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REVIEW

Redefining cerebellar ataxia in degenerative ataxias: lessons from recent research on cerebellar systems

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ABSTRACT

Recent advances in our understanding of neurophysiological functions in the cerebellar system have revealed that each region involved in degenerative ataxias contributes differently. To regulate voluntary movements, the cerebellum forms internal models within its neural circuits that mimic the behaviour of the sensorimotor system and objects in the external environment. The cerebellum forms two different internal models: forward and inverse. The forward model is formed by efference copy signals conveyed by the corticopontocerebellar system, and it derives the estimated consequences for action. The inverse model describes sequences of motor commands to accomplish an aim. During motor learning, we improve internal models by comparing the estimated consequence of an action from the forward model with the actual consequence of the action produced by the inverse model. The functions of the cerebellum encompass the formation, storage and selection of internal models. Considering the neurophysiological properties of the cerebellar system, we have classified degenerative ataxias into four types depending on which system is involved: Purkinje cells, the corticopontocerebellar system, the spinocerebellar system and the cerebellar deep nuclei. With regard to their respective contributions to the internal models, we speculate that loss of Purkinje cells leads to malformation of the internal models, whereas disturbance of the afferent system, corticopontocerebellar system or spinocerebellar system leads to mis-selection of the proper internal model. An understanding of the pathophysiological properties of ataxias in each degenerative ataxia enables the development of new methods to evaluate ataxias.

INTRODUCTION

Ataxia, originally derived from the Greek word meaning 'lack of order', signifies a disturbance of voluntary motor coordination. In 1899, Babinski proposed the concept of 'asynergy', a disturbance of coordination of the muscles involved in specific voluntary movements.[1] He also used the terms 'decomposition' and 'adiadochokinesis' to delineate the signs and symptoms of asynergia, as well as 'hypermetria' in movement extending a limb to the target. Holmes highlighted another important feature of ataxia: 'delayed initiation' of voluntary movements. 'Dyschronometria' is a temporal variability in the contraction of individual muscles, including agonists as well as antagonists, which is characteristic of cerebellar symptoms, including asynergia, decomposition and adiadochokinesis.[1] These clinical symptoms have been observed in repeated, discontinuous and visually guided reaching movements. However, the underlying neurophysiology of these symptoms has not been fully explained, and the anatomical regions responsible for these symptoms remain unknown. It is necessary to reconsider ataxias in terms of the physiological functions of the cerebellar system and the neuropathology associated with each type of degenerative ataxia. To address these issues, understanding how the cerebellum regulates our actions is essential.

ATAXIA IN THE CONTEXT OF CEREBELLAR FUNCTION

The cerebellum has multiple functions in the context of motor regulation, learning and behaviour.[1 2] Regarding motor regulation, the cerebellum has been proposed to form 'internal models' that mimic the behaviour of the sensorimotor system and objects in the external environment.[3–6] The internal models enable us to predict the consequences of motor commands and to select the best sequence of motor commands for accomplishing a specific aim.[3–6] The cerebellum forms two different types of internal model: forward and inverse.[7] The forward model is formed by the efference copy signals conveyed by the corticopontocerebellar system, and it derives the estimated consequences of an action.[2–4 7–11] The inverse model provides the best motor command estimated for the desired consequence, and orchestrates individual muscles, including agonists and antagonists, during an action.[3 4 7–13] The information about estimated consequences of the action is necessary to assess the actual consequences.[10] By comparing information about estimated consequences from the forward model with the actual consequences from the inverse model, the internal model can be improved to increase the accuracy and smoothness of the action the next time it is performed.[10–13] The forward and inverse model are suggested to be tightly coupled, with multiple paired forward and inverse models existing in the cerebellum.[3 7] The forward model selection automatically determines the coupled inverse model, from which information is conveyed to the motor cortex by the dentate-thalamic pathway, resulting in an action.[14] This process of formation, storage and selection of internal models is one of the main functions of the cerebellum.[1 4–6 10–13] We speculate that degenerative ataxias may differ from each other in terms of internal model relationships. To discuss this possibility, we first review the function of each part of the cerebellar system.

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THE CEREBELLAR CORTICAL CIRCUIT: INTERNAL MODEL FORMATION

The structure of neuronal circuits is relatively identical in all areas of the cerebellar cortex.[9 15] Two types of neurons, granule and Purkinje cells and at least four types of interneurons compose the network in the cerebellar cortex. These neurons are arranged as repeating units in a highly regular manner, each of which is a basic circuit module. The granule cells receive excitatory signals from mossy fibres arising from neurons in the brainstem or spinal cord, mainly via the middle or inferior cerebellar peduncle. The information from 25 million mossy fibres is dispersed to ~50 billion granule cells.[15] Then the information of the granule cells is conveyed to 15 million Purkinje cells via excitatory signals from parallel fibres arising from the granule cells (figure 1).[15] Each Purkinje cell also receives excitatory signals from a single climbing fibre arising from the inferior olive neurons in the medulla; each climbing fibre contacts 1–10 Purkinje cells.[2] A group of several hundred or thousand Purkinje cells composes a microzone, which is an effective cerebellar functional unit.[2] The Purkinje cells within the same microzone transmit inhibitory signals to the same small cluster of cells within the deep cerebellar nuclei.[2] The initial trace for the memory of a motor sequence is speculated to be stored in the cerebellar cortical circuit; this memory may be consolidated in the cerebellar deep nuclei.[16] This extensive transmission of information from mossy fibres to granule cells and into Purkinje cells is believed to provide a computational benefit for the cerebellar system.[15] The number of microzones might define the quantity of the internal model, while disorder in the cerebellar circuit may affect the quality of the internal model. Therefore, the loss of Purkinje cells may initially affect the quality of the internal model, but not the quantity (figure 2B).[15] A massive depletion of Purkinje cells might eventually decrease the number of microzones, resulting in a quantifiable depletion in the internal model.[2 3 9 10 13 17]

