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Unexpected clinicopathological phenotype linked to small elongation of CAG repeat in SCA1 gene

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Sirs: We report a patient with spinocerebellar ataxia type 1 (SCA1), whose clinical and pathological pictures are not expected from the genetic abnormality [4, 5]. The patient was a 74-year-old man, who developed progressive gait disturbance, dysphagia and dysarthria for several years, as noted in his mother and a sister. He was fully conscious and well oriented. Extraocular movements were restricted in vertical directions and to a lesser extent in horizontal directions. Generalized muscle wasting and weakness involving bulbar muscles were prominent especially in the distal portion of the lower extremities. Patellar tendon reflexes were normal and Achilles tendon reflexes were decreased with positive right Babinski sign. Sensory disturbance, ataxia and extrapyramidal signs were not evident. Needle electromyogram demonstrated neurogenic changes. Laboratory examination was normal except for elevated blood glucose (320 mg/dl) and creatine kinase (1760 U/l). His general condition deteriorated so rapidly that severe respiratory distress led to a fatal outcome. The clinical diagnosis was motor neuron disease.

The brain weighed 1260 g. The pons and spinal cord was atrophic while inferior olives (Fig. 1A) and cerebellum were relatively preserved. The brain was otherwise normal except for nigral discoloration.

Marked degeneration of the pontocerebellar fibers and of pontine neurons, occasionally containing intranuclear inclusions (NIs) immunopositive for ubiquitin and expanded polyglutamine (1C2, Fig. 1B) was noted [1]. By contrast, degeneration was relatively mild but consistently accompanied by a few NIs, in the inferior olives, dentate nucleus, substantia nigra and lower motor neurons including those in the oculomotor nucleus. Depletion of Purkinje cells and glial reactions were mild to moderate. NIs were absent in Pj cells [3]. Dilatation of perineuronal space and mild spongiosis was noted in the cerebral cortex and striatum, where NIs were identified (Fig. 1C). Neurons were mildly degenerated in the subthalamic nucleus, where gliosis was slight (Fig. 1D). Neuronal degeneration and gliosis were evident in the globus pallidus, where difference between its external and internal segments was not apparent. Neither Bunina bodies nor skein-like inclusions were detected and anterior and lateral corticospinal tracts were preserved relative to the spinocerebellar tracts. Skeletal muscles exhibited neurogenic changes. With the consent of the family, genomic DNA was extracted and a small elongation (n = 41, normal < 39) of CAG repeat was noted in SCA1 gene [4], while CAG repeat size was normal in SCA2, SCA3, SCA6, SCA17, DRPLA genes.

Preferential involvement of pontine nucleus, spinocerebellar system, substantia nigra and lower motor neurons in the presence of NIs, noted in this patient are compatible with reported autopsy findings of SCA1 [1]. In typical SCA1 cases, apparent involvement of inferior olivary nucleus and cerebellar cortex and dentate nucleus, is the rule [2], all of which were not evident in this case (Table). Al-

Table Regional distribution of lesions and their severity

Area	representative SCA1 cases [2]	this case
Cerebellar system including its afferents and efferents		
Purkinje cells	±–++	+
Dentatofugal system	+–++	+
Pontocerebellar system	±–++	++
Inferior olivary nucleus	+–++	+
Spinocerebellar tracts	+++	++
Clarke's column	++	+++
Extrapyramidal system		
Substantia nigra	±–++	++
Pallidum interna	–	++
Pallidum externa	±–++	++
Subthalamic nucleus	±–+	+Oculomotor system +–+++ +
Anterior horns	+–++	++
Dorsal column	–++	+

ext external; *int* internal; *nucl.* nucleus; – absent; ± very mild; + mild, ++ moderate; +++ severe

