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

Giovanni Stevanin, PhD; Valérie Hahn, PhD; Ebba Lohmann, MD; Naima Bouslam, MS; Michel Gouttard, MD; Caroline Soumphonphakdy, BS; Marie-Laure Welter, MD; Elisabeth Ollagnon-Roman, MD, PhD; Arnaud Lemainque, PhD; Merle Ruberg, PhD; Alexis Brice, MD; Alexandra Durr, MD, PhD

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.

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.

Arch Neurol. 2004;61:1242-1248

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 protein.

METHODS

PATIENTS

We identified a 4-generation family of French ancestry (Figure 1). Twenty-four individuals were examined, and 23 were sampled with...
their informed and written consent. Results of routine diagnostic testing of the index patient (III-425) for CAG repeat expansions in the SCA1, SCA2, SCA3, SCA6, SCA7, SCA17, and dentatorubropallidoluysian atrophy (DRPLA) genes were negative. All family members were seen at their homes and were carefully examined following a standardized clinical protocol.

Bedside progressive matrix 47 was used in 8 affected members to evaluate their intellectual level and to test the hypothesis of mental deficiency. Progressive matrix 47 is intended to be a “cultural 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 neuropsychological 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 bedside. 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, D19S924, D19S921, D19S926, D19S571, and D19S924.
D19S927, D19S926, D19S418, and D19S605. Genotyping and linkage analyses were performed as described. Age-dependent penetrance was taken into account by assigning individuals to 1 of 5 liability classes with a maximal penetrance of 0.96 after age 55.

**MUTATION ANALYSIS**

All 18 exons of the *PRKCG* gene and their splice junctions were sequenced (BigDye Chemistry v3; Applied Biosystems, Foster City, Calif) in the index patient with primers and amplification conditions as described. We looked for the T-to-C substitution at nucleotide 22 of exon 18 using direct sequencing in all family members and primer extension in 163 French and 42 North African healthy controls using primer TTTTGTGGCCGCAGCGGCGAGAAC and annealing at 57°C (SNaPshot kit; Applied Biosystems). Sequences and extension products were resolved using an ABI Prism 3100 sequencer according to the recommendations of the manufacturer and analyzed using SeqScape or GeneScan/Genotyper software (Applied Biosystems).

**RESULTS**

Positive 2-point lod scores were obtained for 9 markers in the SCA14 candidate interval (Table 1). Lod scores higher than the significance threshold of 3 were obtained with markers D19S180, D19S921, D19S924, and D19S927. Consistent with the linkage analysis, a single haplotype segregated with the disease (Figure 1). The linked haplotype encompassed markers D19S571 to D19S926.

By direct sequencing of the *PRKCG* gene, we identified a single-nucleotide substitution of T to C at position 22 of exon 18. All affected members were heterozygotes and carried a TTT codon as well as the TTT wild-type codon (Figure 2A). This T-to-C missense mutation caused an F643L substitution in protein kinase C γ. The mutation completely cosegregated with the disease, and there was no evidence of reduced penetrance (Figure 1). This mutation was not found in 410 control chromosomes from 163 French and 42 North African individuals.

Multiple alignments of members of the protein kinase family and orthologs of protein kinase C γ 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 C γ 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 ataxia 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 contracture 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-425). Scores on the bedside progressive matrix test 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 already 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 test.
Phenotypes than in humans, which could be related to speciation in mouse and rat models was associated with milder pathology and mild ophthalmoparesis. 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 C\(\gamma\) function in mouse and rat models was associated with milder phenotypes than in humans, which could be related to species differences. However, it is striking that this protein was down-regulated in a mouse model with SCA pathologic features caused by a polyglutamine expansion. 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 C\(\gamma\), suggested to be implicated in the maturation and stability of the enzyme and in interactions with other proteins. More importantly, amino acid F643 of protein kinase C\(\gamma\) corresponds to amino acid F327 in protein kinase A, which responds to amino acid F643 of protein kinase C\(\gamma\) 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. 

Five missense mutations in the cysteine-rich domain 2 region of the regulatory domain of protein kinase C\(\gamma\) encoded by exon 4 were recently reported in 4 SCA14 families and an isolated case. 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 C\(\gamma\) function in mouse and rat models was associated with milder phenotypes than in humans, which could be related to species differences. However, it is striking that this protein was down-regulated in a mouse model with SCA pathologic features caused by a polyglutamine expansion. 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 C\(\gamma\), suggested to be implicated in the maturation and stability of the enzyme and in interactions with other proteins. More importantly, amino acid F643 of protein kinase C\(\gamma\) corresponds to amino acid F327 in protein kinase A, which responds to amino acid F643 of protein kinase C\(\gamma\) 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.

Surprisingly, the mutation reported in this article is located in the same domain of protein kinase C\(\gamma\) as the R659S mutation described in families with retinitis pigmentosa. None of our patients, however, complained of decreased visual acuity. Protein kinase C\(\gamma\) 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.