MOSSY FIBRES: THE AFFERENT PATHWAY FROM THE CEREBRUM AND BODY PERIPHERY

Mossy fibres are the most numerous afferent fibres terminating in the granular layer, where they form moss-like structures. Most mossy fibres arise from neurons located within the brainstem and spinal cord. Mossy fibres convey information from the cerebral cortex, brainstem and spinal cord to the cerebellum (figure 1).[9 15] The corticopontocerebellar system is associated with skilled movements or fine motor movements, such as those involved in writing, speaking or playing an instrument,[10 18 19] whereas the spinocerebellar or vestibulocerebellar systems are associated with actions that require continuous feedback information, such as those involving standing or gait.[12 15 20–22]

The cerebellum receives instruction about an intended action from a higher centre in the cerebrum such as the supplementary motor cortex or premotor cortex via the corticopontocerebellar system (efference copy signal).[8 10] The pontine nuclei, located in the pontine base, relay this information from the higher centre to the contralateral cerebellar cortex of the lateral parts of the hemisphere (cerebrocerebellum) through the middle cerebellar peduncle via mossy fibres.[21] With this information, the cerebellar

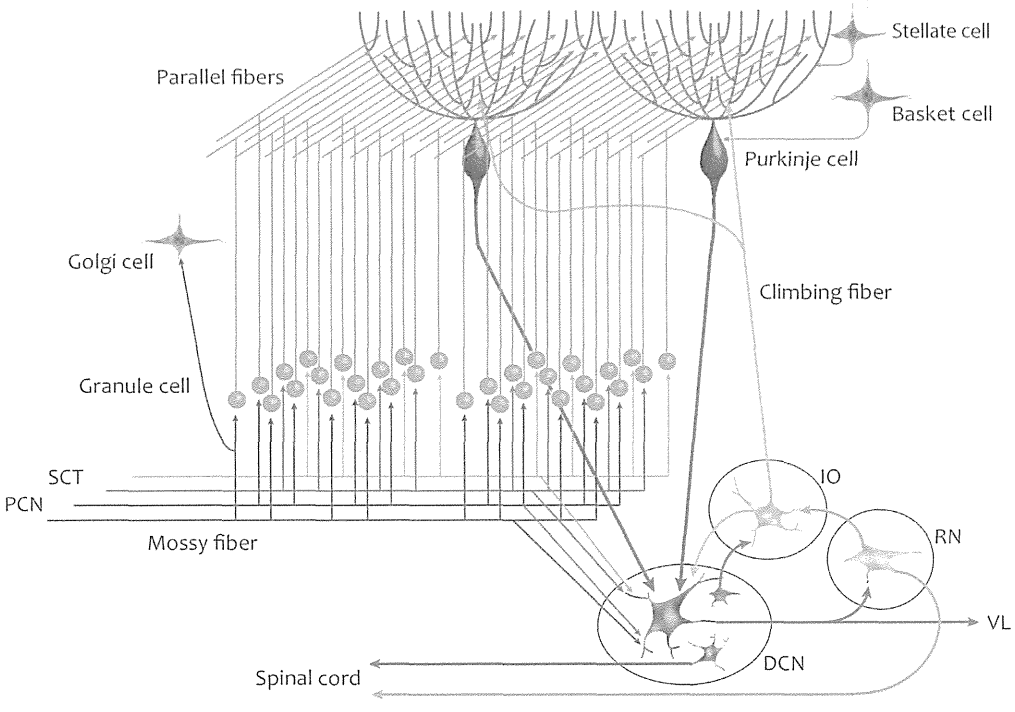


Figure 1 Schematic representation of the neuronal circuit of the cerebellum. The cerebellum contains numerous modules, each consisting of uniformly structured neuronal circuits. There are two main afferent pathways to the cerebellar cortex: mossy fibres, which terminate in the granular layer and form excitatory synaptic contacts mainly with granule cells (but also with Golgi cells), and climbing fibres, which form direct excitatory contacts with Purkinje cells. The stem axons of climbing and mossy fibres also provide collateral to the cerebellar deep nuclei. The ascending axons of the granule cells branch in a T-shaped manner to form parallel fibres, which form excitatory synaptic contacts with Purkinje cells (and molecular layer interneurons, stellate cells and basket cells). With the exception of granule cells, all cerebellar cortical neurons, including the Purkinje cells, form inhibitory synaptic connections with their target neurons. DCN, deep cerebellar nucleus; IO, inferior olive; RN, red nucleus; PCN, precerebellar nucleus; SCT, spinocerebellar tracts; VL, ventrolateral thalamus.

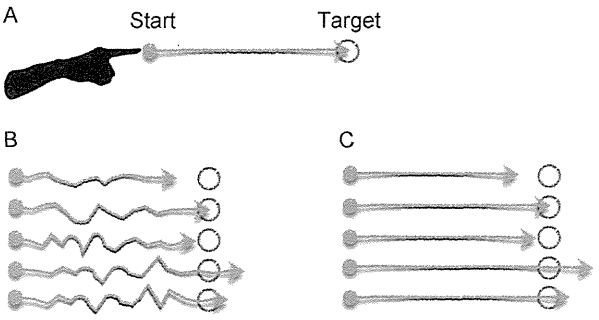


Figure 2 Hand trajectories in a straight-line motion towards a target. (A) Healthy individuals can move their hand smoothly along a straight line towards the target. (B) A defect in the cerebellar cortical neurons including Purkinje cells causes malformation of the cerebellar internal models, consequently leading to uncoordinated, irregular movements, such as decomposition and dysmetria, in a straight-line motion. (C) A defect in afferents to the cerebellar cortex via mossy fibres results in mis-selection of the internal model. This mismatch between the proper internal model and actual body dynamics typically results in dysmetria.

system can predict the consequence of an action and selects the ideal internal model for the given purpose.[3–7 10 14 21]

Information about a body part in space is mainly conveyed by the vestibulopontine pathway or spinocerebellar system through the inferior cerebellar peduncle via mossy fibres (some information from spinal cord via superior cerebellar peduncle). The vestibulocerebellar system conveys information about the situation and location of the head from the vestibular nucleus to the vestibulocerebellum (flocculonodular lobe).[21] This lobe also receives information from the superior colliculus and the posterior lobe, which contributes to the stability of the head in space, accompanied by visual information. The spinocerebellar system conveys proprioceptive information about peripheral areas of the body from the spinal cord to the spinocerebellum (vermis and paravermis).[21 23 24] Therefore, any disturbance in these regions has an ataxic effect on actions that require feedback information from the head or other parts of the body.