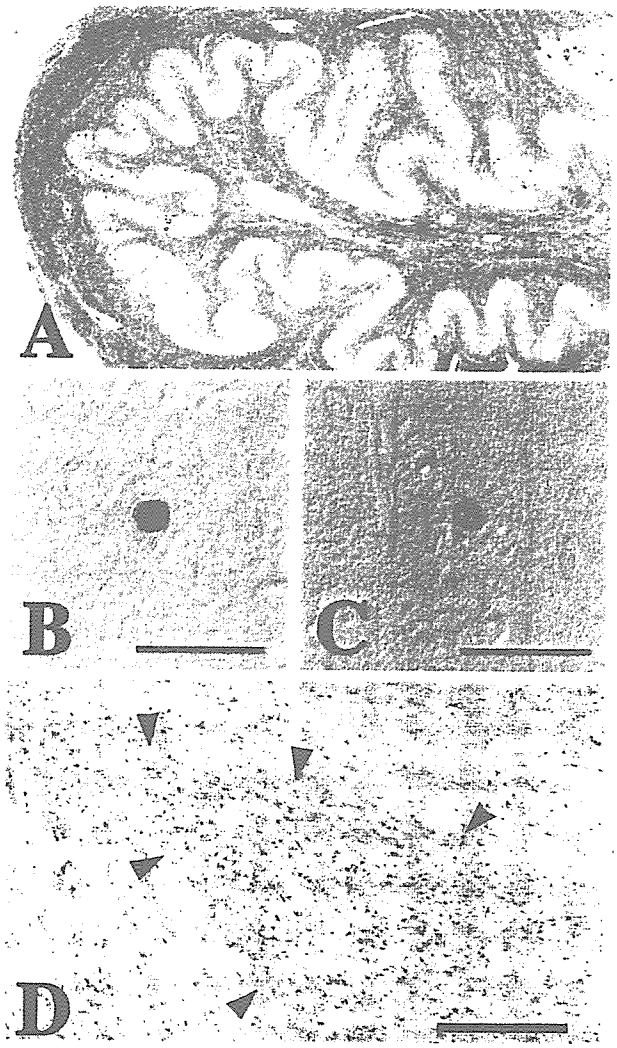

Fig. 1 **A:** Inferior olivary nucleus. The width of ribbon is slightly reduced, but the entire structure is relatively preserved. (Klüver-Barrera stain). **B:** Nuclear inclusion in a pontine neuron (1C2 immunostaining after pretreatment with formic acid, bar = 25μm). **C:** Nuclear inclusion in cerebral cortex (ubiquitin immunostaining, bar = 25μm). **D:** Subthalamic nucleus (arrowheads, GFAP immunostaining, bar = 50μm). Atrophy is not evident and proliferation of GFAP-positive cells is slight

though the lesion in the globus pallidus is one of the pathological features of SCA1, typical SCA1 cases are characterized by preferential involvement of its external segment [2], again not detectable in this case. Mild spongiosis with minimal glial proliferation may represent an influence not directly linked to degeneration but possibly related to circulatory disturbance in the agonal state. However, identification of NIs in these areas (Fig. 1C), as well as in the thalamus indicates that degenerative process with NI is extended to these areas, not described so far in SCA1 brains. Small expansion of the CAG repeat in this patient may be correlated not only with late disease onset [4] but also with relative preservation of these regions, which may explain predominant manifestation of lower motor involvement without apparent abnormality on motor control. Variability of pathological lesion as seen in this case may provide an opportunity to gain further insight into how lesions are engendered in human brains.

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Spinocerebellar Ataxia Type 6

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Spinocerebellar ataxia type 6 (SCA6), one of the autosomal dominant neurodegenerative diseases, is caused by small expansions of CAG repeat that encodes polyglutamine tract for the α_{1A} (P/Q type; alpha 12.1) subunit of the voltage-gated calcium channel (*CACNA1A*, $Ca_v2.1$). Among the CAG repeats and their expansions known to cause human diseases, the length and expansion in SCA6 patients are the smallest and even the expanded length is within a range of normal sizes in other repeats. Clinically, patients with SCA6 show progressive, and rather "pure", cerebellar dysfunctions including gait ataxia, nystagmus, dysarthria and incoordination of the limbs at an average age-of-onset around 45 years. Vertigo with or without down beat nystagmus, typically experienced with rapid change in the head position, is also a characteristic clinical feature that would be important to clinically differentiate SCA6 from other autosomal dominant cerebellar ataxias. Despite that the expansion is small in SCA6, the mutation causes cerebellar dysfunction by the mechanism not clearly known yet. The expanded polyglutamine causes alteration in calcium channel function in cultured cells, suggesting that the mutation causes channel dysfunction other than a simple loss-of-function. In addition, formation of aggregation containing calcium channel protein has been found in Purkinje cells of SCA6 brains. However, protein aggregation is not within the nucleus as in most other polyglutamine diseases. Further studies are needed to elucidate molecular pathomechanism underlying SCA6 and to develop an effective treatment.

I. INTRODUCTION

Spinocerebellar ataxia type 6 (SCA6) is one of the autosomal dominant cerebellar ataxias (ADCAs), and its main clinical feature is slowly progressive ataxia consistent with ADCA type III, namely, pure cerebellar ataxia [1]. SCA6 is caused by the expansion of CAG repeats in the gene for the α_{1A} (P/Q type, α12.1) subunit of the voltage-gated calcium channel (*CACNA1A*, $Ca_v2.1$), which is located on the short arm of chromosome 19 [2]. Before the report of SCA6, a nationwide linkage analysis for patients with pure cerebellar ataxia was performed in Japan, and the locus 19p13 was identified in half of the families collected [3]. This group of patients turned out to have SCA6. A very characteristic feature of SCA6 is the small size of both normal and expanded CAG repeats. Even the expanded repeats are usually within normal range in other polyglutamine diseases. In Japan, no case of Friedreich's ataxia has been idendified by gene analysis, and SCA6 seems to be either the most common or the second most common disease of the hereditary spinocerebellar ataxias (SCAs) [4–7]. The prevalence of SCA6 is lower in Europe and North America [8–11], and other SCAs with pure cerebellar ataxias such as SCA5, SCA10, SCA11, SCA14, SCA15, SCA16, and SCA22 appear uncommon in Japan [12–18]. There is also

another SCA with pure cerebellar ataxia linked to chromosome 16q, the associated gene of which has been identified very recently [19–23]. The remainder of the initial families just mentioned have this type of SCA [3].

