RESEARCH PAPER

Spinocerebellar ataxia type 36 exists in diverse populations and can be caused by a short hexanucleotide GGCCTG repeat expansion

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ABSTRACT

Objective Spinocerebellar ataxia 36 (SCA36) is an autosomal-dominant neurodegenerative disorder caused by a large (>650) hexanucleotide GGCCTG repeat expansion in the first intron of the *NOP56* gene. The aim of this study is to clarify the prevalence, clinical and genetic features of SCA36.

Methods The expansion was tested in 676 unrelated SCA index cases and 727 controls from France, Germany and Japan. Clinical and neuropathological features were investigated in available family members.

Results Normal alleles ranged between 5 and 14 hexanucleotide repeats. Expansions were detected in 12 families in France (prevalence: 1.9% of all French SCAs) including one family each with Spanish, Portuguese or Chinese ancestry, in five families in Japan (1.5% of all Japanese SCAs), but were absent in German patients. All the 17 SCA36 families shared one common haplotype for a 7.5 kb pairs region flanking the expansion. While 27 individuals had typically long expansions, three affected individuals harboured small hexanucleotide expansions of 25, 30 and 31 hexanucleotide repeat-units, demonstrating that such a small expansion could cause the disease. All patients showed slowly progressive cerebellar ataxia frequently accompanied by hearing and cognitive impairments, tremor, ptosis and reduced vibration sense, with the age at onset ranging between 39 and 65 years, and clinical features were indistinguishable between individuals with short and typically long expansions. Neuropathology in a presymptomatic case disclosed that Purkinje cells and hypoglossal neurons are affected.

Conclusions SCA36 is rare with a worldwide distribution. It can be caused by a short GGCCTG expansion and associates various extracerebellar symptoms.

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INTRODUCTION

Spinocerebellar ataxia (SCA) is a group of autosomal dominant neurodegenerative disorders clinically showing adult-onset progressive cerebellar ataxia often complicated with various extracerebellar signs or symptoms.[1–3] SCA36 is caused by a hexanucleotide GGCCTG repeat expansion in the first intron of the *NOP56* gene located on human chromosome 20.[4] In normal individuals, the repeat is a polymorphic and complex sequence containing slightly different hexanucleotides [AGCCTG], [GGCCTG] repeat and [CGCCTG], and the entire hexanucleotide repeats range in length between 3 and 14 units.[4 5] In contrast, this GGCCTG repeat was previously described as being highly expanded in SCA36 patients, reaching between 6.8 kb and ~18 kp pairs in Southern blot analysis, which was roughly estimated to be between 650 and 2500 repeat-units.[4–6] Given that the expansion lies in the first intron (ie, a non-coding region) of *NOP56*, SCA36 is regarded as one of the neurological diseases caused by non-coding microsatellite repeat expansion.[7–10] Clinically, individuals with SCA36 show progressive cerebellar ataxia complicated by motor neuron dysfunction, which is particularly prominent in their tongues.[4] So far, SCA36 has been described only in western regions of Japan[4 6] and the Costa da Morte region of Spain,[5] suggesting that there could be strong founder effects.

We conducted this study to clarify whether SCA36 is seen beyond these founder populations by investigating the SCA36 hexanucleotide repeat in a cohort of 676 families comprising mostly French, German and Japanese SCA index cases collected across these countries. Besides genetic analysis, we investigated clinical and neuropathological features in available family members to clarify how this disease differs from other SCAs.

MATERIALS AND METHODS

Patient and control enrollment

We enrolled 676 index patients with SCA comprising 270 French, 175 German and 231 Japanese families. Eighty-seven per cent of the French families were of French Caucasian origin, while the remaining 13% were the descendants of other ethnic populations. The German cohort was Caucasian, and the Japanese cases were of single ethnicity. These index patients were all excluded for previously known SCA mutations, which

accounted for about 50% of all SCAs in their cohorts.[1 11–13] All patients were collected from diverse areas of their respective countries: Pitié-Salpêtrière University Hospital and through the SPATAX network (France; http://spatax.wordpress.com/), Ludwig-Maximilians-University Munich (Germany), University of Tübingen (Germany) and Tokyo Medical and Dental University (Japan).[12] A half of the Japanese SCA cohort was collected from the Tokyo Metropolitan area, and thus the present Japanese cohort is based on a population distinct from the previous studies.[4 6] Seven hundred and twenty-seven healthy controls comprising 186 French, 304 German and 237 Japanese were also recruited.

From each participant, peripheral blood was drawn after obtaining informed consent following bioethics guidelines from each country. When index patients were found to be positive for *SCA36* expansions, other available family members were further investigated. The study conformed to the tenets of the Declaration of Helsinki, and was ethically approved by each institutional review board.

Mutation screening

The hexanucleotide repeat expansion was screened by both repeat-primed PCR and PCR-fragment analyses using primer pairs flanking the hexanucleotide repeat. The conditions of these reactions were determined using a DNA sample from an original patient with SCA36 (Pedigree B, II-3).[4] Primer sequences are shown online (see online supplementary table). The forward primer used for the repeat-primed PCR and PCR-fragment analyses (5′-TTTCGGCCTGCGTTCGGG-3′) was set between the 6 bp (base-pairs) CGGGCG insertion/deletion polymorphism (rs28970277)[5] and the GGCCTG repeat sequence, allowing us to detect only the hexanucleotide repeat complex containing the GGCCTG repeat. The numbers of repeat-units in selected individuals, both controls and SCA36 individuals with short expansions, were determined by cloning in pCR-TOPO (Invitrogen, California, USA) followed by sequencing analysis. PCR products were finally separated and analysed in an ABI PRISM 3100 (Applied Biosystems) and 2% agarose gel. Segregation of genotype and phenotype was checked in all available family members. For every individual discovered to harbour the expansion, the source of transmission was analysed by tracing and identifying the parent who had transmitted the mutation. Then the bias of parental gender in massive contraction was determined.

Southern blot analysis was carried out in 10 selected SCA36 samples using Avr II (New England Biolabs, Ipswich, Massachusetts, USA) and 0.8% agarose gel.[8] A 900 bp probe was synthesised from genomic DNA by PCR, as shown online (see online supplementary table). Using this probe, normal controls show a single 3.5 kb band.

