Genetics of the SCA6 gene in a large family segregating an autosomal dominant "pure" cerebellar ataxia

Javier García-Planells, Ana Cuesta, Juan J Vílchez, Francisco Martínez, Félix Prieto, Francesc Palau

Abstract

Spinocerebellar ataxia type 6 (SCA6) is an autosomal dominant cerebellar degeneration caused by the expansion of a CAG trinucleotide repeat in the CACNA1A gene. Mutations in patients are characterised by expanded alleles of between 21 and 30 repeat units and by extreme gonadal stability when transmitted from parents to children. We have investigated the SCA6 mutation in a large Spanish kindred in which previously reported spinocerebellar SCA genes and loci had been excluded. We observed a 23 CAG repeat expanded allele in the 13 clinically affected subjects and in three out of 10 presymptomatic at risk subjects. Transmission of the mutant allele was stable in six parent to child pairs and in 29 meioses through the pedigree. Linkage analysis with the SCA6-CAG polymorphism and marker D19S221 confirmed the location of SCA6 on chromosome 19p13. The molecular findings in this large family confirm the expansion of the CAG repeat in the CACNA1A gene as the cause of SCA6 and the high meiotic stability of the repeat.

(*J Med Genet* 1999;**36**:148–151)

Keywords: spinocerebellar ataxia 6; meiotic stability; presymptomatic diagnosis

Unitat de Genètica, Hospital Universitari La Fe, Av Campanar 21, E-46009 Valencia, Spain
J García-Planells
A Cuesta
F Martínez
F Prieto
F Palau

Servei de Neurologia, Hospital Universitari La Fe, Av Campanar 21, E-46009 Valencia, Spain
J J Vílchez

Departament de Genètica, Facultat de Ciències Biològiques, Universitat de València, Dr Moliner 50, Burjassot, E-46100 Valencia, Spain
J García-Planells
F Palau

Correspondence to:
Dr Palau.

Received 5 January 1998
Revised version accepted for publication 10 July 1998

Spinocerebellar ataxia type 6 (SCA6) is the fifth autosomal dominant cerebellar ataxia associated with the expansion of an exonic CAG trinucleotide repeat encoding a polyglutamine tract.[1] The CAG repeat is located in exon 47 of the α_{1A} voltage dependent calcium channel unit gene (CACNA1A gene[2]), mapped on chromosome 19p13.[3] The SCA6 CAG repeat shows some differences from the other polyglutamine repeats associated with spinocerebellar ataxias, SCA1, SCA2, SCA3, and SCA7. (1) SCA6 mutant alleles range between 21 and 30 CAG repeat units,[1 4–6] a number that is within the normal range for SCA1, SCA2, SCA3, and SCA7, in which patients display a range between 36 and 121 repeats.[7 8] (2) SCA6 mutant alleles do not usually show meiotic instability during intergenerational transmission,[1 4–6] the opposite of what is usually observed in SCA1,[9 10] SCA2,[11] SCA3,[12] and SCA7.[13] In these disorders intergenerational CAG repeat expansion is frequently associated with anticipation.

We report the genetic aspects of a large family segregating a "pure" cerebellar ataxia associated with the SCA6 CAG expansion.

Family SRB (fig 1) is originally from Sueca, a Valencian town on the Mediterranean coast of Spain. Genealogical data were obtained directly from members of the family and from civil and Catholic church records. Clinical information was obtained from 57 members, 29 of whom were examined by a neurologist (JJV). Twenty-two were affected, 12 of whom were personally clinically examined. Mean age of onset was 49 years (range 31-60). The clinical picture was that of a "pure" cerebellar ataxia with no ophthalmoplegia, pyramidal signs, extrapyramidal signs, dementia, or peripheral neuropathy. MRI studies showed cerebellar atrophy especially of the vermis. Genotypic analysis of the SCA6 CAG repeat was performed in 13 patients, six males and seven females, from family SRB. All of them showed a common pure 23 CAG repeat as the disease associated expansion and a normal allele varying from 11 to 14 repeats (fig 2) (allele sizes in 120 normal Spanish chromosomes varied between seven and 13 repeat units, the most frequent allele repeats being 11, 12, and 13 units). None of the four unaffected subjects older than 60 years, the upper limit of the age at onset of the disease within the family (full penetrance), showed the 23 repeat allele, suggesting that this allele segregates with the disease as well.