II. ETIOLOGY, PATHOGENESIS, AND NEUROPATHOLOGY

SCA6 is caused by small expansions of CAG repeats in exon 47 of *CACNA1A* ($Ca_v2.1$) [2, 3]. Although the normal range is 5 to 18 repeats, SCA6 patients have 20 to 33 repeats. The 19-CAG repeat has been reported as the lower limit of expansion causing SCA6 [24, 25], although this repeat has been observed in normal alleles including our report [3]. Our three asymptomatic cases with 19 repeats harbored 13 repeats in the other normal chromosome. In contrast, among four individuals with 19/19, 19/13, 19/11, and 19/11 repeats, ataxia was seen in only one case with homozygous 19/19 repeats [25]. Therefore, the total number may have some influence. However, because a SCA6 patient with 19/7 repeats was also reported [24], the significance of a single 19 repeat should be elucidated in the future. The repeat number 20 is definitely pathological because a neuropathological study of a case with 20 repeats revealed specific inclusions in Purkinje cells (unpublished data).

Although there is an inverse correlation between age at onset and repeat length, the age-at-onset range is 36 years, for example, in patients with 22 CAG repeats in the expanded allele (Fig. 25-1) [26]. Factors other than the CAG repeat length of *CACNA1A* may be influencing the age at onset. In contrast to apparent genetic anticipation in other polyglutamine diseases, the repeat length is usually the same within a given SCA6 family, probably due to the small size of the expansion. The repeat length is also the same among various parts of a given SCA6 brain [27]. These facts suggest that the CAG repeat in *CACNA1A* is very stable in both meiotic and mitotic divisions.

Mutations other than the repeat expansion of the gene *CACNA1A* cause familial hemiplegic migraine (FHM: point mutations, deletions, splice abnormalities) and episodic ataxia type 2 (EA2: point mutations) [28]. The mechanism of phenotypic variations between the repeat expansion and other mutations has not yet been clarified. Furthermore, some mutant ataxic mice have resulted from point mutations or a splice abnormality of *CACNA1A*, namely, tottering (*tg*), leaner (tg^{la}), rolling mouse Nagoya (tg^{rol}), and rocker (*rkr*). Although, in humans, SCA6, FHM, and EA2 are autosomal dominant diseases, these mouse ataxias are transmitted as autosomal recessive traits.

Voltage-gated Ca channels are composed of several subunits including α_1, β, and α_2/δ. Because the α_{1A} subunit forms the pore for Ca ions and appears very important for channel functions, mutations of the gene might cause impairment of channel functions. Indeed, a few studies using cultured cells reported alterations of Ca channel functions, but the degree of change was not that large, and the quality of the alteration was different in different reports [29–32]. Therefore, although there may be a functional abnormality of the Ca channel, the contribution to the pathogenesis of SCA6 would not be large enough. On the other hand, neuropathologically, SCA6 brain shows loss of Purkinje cells, milder degeneration of granule cells, and thinning of the molecular layer with astrogliosis [27] (Fig. 25-2). Because α_{1A} Ca

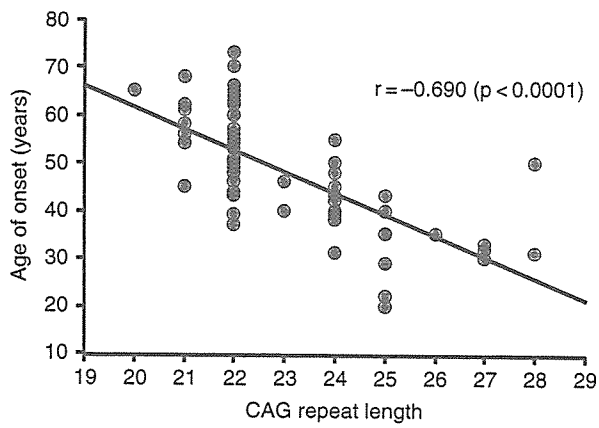

FIGURE 25-1 Age at onset correlates inversely with the number of the CAG repeats of the *CACNA1A* gene, suggesting the expansion is the cause of the illness. Note the wide range of age at onset with the same length of repeats. For example, the age-at-onset range is more than 30 years for a CAG repeat length of 22 [26]. See CD-ROM for color image.