Haplotype analysis

We investigated all available Japanese and French SCA36 individuals for the microsatellite markers *D20S906, D20S179, D20S113, D20S198, D20S842, AFMa049yd1, D20S181* and *D20S193*.[4] The genotypes were expressed with an allele numbering consistent with that of a previously published individual (II-3 in Pedigree 3).[4] In addition, eight informative single-nucleotide polymorphism (SNP) markers within *NOP56* were further tested to assess the founder haplotype. Six SNPs were in the 5′-untranslated region (5′-UTR) and two were in intron 3. No informative SNPs were found in intron 2. We could reconstruct SNP haplotypes in every SCA36 individual with long expansion by using a forward primer 'NOP56-5′UTR-F' in the 5′-UTR and a reverse primer 'NOP56 intron 3-R' in intron 3 (see online supplementary table). This was because the normal allele was specifically amplified in the presence of long hexanucleotide repeat expansions. Allele frequencies were investigated in Japanese (n=9) and French (n=10) control individuals.

Clinical investigations

Data on clinical features were collected by neurologists in charge of each participant with SCA36. The clinical features were retrospectively reviewed for three Japanese patients (Chubu #1, #2 and Chugoku) and four French participants (AAD-508 #5 and #7, AAD-681 #7 and #11), as they had been examined long before the identification of the *SCA36* mutation. The rest of the Japanese and all the French SCA36 patients were clinically re-analysed and summarised in one common format. The correlation between the clinical features and the length of the expansion was statistically analysed by Welch's test.

Neuropathological analysis

An autopsy was undertaken in the individual AAD-508 #7, who died at the age of 83. This participant did not show obvious neurological dysfunction. After formaldehyde fixation, multiple samples of the brain were embedded in paraffin and sectioned at a thickness of 5 μm. The spinal cord was not available. The sections were stained with H&E. Immunohistochemistry was performed using the following primary antibodies: antiubiquitin (Dako, rabbit polyclonal, diluted in phosphate buffered saline: 1/500), anti-TAR DNA-binding protein 43 (TDP-43) (Protein Tech Group, rabbit polyclonal, 1/2000), antifused in sarcoma (FUS) (Sigma, rabbit polyclonal, 10 μg/mL) and anti-p62 (MBL, mouse monoclonal, 1/300). Appropriate positive-control and negative-control specimens were also stained to check the staining conditions. The primary rabbit antibodies were detected with appropriate secondary antibodies using the XT Ultraview DAB system (Ventana, Oro Valley, Arizona, USA). The anti-p62 antibody was detected with the Vectastain ABC mouse IgG kit (Vector Laboratories, Burlingame, California, USA), and visualised by using Histofine Simple Stain DAB (Nichirei Bioscience, Tokyo, Japan) according to the manufacturer's protocol.

RESULTS

Molecular results

The PCR-fragment analysis revealed that normal repeats ranged from 5 to 14 complex hexanucleotide repeat-units in our cohorts of 727 control individuals. The distribution of normal repeats was basically identical among French, German and Japanese controls (see online supplementary figure 1). The most common allele carried 9 complex hexanucleotide repeat-units and the normal upper limit of our cohort was 14 complex hexanucleotide repeats as described by García-Murias *et al*.[5]

The repeat-primed PCR analysis disclosed GGCCTG repeat expansions in 17 index cases from 17 families (12 from the French cohort, including one individual each from Chinese, Portuguese and Spanish families living in France and five Japanese; figures 1 and 2A). Thus, SCA36 accounted for 1.9% of all SCAs of the French cohort and 1.5% of all Japanese SCAs, both including the families with already known mutations in other SCA genes. No expansions were found in the German cohort. Further investigations additionally revealed hexanucleotide expansions in a total of 30 individuals with the SCA36 GGCCTG expansion.

On the PCR-fragment analysis, normal participants often showed two different peaks (figure 2B). On the other hand,

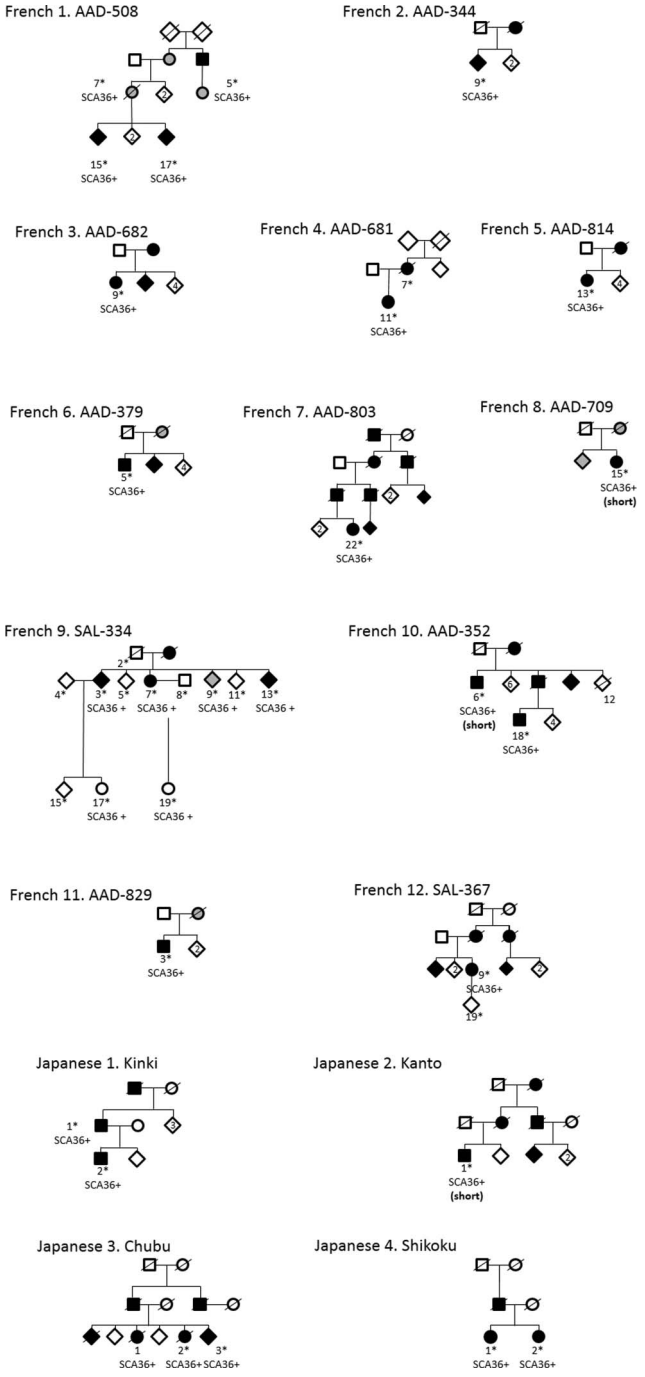

Figure 1 Twelve French and 4 Japanese SCA36 families. The French families consisted of 9 originally French kindred, one for each of Portuguese, Spanish and Chinese descendants (*the origins are not shown to protect from personal identification*). In the family AAD-352, an individual with short expansion (#6) and his nephew (#18) with typically long expansion are observed. Note that all three individuals with short expansions (French AAD-352 #6, French AAD-709 #15 and Japanese Kanto-1) had inherited the disease from their mothers. Owing to ethical reasons, pedigree structures are simplified and genders of some participants are anonymised. The pedigree information on the fifth Japanese family was not available and thus not shown in this figure. *Genetically tested; grey symbols: individuals without any complaints of neurological disturbances, or those not examined by the authors.