To confirm the association of the CAG expansion with the disease phenotype, linkage analysis was performed.[14] Positive lod scores were obtained with the CAG repeat polymorphism (Zmax=6.89, θ=0.00). Pairwise analysis also showed a variable degree of linkage with markers D19S586, D19S221, and D19S714,[15] located in a telomere to centromere order (table 1). Conclusive lod scores in the absence of recombinants using the "only affected" method were also obtained with SCA6-CAG (Z=7.88 at θ=0.00) and D19S221 (Z=4.28 at θ=0.00). Four point linkage analysis was performed to determine the position of the disease phenotype in the fixed map D19S586-(0.08)-D19S221-(0.02)-D19S714. The results suggest that the most probable location of the gene lies between loci D19S221 and D19S714. No crossing over was observed between the SCA6-CAG repeat and marker D19S221. Haplotype analysis showed several recombination events (fig 1). The mutant expansion was associated with the minor D19S221-SCA6-D19S714 haplotype 4-23-4 in pedigree branch A, whereas it was associated with haplotype 4-23-1 in branch B. Thus, it can be inferred that a recombination event between the CAG repeat polymorphism

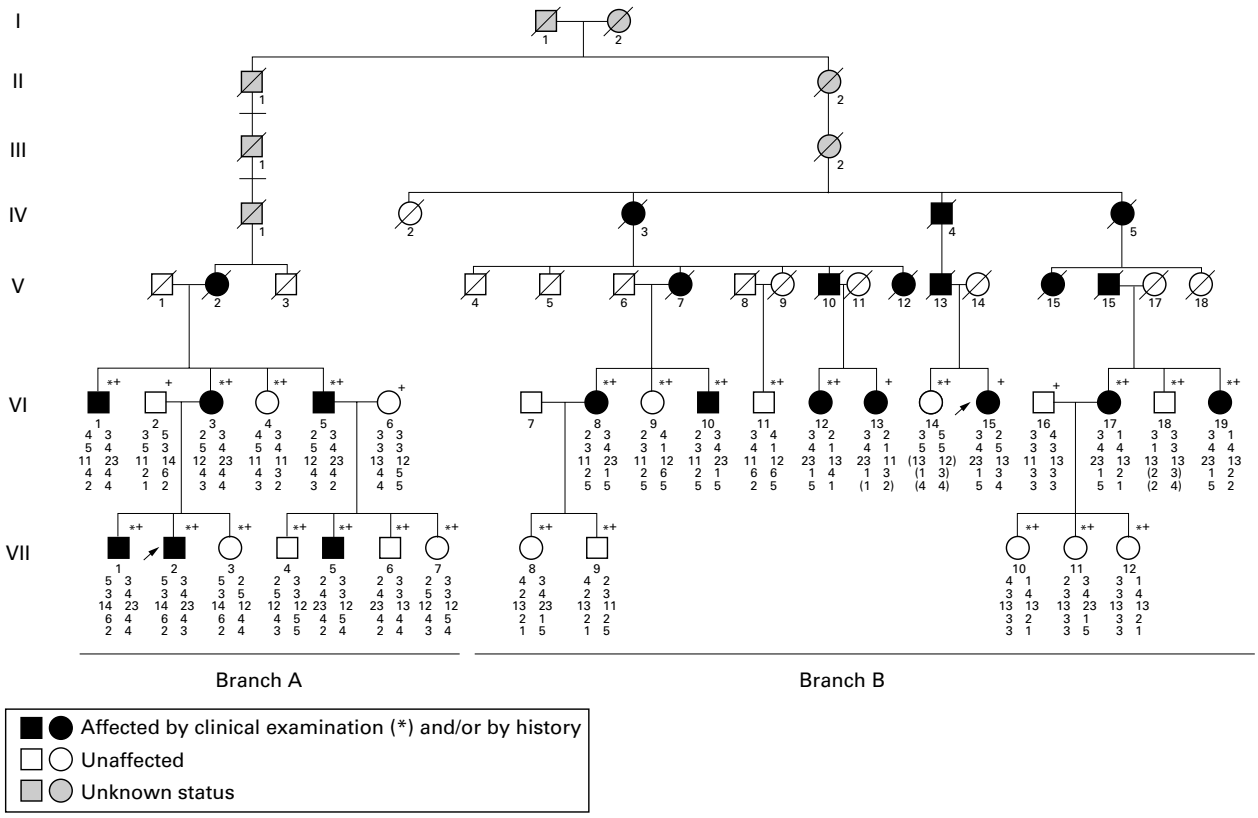

Figure 1 Partial pedigree of family SRB. An asterisk indicates that the subject has been clinically examined by one of us (JJV). After informed consent blood samples were taken from subjects indicated by +. The two probands (VI.15 and VII.2) are indicated by an arrow. Presymptomatic subjects studied are VI.18, VII.3, VII.4, VII.6, VII.7, VII.8, VII.9, VII.10, VII.11, and VII.12. Extended haplotypes pter-D19S586-D19S221-SCA6/CAG-D19S714-D19S433-cen are shown under each subject.

and the D19S714 locus has occurred in an unstudied ancestor.

Direct analysis of parent to child meiotic transmissions was only possible in six instances. In each case the expanded allele was transmitted with no size change (fig 2). However, we took advantage of the pedigree size; the two probands, VII.2 and VI.15, were separated by 11 meiotic events, and through the full pedigree a total of 29 meiotic events could be defined. As only the 23 CAG allele was observed in the 13 patients analysed, we postulate that the mutant allele has not undergone any variation when transmitted in previous generations.

