds to a physical distance of 1 M base. Ten genes have been reported in this interval, in the Human Genome database. We analysed the electronical sequences of the putative proteins encoded by these genes and their predicted function (Table 3). Amino-acid sequence alignments to the human and rodent databases failed to detect any homology to the known

genes involved in motor neuron disorders. In addition, none of these genes displayed specific and/or marked neuronal expression.

Recruitment of novel European families presenting Chronic DSMA and genetic analysis at the 11q13.3 locus will hopefully help narrowing the critical region and excluding some of these genes. Sequence analysis of the candidate sequences will eventually lead to the identification of the gene causing Chronic DSMA.

Acknowledgements

This work was supported by the Association Française contre les Myopathies (9297,LV), We thank the patients and their families for their participation to the study, We also thank MYOBANK-Genethon, Evry for their help in collecting DNA samples.

Electronic database information

Online Mendelian Inheritance in Man (OMIM) (for Chronic DSMA or SMAR MIM *607088, for SMARD MIM *604320, for IGHMBP2 gene MIM *600502): http://www. ncbi.nlm.nlh.gov/Omim

Genethon (for markers): http://www.genethon.fr

Genome database at UCSC (for integrated map): http://ucsc.genome.edu

Acembly/Ace view program (for predicted protein function): http://www.ncbi.nih.gov/IEB/Research/Acembly/index/html

Pfam protein family database (for protein homology): http://www.sanger.ac.uk/Software/Pfam/

References

1 Dyck PJ, Lambert EH: Lower motor and primary sensory neuron diseases with peroneal muscular atrophy. I. Neurologic, genetic and electrophysiologic findings in hereditary polyneuropathies. *Arch Neurol* 1968; **28**: 603–618.

2 Emery AEH: The nosology of spinal muscular atrophies. *J Med Genet* 1971; **8**: 481–495.

3 Harding AE: Inherited neuronal atrophy and degeneration predominantly of lower motor neurons; In Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF (eds) *Peripheral neuropathy*. Philadelphia, PA: WB Saunders; 1993, 2nd edn. pp. 1051–1064.

4 Viollet L, Barois A, Rebeiz J *et al*: Mapping of autosomal recessive chronic distal spinal muscular atrophy to 11q13. *Ann Neurol* 2002; **51**: 585–592.

5 Grohmann K, Wienker TF, Saar K *et al*: Diaphragmatic spinal muscular atrophy with respiratory distress is heterogeneous, and one form is linked to chromosome 11q13–q21. *Am J Hum Genet* 1999; **65**: 1459–1462.

6 Grohmann K, Schuelke M, Diers A *et al*: Mutations in the gene encoding immunoglobulin μ-binding protein 2 cause spinal muscular atrophy with respiratory distress type 1. *Nat Genet* 2001; **29**: 75–77.

7 Schuler GD, Boguski MS, Stewart EA *et al*: A gene map of the human genome. *Science* 1996; **274**: 540–546.

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

9 Gardner-Medwin D, Hudgson P, Walton JN: Benign spinal muscular atrophy arising in childhood and adolescence. *J Neurol Sci* 1967; **5**: 121–158.

10 Harding AE, Thomas K: Hereditary distal spinal muscular atrophy. A report on 34 cases and a review of the literature. *J Neurol Sci* 1980; **45**: 337–348.

11 Pearn J, Hudgson P: Distal spinal muscular atrophy: a clinical and genetic study of 8 kindred. *J Neurol Sci* 1979; **43**: 183–191.