27 of the 30 SCA36 individuals showed single peaks within the normal range, suggesting that these patients harbour typically long expansions that hinder amplification by ordinary PCR (figure 2C). The remaining three (two French [AAD 709 #15 and AAD 352 #6] and one Japanese [Kanto-1]) showed two peaks on the PCR-fragment analysis, the larger one of which was always mosaic and exceeded the normal repeat range (figure 2D, E). Cloning and subsequent sequence analysis revealed that the longer repeats ranged between 25 and 31 complex hexanucleotide repeats containing 21 to 28 GGCCTG repeats: AAD 709 #15 (with 25 hexanucleotide repeats): 5′-[AGCCTG]-$(GGCCTG)_{21}$-[CGCCTG]$_3$-3′; AAD 352 #6 (with 31 hexanucleotide repeats): 5′-[AGCCTG]-$(GGCCTG)_{28}$-[CGCCTG]$_2$-3′; Kanto-1 (with 26 hexanucleotide repeats): 5′-[AGCCCG]-$(GGCCTG)_{23}$-[CGCCCG][CGCCTG]-3′. There was no affected individual without the expansion as far as the available DNA samples were tested, supporting the theory that the expansion segregated with the disease in all families examined. In addition, an individual with a typically long expansion and another affected individual with a short expansion were present in the same French family AAD352 (figure 1). Southern blot analysis disclosed that the typically long expansion ranged between 800 and 2000 repeats (figure 2F). In addition, a broad band was demonstrated from individuals harbouring short expansions, supporting the theory that the expansions in these three participants were indeed very small. All three individuals with short expansions (AAD-709 #15, AAD-352 #6 and Kanto-1) had received the disease from their mothers (figure 1). However, it was not certain whether the short expansions in the three individuals were from contractions, as we could not directly test the transmission in a parent-offspring basis.

As we found SCA36 families from diverse ethnic origins, we tested if SCA36 families harboured different haplotypes. Genotype data from all available participants are summarised in table 1. We found a common haplotype in all SCA36 individuals irrespective of the ethnicity (Japanese, Chinese, Portuguese and French) for the SNP markers flanking the GGCCTG repeat in *NOP56* and for *D20S198*, only 7 kb away from the repeat. On the other hand, haplotypes diverged significantly among the families when we tested distant microsatellite DNA markers. For example, *D20S842*, which showed a conserved allele among Spanish SCA36 families,[5] was discordant within the Japanese as well as the French SCA36 families. These data imply that SCA36, even with different ethnic origins, is associated with a common haplotype close to the repeat. However, the SNP haplotype common to all SCA36 families was also found in 26% of control chromosomes, and the frequency of allele 3 for *D20S198* was 28% in Japanese controls and 55% in French controls.

Clinical results

Clinical information was available from 28 individuals with the expansion: 20 French and 8 Japanese (table 2). Among these, three French individuals (AAD-508 #5, #7 and SAL-334 #9) did not complain of any neurological disturbances, and were thus excluded from the evaluation of clinical features. The age of onset defined by the time when patients started to notice cerebellar signs was 50.4±7.2 (SD) years, with a range of 39–65 years in the remaining 25 individuals. The cardinal clinical feature was progressive cerebellar ataxia in all 25 symptomatic individuals. Other frequent involvements included (1) hearing impairment (6 French, 1 Chinese, 1 Portuguese and 7 Japanese, a frequency of 60% among the 25 individuals), (2) postural tremor (5 French and 2 Japanese; 28%), (3) ptosis (2 French and 4 Japanese; 24%) and (4) cognitive impairment (3 French, 1 Spanish and 2 Japanese; 24%). Reduced vibration sense was seen in 13 (6 French, 1 Portuguese and 6 Japanese; 52%).