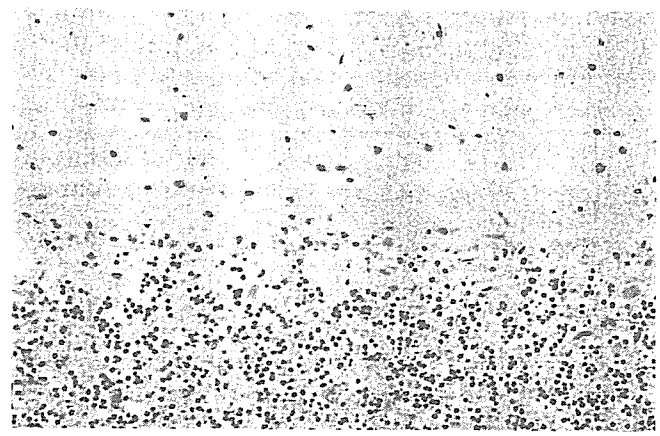

FIGURE 25-2 Cerebellar cortex of a SCA6 patient. Purkinje cells are decreased in number, and the remaining cells are atrophic, with milder loss of granule cells. There is astrogliosis of Bergman's glia in the Purkinje cell layer and astogliosis in the molecular layer. Hematoxylin and eosin stain. ×80. See CD-ROM for color image.

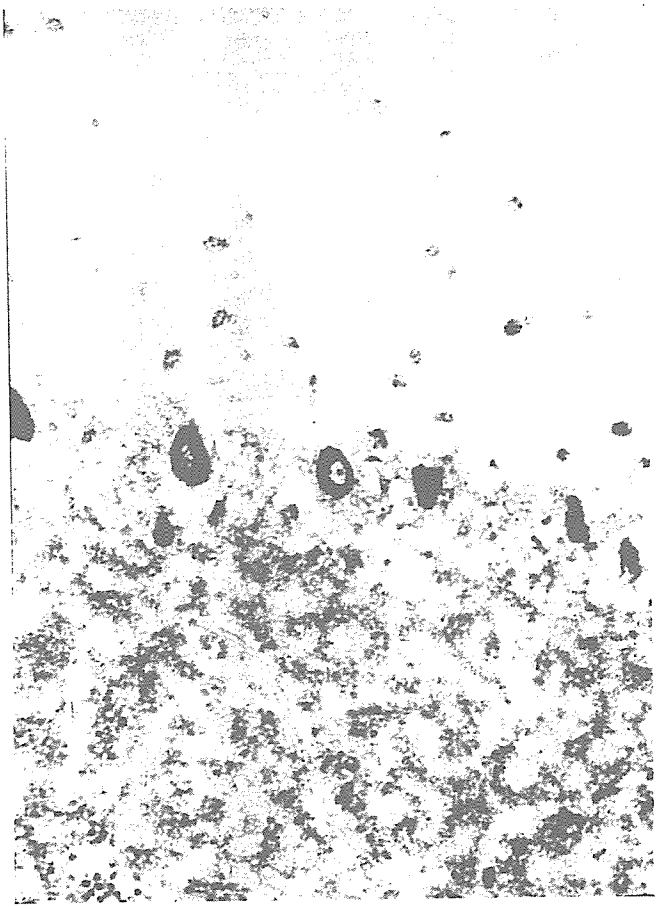

FIGURE 25-3 *In situ* hybridization of mRNA of the *CACNA1A* gene. The cerebellum, particularly Purkinje cells, shows a very strong reaction followed by a less marked reaction of granule cells. ×100. See CD-ROM for color image.

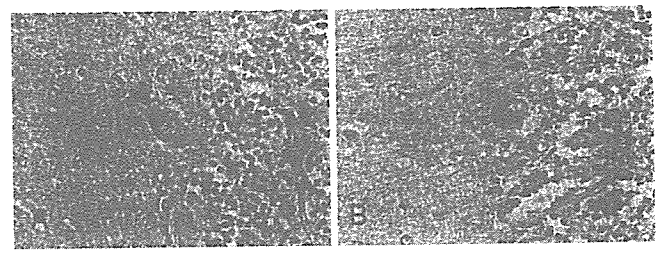

FIGURE 25-4 Two types of cytoplasmic inclusions specific to both SCA6 and Purkinje cells. Remaining Purkinje cells contain α_{1A} Ca channel protein-positive inclusions in the cytoplasm (A). Finer cytoplasmic inclusions positive with 1C2 antibody suggesting the presence of elongated polyglutamine tracts are also seen in Purkinje cells (B). ×150. See CD-ROM for color image.