CLARKE'S COLUMN: A CENTRE FOR LOCOMOTION AND STANCE?

One of the regions frequently involved in spinocerebellar ataxias (SCAs) is Clarke's column, which is located in the dorsolateral part of the T1–L2 spinal cord.[23 24] It conveys proprioceptive information from the lower extremities and body to the ipsilateral cerebellum through the dorsal spinocerebellar tracts (figures 1 and 3A). Although the physiological function of this pathway is not fully understood, recent findings indicate that the neurons in Clarke's column have direct sensorimotor communication and receive information from the central pattern generator of the spinal cord, which generates rhythmic motor activity such as that used in locomotion.[23–25] These facts suggest that Clarke's column does not simply relay peripheral sensory information to the cerebellum, but it also acts as a centre for specific actions.[23–25] Information from the central pattern generator of the spinal cord may play a role in distinguishing sensory inputs that are a consequence of active locomotion from those attributable to perturbations in the external world.[26] Therefore, an abnormality of Clarke's column may contribute to gait disturbance in two ways: (1) interference with actions that compensate for perturbations in rhythmic movement, and (2) delivery of erroneous proprioceptive information from the body periphery.

For forelimb movement, the center for direct sensorimotir communication also exist in brainstem and spinal cord.[22 27 28] These circuits project to the cerebellum via mossy fibres and are associated with highly specific skilled forelimb movements, suggesting the existence of a centre for forelimb movement in these areas.[22 27 28]

THE CEREBELLAR DEEP NUCLEI: EFFERENT PATHWAY

Purkinje cells convey the results of analysis of afferent information somatotopically to neurons of cerebellar deep nuclei, including the fastigial, interposed and dentate nuclei (figures 1 and 3A).[9 15] Most of the output information from the cerebellum originates from the cerebellar deep nuclei. The number of neurons in the cerebellar deep nuclei is striking low relative to the number of Purkinje cells, and therefore these nuclei gather information from Purkinje cells.[15] It has been speculated that the cerebellar deep nuclei are related to the control of timing, rather than modality or topography,[17 29] and these interposed nuclei consolidate procedural memory.[16] Interestingly, perturbation of the cerebellar deep nuclei has been reported to be responsible for the postural instability seen in patients with metronidazole intoxication and progressive supranuclear palsy.[30] Disturbance of the outflow pathway from the cerebellar deep nuclei, or the thalamocortical or rubrospinal pathway, due to cerebrovascular accidents sometimes causes severe postural instability or intentional tremor in the upper extremities.

Although the structure of the cortical circuit is identical in each part of the cerebellum, the input and output pathways differ according to the part. The Purkinje cells in the vermis and in the intermediate part of the cerebellar hemisphere receive sensory information from the spinal cord and brainstem mainly via inferior cerebellar peduncle (some information is received from the spinal cord via superior cerebellar peduncle).[21 23 24] Purkinje cells in the vermis send projections to the fastigial nucleus, which projects bilaterally to the reticular formation and lateral vestibular nuclei, followed by composing the medial reticulospinal tract and the lateral vestibule-spinal tract, respectively.[2 21] The pathways are important for movements of the head and neck, and for balance and postural control during voluntary motor tasks. Purkinje cells in the intermediate part of the cerebellar hemisphere project to the interposed nuclei, which in turn project to the contralateral red nucleus, followed by composing the contralateral rubrospinal tract. This pathway is important for integration of sensory input from body parts with given action to adjust postures or movements to environments.[9 10 12 24]

In contrast to other cerebellar regions, the lateral hemispheres receive afferent signals exclusively from the cerebral cortex via the pontine nucleus.[10 21 31] Purkinje cells in the lateral cerebellar hemispheres project to the dentate nucleus, which is markedly enlarged in humans. The nucleus projects to both the contralateral ventrolateral thalamus and red nucleus. The parvocellular red nucleus, the part receiving inputs from the dentate nucleus, projects to the inferior olivary nucleus, which projects and terminates to Purkinje cells in the contralateral cerebellum, thus forming a feedback loop.[29] The efferent pathways via the dentate nucleus are responsible for the planning, initiation and control of well-trained voluntary movements.[17 29]

Purkinje cells in the flocculonodular lobes, which receive input from the vestibular nuclei and superior colliculi, directly provide output to the medial and lateral vestibular nuclei in the brainstem.[21] The efferent pathway via the lateral vestibular nucleus controls axial muscles and limb extensor muscles, stabilising balance during standing and walking. The efferent

