Genetic mapping of a susceptibility locus for disc herniation and spastic paraplegia on 6q23.3-q24.1

M Zortea, A Vettori, C P Trevisan, S Bellini, G Vazza, M Armani, A Simonati, M L Mostacciuolo

It has been suggested that a genetic factor(s) or a familial predisposition may contribute to the clinical manifestations of disc herniation; moreover, no genetic linkage between spinal disc herniation and spastic paraplegia has ever been described. A family with consanguineous parents and four of eight sibs affected by multiple disc herniations and spastic paraplegia was clinically and genetically analysed. Surgery caused partial improvement in all of them. After the exclusion of type II collagen and vitamin D receptor genes and the recessive loci for HSPs, a genome wide search was performed with about 500 fluorescent markers. Positive lod score values were obtained for chromosome 6q22.31-q24.1, with evidence of three homozousy intervals. The maximum multipoint lod score of 3.28 was obtained in only one interval, between markers D6S1699 and D6S314. On the whole, a susceptibility locus for disc herniation and autosomal recessive spastic paraplegia was found on chromosome 6q23.3-q24.1. This is the first time that disc herniation and the associated neurological syndrome has been linked to a human chromosomal region.

**SUBJECTS AND METHODS**

**Clinical analysis**

The family analysed came from the north east of Italy. Four patients (IV.3, IV.4, IV.5, and IV.7, fig 1), belonging to a sibship of eight, were affected by different types of spinal disc herniations and spastic paraplegia. Their unaffected parents were first cousins. No other family members had either disc herniation or paraparesis in the previous generations or in the descendants of the cases considered in this study. All the living members in the fourth generation were neurologically examined by two of us (CPT and MA) as inpatients or outpatients at the Neurological Clinic of Padua University. The clinical features of the four affected subjects are summarised in table 1. In the patients, the onset of the pyramidal symptoms in the lower limbs was of late type with a variation in the age of onset, ranging from 30 (IV.4, fig 1) to 46 years (IV.7). In all of them the paresis was accompanied or preceded by different manifestations of spinal pain irradiating to the upper (IV.4 and IV.5) or the lower limbs (IV.3 and IV.7). All the patients underwent neuroimaging evaluations (CT scan and MRI) in the first years of progression of their paraparesis, which showed different sites of spinal disc herniations: cervical disc herniation in IV.3 and IV.4, and cervical in IV.5, and thoracolumbar in IV.7. X rays of the shoulders, elbows, hips and knees showed no major abnormalities in any of them, while X rays of the spinal column detected minor spondylosis, mainly in the cervical and the lumbar spine in IV.3 and IV.4, only in the cervical spine in IV.5, and in the whole spine in IV.7. All of them underwent surgical treatment of the disc herniations and they showed a clear, even though partial, improvement of the motor symptoms and complete remission of the neck and back pain. They did not show clear signs of amyotrophy or of sensory deficit of the limbs; however, electromyography showed evidence of mild motor-sensory (IV.3), or sensory (IV.5), or motor (IV.7) neuropathy; without radiologic involvement. In these three patients, nerve conduction studies showed only minor abnormalities. In patient IV.4, the same electrophysiological investigations were normal. The variable...
Informed consent was previously obtained, in accordance with current privacy legislation. In order to exclude a possible involvement of the COL2A1 and VDR genes, we used two fluorescent markers, D12S85 and D12S368, flanking both these genes.

All the microsatellites relative to the known AR-HSP loci (D8S260 and D8S285 for SPG5, D16S303 and D16S3023 for SPG7, D15S1007 and D15S1012 for SPG11, D3S1294 and D3S2747 for SPG14, and D14S77 and D14S61 for SPG15) were PCR amplified, analysed on a 9% denaturing polyacrylamide gel, and silver stained.

A genome wide search was performed using 485 fluorescent markers on autosomal chromosomes. Three hundred and eighty-two were from the ABI PRISM Linkage Mapping Set Version 2 (LMS2, Perkin Elmer) and a further 103 fluorescent markers were selected from the ABI PRISM Linkage Mapping Set High Density 5 (LMS HD5) in order to obtain a better resolution. All these markers were amplified in a PTC-225 Pelletier Thermal Cycler (MJ Research). The PCR products were evaluated using two fluorochromes (Int 1, Int 2, and Int 3) represent the homozygous intervals.