channel mRNA and protein are widely expressed in the central nervous system, particularly in cerebellar Purkinje cells [33] (Fig. 25-3), it seems quite reasonable that the Punkinje cell, which seems to be the strongest expressor of the causative gene *CACNA1A*, would be affected most profoundly. In fact, there are two types of cytoplasmic inclusion bodies specific to both Purkinje cells and SCA6 (Fig. 25-4) [33, 34]. Larger inclusions are immunostained by antibodies against α_{1A} Ca channel protein and appear to be composed of the channel protein. Finer inclusions are positively immunostained with antibody 1C2, suggesting that the latter finer inclusions contain expanded polyglutamine tracts with conformational changes. Interestingly, intranuclear inclusions, which are a hallmark common to other polyglutamine diseases, are not apparent in SCA6, at least by light microscopy. However, the presence of the inclusions implies that the mutant protein may have toxic effects on Purkinje cells, as in other polyglutamine diseases. Our study on cultured HEK cells further suggested that the α_{1A} Ca channel with expanded polyglutamine may be prone to fragmentation and cell death [35]. This is a striking feature because the result may suggest that the proteolytic cleavage seen in other polyglutamine diseases could operate with small polyglutamine expansions. Matsuyama et al. also showed that a Ca channel with expanded polyglutamine repeats may disturb the antiapoptotic role of normal Ca channels [36]. The neuropathological changes were usually pronounced in the superior vermis and milder in the hemisphere in most patients [27, 37–40]. When cerebellar pathology is marked, the inferior olivary nucleus exhibits neuronal loss to some extent, with some cases exhibiting the pathology of cerebello-olivary atrophy. The fact that the two types of inclusions are found exclusively in Purkinje cells indicates the Purkinje cell may be the primary target in SCA6.

III. CLINICAL MANIFESTATION

Clinically, SCA6 is a slowly progressive cerebellar impairmant including nystagmus, cerebellar dysarthria, incoordination of the extremities, ataxic gait, and muscle hypotonia. According to our study of 140 patients, the mean age at onset is 47.2 ± 11.5, the latest age at onset of the various spinocerebellar ataxias [26]. It is, however, important to note that some patients become ill in their twenties. Unsteadiness of gait or gait ataxia was the most frequent initial symptom in our and other reports [8, 9, 37, 41]. The second most frequent initial symptom was "vertigo" or oscillopsia. Yabe et al. (2003) reported that vertigo or oscillopsia was experienced by 68% of their patients [42], whereas only 12% of the patients in our cohort exhibited these symptoms [26]. The difference could be due to the confusion of vertigo with unsteadiness: patients might complain of "vertigo" when they suffer from unsteady gait and vice versa. Vertigo, however is

one of the clinical features characteristic of SCA6 among the various SCAs [37]. In particular, vertigo and oscillopsia are induced by changes in head position (positioning vertigo) [8, 37, 42–45]. Symptomatic vertigo was present in only 8.8% of the subjects, but neuro-otological testing revealed positioning vertigo in 38.9% of the subjects examined. This difference suggests that daily activities may not provide strong enough stimulation to induce vertigo, or patients may unconsciously avoid stimulation inducing vertigo. Vertigo/oscillopsia and positioning vertigo/oscillopsia tend to be accompanied by downbeat positioning nystagmus (DPN) with or without downbeat gaze nystagmus. Various episodic features, including marked vertigo and episodic ataxia, were reported in previous studies of SCA6 [8, 37, 42], but only a small number of our subjects had true and significant episodic symptoms as in others [4]. Episodic symptoms may be frequent in foreign reports, and this variability may be due to differences in modifying gene's effect among various ethnic groups.

Although extracerebellar signs such as pyramidal tract signs, abnormal involuntary movements, parkinsonism, hyporeflexia, sensory disturbances, intellectual impairment, and urinary incontinence have also been reported, the frequencies of these signs are very low in our cohort and not constant among various reports [4, 5, 8–10, 37, 41, 46]. In addition, there were no anatomical substrates responsible for these extracerebellar signs. Extracerebellar signs do not appear to be significant at present.

IV. EXAMINATION AND DIAGNOSIS

Routine laboratory examinations of blood, urine, and feces, as well as cerebrospinal fluid, are all normal. Brain MRI of patients with SCA6 is the most important examination and reveals atrophy of the cerebellum, particularly the superior vermis, without atrophy of the brainstem and cerebrum (Fig. 25-5) [47, 48]. Rarely, brainstem atrophy may be observed [49]. These features on MRI, however, do not differentiate SCA6 from other SCAs presenting with pure cerebellar ataxia, such as sporadic late cortical cerebellar atrophy and autosomal dominat cerebellar ataxia linked to chromosome 16 [19].

