Motor system abnormalities in hereditary spastic paraparesis type 4 (SPG4) depend on the type of mutation in the spastin gene

D Bönsch, A Schwindt, P Navratil, D Palm, C Neumann, S Klimpe, J Schickel, J Hazan, C Weiller, T Deufel, J Liepert

Background: Hereditary spastic paraparesis (HSP) denotes a group of inherited neurological disorders with progressive lower limb spasticity as their clinical hallmark; a large proportion of autosomal dominant HSP belongs to HSP type 4, which has been linked to the SPG4 locus on chromosome 2. A variety of mutations have been identified within the SPG4 gene product, spastin.

Objective: Correlation of genotype and electrophysiological phenotype.

Material: Two large families with HSP linked to the SPG4 locus with a very similar disease with respect to age of onset, progression, and severity of symptoms.

Methods: Mutation analysis was performed by PCR from genomic DNA and cDNA, and direct sequencing. The motor system was evaluated using transcranial magnetic stimulation.

Results: Patients differ in several categories depending on the type of mutation present.

Conclusions: For the first time in hereditary spastic paraparesis, a phenotypic correlate of a given genetic change in the spastin gene has been shown.

The identification of disease genes and their alteration is likely to define more precisely clinically heterogeneous genetic disorders, yet, examples are scarce where a clear cut correlation can be shown between defined mutation genotypes and a specific clinical phenotype. Hereditary spastic paraparesis (HSP) denotes a group of inherited neurological disorders with progressive lower limb spasticity as their clinical hallmark; pedigrees show autosomal dominant, autosomal recessive as well as X linked inheritance, and up to now, at least 15 genetic loci have been identified for clinically “pure” HSP and “complicated” forms of HSP. Clinical presentation may vary to a large degree, even in patients within the same pedigree, and this is reflected in variable and inconsistent results of electrophysiological studies that, so far, do not contribute significantly to our understanding of the underlying disturbance of neurological function.

A large proportion of autosomal dominant HSP belongs to HSP type 4, which has been linked to the SPG4 locus on chromosome 2; the gene was recently cloned and a variety of mutations, many of them private, have been identified. The gene product, spastin, belongs to the family of AAA proteins, and has been shown to be involved in microtubule dynamics.

We report on two pedigrees with pure HSP where we have identified different mutations in the spastin gene and where, clinically, affected members present with a very similar disease with respect to age of onset, progression, and severity of symptoms. The motor system of both clinically affected and unaffected subjects in both pedigrees was investigated using transcranial magnetic stimulation (TMS).

METHODS
Twenty eight people from pedigree SPG 182, and 21 from pedigree SPG 0189, both of German origin and each extending over three generations, have been examined by one of us (JL, AS, or PN). Clinically affected subjects presented with increased reflexes in the lower compared with upper limbs plus at least one pyramidal sign; apart from pyramidal signs, most patients showed at least mild sensory loss such as diminished vibratory sense in the feet. Pes cavus was found in nearly all patients; urge incontinence was the only urinary symptom and it was seen in only one patient from pedigree SPG182 and three patients in pedigree SPG189. None of the family members showed any signs of complicated HSP (table 1).

In each of the two pedigrees, the HSP phenotype segregated in a pattern consistent with autosomal dominant inheritance; linkage to the SPG4 locus was established in both with calculated maximum two point lod scores of 4.70 in pedigree SPG182, and of 3.94 in pedigree SPG189, respectively, for the marker D2S352 (data not shown).

Mutation analysis was performed using total RNA extracted from patient lymphoblastoid cell lines with the QiAamp RNA Blood mini kit (Quiagen); cDNA synthesis was performed on about 1 µg of each RNA sample with 100 pmol of random primers (Quiagen, Advantage RT-for-PCR kit) and 200 U of MMLV reverse transcriptase according to standard procedures. Four overlapping PCR products spanning the SPG4 open reading frame were obtained from patient cDNAs and were subsequently sequenced on a LICOR sequencer. Additionally, in pedigree 182, all coding exons from 11 through 17, as well as intron 12, were amplified by PCR from 100 ng of genomic DNA and sequenced on a LICOR sequencer. Primers used to amplify and sequence both the SPG4 exons and SPG4 cDNA are available at the web site (http://www.genoscope.cns.fr). Co-segregation of mutations identified was then ascertained in the remainder of the pedigree.