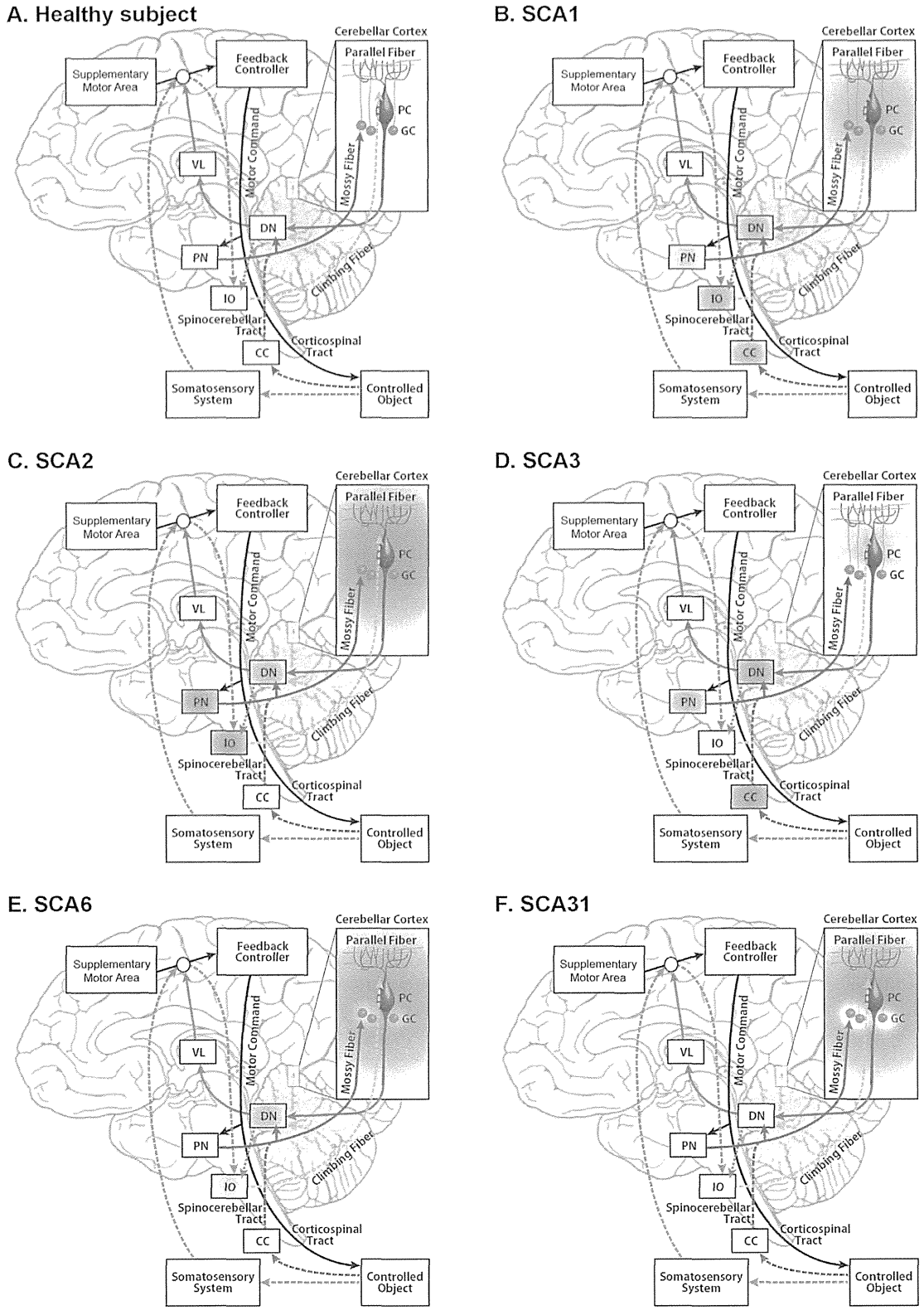
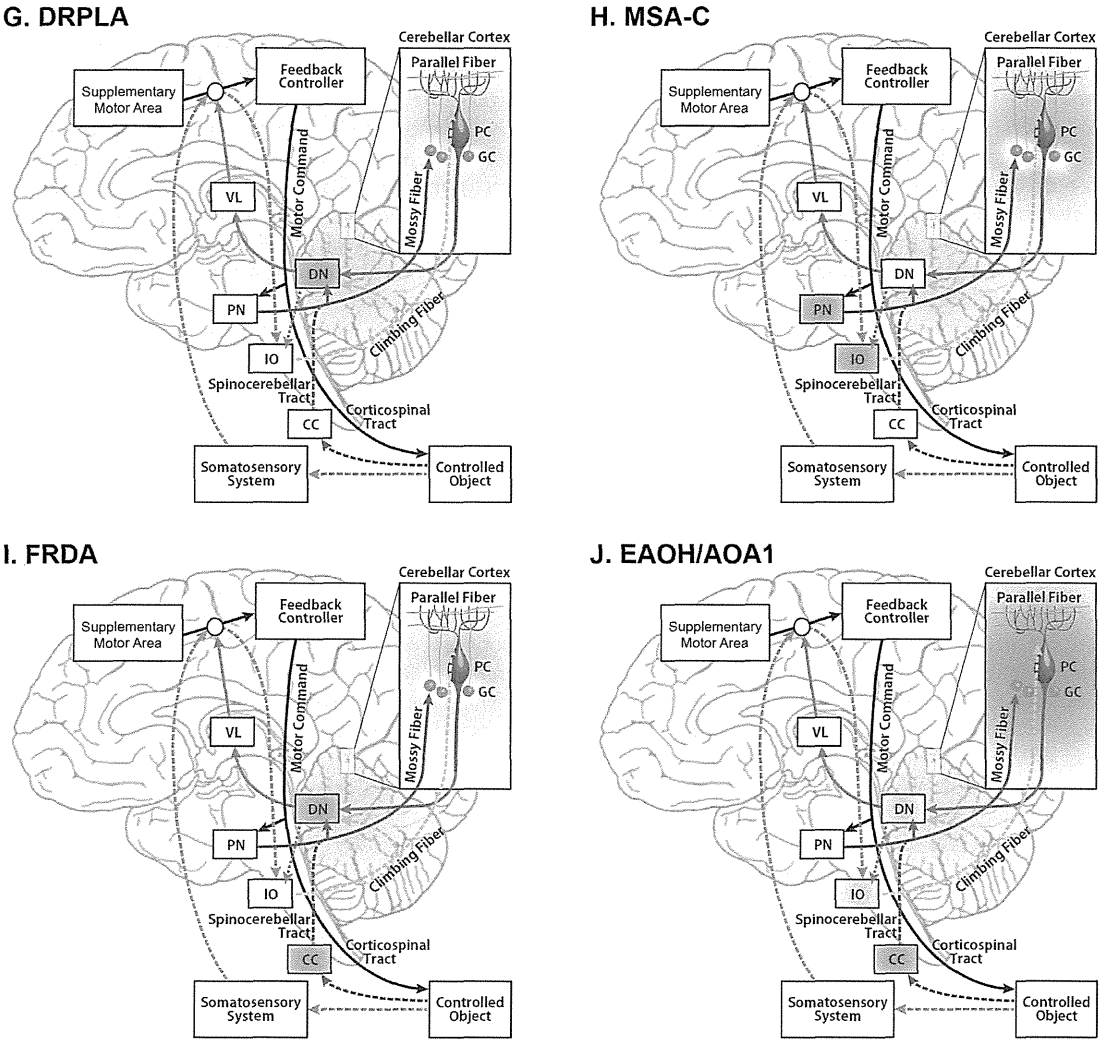