Affected patients with the F643L mutation developed cerebellar signs resembling those of other SCA14

<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, Mental confusion</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>Nystagmus</td>
</tr>
<tr>
<td>IV-124</td>
<td>30</td>
<td>25</td>
<td>Mild</td>
<td>Difficulty in understanding</td>
<td>87 (&gt;90)</td>
<td>Nystagmus</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>IV-20†</td>
<td>63</td>
<td>About 60</td>
<td>Mild</td>
<td>Normal</td>
<td>Not done</td>
<td>Nystagmus</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>Nystagmus, decreased vibration sense at ankles, facial myokimia</td>
</tr>
<tr>
<td>III-59‡</td>
<td>54</td>
<td>?</td>
<td>?</td>
<td>Dementia</td>
<td>Not done</td>
<td>Cognitive decline worsened after suicide attempt by hanging, parkinsonism</td>
</tr>
<tr>
<td>IV-106‡</td>
<td>31</td>
<td>?</td>
<td>Doubtful</td>
<td>Normal</td>
<td>Not done</td>
<td>Deviation from line while tandem walking</td>
</tr>
<tr>
<td>IV-99/H11022</td>
<td>Childrenhood</td>
<td>Mild</td>
<td>None</td>
<td>88† (&gt;90)</td>
<td>Mild head tremor, facial myokimia, nystagmus</td>
<td></td>
</tr>
<tr>
<td>IV-117/H11022</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/H11022</td>
<td>38</td>
<td>18</td>
<td>Moderate</td>
<td>Memory loss</td>
<td>99 (&gt;90)</td>
<td>Nystagmus</td>
</tr>
<tr>
<td>IV-124/H11022</td>
<td>30</td>
<td>25</td>
<td>Mild</td>
<td>Difficulty in understanding</td>
<td>87 (&gt;90)</td>
<td>Nystagmus</td>
</tr>
<tr>
<td>IV-136/H11022</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†/H9253</td>
<td>About 60</td>
<td>Mild</td>
<td>Normal</td>
<td>Not done</td>
<td>Nystagmus</td>
<td></td>
</tr>
<tr>
<td>III-27‡/H9253</td>
<td>60</td>
<td>?</td>
<td>None</td>
<td>Memory loss</td>
<td>91 (&gt;90)</td>
<td>Nystagmus, decreased vibration sense at ankles, facial myokimia</td>
</tr>
<tr>
<td>III-71‡/H9253</td>
<td>54</td>
<td>?</td>
<td>?</td>
<td>Dementia</td>
<td>Not done</td>
<td>Cognitive decline worsened after suicide attempt by hanging, parkinsonism</td>
</tr>
<tr>
<td>IV-106‡/H9253</td>
<td>31</td>
<td>?</td>
<td>Doubtful</td>
<td>Normal</td>
<td>Not done</td>
<td>Deviation from line while tandem walking</td>
</tr>
</tbody>
</table>

Abbreviations: PM, progressive matric; †, 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.
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 C γ 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 expanding 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 C γ 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.

Accepted for Publication: February 23, 2004.

Author Affiliations: INSERM U289, Institut Fédératif de Recherche en Neurosciences (Drs Stevanin, Lohmann, Ruberg, Brice, and Durr and Mss Bouslam and Soumpophonphakdy), and Fédération de Neurologie (Drs Hahn, Lohmann, Welter, and Brice) and Département de Géné-tique Cytogénétique et Embryologie (Drs Stevanin, Brice, and Durr); Assistance Publique Hôpitaux de Paris, Hôpital de la Salpêtrière, Paris, France; Service de Neurologie, Hôpital de Bourg-en-Bresse, Bourg-en-Bresse, France (Dr Gouttard); Consultation de Génétique, Hôpital de la Croix-Rousse, Lyon, France (Dr Ollagnon-Roman); and Centre National de Génoty wholey, Evry, France (Dr Lemainque).

Correspondence: Alexis Brice, MD, INSERM U289, Groupe Hospitalier Pitié-Salpêtrière, 47 Boulevard de l'Hôpital, 75651 Paris CEDEX 13, France (brice@ccr.jussieu.fr).

Author Contributions: Study concept and design: Stevanin, Brice, and Durr. Acquisition of data: Stevanin, Hahn, Lohmann, Welter, Ollagnon-Roman, Lemainque, Durr, Bouslam, and Soumpophonphakdy. Analysis and interpretation of data: Stevanin, Hahn, and Durr. Drafting of the manuscript: Stevanin, Ruberg, and Durr. Critical revision of the manuscript for important intellectual content: Hahn, Ruberg, and Brice. Obtained funding: Brice and Durr. Administrative, technical, and material support: Stevanin, Brice, and Durr. Study supervision: Stevanin and Durr.

Funding/Support: This study was supported by the...
ACKNOWLEDGMENTS: We thank the members of the family for their kind cooperation. The contributions of Cécile Jeannequin, Agnès Camuzat, Lucas Ravaux, Hamid Azzedine, and Thierry Maisinobe, MD, are gratefully acknowledged. We also thank the DNA and cell bank of the Institut Fédératif de Recherche (IFR-70) de Neurosciences (Paris) for technical assistance.

REFERENCES


13. Brice