Mutation in the Catalytic Domain of Protein Kinase Cγ and Extension of the Phenotype Associated With Spinocerebellar Ataxia Type 14

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Background: Autosomal dominant cerebellar ataxias comprise a clinically, neuropathologically, and genetically heterogeneous group of neurodegenerative disorders. The vast majority of cases are caused by trinucleotide or pentanucleotide repeat expansions in 9 different genes. Spinocerebellar ataxia type 14 (SCA14) is a relatively pure form of autosomal dominant cerebellar ataxia mapped to chromosome 19q and caused by missense mutations in the gene encoding protein kinase Cγ (PRKCG), which are all located in the regulatory domain.

Objectives: To identify new SCA14 families and to describe the associated phenotype.

Methods: We describe a new SCA14 family of French ancestry with 14 patients and 4 probably affected individuals. Linkage to the SCA14 locus was evaluated according to standard procedures using 5 markers covering the SCA14 candidate interval. All 18 exons of the PRKCG gene and splice junctions were screened with direct sequencing in the index patient.

Results: Linkage to the SCA14 locus was established with lod scores greater than 3 in the interval between DNA segments D19S571 and D19S926. Direct sequencing of the PRKCG gene revealed a T-to-C transition in exon 18 responsible for a novel missense mutation, F643L, which mapped to a highly conserved amino acid of the catalytic domain of protein kinase Cγ. The mutation showed complete segregation with the disease phenotype, was present in all affected and probably affected individuals, and was not observed on 410 control chromosomes from healthy white subjects. Age at onset, assessed in 14 affected individuals, was broader than in previous reports and ranged from childhood to age 60 years. All affected patients had slowly progressive cerebellar ataxia frequently associated with brisk reflexes. Cognitive impairment was also a striking feature in this family and has not been reported previously. Interestingly, there was no axial myoclonus as reported in a Japanese SCA14 family, but electrophysiological recordings in a single patient showed diffuse myoclonus in the arms and legs.

Conclusions: We have identified a new SCA14 family with the first mutation (F643L) located in the catalytic domain of the enzyme. The wide range of ages at onset, the presence of myoclonus in the limbs, and the presence of cognitive impairment extend the phenotype associated with this genetic entity.

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Inherited cerebellar ataxias are clinically and genetically heterogeneous. For the autosomal dominant forms, at least 23 loci have been implicated: spinocerebellar ataxia (SCA) 1-8, SCA10-18, SCA19/22, SCA20, SCA21, SCA23, SCA25, and the FGF14 gene–related SCA.1-3 The SCA14 locus was mapped to chromosome 19q in 2 families with different phenotypes: a Japanese family with cerebellar ataxia associated with axial myoclonus in patients with early onset4 and an American family of Dutch and English origin who developed pure cerebellar ataxia.5 Very recently 5 missense mutations (H101Y, G118D, S119P, G127R, and G128D) were found in the protein kinase Cγ gene (PRKCG) in 5 different families, all located in exon 4, which encodes part of the regulatory domain of the protein.6-8 Protein kinase Cγ is abundant in the brain, particularly in Purkinje cells, and is thought to play important roles in signal transduction, cell differentiation and proliferation, and synaptic transmission.

In this article, we describe a French family linked to the SCA14 locus with the first mutation (F643L) in the catalytic domain of the enzyme.

Methods

Patients

We identified a 4-generation family of French ancestry (Figure 1). Twenty-four individuals were examined, and 23 were sampled with

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tal deficiency. Progressive matrix 47 is intended to be a "culture-
their informed and written consent. Results of routine diag-
scans in the SCA1, SCA2, SCA3, SCA6, SCA7, SCA17, and
single-nucleotide polymorphism and the T-to-C transition located in the protein kinase C-α gene in intron 6 and exon 18, respectively. Asterisks indicate sampled
individuals; black circles, affected women; black squares, affected men; diamonds, nonaffected family members; gray symbols, probably affected individuals; and
arrow, index case. The haplotype assumed to carry the disease allele is boxed, and inferred haplotypes are bracketed. The genetic distances are presented in
centimorgans according to the Marshfield database (www.marshfieldclinic.org/genetics).

