Large scale deletions of the 5q13 region are specific to Werdnig-Hoffmann disease

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Abstract

Spinal muscular atrophy (SMA) is characterised by degeneration of anterior horn cells of the spinal cord and represents the second most common, lethal, autosomal recessive disorder after cystic fibrosis. Based on the criteria of the International SMA Consortium, childhood SMAs are classified into type I (Werdnig-Hoffmann disease), type II (intermediate form), and type III (Kugelberg-Welander disease). Recently, two genes have been found to be associated with SMA. The survival motor neurone gene (SMN) is an SMA determining gene as it is absent in 98.6% of patients. A second gene, XS2G3, or the highly homologous neuronal apoptosis inhibitory protein gene (NAIP) have been found to be more frequently deleted in type I than in the milder forms (types II and III). We investigated the correlation between the clinical phenotype and the genotype at these loci. A total of 106 patients were classified into type I (44), type II (31), and type III (31) and analysed using SMN, markers C212 and C272, and NAIP mapping upstream and downstream from SMN respectively. The combined analysis of all markers showed that a large proportion of type I patients (43%) carried deletions of both SMN and its flanking markers (C212/C272 and NAIP exon 5), as compared with none of the patients with type II or III SMA. The presence of large scale deletions involving these loci is specific to Werdnig-Hoffmann disease (type I) and allows one to predict the severity of the disease in our series.

(J Med Genet 1996;33:281-283)

Hoffmann disease, can be recognised at birth or within 6 months of age. The children are unable to sit unaided and respiratory involvement is usually responsible for death before 2 years of age. In type II SMA, the intermediate form, children are able to sit but unable to stand or walk unaided, and they live beyond 2 years of age. In type III SMA, the mildest form known as Kugelberg-Welander disease, children have an onset after the second year of life and the course of the disease is usually chronic.

The underlying biochemical defect remains unknown. Linkage analysis has been used to localise the disease gene. All three forms map to chromosome 5q13.3, suggesting that they are the result of allelic mutations.⁷⁻¹⁰ The genomic region encompassing the disease gene is particularly unstable and prone to large scale deletions.¹¹ Two genes associated with SMA have been identified in this region. The neuronal apoptosis inhibitory protein (NAIP) gene and the highly homologous gene XS2G3 have been found to be more frequently deleted in type I (45%) than in the milder forms (types II and III, 18%).¹²⁻¹⁴ The survival motor neurone (SMN) gene was either absent or interrupted in the majority of patients (98.6%), independent of the type of SMA.¹⁵ Moreover, the observation of intragenic mutations in three patients retaining the gene supported the view that SMN is the SMA determining gene.¹⁵

We have compared clinical data from a group of 106 unrelated SMA patients with their genotype using the SMN and NAIP genes, and markers C212 and C272. Here, we show that large scale deletions involving these loci are specific to Werdnig-Hoffmann disease.

Methods

FAMILY STUDIES

A total of 106 unrelated SMA patients were placed into one of three subgroups according to the criteria of the International SMA Consortium⁶: 44 belonged to type I, 31 to type II, and 31 to type III SMA. Among them, 14/44 type I, 3/31 type II, and 9/31 type III SMA patients were born to consanguineous parents of various geographical origins. A total of 66 parents and 66 unrelated healthy subjects were also tested.

DNA ANALYSES

Dinucleotide repeat polymorphism analysis

DNA was extracted from either peripheral blood leucocytes or lymphoblastoid cell

Key words: Werdnig-Hoffmann disease; large scale deletions.

Proximal spinal muscular atrophy (SMA) represents the second most common lethal autosomal recessive disorder after cystic fibrosis (incidence 1 in 6000 live births).¹⁻³ SMA is characterised by degeneration of anterior horn cells of the spinal cord. The diagnosis is based on proximal and symmetrical weakness with muscular atrophy. Pathological and electrophysiological evidence of muscle denervation is also found with, in most patients, normal nerve conduction velocities.^{4,5} The childhood SMAs are classified into three major groups on the basis of age of onset, milestones of development, and age of survival.⁶ Type I SMA, the most severe form known as Werdnig-

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Received 4 October 1995
Revised version accepted
for publication
19 December 1995

SMN, NAIP, and C212/C272 deletions in the clinical subtypes of SMA (A,B,C). The numbers of subjects (%) showing a deletion of SMN exon 7 (A), NAIP exon 5 (B), or a marked reduction of C212 (grey) or C272 loci (black, C) on both chromosomes are indicated. (D) Frequency of large scale deletions in the various clinical subtypes of SMA. The numbers of subjects (%) showing large scale deletions including the C212-C272 loci, SMN and NAIP genes are indicated.

lines. 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 one minute each, for 30 cycles.¹¹