Abbreviations: HSP, hereditary spastic paraparesis; CMCT, central motor conduction time; PMCT, peripheral motor conduction time; MEP, motor evoked potential; TMS, transcranial magnetic stimulation.
Central (CMCT) and peripheral (PMCT) motor conduction time, amplitudes of motor evoked potentials (MEPs) after TMS, motor thresholds, duration of the silent period evoked by TMS, and amplitudes of motor responses evoked by electrical nerve stimulation (M waves) were recorded. PMCT was calculated according to the formula (F wave latency + distal motor latency –0.5), CMCT was calculated by subtracting PMCT from the overall latency. TMS was performed with increasing intensities (110%, 120%, 130%, 140%, and 200% of motor threshold at rest) to obtain a stimulus response curve. MEP amplitudes were expressed in percentages of the corresponding M wave amplitude. The silent period was produced with a stimulus intensity of 120% motor threshold at rest; for each condition, eight stimuli were applied. All response amplitudes were measured peak to peak, the duration of the silent period was measured from the beginning of the MEP until the re-occurrence of ongoing muscle activity. Responses were recorded from the right first dorsal interosseous muscle and the right tibial anterior muscle, respectively, stored on an EMG machine and analysed off line. TMS was performed with a Dantec maglite 25 and a circular coil.

Affected subjects from family 182 (n=5) and family 189 (n=5) (mean (SD) age 36.6 (11.6) years, range 20–54) were compared with a group of 16 age matched, healthy control subjects (mean age 35.2 (11.1) years; range 20–54). Our healthy control group was unrelated to the patients. In each family, we also had the opportunity to perform electrophysiological studies in three clinically and genetically unaffected members. Their results did not differ from the unrelated control group for any TMS parameter and data are therefore not presented here.

For statistics, an analysis of variance and post-hoc Tukey tests were performed. The level of significance was assumed at 5%.

RESULTS
Sequencing of the complete spastin cDNA of an affected person from pedigree 189 showed a micro-deletion of a single G in position 1299 resulting in a premature stop codon in exon 9 (fig 1B). In pedigree 182, PCR and cDNA sequencing showed an in-frame deletion of exons 13 to 16 (fig 1C); subsequent

### Table 1 Clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>Family 189</th>
<th>Family 182</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>f</td>
<td>f</td>
</tr>
<tr>
<td>Age at onset</td>
<td>60 55 41</td>
<td>57 38 25</td>
</tr>
<tr>
<td>Age at research</td>
<td>72 73 52</td>
<td>56 50 48</td>
</tr>
<tr>
<td>Disease duration</td>
<td>12 18 11</td>
<td>19 12 25</td>
</tr>
<tr>
<td>Upper limbs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased reflexes</td>
<td>– – – – – + – – – – – + – – – – +</td>
<td>– – – – – + – – – – – + – – – – +</td>
</tr>
<tr>
<td>Lower limbs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased tone</td>
<td>++ ++ ++ + + ++ ++ ++ + – + ++ + ++ ++ ++ ++</td>
<td>++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++</td>
</tr>
<tr>
<td>Pyramidal weakness</td>
<td>++ ++ + + + + ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++</td>
<td>++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++</td>
</tr>
<tr>
<td>Ankle clonus</td>
<td>++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++</td>
<td>++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++</td>
</tr>
<tr>
<td>Babinski</td>
<td>+ + – – + – + + ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++</td>
<td>++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++</td>
</tr>
<tr>
<td>Adductor spasm</td>
<td>++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++</td>
<td>++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++</td>
</tr>
<tr>
<td>PSR zone enlargement</td>
<td>++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++</td>
<td>++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++</td>
</tr>
<tr>
<td>Sensory loss</td>
<td>++ ++ + + + – + + – – + ++ + – ++ + + – + + + + ++ + + + + +</td>
<td>++ ++ + + + – + + – – + ++ + – ++ + + – + + + + ++ + + + + +</td>
</tr>
<tr>
<td>Urinary symptoms</td>
<td>+ + – – + – – – – – + – – – – – + – – – – – + – – – – –</td>
<td>+ + – – + – – – – – + – – – – – + – – – – –</td>
</tr>
<tr>
<td>Pes cavus</td>
<td>++ ++ ++ + – + ++ ++ ++ + – + ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++</td>
<td>++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++</td>
</tr>
</tbody>
</table>

Analysed probands are in bold type.

Affected subjects from family 182 (n=5) and family 189 (n=5) (mean (SD) age 36.6 (11.6) years, range 20–54) were compared with a group of 16 age matched, healthy control subjects (mean age 35.2 (11.1) years; range 20–54). Our healthy control group was unrelated to the patients. In each family, we also had the opportunity to perform electrophysiological studies in three clinically and genetically unaffected members. Their results did not differ from the unrelated control group for any TMS parameter and data are therefore not presented here.