Bedside progressive matrix 47 was used in 8 affected members to
evaluate their intellectual level and to test the hypothesis of men-
tal deficiency. Progressive matrix 47 is intended to be a "cultur-
fair" test of general ability; it does not require language or academic
skills influenced by education. In addition, 2 patients were seen at
the Salpêtrière Hospital (Paris, France) for extensive neuropsy-
chological testing. Global cognitive efficiency was assessed with
the Mini-Mental State Examination10 and Mattis Dementia Rating
Scale.11 Attention was assessed using the mental control and the
digit and visual span subtests.12 Executive functions were assessed
with the frontal score13 and Frontal Assessment Battery14 at bed-
side. Memory efficiency was evaluated with the test developed by
Grober et al,15 which distinguishes between the different stages of
memory (encoding, storage, and retrieval) to confirm the existence
of subcortical dementia. Depression was evaluated using the
Montgomery-Asberg depression rating scale.16

**LINKAGE ANALYSIS**

Following a genomewide scan, linkage was established with 11
markers covering the **SCA14** candidate interval: D19S904,
D19S246, D19S206, D19S571, D19S180, D19S21, D19S24,

![Figure 1. Partial pedigree of the spinocerebellar ataxia type 14 family. Haplotype reconstruction is shown for 11 microsatellite markers and for the rs3745405 single-nucleotide polymorphism and the T-to-C transition located in the protein kinase C-α gene in intron 6 and exon 18, respectively. Asterisks indicate sampled individuals; black circles, affected women; black squares, affected men; diamonds, nonaffected family members; gray symbols, probably affected individuals; and arrow, index case. The haplotype assumed to carry the disease allele is boxed, and inferred haplotypes are bracketed. The genetic distances are presented in centimorgans according to the Marshfield database (www.marshfieldclinic.org/genetics).](image-url)
Multiple alignments of members of the protein kinase family and orthologs of protein kinase Cγ in various species using the Pfam database (Sanger Centre, Hinxton, England; http://www.sanger.ac.uk) or ClustalW software (http://www.ebi.ac.uk/clustalw/) showed that the mutation is located in a highly conserved region of the catalytic domain (C4) of the protein. As shown in Figure 2B in a selected subset of these sequences, amino acid F at position 643 of protein kinase Cγ is present in orthologs of this protein, in all members of the human protein kinase C family, as in other protein kinases.

CLINICAL FINDINGS

The clinical characteristics of affected and probably affected patients are summarized in Table 2. Ages at onset in the 14 affected subjects were variable and ranged from childhood to age 60 years with a mean±SD of 33.9±9.7 years, excluding 2 patients for whom age at onset could not be precisely determined. Cerebellar signs were mild in 7 patients (after a mean disease duration of 7.3 years), moderate in 6 (17.6 years’ duration), and severe in 2 (29.5 years’ duration). However, functional impairment was moderate; only 1 patient could not walk without help (22 years’ duration). Cerebellar dysarthria was present in all but 3 patients. Reflexes were increased in 12 of 14 affected individuals, but plantar responses were extensor in only 2. They were flexor in 7 and indifferent in 5.

Additional signs are also listed in Table 2. The most frequent associated sign was nystagmus in 7 patients, whereas limited eye movements were present in only 2 patients and diplopia in 1. Facial contraction fasciculations or myokymia were present in 4 patients. Decreased vibration sense at the ankles was noted in 4 patients. Rare signs included chorea in the hands and head tremor in 2 patients each. Since axial myoclonus was described in Japanese patients with SCA14, surface muscle activity has been recorded in patient IV-97. A pattern typical of myoclonus was observed in both the upper and lower limbs despite the absence of detectable jerks during clinical examination (Figure 3). Results of electromyographic and nerve conduction studies in patient IV-97...
were normal. Sagittal brain magnetic resonance imaging (patient IV-97) showed atrophy of the cerebellar vermis, but all other brain structures were spared (Figure 4).

The most striking association was the presence of cognitive impairment. Eight patients had memory complaints. Overt frontal signs were noted during examination in the index patient (III-423). Scores on the bedside progressive matrix 47 test were lower than expected in 4 of 7 subjects. This indicated a low IQ and difficulty with abstract thinking, which was confirmed by detailed neuropsychological examination in patient III-52 and was suspected in patient IV-97 (Table 3). The neuropsychological pattern was an isolated executive function deficit, reflecting subcortical impairment. There were difficulties with memory encoding and retrieval (but not storage), an attention deficit, and cognitive slowing as well as impaired working memory, concept shifting, abstract thinking, resistance to interference, and inhibitory control. The younger patient, IV-97, showed the beginning of impaired disexecutive functions reflected by a tendency to perseverate, an attention deficit, and lack of inhibitory control (go–no go test).