**Molecular genetic analysis**

For molecular analysis, blood samples from the eight members of the fourth generation (fig 1) were collected and the DNA extracted from 10 ml of blood by standard methods. Informed consent was previously obtained, in accordance with current privacy legislation. In order to exclude a possible involvement of the COL2A1 and VDR genes, we used two fluorescent markers, D12S85 and D12S368, flanking both these genes.

All the microsatellites relative to the known AR-HSP loci (D8S260 and D8S285 for SPG5, D16S303 and D16S3023 for SPG7, D15S1007 and D15S1012 for SPG11, D3S1294 and D3S2747 for SPG14, and D14S77 and D14S61 for SPG15) were PCR amplified, analysed on a 9% denaturing polyacrylamide gel, and silver stained.

A genome wide search was performed using 485 fluorescent markers on autosomal chromosomes. Three hundred and eighty-two were from the ABI PRISM Linkage Mapping Set Version 2 (LMS2, Perkin Elmer) and a further 103 fluorescent markers were selected from the ABI PRISM Linkage Mapping Set High Density 5 (LMS HD5) in order to obtain a better resolution. All these markers were amplified in a PTC-225 Pelletier Thermal Cycler (MJ Research). The PCR products were evaluated using two fluorochromes (Int 1, Int 2, and Int 3) represent the homozygous intervals.
A susceptibility locus for disc herniation and spastic paraplegia

To analyse the critical region (6q22.31-q24.1) more precisely, 26 evenly distributed additional markers were chosen from the Genome Browser database (http://genome.ucsc.edu). Owing to a lack of informative markers, two CA repeats, which we named SP/H1 and SP/H2, were also selected to define the homozygous regions further, belonging to GenBank clones AL078600 and AL050388, respectively. These two clones are contiguous to the ones containing the homozygous markers D6S1696 and D6S1684, respectively. The two point lod scores obtained for all the analysed markers were negative, with values < -2 at recombination fraction (θ) = 0.0 (data not shown).

By a genome wide analysis, almost the entire genome (≈90%) was excluded. Significant positive lod score values were obtained in region 6q22.31-q24.1 for three consecutive fluorescent markers: D6S287 (Zmax = 2.81 at θ = 0.0), D6S262 (Zmax = 1.40 at θ = 0.1), and D6S308 (Zmax = 1.38 at θ = 0.1). Because of the homozygous status shared only by the affected subjects for the alleles of marker D6S287 and the closeness of the lod score of this marker to the computer simulated one (simulated Zmax = 2.88), we decided to investigate this region further by means of 26, evenly distributed, additional markers. Table 2 shows the lod score values for only 24 markers, because D6S1705 and D6S1569 are not informative. The haplotype analysis carried out for this region (about 26 cM) confirmed homozygous status shared only by the affected subjects in the three intervals, as shown in fig 1. Interval 1 spans 0.9 cM between the phenotype peculiar to this family and region 6q22.31-q24.1; interval 2 spans 5.5 cM between markers D6S304 and D6S384 on 6q22.31-q24.1, and interval 3 spans 6 cM between markers D6S1958 and D6S1572 on 6q22.33-q23.1, and interval 3 spans 5.5 cM between markers D6S1958 and D6S1572 on 6q22.33-q23.1, and interval 3 spans 6 cM between markers D6S1958 and D6S1572 on 6q22.33-q23.1, and interval 3 spans 6 cM between markers D6S1958 and D6S1572.

### RESULTS

We began our study by excluding COL2A1 and VDR and the known AR-HSP loci (SPG5, SPG7, SPG11, SPG14, and SPG15). The two point lod scores obtained for all the analysed markers were negative, with values < -2 at recombination fraction (θ) = 0.0 (data not shown).