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RESULTS
Sequencing of the complete spastin cDNA of an affected person from pedigree 189 showed a micro-deletion of a single G in position 1299 resulting in a premature stop codon in exon 9 (fig 1B). In pedigree 182, PCR and cDNA sequencing showed an in-frame deletion of exons 13 to 16 (fig 1C); subsequent
primers from intron 12 and intron 16, respectively, produced a fusion product in affected subjects only, and its sequence confirmed a genomic deletion with breakpoints within introns 12 and 16 (intron 12: 3611, intron 16: 391). The position of both mutations is shown in figure 1A.

MEP induced by TMS failed to show significant differences from age-matched controls in pedigree 189; in pedigree 182, however, affected members differed significantly, both from those in family 189 and from the age-matched controls:

1. MEP amplitudes obtained from FDI muscle during contraction with a stimulus intensity of 200% motor threshold at rest were significantly smaller than in controls (p=0.028) and tended to be lower than in family 189 (p=0.065) (fig 2A);

2. CMCTs, recorded from the tibial anterior muscle, were significantly longer when compared with the control group (p<0.001) as well as with patients in pedigree 189 (p=0.001) (fig 2B);

3. In pedigree 182, motor thresholds for tibial anterior muscle were significantly increased as compared with the control group (p=0.021); in family 189 motor thresholds were non-significantly higher than in the control group, but lower than in family 182. Therefore, the mean motor threshold of pedigree 189 was not significantly different from controls or from family 182 (fig 2C).

Motor thresholds and central motor conduction times to FDI muscle, silent periods in FDI and AH, stimulus response curves (stimulus intensities from 110% to 140% motor threshold), peripheral motor conduction times, and M wave amplitudes were not significantly different in family 182, family 189, and in the controls (table 2).

DISCUSSION

Heterogeneity, both genetically and phenotypically, is a key feature of HSP and clinical phenotypes are not consistent even within pedigrees where a broad range, for example, if the age of onset is not a result of anticipation, as once discussed, but rather reflects variations in expression; accordingly, previous studies have failed to pinpoint defined clinical features to a certain genetic subtype or mutation. The results of electrophysiological investigations vary between studies and have not contributed significantly to the elucidation of possible differences in the aetiology and pathogenesis of the disorder. TMS studies have demonstrated that CMCTs to lower extremities are delayed in most but not all patients. CMCTs to upper extremities are usually normal but may be prolonged in a minority of patients, and intracortical facilitation may be increased. In none of these studies, electrophysiological findings have been combined with genotypes. In our study, CMCTs to FDI were normal in all patients; the smaller MEP amplitudes in FDI in affected members of family 182, however, indicate a reduced neuronal recruitment in the motor system that was only apparent with high stimulus intensities. This result was obtained in a hand muscle that was

Figure 2  [A] MEP amplitudes from right FDI muscle obtained during voluntary contraction with a 2.0 motor threshold stimulus intensity. Error bars indicate standard deviations. (B) Central motor conduction times to right tibial anterior muscle. Error bars indicate standard deviations. (C) Motor thresholds to right tibial anterior muscle. Error bars indicate standard deviations.

Table 2  TMS results obtained in the healthy control group, family 189 and family 182

<table>
<thead>
<tr>
<th>Threshold</th>
<th>CMCT (ms)</th>
<th>PMCT (ms)</th>
<th>M wave (mV)</th>
<th>S-R-C (110%)</th>
<th>S-R-C (120%)</th>
<th>S-R-C (130%)</th>
<th>S-R-C (140%)</th>
<th>SP (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>64.3 (7.7)</td>
<td>6.3 [0.9]</td>
<td>16.1 (1.6)</td>
<td>16.6 (3)</td>
<td>1.9 (1)</td>
<td>4.8 (1.3)</td>
<td>8.2 (2.8)</td>
<td>12.9 (4)</td>
</tr>
<tr>
<td>Fam 189</td>
<td>63.4 (13.8)</td>
<td>7.2 [0.8]</td>
<td>14.7 (1.8)</td>
<td>17.6 (3.7)</td>
<td>3.4 (2)</td>
<td>8.5 (3.4)</td>
<td>16.1 (8.1)</td>
<td>23.7 (13.1)</td>
</tr>
<tr>
<td>Fam 182</td>
<td>63.6 (7.6)</td>
<td>5.6 [1.3]</td>
<td>17.1 (1.1)</td>
<td>19.5 (2.9)</td>
<td>1.9 (1.1)</td>
<td>5.4 (3.5)</td>
<td>7.4 (5.8)</td>
<td>12.7 (6.4)</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>86.9 (10)</td>
<td>11.2 [2]</td>
<td>17.4 (2.1)</td>
<td>7.4 (1.8)</td>
<td>3.1 (1.3)</td>
<td>4.7 (2.7)</td>
<td>8.9 (6.9)</td>
<td>12.5 (10.2)</td>
</tr>
<tr>
<td>Fam 189</td>
<td>92.3 (17.2)</td>
<td>13.2 [2]</td>
<td>18.5 (1.8)</td>
<td>7.7 (2.2)</td>
<td>4.7 (4.1)</td>
<td>11.8 (7.8)</td>
<td>15.3 (10)</td>
<td>14.4 (11.9)</td>
</tr>
<tr>
<td>Fam 182</td>
<td>111.6 (26.5)</td>
<td>19.5 (3.4)*</td>
<td>18.8 (1.9)</td>
<td>7.9 (3)</td>
<td>2.1 (8)</td>
<td>7.3 (8.1)</td>
<td>11.2 (10.4)</td>
<td>No data</td>
</tr>
</tbody>
</table>