Figure 3 Distribution of neurodegeneration in the brain of patients with spinocecerebellar ataxias. (A) Healthy subjects, neuroal circuit for cerebellar system. (B) SCA1, spinocerebellar ataxia type 1. (C) SCA2, spinocerebellar ataxia type 2. (D) SCA3, spinocerebellar ataxia type 3. (E) SCA6, spinocerebellar ataxia type 6. (F) SCA31, spinocerebellar ataxia type 31. (G) DRPLA, dentatorubro-pallidoluysian atrophy. (H) MSA-C, multiple system atrophy-cerebellar type. (I) FRDA, Friedreich's ataxia. (J) EAOH/AOA1, early-onset ataxia with ocular motor apraxia and hypoalbuminaemia/ataxia with ocular motor apraxia type 1. (B)-(J) The degree of neurodegeneration in the anatomical regions related to the cerebellar systems is indicated by red (severe) or light red (moderate, or mild) colouration. (K) Summery of distribution of neurodegeneration in each degenerative ataxia, +++, severly affetced; ++, moderately affected; +, mildly affected; −, reratively spared. CC, Clarke's column; DN, dentate nucleus; DRG, dorsal root ganglia; IO, inferior olive; GC, granule cell; PC, Purkinje cell; PN, pontine nucleus; RN, red nucleus; VL,ventrolateral thalamus; VN, vestibular nucleus.



K. Summary of distribution of neurodegeneration in each degenerative ataxia

Disease	DRG	CC	VN	PN	GC	PC	DN	RN	IO
DRPLA	−	−	−	−	−	+	+++	+	−
SCA6	−	−	−	−	+	+++	++	−	+
SCA31	−	−	−	−	−	+++	−	−	−
MSA	−	−	+	+++	−	+	−	−	+++
SCA2	−	−	++	+++	+++	+++	++	+	+++
SCA1	−	++	+	+	++	++	++	−	++
SCA3	−	+++	++	++	−	+	+++	−	−
FRDA	+++	+++	++	−	−	+	+++	−	−
EAOH	+++	+++	−	−	+	+++	+	−	+

Figure 3 Continued.

pathway via the medial vestibular nucleus controls eye movements, and adjusts movements of the head and eyes.[29]

THE INFERIOR OLIVES: A SUPERVISION OF CEREBRAL CIRCUITS

The olivary nucleus, as well as the dentate nucleus and cerebellar hemisphere, is very well developed in humans, suggesting that this system may play an important role in actions that are characteristic of humans, such as hand movement, gait and speech. Neurons from the inferior olive send several climbing fibres to Purkinje cells and each fibre forms numerous synaptic contacts with the dendrites of a single Purkinje cell.[2] Signals from climbing fibres induce long-term depression (LTD) in conjunctively activated parallel fibre–Purkinje cell synapses.[13] LTD in these synapses forms the basis for motor learning in the cerebellum. Long-term potentiation, the long-lasting increase of

synaptic strength, is the opposing process to LTD. It occurs in parallel fibre–Purkinje cell synapses when parallel fibres are stimulated at high frequency, without being paired with climbing fibre activation.[32]

The inferior olives receive signals from the cerebellar deep nuclei, spinal cord and cerebrum. Although the precise physiological function of the inferior olive is still controversial, it may function as a comparator and send teaching signals representing errors to Purkinje cells via the climbing fibres.[10] This error learning based on teaching signals would be a plausible basis for motor learning in the cerebellum.[14 33]

Inactivation of the inferior olive nuclei in cats has been shown to not cause limb ataxia immediately[34]; limb ataxia develops over the course of several months without any degeneration of Purkinje cells or the cerebellar deep nuclei. These results reveal that loss of the inferior olives affects limb movement in a long-term manner, supporting the hypothesis that climbing fibres convey teaching signals. Erroneous teaching signals or lack of prompt feedback from actions may prevent effective reformation of internal models or lead to formation of erroneous models in the cerebellar circuit. However, several studies have demonstrated that LTD in parallel fibre–Purkinje cell synapses is not necessarily needed for motor learning.[35 36] Impulses from climbing fibres are regular and have a low frequency, unlike those from parallel fibres, suggesting that only a specific type of impulse is suitable as a teaching signal.[36] Therefore, it is still unclear whether the major function of the inferior olive is transmission of teaching error signals. Another hypothetical function of the inferior olive is as a generator of timing[17 36]; it may possess a set of system properties that allow temporal or rapid correction of action in response to unexpected events.