Table 2 shows the lod score values for only 24 markers, because D6S1705 and D6S1569 are not informative. The haplotype analysis carried out for this region (about 26 cM) confirmed homozygous status shared only by the affected subjects in the three intervals. Because of the homozygous status shared only by the affected subjects in the three intervals, the well known primary degeneration of the cervical spinal tracts, without spinal abnormalities. Even though familial aggregation has

### DISCUSSION

A major disc herniation of the cervical or dorsal column causes compression of the spinal cord with the clinical consequence of a pyramidal disturbance affecting the lower limbs. The whole pyramidal syndrome, including spasticity, deep tendon hyperreflexia, Babinski's sign and partial (paraparesis) or complete (paraplegia) impairment of intentional movement of the lower limbs, is also the characteristic clinical phenotype of hereditary spastic paraplegia (HSP), the well known primary degeneration of the cortical spinal tracts, without spinal abnormalities. Even though familial aggregation has
been observed in intervertebral disc degeneration, from a genetic point of view the few available reports on spinal disc herniation were association studies with polymorphisms found in two genes: an exonic VNTR in the AGCI and an intronic TaqI polymorphism in the VDR gene. This approach, however, is of non-parametric type; indeed, cohorts of patients have always been considered instead of families with clear mendelian heredity. For this reason, no linkage studies have ever been published.

In the family analysed in this study, two particular traits were evident: (1) clear mendelian transmission (autosomal recessive) and (2) a compound phenotype. The four affected cases had consanguineous parents and showed a paraparetic syndrome associated with upper and lower spine disc herniations. Because of this particular phenotype, never previously described, the first, compulsory step of our analysis was the exclusion of the COL2A1 and VDR genes (involved in disc degeneration) and the AR-HSP loci. On the basis of these preliminary results, we continued with the analysis using the homozygosity mapping method, finding positive lod score values spread over a large region (about 26 cM) only on chromosome 6q22.31-q24.1. A better definition of the whole region showed three homozygous intervals of varying size. The multipoint analysis yielded significant results for interval 3 (6 cM), with a peak of Zma=3.28, whereas for interval 2 (5.5 cM) this value was 2.58. Given that for this kind of analysis the accepted threshold value is 3, we initially focused our attention on interval 3. On the other hand, in our opinion, a value of 2.58 still warrants further analysis, should the search within interval 3 not yield any results.

The identification of a candidate region is a first step in the search for a gene linked to a disease. The subsequent step is the analysis of the physiological and structural traits of the tissue involved in the disease, in order clearly to identify what gene(s) may play a crucial role in its pathogenesis. As far as disc herniation is concerned, the intervertebral discs consist mainly of collagens and proteoglycans. The latter confer rigidity and resistance to compression and are responsible for the high water content, while the former prevent breakage. On these bases, it is reasonable to consider these genes as playing a role in the genetic predisposition to disc herniation.

Another important step in the identification of candidate genes is also to consider the structural and functional features shared by the products of the genes previously described and linked to other variants of the same disease. In the case of autosomal HSPs, it should be noted that, even though the three genes mapped so far (paraplegin,\(^{16}\) spastin,\(^{16}\) and atlastin\(^{17}\)) encode proteins belonging to different families, the products of two genes (paraplegin and spastin) both contain an AAA motif. The third autosomal gene (atlastin) does not contain this motif, but instead shows great homology to gynaecyl binding protein 1 (GBP1); for this reason, atlastin may belong to the dynamin family of large GTPases. Dynamins are important for the rapid and efficient recycling of the synaptic vesicles and have been found associated with cytосkeletal elements, including microtubules.\(^{18}\)

For the analysis of the candidate region (interval 3) in the family reported in this study, we referred to MapViewer (http://www.ncbi.nlm.nih.gov/) and the Genome Browser Database; in this region spanning about 5 Mb, 16 known genes and four Unigene EST clusters have been reported. On the basis of a preliminary analysis of the characteristics of these genes, none of them seems to have functional or structural homology to proteoglycans or collagens, while a gene, MAP7, microtubule associated protein 7, may be a candidate gene for HSP.

All together, these data provided evidence of a susceptibility locus for multiple herniation and paraplegia on chromosome 6q23.3-q24.1. In conclusion, studying other families with similar clinical and genetic characteristics may provide further information to help define this disease locus better. This study also indicates that possible overlooked spinal column pathology should be considered in patients with apparent primary autosomal recessive HSP.

**ACKNOWLEDGEMENTS**

This work was supported by Telethon grant No 797 to MLM and by “Legato Destro” grant to CPT. We wish to thank the members of the family for their cooperation in this study.

**REFERENCES**