Sixteen clinically unaffected subjects were considered at risk because they were under 60 and over 18 years old and they had an affected parent. After informed consent, 10 of them at 50% prior risk were sampled and presymptomatic diagnosis was performed. The disease associated 23 repeat allele was observed in three subjects, aged 27, 28, and 47 years. The other seven subjects carried two alleles within the normal range. Presymptomatic diagnosis was also confirmed by linkage analysis; no recombinant events were observed with marker D19S221, being the three positive cases associated with allele 4.

In this study we report the genetic analysis of a large family segregating an autosomal dominant cortical cerebellar atrophy. We have shown that a 23 CAG repeat allele in the CACNA1A gene is associated with the disease phenotype. This allele is within the expanded range between

Table 1 Pairwise lod scores between the disease and 19p13 markers

	Lod score at θ							
Locus	*0.00*	*0.01*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*Zmax*
D19S586	1.88	1.86	1.75	1.58	1.14	0.68	0.27	1.88
D19S221	3.29	3.23	2.97	2.63	1.89	1.13	0.46	3.29
SCA6/CAG	6.89	6.78	6.33	5.74	4.46	3.03	1.47	6.89
D19S714	−0.89	1.32	1.77	1.73	1.30	0.73	0.22	1.77
D19S433	−∞	−4.94	−2.24	−1.20	−0.39	−0.10	−0.001	−0.001

Pairwise linkage analysis was performed using the FASTLINK computer package version 2.1.[14] A gene frequency of 0.0001 and equal female and male recombination values were considered. Each asymptomatic at risk subject was assigned to one of the six age dependent liability classes obtained from family data according to the age at onset curve: class 1 = 0 (<40), class 2 = 0.12 (40–45), class 3 = 0.37 (46–50), class 4 = 0.70 (51–55), class 5 = 0.96 (56–60), and class 6 = 1.0 (>60). A disease penetrance of 99% was reached at 60 years.