REDEFINING CEREBELLAR ATAXIA IN THE CONTEXT OF CEREBELLAR SYSTEM FUNCTION

With regard to its relationship with the internal models, we attempt to classify cerebellar ataxia into two categories: ataxia caused by a malformation of internal models and ataxia caused by mis-selection of internal models. Loss of cerebellar cortical neurons, involving Purkinje cells, may create inappropriate internal models,[1 2 4–6 9–13 15] resulting in uncoordinated irregular movements, such as decomposition and dysmetria, in the trajectory comprising a specific action (figures 1 and 2A,B). Input of incorrect afferent information to the cerebellum via mossy fibres from the cerebropontine pathway or the spinocerebellar or vestibulocerebellar systems may result in mis-selection of internal models.[23–26] This in turn could result in erroneous estimation of a desired action, represented as dysmetria, or increased variability of each interval in a repeated set of movements, represented as dysrhythmia (figures 1 and 2A,C). Although it has been proposed that the cerebellar ataxia are improved by the repeated manoeuvre, it would be interesting to investigate whether the accuracy and smoothness in the action are improved by the repeated manoeuvre in the situations with disturbance of afferent information system.

The corticopontocerebellar system is associated with skilled limb movements,[10 18 19] whereas the spinocerebellar or vestibulocerebellar system is associated with actions that require continuous feedback information, involving standing or gait.[12 15 20–22] The difference in the action contributed by each afferent information system in the cerebellum is well supported by the ataxia arising from cerebral infarction in each system. The damage to the corticopontocerebellar system at the pontine base causes dysarthria or clumsy hand syndrome in the contralateral side, which includes dysarthria; clumsiness, awkwardness and retarded fine movements of the affected hand; and difficulty in writing; however, a wavering ataxia on the finger–nose test is not clearly cerebellar in type.[18 19] In addition, Schmahmann *et al*[18] reported that dysmetria occurs in the ipsilateral side when the hemipontine lesion is extensive and interrupts pontocerebellar fibres traversing from the opposite side of the pons. Dorsolateral medullary infarction involves the dorsal spinocerebellar tract and the inferior cerebellar peduncle, which convey afferent information from the ipsilateral body and results in an ataxic gait.[20]

Degenerative ataxias are progressive neurodegenerative diseases involving the cerebellum as well as the brainstem, spinal cord and cerebrum.[37 38] Although degenerative ataxias involve several systems in the central nervous system (CNS), the distribution of the pathological lesions is unique to each degenerative ataxia. The regions of cerebellar circuits that are primarily affected in degenerative ataxias are summarised in figure 3A–K.[37 38] Based on knowledge about neuropathological findings in degenerative ataxias and the function of the cerebellar circuit, degenerative ataxias can be classified into four types based on the anatomical regions affected: Purkinje cells, the corticopontocerebellar system, the spinocerebellar system and the cerebellar deep nuclei (figure 3).

The neuropathological involvement in SCA31 is relatively restricted to Purkinje cells (figure 3F),[37] which may result in coarse internal models. Patients with SCA31 often show scanning speech and decomposition, which represent loss of smooth movement. However, because the patients' afferent and efferent systems are mostly preserved, it may be possible for them to reconstruct a new internal model using their residual cerebellar cortical circuits if correct movements are undertaken carefully and repeatedly. Indeed, patients with pure cerebellar ataxia respond well to rehabilitation, and this effect persists for more than several months.[39] In advanced stages, individuals with marked depletion of Purkinje cells lose their acquired internal models and are unable to learn a new internal model, resulting in marked ataxia. Although Purkinje cells are also predominantly involved in SCA6, degeneration of granule cells, inferior olive neurons and the dentate nucleus is evident (figure 3E).[37 38] Therefore, patients with SCA6 show more severe ataxia than those with SCA31.

In contrast, the disturbance of afferent systems involving the corticopontocerebellar or spinocerebellar system may disturb the selection of an appropriate internal model. Cerebellar-type multiple system atrophy (MSA-C) and SCA2 predominantly affect the pontine nuclei, which are involved in the corticopontocerebellar system and the inferior olives (figure 3C,H).[37 38] In the early stages of disease, disturbance of the pontocerebellar system may hinder selection of a proper internal model for well-trained limb movements in writing or playing instruments,[10 18 19] while an action that depends on the spinocerebellar tract might be relatively preserved at their onset.[12 15 20–22] Thereafter, however, the internal models might become progressively disordered because of erroneous information from the cerebral cortex. Furthermore, involvement of the inferior olive could disturb internal models in response to teaching error signals, leading to ataxic symptoms.

SCA1, SCA3, Friedreich's ataxia (FRDA) and early-onset ataxia with ocular motor apraxia and hypoalbuminaemia/ataxia with ocular motor apraxia type 1 (EAOH/AOA1) involve Clarke's column (figure 3B,D,I,J).[37 38] In these disorders, patients receive incorrect afferent information from somatosensory systems. Indeed, these patients show marked disability in

stance and gait that requires continuous feedback information from the body periphery.[12 15 20–22] Increasing the intensity of input signals from the lower limbs may stabilise these actions. It has been well recognised that heavily weighted shoes are of some benefit for correcting the disabilities of stance and locomotion seen in patients with ataxia. However, this approach would not be useful for diseases involving the corticopontocerebellar system, rather than the spinocerebellar system.