*Threshold indicates stimulus intensity which represents motor threshold; CMCT, central motor conduction time; PMCT, peripheral motor conduction time; M wave, amplitude of the muscle potential obtained by electrical stimulation of ulnar nerve (A) or peroneal nerve (B); S-R-C, stimulus-response-curve, MEP amplitudes are presented as percentage of M wave amplitude; SP, silent period. *p<0.05.
Clinically not affected, which may be explained by a malfunction of a subgroup of neurons that have high depolarisation thresholds or an axonal damage to these neurons. Similar to earlier reports, our results do not allow to differentiate between a cortical and a subcortical origin of this disturbance: the prolongation of CMCT to the lower extremity, too, could be attributable to cortical or subcortical lesions. Neuropathological findings, however, support the hypothesis of lesions occurring mainly at the level of the spinal cord. Again in accordance with previous reports, we did not find any abnormalities in the peripheral nervous system.

When comparing patients from two pedigrees with different mutations in the spastin gene, our results, surprisingly, show that they differ with respect to CMCTs, motor thresholds, and maximal neuronal recruitments depending on the type of mutation present. In addition to these deficits in excitatory mechanisms, patients in family 182 also had a non-significant prolongation of the silent period obtained from tibial anterior muscle. Silent periods are supposed to reflect the excitability of inhibitory cortical and spinal neuronal circuits. This finding further supports the idea of a dysbalance between excitation and inhibition in the motor system of these patients. It provides a phenotypic correlate of the genetic change in the spastin gene suggesting that excitatory mechanisms in the motor system of HSP patients may be affected to different degrees depending on the type of mutation and, thus, the resulting spastin variant expressed in the patient. Furthermore, with MEP results indistinguishable from normal controls in one pedigree and significantly abnormal findings in the other, we are able to show that it is the type of mutation that determines severity of MEP disturbances rather than the fact whether or not spastin is mutated at all.

Our findings help to explain the variability of electrophysiological changes as reported in other studies on HSP; they also may lead the way to outline in more detail the, yet largely unknown, function of spastin. The mutations in the spastin gene, identified as the underlying molecular defects resulting in HSP in the two pedigrees investigated, are predicted to differ at the protein level. A single base deletion in exon 9 found in pedigree 189 will result in a stop codon and thus in a very short truncated protein; a large genomic deletion seen in pedigree 182, would predict a protein missing those parts encoded by exons 12 to 16 with a stop codon as the first base in exon 17. Only exon 17 is associated with higher motor thresholds and prolonged CMCTs; the single base deletion resulting in a stop codon in exon 9 is not reflected in a similar electrophysiological abnormality.

As has been shown recently, spastin is involved in microtubule dynamics, and both mutations are within the AAA domain. We cannot speculate as to the functional impact that cDNA alterations will have at the protein level. We have, though, observed electrophysiological differences that reflect differential degrees of disturbance in the motor system excitability and these findings are consistent within a pedigree, but vary between pedigrees; they are therefore likely to depend on the mutation present in the pedigree. TMS can therefore be used as a phenotypic feature to stratify HSP patients with respect to the functional impact of their underlying spastin mutation as we show, for the first time, that the type of the genetic abnormality in the spastin gene determines the degree of malfunction associated with the mutated gene product.

Grouping mutations according to the severity of MEP changes in patients, as our next step, will enable us to define functionally distinct spastin mutants and, possibly, to map functionally relevant domains in spastin. Eventually, this may help to elucidate its role both in the pathology of HSP and in the physiological sequence of events leading to intact function of the corticospinal tract.

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REFERENCES