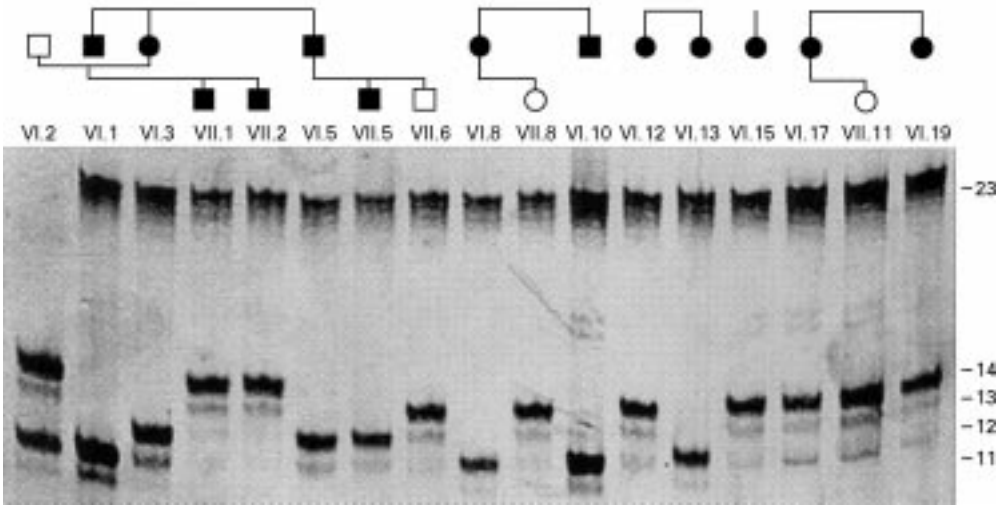

Figure 2 Analysis of the PCR amplified products containing the SCA6 CAG trinucleotide repeat in 13 patients (black symbols) and the three positive presymptomatic subjects (white symbols). Six parent to child transmissions are shown. Size alleles are represented on the right side. A normal subject in the first lane is carrying two normal 11 and 14 repeats. The rest of the subjects show an identical 23 repeat allele. SCA6 CAG trinucleotide repeat was analysed by the polymerase chain reaction (PCR) in a final volume of 50 µl containing 250 ng of DNA, 25 pmol each primer, S5-F1 and S5-R1[1], 250 µmol/l each dNTP, 6.7 mmol/l Tris-HCl (pH 8.8), 1.5 $MgCl_2$, 16 mmol/l $(NH_4)_2SO_4$, 0.1% Tween-20, and 5% DMSO. Three µl of the product were mixed with 2 µl of loading buffer and run on a 6% polyacrylamide/6.7 mol/l urea gel and alleles were visualised by silver staining.

21 and 30 repeats that defines the SCA6 type of adult onset cerebellar ataxias.[1 4–6]

SCA6 expansion shows high stability during meiotic transmission. More than 50 parent to child transmissions have been evaluated and only one unstable transmission has been reported.[5] This fact has been explained by the small size of the repeat block in mutant alleles, within the normal range for other neurodegenerative diseases associated with exonic CAG expansions.[7 8] Taking into account the presymptomatic positive members, we observed six parent child pairs and inferred 23 additional meiotic events in family SRB. The mutant allele size remained unchanged in all of them. This finding confirms the high intergenerational stability of the SCA6 mutation.

Different mutations in the CACNA1A gene are expressed as distinct disease phenotypes: missense mutations have been identified in familial hemiplegic migraine (FHM), mutations disrupting the reading frame have been observed in patients with episodic ataxia type 2 (EA2),[3] and CAG expansions at exon 47 are associated with cerebellar ataxia but also with EA2 in a family.[16] The clinical picture was homogeneous in the 13 patients examined from family SRB. Typical findings were progressive, unremitting gait and truncal ataxia and upper and lower dysmetria, dysarthria, and nystagmus. In some patients, hypotonia and hyperreflexia were observed. None of the patients showed the phenotype associated with EA2 or FMH.

Presymptomatic diagnosis in neurodegenerative disorders has ethical and psychological implications. The finding of the mutation in a family allows molecular diagnosis to be performed without involvement of other family members for linkage analysis.[10] We have performed presymptomatic diagnosis in 10 members of the family. In three cases the expanded mutant allele was found. Moreover, another seven at risk subjects under 18 years, five at 50%, and two at 25% prior risk, may still ask for presymptomatic diagnosis in the future. Anticipation has been observed in SCA6[6 17] but no relationship with CAG repeat instability has been shown. Family SRB provides a model for presymptomatic diagnosis of CAG expansion associated disorders with unknown genetic factors related to clinical anticipation.