In addition, the 4 patients considered probably affected also carried the F643L mutation (Table 2). Despite the absence of clear cerebellar signs, memory loss was already evident in 2 of these patients, bringing the total number of patients with abnormal cognition to 13 (68%) of 19. It is difficult to establish whether cognitive changes were progressive because they were almost present early in the course of the disease in several patients, and prospective evaluations were not performed. However, gradual cognitive decline was suspected in patient III-117 because his level of education was not compatible with his poor performance on the progressive matrix 47 test.
Table 2. Affected and Probably Affected Members of the SCA14 Family

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at Examination, y</th>
<th>Age at Onset, y</th>
<th>Cerebellar Signs</th>
<th>Cognitive Complaint</th>
<th>Intellectual Level by the PM47 Score (Normal Value*)</th>
<th>Additional Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>III-22</td>
<td>62</td>
<td>40</td>
<td>Severe</td>
<td>None</td>
<td>Not done</td>
<td>Facial myokimia, decreased vibration sense at ankles, nystagmus, intermittent diplopia, choreic movements of the hands</td>
</tr>
<tr>
<td>III-37</td>
<td>67</td>
<td>30</td>
<td>Severe</td>
<td>Memory loss</td>
<td>Not done</td>
<td>Decreased vibration sense at ankles, nystagmus, limited upward gaze, swallowing difficulties, hearing impairment</td>
</tr>
<tr>
<td>III-39</td>
<td>58</td>
<td>30</td>
<td>Moderate</td>
<td>Memory loss</td>
<td>Not done</td>
<td>Nystagmus, limited upward gaze, left arm rigidity (Froment sign)</td>
</tr>
<tr>
<td>III-41</td>
<td>55</td>
<td>40</td>
<td>Mild</td>
<td>Memory loss</td>
<td>81‡ (&gt;85)</td>
<td>Limited upward gaze, choreic movements of the hands</td>
</tr>
<tr>
<td>III-52</td>
<td>60</td>
<td>30</td>
<td>Moderate</td>
<td>Attention deficit,</td>
<td>Not done</td>
<td>Swallowing difficulties</td>
</tr>
<tr>
<td>III-54</td>
<td>58</td>
<td>51</td>
<td>Mild</td>
<td>None</td>
<td>Not done</td>
<td>Nystagmus</td>
</tr>
<tr>
<td>III-59</td>
<td>54</td>
<td>50</td>
<td>Moderate</td>
<td>Memory loss</td>
<td>81‡ (&gt;85)</td>
<td>Swallowing difficulties</td>
</tr>
<tr>
<td>III-425</td>
<td>56</td>
<td>30</td>
<td>Moderate</td>
<td>Frontal behavior</td>
<td>Not done</td>
<td>Head tremor, facial myokimia, decreased vibration sense at ankles</td>
</tr>
<tr>
<td>IV-97</td>
<td>32</td>
<td>Childhood</td>
<td>Moderate</td>
<td>Attention deficit</td>
<td>95 (&gt;90)</td>
<td>Nystagmus, decreased vibration sense at ankles, facial myokimia</td>
</tr>
<tr>
<td>IV-99</td>
<td>32</td>
<td>Childhood</td>
<td>Mild</td>
<td>None</td>
<td>88‡ (&gt;90)</td>
<td>Mild head tremor, facial myokimia, nystagmus</td>
</tr>
<tr>
<td>IV-117</td>
<td>27</td>
<td>27</td>
<td>Mild</td>
<td>None</td>
<td>87‡ (&gt;90)</td>
<td>Nystagmus</td>
</tr>
<tr>
<td>IV-130</td>
<td>38</td>
<td>18</td>
<td>Moderate</td>
<td>Memory loss</td>
<td>99 (&gt;90)</td>
<td></td>
</tr>
<tr>
<td>IV-124</td>
<td>30</td>
<td>25</td>
<td>Mild</td>
<td>Difficulty in understanding</td>
<td>97 (&gt;90)</td>
<td></td>
</tr>
<tr>
<td>IV-136</td>
<td>36</td>
<td>30</td>
<td>Mild</td>
<td>None</td>
<td>Not done</td>
<td>Nystagmus</td>
</tr>
<tr>
<td>III-20†</td>
<td>63</td>
<td>About 60</td>
<td>Mild</td>
<td>Normal</td>
<td>Not done</td>
<td></td>
</tr>
<tr>
<td>III-27‡</td>
<td>60</td>
<td>?</td>
<td>None</td>
<td>Memory loss</td>
<td>91 (&gt;90)</td>
<td>Cognitive decline worsened after suicide attempt by hanging, parkinsonism</td>
</tr>
<tr>
<td>III-71†</td>
<td>54</td>
<td>?</td>
<td>Dementia</td>
<td>Not done</td>
<td>Not done</td>
<td></td>
</tr>
<tr>
<td>IV-106‡</td>
<td>31</td>
<td>Doubtful</td>
<td>Normal</td>
<td>Not done</td>
<td>Deviation from line while tandem walking</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: PM, progressive matrix; ?, unknown.
*Normal PM47 value according to the level of education.
†Abnormal score.
‡Probably affected member.