Neuro-otological examination reveals DPN, particularly in those with positioning vertigo [26, 42, 50, 51]. In contrast, DPN is uncommon or variable in multiple-system atrophy [42, 51, 52] and other autosomal dominant SCAs, particularly Machado–Joseph disease or SCA3 [42, 51]. Positioning vertigo and DPN tend to

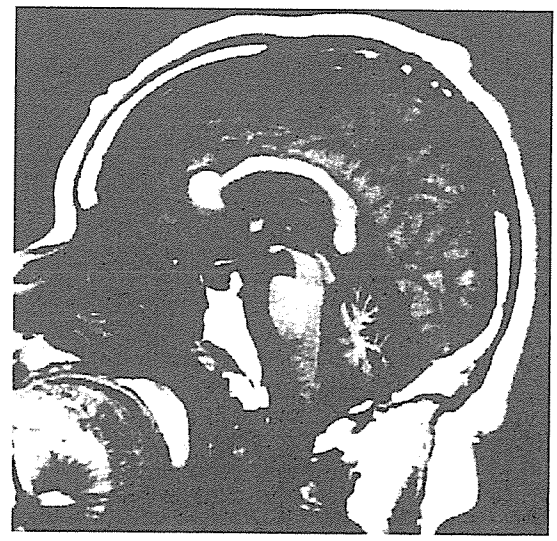

FIGURE 25-5 Brain MRI scan of a SCA6 patient. The cerebellum, particularly the vermis, is atrophied, with widening of sulci and the fourth ventricle. The brainstem and the cerebrum appear normal. T1WI, midsagittal view.

be present at a later stage in SCA6. DPN was reported to be related to impaired cancellation of the vestibulo-ocular reflex (VOR); namely, the inhibitory effect of Purkinje cells in the flocculus and nodulus on the vestibular nuclei is impaired, on the basis of a hyperactive VOR [53–55]. Extensive Purkinje cell loss including the flocculus has been reported in SCA6 [37]. These findings may be accounted for by the pathology in the vermis, flocculus, paraflocculus, or nodulus, with sparing of the paramedian pontine reticular formation, in consonance with previous reports in the literature [56–63].

Although the neuro-otological findings are characteristic of SCA6, they are not specific to the disease. Many autosomal dominant SCAs present with pure cerebellar ataxia, as do sporadic cases with SCA6 gene mutation. Furthermore, some SCA patients with involvement of multiple systems, as in Machado–Joseph disease/SCA3, may present with pure cerebellar phenotype [64]. The diagnosis of SCA6 should be confirmed by gene analysis.

V. TREATMENT, PROGNOSIS, AND PERSPECTIVE

In Japan, the TRH derivatives taltirelin hydrate and protirelin tartate have been approved by the Ministry of Health, Welfare and Labor as effective for ataxic patients with various spinocerebellar ataxias including olivopontocerebellar atrophy or MSA-C

and are widely used. In addition, taltirelin hydrate may have other effects on the nervous system, such as a neurotrophic effect [65]. However, general improvement with use of taltirelin was observed in only 22.6% of the patients, twice that in control. In some, episodic symptoms of SCA6 as well as EA2 may be improved by acetazolamide, but the effect is usually limited. The present status of treatment of SCA6 is quite insufficient, although SCA6 patients tend to be ambulatory until relatively late ages, and life span does not seem to be shortened [66].

SCA6 is one of a few neurological diseases in which the functions of causative proteins have been explored, for example, SOD1 causing ALS1, androgen receptor causing spinal and bulbar muscular atrophy, and protein kinase Cγ (PKCγ) causing SCA14 [15]. These diseases may be of some advantage in elucidation of the pathomechanisms. However, research related to Purkinje cells, the main target of SCA6, appears to suffer from the fact that cultured cells into which α_{1A} Ca channels are introduced show only Q-type properties, whereas Purkinje cells exhibit exclusively P-type channel properties. To overcome this difficulty, a suitable animal model is essential. A great deal of effort should be expended in elucidation of the SCA6 pathomechanism, and the development of new therapies based on the mechanism should be continuously pursued.

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●常染色体優性遺伝性脊髄小脳変性症

第16番染色体長腕に連鎖する優性遺伝性脊髄小脳変性症

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第16番染色体長腕に連鎖する優性遺伝性脊髄小脳変性症*

石川欽也** 融 衆太** 水澤英洋**

われわれは原因未同定の優性遺伝性脊髄小脳変性症のうち，小脳症状のみを呈する高齢発症の常染色体優性遺伝性脊髄小脳変性症の原因遺伝子を同定すべく，ポジショナルクローニングを行った。連鎖解析によって解明された第16番染色体長腕(16q22.1)遺伝子座での詳細なハプロタイプ解析を行い，候補領域を限定化後，候補領域内の21個の候補遺伝子を逐次スクリーニングしたところ，新しい遺伝子(“*puratrophin-1*”と命名)の翻訳開始点上流16塩基の位置に，一塩基置換(C→T)があることを解明した。この遺伝子変化は検索した範囲では，健常日本人にはない患者特有の変化であり，遺伝子産物は神経細胞などで発現し患者脳では凝集体を形成していた。さらにpuratrophin-1蛋白が機能的に関連していることが予想されたGolgi装置膜蛋白も，同様に凝集していた。*puratrophin*-1遺伝子の一塩基置換を用いて，疾患頻度を解析したところ，本病型はSCA6やMJD(マシャド・ジョセフ病)に並んで頻度の高い病型であることが推測された。