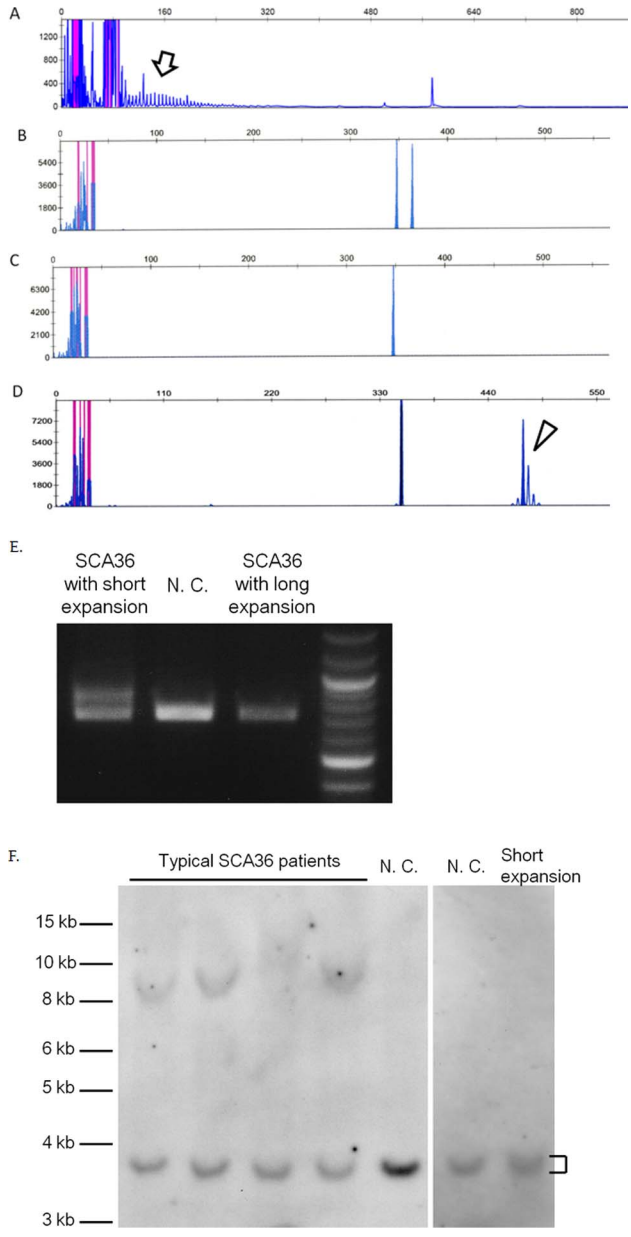

Figure 2 SCA36 hexanucleotide repeat expansions. (A) Typical attenuating peaks (arrow) of a hexanucleotide (GGCCTG) repeat expansion detected by the repeat-primed PCR analysis. (B) The PCR fragment analysis in a normal control showing two peaks corresponding to normal heterozygous alleles. (C) The same PCR fragment analysis on an SCA36 individual with a heterozygous long expansion showing only a single peak from a normal allele. The PCR reaction fails to amplify a typically long expansion, and thus the expansion is not detected by this method. (D) Demonstration of a short expansion (an arrowhead). Notice that the short expansion is mosaic, having six different peaks. (E) Short expansion in a Japanese individual (Kanto-1) (middle lane, upper band) is clearly seen in 2% agarose gel electrophoresis. The 100 base-pair size marker is separated in the right lane. Normal control (NC). (F) Southern blotting analysis detects typically long GGCCTG expansions in 4 SCA36 participants ('typical SCA36 patients') ranging between 8 and 15 kp pairs as well as short clear bands at approximately 3.5 kb. NC showed only a single band at 3.5 kb. The expanded allele in the third SCA36 sample from the right is faint, which may suggest a highly mosaic expansion. An individual with short expansion (Japanese Kanto-1) shows a blurred and slightly broader band of normal size.

Evidence of lower motor neuron signs, such as tongue atrophy and bulbar signs, was present in two French symptomatic participants and five Japanese individuals (28%). On ancillary investigations, eight patients (4 French and 4 Japanese) who were tested by peripheral nerve conduction study revealed reduced sensory action potentials (SNAPs) with an overall frequency of 32%, suggesting that peripheral nerves can be affected by this disease. Among the 14 participants examined by MRI, all showed cerebellar atrophy (100%), with some cases also exhibiting additional atrophy in the cerebrum (n=2; 14.3%) and in the brainstem (n=4; 28.6%).

Regarding the genotype-phenotype correlations, the average age at onset in the three individuals with short expansions (57.3 years) tended to be later than that of patients with long expansions (49.4 years, n=22). However, this difference was not significant (p=0.408, Welch's test).

Neuropathological findings

The neuropathological examination of patient AAD508#7 revealed diffuse cortical cerebellar atrophy. Histology demonstrated a mild Purkinje cell loss with Bergmann's gliosis (figure 3A, black arrow), distorted dendrites and atrophic cell body of the Purkinje cells (figure 3B, white arrows) and swellings of Purkinje cell axons called 'torpedo' (figure 3B, a black arrow). The hypoglossal nucleus (outlined by four arrows in figure 3C) showed a mild neuronal loss and gliosis (figure 3D). No alteration was evident in the cochlear and pontine nuclei. Ubiquitin, TDP43, FUS and p62 immunohistochemistry were all negative. Neuropathological changes compatible with Alzheimer's disease, such as numerous amyloid plaques of the Braak and Braak Stage IV,[14] were also seen.

DISCUSSION

The present study disclosed that SCA36 is not confined to the western region of Japan[4] and the Costa da Morte region of Spain,[5] but instead shows a global distribution, including individuals of French, Portuguese and Chinese ancestry. Nevertheless, the frequency of SCA36 was low in both Japanese (1.5%) and French (1.9%) SCA cohorts: this level was much lower than the prevalence in Okayama, Japan (3.6%)[4] and in Galicia, Spain (6.3%).[5] The absence of SCA36 patients in the present German cohort may suggest that SCA36 has an uneven distribution in Europe. Despite their diverse ethnicity, all the present SCA36 families showed a single haplotype for the tested SNPs in *NOP56* and the nearby microsatellite *D20S198*, whereas their haplotypes diverged for distant microsatellite markers including *D20S842*, which was conserved in the Spanish families in Galicia.[5] This suggests that SCA36 repeat expansions arose from one or a few founder chromosomes in ancient times. Compared to SCA10, another non-coding repeat expansion disorder with a strong founder effect particularly prevalent in Central and South American countries,[15] SCA36 repeat expansion might have arisen in a much ancient era. However, we noted that the founder SNP haplotype was still common with a frequency of 26% of our control French and Japanese chromosomes. Therefore, it is necessary to find markers that are tightly linked to SCA36 individuals and then to test them on larger numbers of SCA36 families in order to draw a more definitive conclusion on founder effects.

This study also disclosed that SCA36 shows Purkinje cell dropout and neuronal loss of the hypoglossal nucleus, consistent