Figure 3. Surface electromyographic recordings from the extensors, flexors, and biceps of the right arm in patient IV-97 during an isometric contraction. Note the presence of brief muscle jerks or myoclonus (20- to 60-millisecond duration) in the top 2 recordings.

Five missense mutations in the cysteine-rich domain 2 region of the regulatory domain of protein kinase Cγ encoded by exon 4 were recently reported in 4 SCA14 families and an isolated case.2-8 In our study of a 4-generation French SCA14 family, we detected the first mutation (F643L) in the catalytic domain of the protein.

The pathogenicity of the F643L mutation is supported by the cosegregation of the mutation with the disease in the family and its absence in a large control population. Whether the pathologic effect of the mutation is due to haploinsufficiency or a toxic gain of function remains to be determined. Loss of protein kinase Cγ function in mouse and rat models was associated with milder phenotypes than in humans, which could be related to species differences.17,18 However, it is striking that this protein was down-regulated in a mouse model with SCA pathologic features caused by a polyglutamine expansion.19 In addition, the F643L mutation is situated in the carboxy terminus of the conserved C4 domain encoding the “turn motif” of the catalytic region of protein kinase Cγ, suggested to be implicated in the maturation and stability of the enzyme and in interactions with other proteins.20 Moreover, it affects an amino acid that is conserved in members of the protein kinase family and in orthologs of protein kinase Cγ in distantly related species. The mutation might alter the autophosphorylation at position T655. On the other hand, amino acid F643 of protein kinase Cγ corresponds to amino acid F327 in protein kinase A, which is part of a group of approximately 20 residues suspected to form a gate modulating the entry-exit of adenosine triphosphate into the hydrophobic active site.21,22

Surprisingly, the mutation reported in this article is located in the same domain of protein kinase Cγ as the R659S mutation described in families with retinitis pigmentosa.23 None of our patients, however, complained of decreased visual acuity. Protein kinase Cγ is abundantly expressed in Purkinje cells, suggesting a reason for the association of PRKCG mutations with cerebellar ataxia. This protein has not been detected in photoreceptors.23,24

Affected patients with the F643L mutation developed cerebellar signs resembling those of other SCA14...
families in that age at onset was variable, although the range was broader than previously reported (childhood to age 60 years). The progression of the disease was slow; all but 1 of our patients, who had a 37-year disease duration, still walked without assistance. We did not observe reduced penetrance as reported, but 4 carriers had mild signs (Table 2).

The fact that the F643L mutation is the first to be found in the catalytic domain of protein kinase Cγ might explain why the clinical features of the patients differed in some aspects from those previously described. Myoclonus, which was overt and axial in the Japanese family, affected the limbs and was clinically very mild in our French family, indicating that this sign might have been overlooked in other studies. In addition, we observed the presence of choreic movements, diplopia and limited gaze, and facial myokimia in our patients, considerably expanded the phenotype associated with SCA14 mutations and bringing it closer to the phenotype observed in SCA3. Parkinsonian rigidity, already reported in 1 case by van de Warrenburg et al, was also observed in the French family. Although carriers of 3 of the previously reported SCA14 mutations were described as having uncomplicated SCA including cerebellar ataxia with increased or decreased reflexes, additional features such as dystonia, deep sensory loss, and slow saccades have been reported.

The most consistent new feature in this family was mild to moderate cognitive impairment, suspected in 63% of the patients because of an abnormal result on the progressive matrix 47 examination along with a low IQ, reflecting deficient abstract thinking in 5 patients. This was confirmed by neuropsychological testing in 2 patients, 1 of whom had a complete pattern of executive dysfunction.

Our study has practical consequences because it demonstrates that causative mutations of the PRKCG gene may be located outside the regulatory region encoded by exon 4 and that mutation screening in SCA families should not be restricted to this region. It also shows that despite the frequently observed slow progression of cerebellar ataxia, the clinical spectrum associated with SCA14 is large and includes both complicated and uncomplicated forms of SCA. It also raises the possibility that subcortical deficits may be specifically associated with mutations in the catalytic domain of the enzyme. Finally, the identification of causative mutations in protein kinase Cγ suggests that this enzyme may participate in a signaling pathway that is affected in other SCAs as well and may open new avenues of research into the pathologic mechanisms involved in these disorders.

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