We are very grateful to the family members for their kind collaboration and Dr G Stevanin and Dr A Brice for providing SCA6 expanded and normal controls. This work was supported by the Fondo de Investigación Sanitaria (FIS) grants 95/1824 and 98/1155, and Fundació "la Caixa" grant 97/134 (Fr P). JG-P is a FIS fellow.

1 Zhuchenko O, Bailey J, Bonnen P, *et al*. Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the α_{1A}-voltage-dependent calcium channel. *Nat Genet* 1997;**15**:62-9.
2 Lory P, Ophoff RA, Nahmias J. Towards a unified nomenclature describing voltage-gated calcium channel genes. *Hum Genet* 1997;**100**:149-50.
3 Ophoff RA, Terwindt GM, Vergouwe MN, *et al*. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca^{2+} channel gene CACNL1A4. *Cell* 1996;**87**:543-52.
4 Ishikawa K, Tanaka H, Saito M, *et al*. Japanese families with autosomal dominant pure cerebellar ataxia map to chromosome 19p13.1-p13.2 and are strongly associated with mild CAG expansions in the spinocerebellar ataxia type 6 gene in chromosome 19p13.1. *Am J Hum Genet* 1997;**61**:336-46.
5 Matsuyama Z, Kawasaki H, Maruyama H, *et al*. Molecular features of the CAG repeats of spinocerebellar ataxia 6 (SCA6). *Hum Mol Genet* 1997;**6**:1283-7.
6 Riess O, Shöls L, Böttger H, *et al*. SCA6 is caused by moderate CAG expansion in the α_{1A}-voltage-dependent calcium channel gene. *Hum Mol Genet* 1997;**6**:1289-93.
7 Ashley AT, Warren ST. Trinucleotide repeat expansion and human disease. *Annu Rev Genet* 1995;**29**:703-28.
8 Zoghbi HY. The expanding world of ataxins. *Nat Genet* 1996;**14**:237-8.
9 Jodice C, Malaspina P, Persichetti F, *et al*. Effect of trinuclotide repeat length and parental sex on phenotypic variation in spinocerebellar ataxia I. *Am J Hum Genet* 1994;**54**:959-65.
10 Matilla T, Volpini V, Genís D, *et al*. Presymptomatic analysis of spinocerebellar ataxia type 1 (SCA1) via the expansion of the SCA1 CAG-repeat in a large pedigree displaying

anticipation and parental male bias. *Hum Mol Genet* 1993; **2**:2123-8.
11 Geschwind DH, Perlman S, Figueroa C, Treiman LJ, Pulst SM. The prevalence and wide clinical spectrum of the spinocerebellar ataxia type 2 trinucleotide repeat in patients with autosomal dominant cerebellar ataxia. *Am J Hum Genet* 1997;**60**:842-50.
12 Maciel P, Gaspar C, DeStefano AL, *et al*. Correlation between CAG repeat length and clinical features in Machado-Joseph disease. *Am J Hum Genet* 1995;**57**:54-61.
13 David G, Abbas N, Stevanin G, *et al*. Cloning of the SCA7 gene reveals a highly unstable CAG repeat expansion. *Nat Genet* 1997;**17**:65-70.
14 Schäffer AA, Gupta SK, Shriram K, Cottingham CN. Avoiding recomputation in linkage analysis. *Hum Hered* 1994;**44**:225-37.
15 Dib C, Fauré S, Fizames C, *et al*. A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 1996;**380**:152-4.
16 Jodice C, Mantuano E, Veneziano L, *et al*. Episodic ataxia type 2 (EA2) and spinocerebellar ataxia type 6 (SCA6) due to CAG repeat expansion in the CACNL1A4 gene on chromosome 19p13. *Hum Mol Genet* 1997;**6**:1973-8.
17 Stevanin G, Dürr A, David G, *et al*. Clinical and molecular features of spinocerebellar ataxia type 6. *Neurology* 1997;**49**: 1243-6.