Dentatorubral-pallidoluysian atrophy mainly affects the outflow system without marked involvement of Purkinje cells (figure 2G).[37] Severe neuronal loss is evident in the dentate nucleus, with mild degeneration in the red nucleus. Degeneration in the dentatorubral system is relatively marked in cases with onset in late adulthood, while degeneration in the pallidoluysian system is relatively marked in juvenile cases.[37] SCA3 and FRDA also involve the dentate nucleus.[37] Involvement of the efferent system of the cerebellum may hamper its overall function, resulting in marked postural instability as well as limb ataxia.[16 30]

PERSPECTIVES

The degenerative ataxias also involve the cerebrum, brainstem and peripheral nervous system. For example, the medial and lateral vestibular nuclei are also involved in SCA2, SCA3, FRDA and MSA, resulting in marked disability in stance and gait.[37 38] Furthermore, degeneration of a part of cerebellar system may also cause degeneration in other parts of the cerebellum. In addition, the cerebellum is connected with other parts of the CNS, including the basal ganglia.[31] Therefore, these multiple reciprocal interconnections within the cerebellar system and other CNS structures may cause ataxias in each disease rather than an isolated disruption of individual structures and/or pathways in the cerebellum. Although we do not deny this possibility and we understand the difficulty in detailing the neurophysiological background in each disease, we would like to emphasise the need to interpret the ataxia within the proposed framework. The recent finding of differing speech characteristics in SCA3 and SCA6 supports our hypothesis.[40] SCA3 was found to have an increased temporal variability in syllable repetition compared with SCA6. In contrast, speech rate and prosody were more severely affected in SCA6 than in SCA3. Our approach to defining the characters of ataxias associated with each disorder may lead to the development of new rating instruments for evaluating degenerative ataxias in future.

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Redefining cerebellar ataxia in degenerative ataxias: lessons from recent research on cerebellar systems

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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,

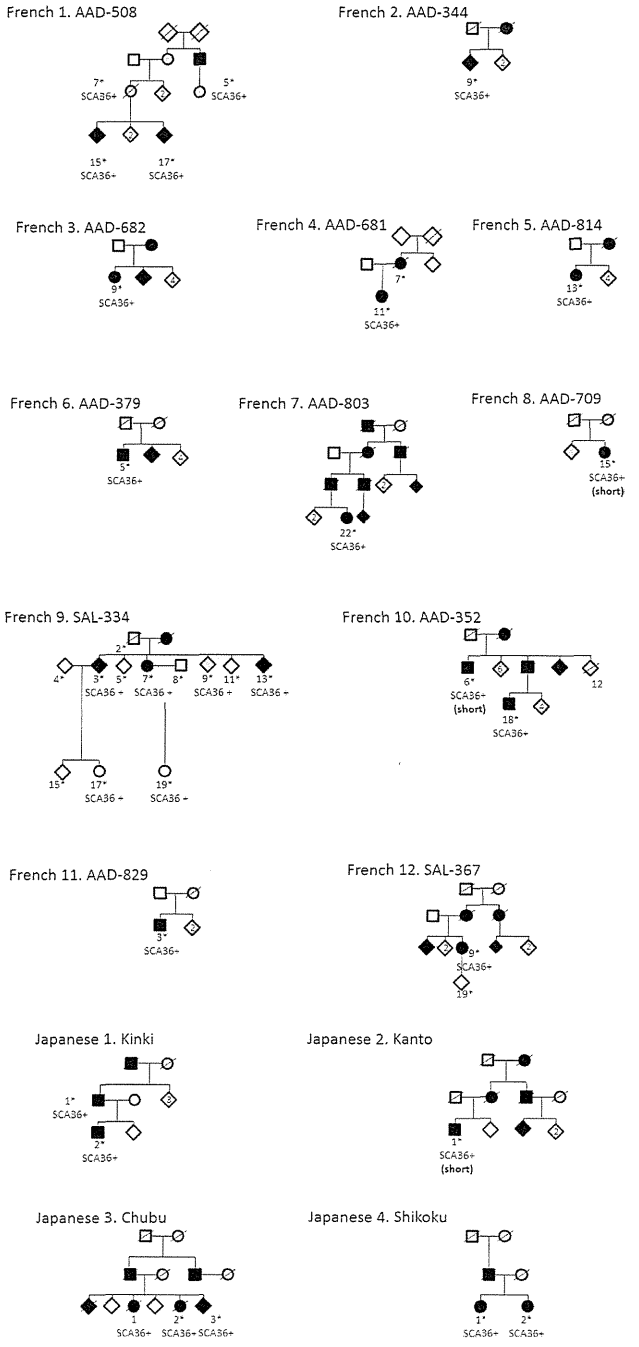

Figure 1 Twelve French and 4 Japanese SCA36 families. The French families consisted of 9 originally French kindred, one for each of Portuguese, Spanish and Chinese descendants (*the origins are not shown to protect from personal identification*). In the family AAD-352, an individual with short expansion (#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%).