本遺伝子変化は非翻訳領域にある変化としてユニークであるが，その病的意義，すなわち本遺伝子変化が神経細胞変性にどのように関係するかなど，今後さらに解明すべき点が残っている。

キーワード：脊髄小脳変性症(spinocerebellar degeneration)，小脳Purkinje細胞(Purkinje cell)，RhoGEF，Golgi装置(Golgi apparatus)

はじめに

脊髄小脳変性症は，臨床的に進行性の平衡機能障害や四肢協調運動障害などの小脳失調を主徴とし，病理学的な面では小脳はもちろん，脳幹や大脳基底核，あるいは脊髄などの系にも障害が及びうる神経変性疾患の総称と言える[1]。脊髄小脳変性症はさらに細かく病型によって分類でき，常染色体優性遺伝性脊髄小脳変性症だけでも，少なくとも30にのぼる病型があることがわかっている[2,3]。このため，遺伝子診断や，大きな家系であれば連鎖解析によって病型診断が可能となり，脊髄小脳変性症が正確な疾患分類に基づいて整理されつつある。将来根本的治療を可能にするためには，このように原因に基づいて正確に病態を解明する必要がある。

筆者らは，これまで本邦に存在する原因不明の遺伝性脊髄小脳変性症について研究を進め，最近その1つの病型に深く関わる遺伝子変化を見出した[4]。この病型(16q-ADCA)は，当初の予想を上回って本邦でかなり頻度が高く，筆者らの解析結果では，脊髄小脳失調症6型(SCA6)やMachado-Joseph病に次いで3位と多かった。本稿では，16q-ADCAが見出された経緯とその臨床徴候について触れ，最後にこの病型に深く関わると考えられる遺伝子変化についてご紹介したい。

2006年4月18日受稿

* Molecular genetics and clinical aspects of 16q-ADCA.

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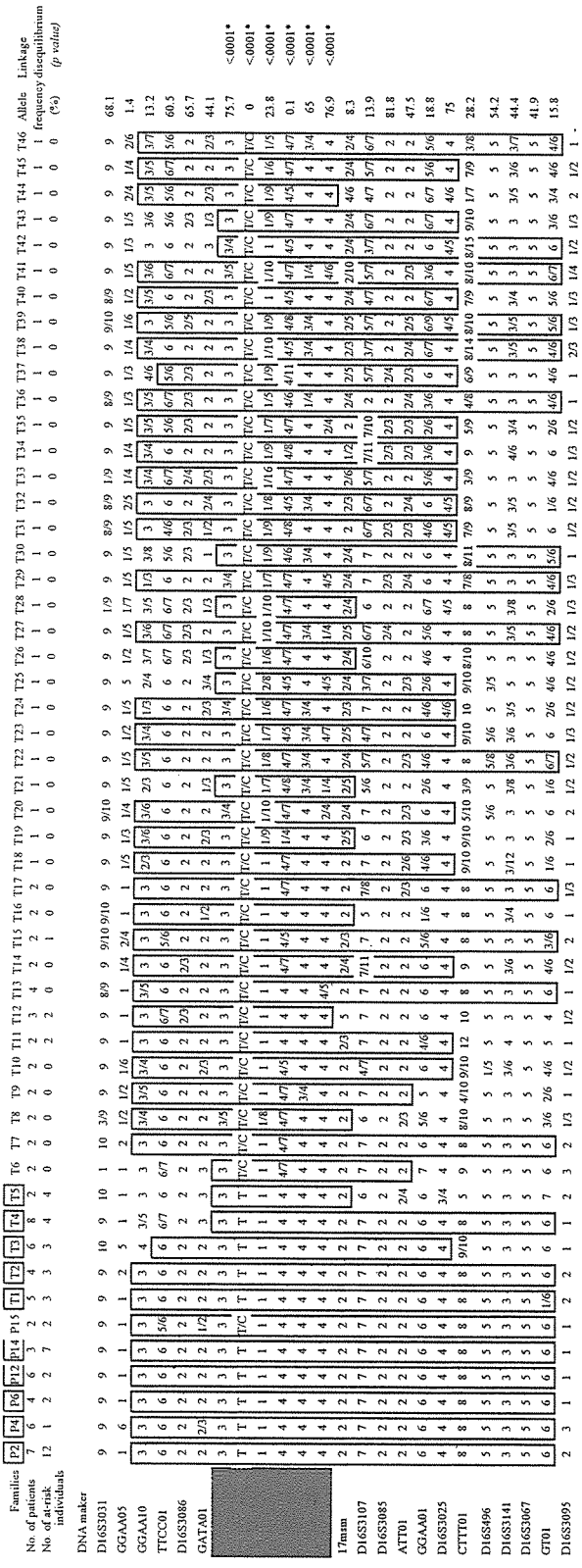