Table 1 Haplotype information on Japanese and French SCA36 families

Distance from the mutation		−1130 kb	−660 kb	−600 kb	−429 bp	−348 bp	−289 bp	−272 bp	−170 bp	−161 bp	0	+ ″705 bp	+ ′801 bp	+7 kb	+52 kb	+460 kb	+640 kb	+780 kb
Marker in the chromosome 20p13	**telomere**	**D20S906**	**D20S179**	**D20S113**	**rs6083954**	**rs2073196**	**rs6083956**	**rs2073195**	**rs6115305**	**rs4815467**	**GGCCTG repeat**	**rs6050911**	**rs78833048**	**D20S198**	**D20S842**	**AFMa049yd1**	**D20S181**	**D20S193 centromere**
The common haplotype in the Okayama SCA36 family ref. 4 (#II-3)		2/3 not shared	2	3	T	G	T	G	G	C	long	C	G	3	3	3	1	4/5 not shared
Japanese SCA36 individuals																		
1	Kinki-1	2/3	2/1	3/3	T	G	T	G	G	C	long	C	G	3/8	3/13	3/3	1/1	4/7
2	Kanto-1	1/3	2/5	3/5	T	G	T	G	G	C	**short**	C	G	3/2	3/8	3/2	1/10	5/5
3	Chubu-2	2/2	1/1	3/6	T	G	T	G	G	C	long	C	G	3/3	3/1	3/2	1/6	3/7
4	Shikoku-1	2/2	1/8	1/2	T	G	T	G	G	C	long	C	G	3/3	4/5	2/3	1/4	7/8
French SCA36 families																		
	AAD508–5	2/4	1/1	1/1	T	G	T	G	G	C	long	C	G	3/3	4/5	3/7	3/11	8/7
1	AAD508-7	2/2	1/1	1/1	T	G	T	G	G	C	long	C	G	3/7	4/4	3/6	3/11	8/6
	AAD508-15	2/2	1/1	1/1	T	G	T	G	G	C	long	C	G	3/4	4/2	3/2	3/2	8/4
	AAD508-17	2/2	1/1	1/1	T	G	T	G	G	C	long	C	G	3/7	4/4	3/6	3/3	8/5
2	AAD344-9	2/5	1/1	1/1	NA	NA	NA	NA	NA	NA	long	NA	NA	3/7	4/12	3/2	3/4	8/5
3	AAD682-9	2/3	1/1	1/4	NA	NA	NA	NA	NA	NA	long	NA	NA	3/3	4/4	3/6	3/11	8/7
4	AAD681-11	2/3	1/1	1/1	NA	NA	NA	NA	NA	NA	long	NA	NA	3/2	4/5	3/3	3/11	5/9
5	AAD814-13	3/5	1/1	1/5	NA	NA	NA	NA	NA	NA	long	NA	NA	3/7	4/2	3/3	3/3	5/6
6	AAD379-5	2/8	1/1	1/1	NA	NA	NA	NA	NA	NA	long	NA	NA	3/2	4/5	4/6	3/3	5/6
7	AAD803-22	2/3	1/6	1/6	NA	NA	NA	NA	NA	NA	long	NA	NA	3/2	6/9	4/6	3/3	5/5
8	AAD709-15	3/4	1/6	1/5	NA	NA	NA	NA	NA	NA	**short**	NA	NA	3/3	6/6	4/2	3/3	5/7
	SAL334-3	5/2	1/1	1/1	T	G	T	G	G	C	long	C	G	3/8	6/8	7/2	11/1	7/5
	SAL334-7	5/3	1/2	1/1	NA	NA	NA	NA	NA	NA	long	NA	NA	3/3	6/4	7/3	11/3	7/5
	SAL334-9	5/3	1/2	1/1	NA	NA	NA	NA	NA	NA	long	NA	NA	3/3	6/4	7/3	11/3	7/5
9	SAL334-13	5/2	1/1	1/1	NA	NA	NA	NA	NA	NA	long	NA	NA	3/8	6/8	7/2	11/1	7/5
	SAL334-17	5/3	1/2	1/1	NA	NA	NA	NA	NA	NA	long	NA	NA	3/3	6/2	7/6	11/11	7/6
	SAL334-19	5/3	1/1	1/5	NA	NA	NA	NA	NA	NA	long	NA	NA	3/3	6/8	7/6	11/3	7/7
10	AAD352-6	3/4	1/6	5/4	T	G	T	G	G	C	**short**	C	G	3/3	8/4	3/4	1/3	4/10
	AAD352-18	3/4	1/6	5/3	T	G	T	G	G	C	long	C	G	3/7	8/5	3/6	1/3	5/11
11	AAD829-3	2/2	1/1	3/3	T	G	T	G	G	C	long	C	G	3/6	5/6	2/2	3/5	3/8

The location of the GGCCTG repeat is shaded in light blue. The Japanese founder haplotype in the Okayama family (see reference #4) and its extent shared in other families are shown by bold boxes. The haplotype shared by Japanese families is coloured in grey. The French haplotypes are coloured in yellow (the most common one), green and purple. The allele frequency of '3' in D20S198 is 28% in the Japanese (+) control population and 55% in the French (*) control population.
NA stands for single-nucleotide polymorphic markers that have not been analysed.
The 5th Japanese family and the 12th French SCA36 family (SAL-367) were not able to assess their haplotype and therefore are not listed in table 1.
SCA36, spinocerebellar ataxia 36.

Table 2 Clinical features of the 20 French and 8 Japanese individuals with SCA36 hexanucleotide repeat expansions

Family code French families	ID	Approximate repeat length	sex	Age at examination years (onset)	Sign at onset	stage	SARA (examined age)	Cerebellar gait (age when confirmed)	Dysarthria	Reflexes in lower limbs	Vibration at ankles	Ocular findings	Hearing impairment	Postural tremor	Ptosis	Cognitive impairment	Lower motor neuron sign	Electrophysiology (examined age)	Other features
AAD-508	5	NA	F	80	No complaint	0	NA	Absent	Absent	Increased	Normal	Limited gaze	No	No	Yes	No	Not evident	NA	
	7	NA	F	83	No complaint	0	NA	Absent	Absent	Normal	Normal	Normal	No	No	No	No	Not evident	NA	Autopsy Case
	15	1080	M	56 (52)	Unknown	4	NA	Two canes	Absent	Increased	Normal	Normal	Yes	No	No	No	Not evident	Mild sensory motor neuropathy (54)	
	17	NA	F	62 (59)	Instability dysarthria	3	NA	Cannot run	Absent	Increased	Decreased	Limited gaze	Deafness	No	No	No	Not evident	NA	
AAD-344	9	NA	M	63 (53)	Instability	4	NA	Walking with aids (62)	Moderate	Abolished	Decreased	Diplopia saccadic pursuit nystagmus	Deafness	No	No	No	Not evident	NA	
AAD-682	9	2000	F	54 (40)	Cramps Instability	3	NA	Cannot run	Mild	Increased	Normal	Saccadic pursuit, slow saccades	No	Yes	No	No	Not evident	NA	
AAD-681	7	NA	F	79 (42)	Instability	Unknown	NA	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	NA	NA	NA	Unknown	NA	
	11	1500	F	59 (56)	Instability	0	NA	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	NA	N.A	Yes	Unknown	NA	
AAD-814	13	NA	F	53 (47)	Instability decreased hearing	3	25 (57)	Cannot run	Mild	Increased	Decreased	Hypometric saccades ptosis	Yes	No	Yes	Yes	Not evident	Bilateral carpal tunnel syndrome (53)	
AAD-379	5	NA	M	54 (51)	Instability dysarthria	2	NA	Mild	Mild	Increased	Normal	Saccadic pursuit	Yes	No	No	Yes	Not evident	NA	
AAD-803	22	1000	F	60 (59)	Instability	4	18 (60)	Walking with aids (53)	Moderate	Increased	Decreased	Saccadic pursuit	Yes	Yes	Yes	No	Not evident	NA	
AAD-709	15	21	F	54 (44)	Diplopia	3	NA	Cannot run (52)	Mild	Abolished	Decreased	Strabismus	Deafness	Yes	No	No	Fasciculation in tongue	Mild sensory neuropathy (55)	
SAL-334	3	NA	M	63 (50)	Instability	3	13/14 (ICARS posture and gait score) (73)	Cannot run	Mild	Increased	Normal	Hypermetric saccades	No	No	No	No	Not evident	Mild sensory motor neuropathy (61)	Rigidity
	7	NA	F	59 (52)	Instability	2	NA	Mild gait	Absent	Abolished	Normal	Normal	No	No	No	No	Not evident	NA	
	9	NA	F	53	No complaint	1	NA	Mild	Absent	Increased	Normal	Normal	No	Yes	No	No	Not evident	NA	
	13	NA	M	49 (46)	Tremor	1	NA	Mild	Absent	Increased	Decreased	Normal	No	Yes	No	No	Not evident	NA	Cramps
AAD-352	6	28	M	73 (63)	Instability	2	NA	Mild	Severe	Increased	Decreased	Normal	No	No	No	No	Not evident	NA	
	18	NA	M	50 (43)	Instability	4	NA	Walking with aids (47)	Severe	Normal	Normal	Nystagmus	No	Yes	No	No	Not evident	NA	
AAD-829	3	NA	M	62 (53)	Instability diplopia	Unknown	10.5 (62)	Unknown	Mild	Increased	Unknown	Hypometric saccades, saccadic pursuit	Yes	No	No	No	Not evident	NA	
SAL-367	9	NA	F	59 (40)	Abnormal behaviour	5	NA	Wheel chair	Severe	Abolished	Normal	Limited gaze optic pallor	No	No	No	Dementia hallucination	Not evident	NA	