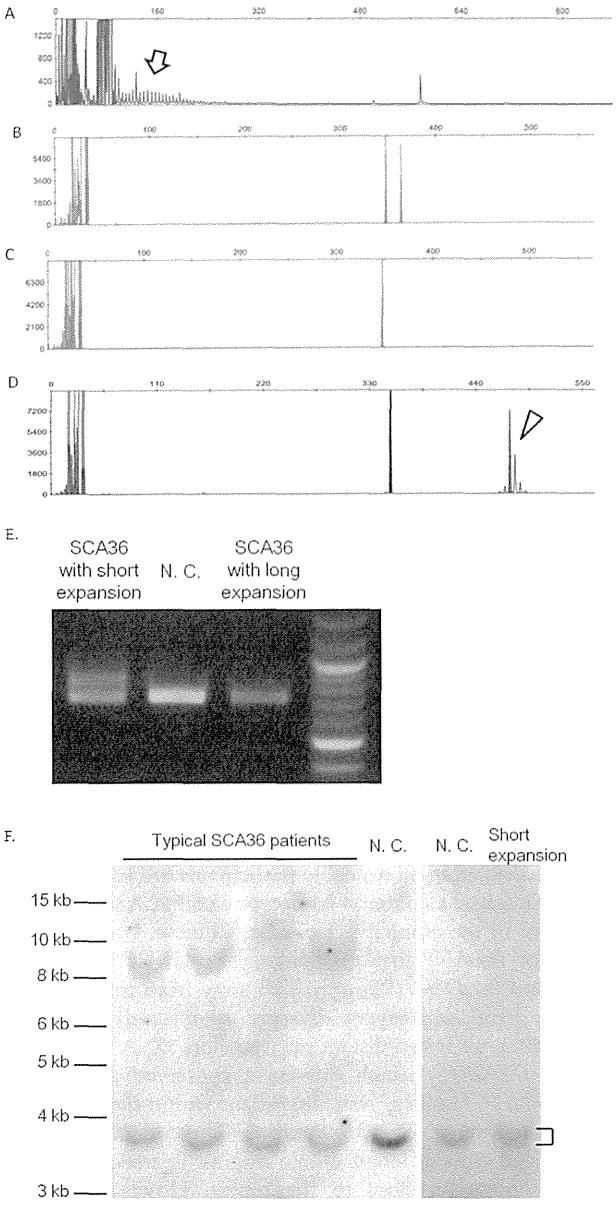

Figure 2 SCA36 hexanucleotide repeat expansions. (A) Typical attenuating peaks (arrow) of a hexanucleotide (GGCCTG) repeat expansion detected by the repeat-primed PCR analysis. (B) The PCR fragment analysis in a normal control showing two peaks corresponding to normal heterozygous alleles. (C) The same 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.

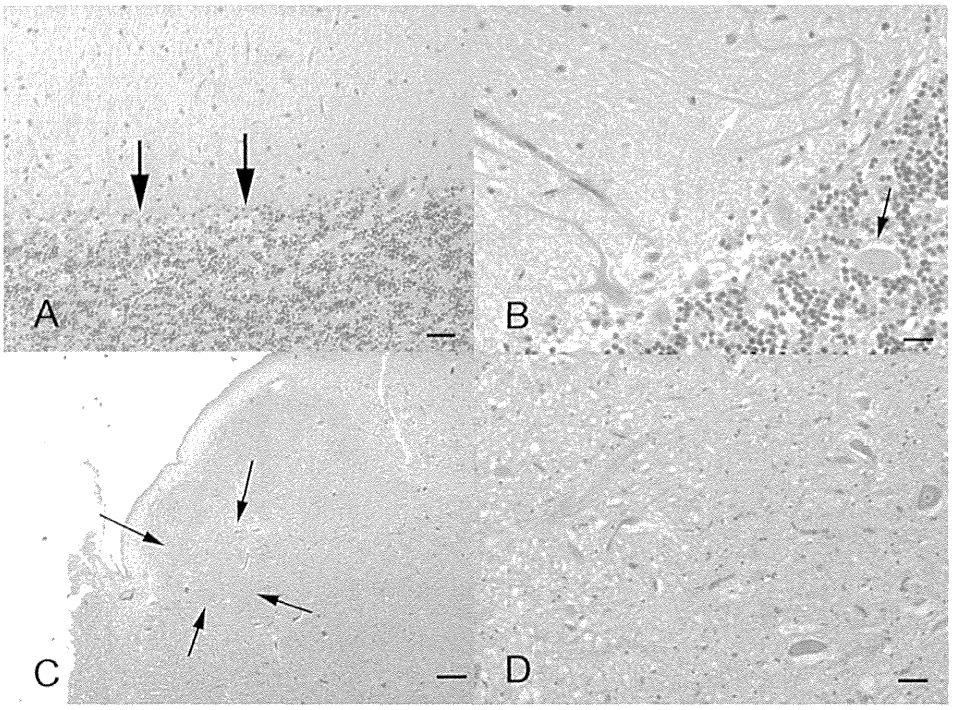

Figure 3 Neuropathology of an asymptomatic 83-year-old female carrier. The cerebellum (A and B) and the tegmentum of the medulla oblongata (C and D) stained with H&E. (A) A mild depletion of Purkinje cells is seen. The black arrows indicate Bergmann's gliosis in a region where Purkinje cells are depleted. (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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JNNP

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

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Case Report

CADASIL with a Novel *NOTCH3* Mutation (Cys478Tyr)

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Recently, an increasing number of *NOTCH3* mutations have been described to cause cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Here, we report 2 CADASIL patients from a Japanese family, who were found to possess a novel *NOTCH3* mutation. The proband only had chronic headache, and her mother had previously suffered a minor stroke. Although the patients' clinical symptoms were mild, their distinctive magnetic resonance imaging (MRI) features suggested CADASIL. Genetic analysis revealed that both patients had a novel heterozygous *NOTCH3* mutation (p.Cys478Tyr) leading to stereotypical cysteine loss. The present finding suggests that genetic testing for *NOTCH3* mutations in patients with distinctive MRI features, even if the symptoms are as mild as chronic headache, should help to broaden the mutational and clinical spectrum of CADASIL. **Key Words:** CADASIL—cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy—NOTCH3—mutation—Cys478Tyr—C478Y.

Case Report

A 46-year-old Japanese woman (II-2 in Fig 1, A) suffered from chronic headache for years. The headache was slightly throbbing in nature, but very mild and was not accompanied by any other features of migraine. The patient did not smoke nor have any cardiovascular risk factors. Neurologic examination was unremarkable. The patient's mother (I-2 in Fig 1, A) did not suffer from headache, but had a history of hypertension, hyperlipidemia, and diabetes, and she suffered a mild hemiparetic stroke at the age of 65 years. T2-weighted and fluid-attenuated inversion recovery magnetic resonance imaging (MRI) of the patient's brain revealed symmetric hyperintensities in the external capsules, anterior temporal poles, and periventricular and subcortical white matter (Fig 1, B-D). Magnetic resonance angiography was normal. Laboratory tests excluded hypercoagulable disorders and vasculitides. The patient's mother also had similar but more pronounced MRI lesions, in addition to an old lacunar infarction in the right internal capsule.

Characteristic MRI abnormalities led us to consider a diagnosis of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). After obtaining written informed consent, blood and skin samples were obtained from the patient. Although electron microscopic examination of the skin biopsy did not detect granular osmiophilic material (GOM) in the arterioles, nucleotide sequencing of exon 9 of the *NOTCH3* gene, unveiled a novel mutation, c.1433G>A

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