SMN gene analysis

SMN exons 7 and 8 were studied by single strand conformation polymorphism (SSCP) analysis after PCR amplification of genomic DNA (200 ng) using unlabelled primers (20 µmol/l) in an amplification mixture (25 µl) containing 200 µmol/l dNTPs, 1 unit *Taq* polymerase (Gibco-BRL), and 0.1 µl α^{32} P dCTP (10 mCi/ml, NEN). The oligonucleotide pairs R111-541C770 and 541C960-541C1120 were used to amplify genomic DNA containing the divergent exons 7 and 8 respectively.¹⁵

NAIP gene analysis

NAIP gene analysis was performed by PCR amplification of exon 5 using primers 1863 and 1864. Amplified DNA was electrophoresed onto a 2% Seaplaque agarose gel and stained with ethidium bromide.¹² PCR amplification and Southern blot analysis showed that NAIP

exon 5 is specific to the telomeric element of the duplication containing the SMN gene (data not shown, available on request).

Results

The figure shows that the SMN gene is absent or interrupted in 102/106 SMA patients, independent of the type of SMA (96.2%). All the controls, the parents (100%), and four SMA patients carried SMN exons 7 and 8. Three patients have been previously shown to carry intragenic mutations of the SMN gene.¹⁵ Analysis using the C272 marker showed that 22/44 type I patients (50%) had a heterozygosity deficiency compared with 2/32 (6.4%), 3/31 (9.6%), 3/66 (4.5%), and 0/66 (0%) in type II, type III, parents, and controls respectively (figure). Similar results were obtained with marker C212 (figure). The heterozygosity deficiency has been previously ascribed to the deletion of one of the two loci detected by these markers.¹¹ Studying the NAIP gene by testing exon 5, we found that 29/44 type I patients (66%) lacked the NAIP gene, compared with 2/31 (6.5%), 4/31 (13%), 1/66 (1.5%), and 0/66 (0%) in types II, III, parents, and controls respectively (figure).

The combined analysis of all markers showed that a large proportion of type I patients (43.5%) carried deletions of both SMN and its flanking markers (C212/C272 and NAIP exon 5), as compared with none of the patients suffering type II or III SMA (table, figure). By contrast, most of type II (28/31, 90.5%) and type III SMA patients (26/31, 84%) lacked SMN but retained NAIP exon 5 and the C212/C272 loci, as compared with 10/44 type I SMA (23%, table).

Discussion

Deletions of the 5q13 region have been found to be statistically associated with type I SMA, as 18% of patients showed a loss of loci detected by markers C212 and C272.¹¹ The SMN gene which encodes a hitherto unknown protein has been identified close to these markers.¹⁵ Recently, we and others showed that the SMN gene is either absent or interrupted in the majority of patients (92 to 98.6% of patients), independent of the type of patient, suggesting that SMN is the SMA determining gene.¹⁵⁻¹⁷ On the other hand, the telomeric version of the NAIP gene mapping close to SMN has been found to be more frequently deleted in the severe form (66%) than in the milder forms of the disease (6.5% and 13% in types II and III respectively). However, the observation of homozygous NAIP gene deletions only in healthy parents favours the view that NAIP mutations modify rather than trigger the SMA phenotype.¹²

Here, the combined analysis of the SMN gene and its flanking markers (C212-C272 and NAIP mapping upstream and downstream from SMN, respectively) in a large cohort of SMA patients shows that the presence of large scale deletions involving these loci is specific to Werdnig-Hoffmann disease in our series

Characteristics of deletions according to the type of SMA

C212-C272	SMN exon 7	NAIP exon 5	Type I (n=44)	Type II (n=31)	Type III (n=31)	Parents (n=66)	Controls (n=66)
-*	-	-	43.5†	0	0	0	0
-	-	+	2	0	0	0	0
+†	-	+	27	90.5	84	0	0
+	-	-	23	6.5	13	0	0
+	+	-	0	0	0	1.5	0
+	+	+	4.5(m)	3(m)	3(nd)	98.5	100

* Minus indicates the presence of heterozygosity deficiency at C212-C272 loci or deletion of gene loci (NAIP and SMN).

† Plus indicates the presence of at least one gene locus.

‡ % of subjects.

(m) indicates the presence of point mutation of the SMN gene.

(nd) indicates that the search for point mutation was not done.

(type I, 43.5%). This study can help to predict the severity of the disease when large scale deletions are observed.

It is tempting to hypothesise therefore that NAIP, along with other genes mapping close to SMN, modify the SMA phenotype thus accounting for the different clinical subtypes of the disease. However, smaller rearrangements can still result in a severe phenotype as 27% of SMA type I patients lacked the SMN gene but not the C212-C272 or NAIP loci. Since deletion or intragenic mutation of SMN alone is sufficient to produce very mild and very severe disease,^{15,18-20} these data suggest that other genetic mechanisms might be involved in the variable clinical expression of the disease. Elucidating the function of the gene products will be important for the understanding of the pathogenesis of SMA.

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

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