Continued

Table 2 Continued

Family code Japanese families	ID	Approximate repeat length	sex	Age	Sign at onset	stage	SARA	Cerebellar gait	Dysarthria	Reflexes in lower limbs	Vibration at ankles	Ocular findings	Hearing impairment	Postural tremor	Ptosis	Cognitive impairment	Lower motor neuron sign	Electrophysiology	Other features
Shikoku	1	850	F	58 (54)	Instability	3	15 (59)	Walking with aids (57)	Mild	Increased	Normal	Saccadic pursuit diplopia	Yes	No	Yes	noticed at age 57	Mild tongue atrophy	(1) Neurogenic change in EMG, (2) Mild sensory axonopathy in median nerve	
	2	NA	F	64 (49)	Instability	6	20 (64)	Unable to walk (62)	Moderate	Normal	Normal	Saccadic pursuit	Yes	No	Yes	noticed at age 63	Mild tongue atrophy	NA	
Kinki	1	1250	M	67 (42)	Instability	6	18 (68)	Cannot stand	Moderate	Increased	Decreased	Facial weakness	Yes	No	Yes	No	Fasciculation and atrophy in tongue and hypothenar region	(1) Neurogenic change in EMG, (2) motor and sensory axonopathy in median nerve (68)	
	2	NA	M	43 (39)	Instability	5	11 (44)	Walking with canes	Mild	Increased	Decreased	Normal	No	No	No	No	Not evident	NA	
Kanto	1	27	M	66 (65)	Dizziness	2	8 (65)	Very mild ataxia	Mild	Increased	Decreased	Slightly saccadic pursuit	Yes	No	No	No	Not evident	NA	
Chubu	1	NA	F	65 (57)	Instability	4	NA	One cane (65)	Moderate	Decreased	Decreased	Hypometric saccades, saccadic pursuit	Deafness	No	NA	NA	Tongue fasciculation and atrophy	NA	
	2	1000	F	57 (54)	Instability	3	NA	Cannot run (54)	Moderate	Increased	Decreased	Hypometric saccades, saccadic pursuit	Yes	Yes	NA	noticed at age 62	Mild bulbar sign with dysphagia	Sensory axonopathy in median and ulnar nerves	Dysesthesia in limbs (66), forced laughing
	3	NA	M	68 (58)	Instability	2	10 (68)	One cane (68)	Mild	Decreased	Decreased	Ptosis, Gaze nystagmus	Yes	Yes	NA	NA	Not evident	Sensory axonopathy in medial plantar nerve	

The three participants without complaints (AAD-508 #5, #7 and SAL-334 #9) are shaded and are excluded for calculating frequencies of neurological signs.
Stage=0: normal; 1: no functional handicap but signs at examination; 2: mild, able to run, walking unlimited; 3: moderate, unable to run, limited walking without aid; 4: severe, walking with one stick; 5: walking with two sticks; 6: unable to walk, requiring wheelchair; 7: confined to bed.
The confirmed age is the age at examination unless described specifically in parentheses.
NA, not assessed.
ICARS, International Cooperative Ataxia Rating Scale; EMG, electromyogram.

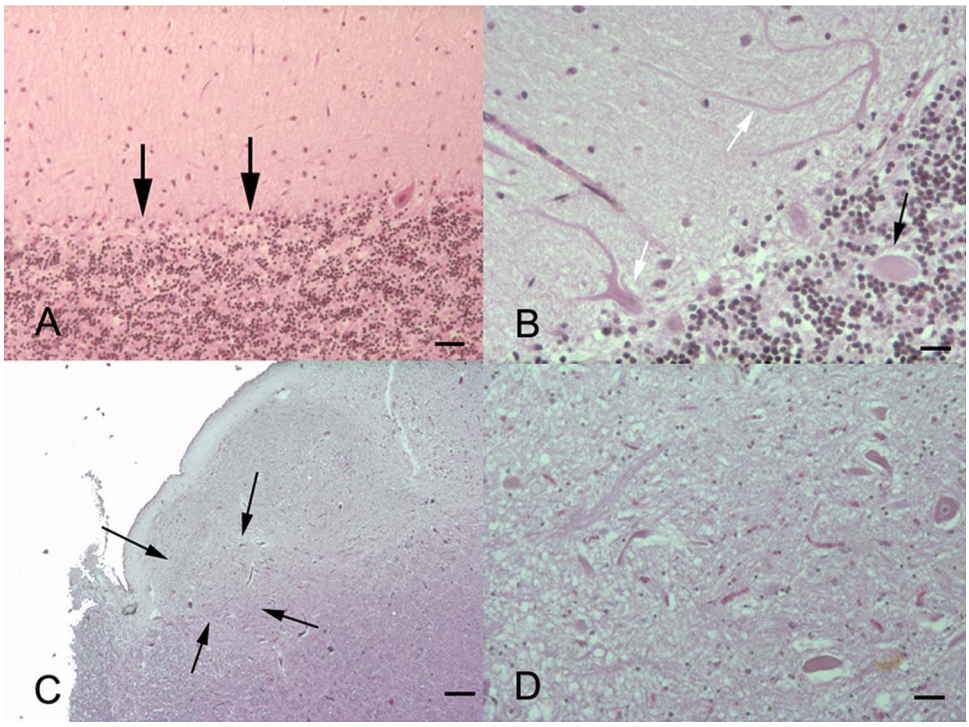

Figure 3 Neuropathology of an asymptomatic 83-year-old female carrier. The cerebellum (A and B) and the tegmentum of the medulla oblongata (C and D) stained with H&E. (A) A mild depletion of Purkinje cells is seen. The black arrows indicate Bergmann's gliosis in a region where Purkinje cells are depleted. (B) A higher magnification view showing dendrites and the cell body of Purkinje cells (white arrows). A torpedo in the granule cell layer is indicated by a black arrow. (C) A low magnification view showing the hypoglossal nucleus (surrounded by black arrows). (D) A high magnification view of the hypoglossal nucleus. Motor neurons are reduced in number. Size bar: A and C=200 μm; B and D=20 μm.

with the first autopsy case of SCA36.[16] What is important from the present case is that the patient presented evidence of neurodegeneration in the Purkinje cell and hypoglossal nucleus without showing obvious neurological signs. It would become important in future to accumulate knowledge on the extent of neurodegeneration in a pre-manifesting stage for deciding when to administer fundamental treatment. Another important finding is that there were no obvious neuronal cytoplasmic inclusions (NCIs) or nuclear inclusions (NNIs) characterising the neuropathology of amyotrophic lateral sclerosis (ALS)[17] as far as we examined by ubiquitin, TDP43, FUS and p62 immunohistochemistry. It has been shown that the frontotemporal lobar dementia and ALS (FTD-ALS) caused by *C9orf72* hexanucleotide GGGGCC repeat expansion shows the accumulation of ubiquitin-immunoreactive and p62-immunoreactive NCIs, not only in the anterior horn cells in the spinal cord, but also in the cerebellar granule cells.[18] Large ubiquitin-positive NIIs seen in fragile X-associated tremor/ataxia syndrome (FXTAS)[19] were also not detected in this case. These findings may suggest that SCA36 pathological features are distinct from those of non-coding repeat expansion disorders. Future investigations should search for abnormal RNA structures ('RNA foci') in these affected neuronal cells, as have been detected in other related diseases caused by repeat expansions in introns: myotonic dystrophy type 2 (DM2),[7] SCA10[20] and SCA31.[8 21]

The most intriguing finding in this study is that the short hexanucleotide expansion of 25–31 repeat-units, slightly exceeding the upper limit in controls (14 repeat-units), is seen in some affected individuals. This indicates that such short expansions could cause the disease, although we cannot exclude a possibility that repeat expansions are much longer in the nervous system than in the blood. As the number of patients with short expansions was very small in the present cohort, the difference in the age of onset between those with short and typically long expansions did not reach a significant level. Further studies including larger numbers of individuals with short expansions are thus necessary. In all the three individuals with short expansions, it was conceivable that the disease had been transmitted from their mothers, as in a previous description.[5] However, we could not directly investigate parent-offspring pairs to confirm maternal bias for repeat contraction. If this was the case, SCA36 would be another example of such contraction after DM1[22] and a mother-to-daughter repeat contraction in Huntington's disease.[23 24] Precise knowledge of such parental bias is important for clinical situations, such as genetic counselling, as well as for determining the mechanism of repeat expansion.

Interestingly, short GGGGCC repeat expansion in *C9orf72* has been identified in patients with FTD[25] and Parkinson's disease,[26] suggesting a common feature in *C9orf72* and *NOP56* repeat expansions. What seems distinct from the *C9orf72*-associated neurological diseases is the fact that patients with short expansions in *NOP56* do not obviously differ in their phenotypes from those with long expansions, while patients associated with expansions in *C9orf72* show a wide spectrum of clinical symptoms depending on the length of the expansion.[9 10 25 26] As such, how can we explain that the short and long GGCCTG expansions in *NOP56* cause similar phenotypes? We investigated whether a short expansion of 21 GGCCTG repeats shows any difference in the likelihood of forming hairpin structures compared with the normal 14 repeats using a computer prediction algorithm.[27] The 21-repeat allele was indeed predicted to form a double-stranded hairpin more efficiently than the 14-repeat allele, but with only a small difference. We need to recognise that the threshold of being a

pathogenic repeat is much lower than was previously being thought. It is also possible that the mechanism underlying SCA36 is similar to the RAN translation mechanism proposed in DM1, SCA8[28] and recently confirmed in *C9orf72*[29 30]-associated FTD/ALS and FXTAS.[31]

In summary, SCA36 is present in various ethnic backgrounds, by the sharing of a common linked haplotype. Clinical variations with regard to lower motor neuron involvement, ptosis, hearing and cognitive impairments, tremor and reduced vibration sense suggest that this disease should be tested in all cases with progressive late-onset cerebellar ataxia. To do this, the PCR-fragment analysis as well as the repeat-primed PCR test are needed. Knowledge that a short expansion of at least 25 hexanucleotide repeats containing a stretch of 21 GGCCTG can cause the disease is an important source of information regarding genetic diagnosis and for future deciphering of SCA36 pathogenesis.

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Contributors MO examined the patients, investigated the DNA samples and wrote the manuscript. GS managed the French DNA samples, analysed the genetic data and wrote the manuscript. MS examined the Tübingen-based patients, collected the DNA, selected the patients for screening and wrote the manuscript. M-L, Monin, AV, VD, YK and YI examined the patients. CD performed the neuropathological study and partially wrote the manuscript. NS examined the patients and investigated the DNA samples with MO. NS performed the neuropathological study. CT managed the DNA samples and analysed the genetic data. H-EW collected the Munich-based German control samples with TI. TI collected the Munich-based German control samples with H-EW. JH analysed the large share of German DNA samples. HM co-ordinated the study. LS examined the Tübingen-based patients and collected the DNA. TK collected the German samples in Munich and wrote the manuscript. AB collected the French samples with GS and AD, coordinated the whole study and wrote the manuscript. KI collected the Japanese samples, examined the patients, analysed the DNA samples with MO and NS, coordinated the whole study and wrote the manuscript. AD examined the patients, collected and arranged the French samples and wrote the manuscript. All authors have read and approved the content of the manuscript.

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REFERENCES

1 Dürr A. Autosomal dominant cerebellar ataxias: polyglutamine expansions and beyond. *Lancet Neurol* 2012;9:885–94.
2 Lee YC, Dürr A, Majczenko K, *et al*. Mutations in KCND3 cause spinocerebellar ataxia type 22. *Ann Neurol* 2012;72:859–69.
3 Serrano-Munuera C, Corral-Juan M, Stevanin G, *et al*. New subtype of spinocerebellar ataxia with altered vertical eye movements mapping to chromosome 1p32. *JAMA Neurol* 2013;70:764–71.
4 Kobayashi H, Abe K, Matsuura T, *et al*. Expansion of intronic GGCCTG hexanucleotide repeat in *NOP56* causes SCA36, a type of spinocerebellar ataxia accompanied by motor neuron involvement. *Am J Hum Genet* 2011;89:121–30.
5 García-Murias M, Quintáns B, Arias M, *et al*. 'Costa da Morte' ataxia is spinocerebellar ataxia 36: clinical and genetic characterization. *Brain* 2012;135:1423–35.
6 Sugihara K, Maruyama H, Morino H, *et al*. The clinical characteristics of spinocerebellar ataxia 36: a study of 2121 Japanese ataxia patients. *Mov Dis* 2012;27:1158–63.
7 Wojciechowska M, Krzyzosiak WJ. Cellular toxicity of expanded RNA repeats: focus on RNA foci. *Hum Mol Genet* 2011;20:3811–21.
8 Sato H, Amino T, Kobayashi K, *et al*. Spinocerebellar ataxia type 31 is associated with "inserted" penta-nuclotide repeats containing $(TGGAA)_n$. *Am J Hum Genet* 2009;85:544–57.
9 DeJesus-Hernandez M, Mackenzie IR, Boeve BF, *et al*. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9orf72 causes chromosome 9p-linked FTD and ALS. *Neuron.* 2011;72:245–56.
10 Renton AE, Majounie E, Waite A, *et al*. A hexanucleotide repeat expansion in C9orf72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 2011;72:257–68.
11 Ishikawa K, Dürr A, Klopstock T, *et al*. Pentanucleotide repeats at the spinocerebellar ataxia type 31 (SCA31) locus in Caucasians. *Neurology* 2011;77:1853–5.
12 Obayashi M, Ishikawa K, Izumi Y, *et al*. Prevalence of inositol 1, 4, 5-triphosphate receptor type 1 gene deletion, the mutation for spinocerebellar ataxia type 15, in Japan screened by gene dosage. *J Hum Genet* 2012;57:202–6.
13 Synofzik M, Beetz C, Bauer C, *et al*. Spinocerebellar ataxia type 15: diagnostic assessment, frequency, and phenotypic features. *J Med Genet* 2011;48:407–12.
14 Braak H and Braak E. Diagnostic criteria for neuropathologic assessment of Alzheimer's disease. *Neurobiol Aging* 1997;18(4 Suppl):S85–8.
15 Almeida T, Alonso I, Martins S, *et al*. Ancestral origin of the ATTCT repeat expansion in spinocerebellar ataxia type 10 (SCA10). *PLoS ONE* 2009;4:e4553.
16 Ikeda Y, Ohta Y, Kobayashi H, *et al*. Clinical features of SCA36. A novel spinocerebellar ataxia with motor neuron involvement (Asidan). *Neurology* 2012;79:333–41.
17 Al-Chalabi A, Jones A, Troakes C, *et al*. The genetics and neuropathology of amyotrophic lateral sclerosis. *Acta Neuropathol* 2012;124:339–52.
18 Boeve BF, Boylan KB, Graff-Radford NR, *et al*. Characterization of frontotemporal dementia and/or amyotrophic lateral sclerosis associated with the GGGGCC expansion in C9orf72. *Brain* 2012;135:765–83.
19 Greco CM, Berman RF, Martin RM, *et al*. Neuropathology of fragile X-associated tremor/ataxia syndrome (FXTAS). *Brain* 2006;129:243–55.
20 White MC, Gao R, Xu W, *et al*. Inactivation of hnRNP K by expanded intronic AUUCU repeat induces apoptosis via translocation of PKC[delta] to mitochondria in spinocerebellar ataxia 10. *PLoS Genet* 2010;6:e1000984.
21 Niimi Y, Takahashi M, Sugawara E, *et al*. Abnormal RNA structures (RNA foci) containing a penta-nucleotide repeat $(UGGAA)_n$ in the Purkinje cell nucleus is associated with spinocerebellar ataxia type 31 pathogenesis. *Neuropathology* 2013;33:600–11.
22 Ashizawa T, Anvret M, Baiqet M, *et al*. Characteristics of intergenerational contractions of the CTG repeat in myotonic dystrophy. *Am J Hum Genet* 1994;54:414–23.
23 Wheeler VC, Persichetti F, McNeil SM, *et al*. Factors associated with *HD* CAG repeat instability in Huntington disease. *J Med Genet* 2007;44:695–701.
24 Aziz NA, van Belzen MJ, Coops ID, *et al*. Parent-of-origin differences of mutant HTT CAG repeat instability in Huntington's disease. *Eur J Med Genet* 2011;54: e413–18.
25 Gómez-Tortosa E, Gallego J, Guerrero-López R, *et al*. C9orf72 hexanucleotide expansions of 20–22 repeats are associated with frontotemporal deterioration. *Neurology* 2013;80:366–70.
26 Lesage S, Le Ber I, Condroyer C, *et al*. *C9orf72* repeat expansions are a rare genetic cause of parkinsonism. *Brain* 2013;136:385–91.
27 Parisien M, Major F. The MC-Fold and MC-Sym pipeline infers RNA structure from sequence data. *Nature* 2008;452:51–5.
28 Zu T, Gibbens B, Doty NS. *et al*. Non-ATG-initiated translation directed by microsatellite expansions. *Proc Natl Acad Sci USA* 2011;108:260–5.
29 Mori K, Weng SM, Arzberger T, *et al*. The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. *Science* 2013;339:1335–8.
30 Ash PEA, Bieniek KF, Gendron TF, *et al*. Unconventional translation of C9orf72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. *Neuron* 2013;77:639–46.
31 Todd PK, Oh SY, Krans A, *et al*. CGG repeat-associated translation mediates neurodegeneration in fragile X tremor ataxia syndrome. *Neuron* 2013;78:440–55.

Spinocerebellar ataxia type 36 exists in diverse populations and can be caused by a short hexanucleotide GGCCTG repeat expansion

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