図1 16q-ADCA家系のハプロタイプ解析（文献4より）

Ⅰ．16q連鎖型脊髄小脳変性症発見の経緯

筆者らは，本邦に存在する原因不明の脊髄小脳変性症の家系を集積し，責任遺伝子を同定する研究に携わってきた過程で，発症後10年以上経過しても小脳失調以外にはあまり大きな神経障害を呈さない，いわゆる純粋小脳失調症（pure cerebellar ataxia）型[5]の家系が多いことに気づき，その家系を集積した[6]。連鎖解析によって約半数が第19番染色体長腕に連鎖することを見出し[7]，それが脊髄小脳失調症6型（SCA6）[8]であることが判明した。その後，残りの家系についてもう一度全ゲノムにわたる連鎖解析を行ったところ，16番染色体長腕に連鎖することを見出した[9]。驚いたことに，この遺伝子座はSCA4という，末梢神経障害が著明で，必ず深部感覚障害がみられる病型の原因遺伝子が存在すると考えられていた領域であった[10]。筆者らの家系は先述のように純粋小脳失調型で，感覚障害は目立たず，振動覚低下はあっても年齢相応の範囲である。また，末梢神経伝道検査での腓腹神経の感覚神経活動電位（SNAP）は，SCA4では導出不能であるのに対して筆者らの家系では異常はみられず，SCA4と筆者らの病型には明瞭な違いがあった。この他にも錐体路徴候の有無や発症年齢の違いなどもみられたことから，筆者らの家系をSCA4と呼ぶのではなく，16q-linked ADCA typeⅢ（純粋小脳失調型）と呼ぶことにした[9]。

Ⅱ．16q連鎖型脊髄小脳変性症の分子遺伝学的研究

先述のように遺伝子座が第16番染色体長腕（16q13.1-q22.1）に存在することを見出した[9,10]あと，臨床的に類似する2家系を加え，詳細に連鎖領域との関連を調べた。その結果，16q22.1領域に存在する多型性DNAマーカーGGAA05とD16S3095で挟まれた3.8 Mb（メガベース）の範囲で，全家系のハプロタイプが一致することを見出した[11]。すなわち，この範囲内に原因遺伝子が存在する可能性が高い。

次に筆者らは，全国の広い範囲の施設の先生方にご協力をいただいて，高齢発症で既知の遺伝子変異のない家系を集積した。最終的に合計52家系について，候補領域を多数の多型性DNAマーカーで解析したところ，全ての家系が

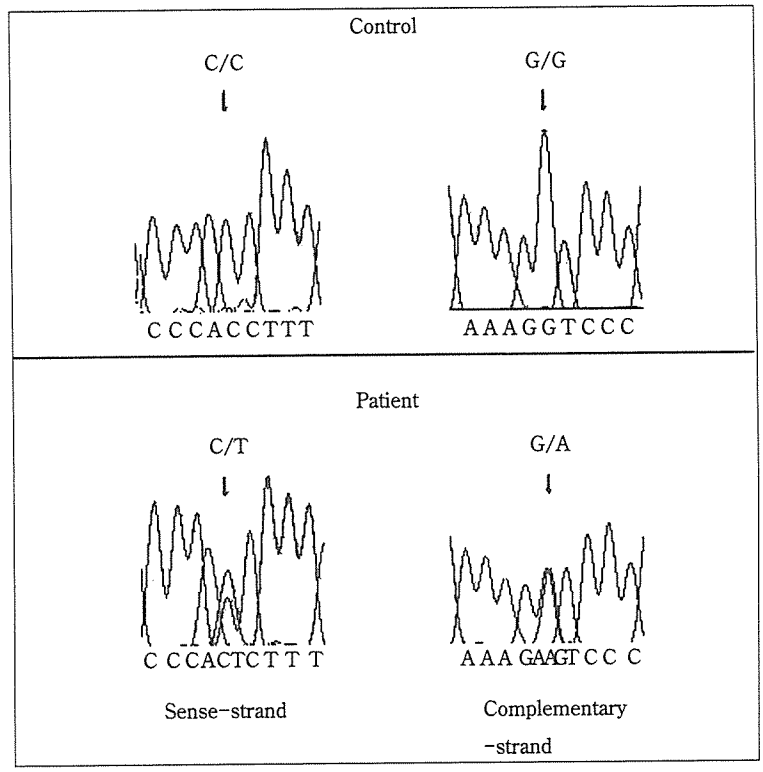

図2 ***puratrophin-1*遺伝子に見出した患者に特異的な一塩基置換(文献4より)**
患者では翻訳開始点より16塩基上流に位置するcytosine (C) がthimine (T) に置換していた。

同じハプロタイプを取る領域は，マーカーGATA01から17 msmまでの，さらに狭い600 kb(キロベース)領域であることを見出した(図1)[4]。逆にこの外の領域では家系間で発症者のアレルが必ずしも一致しなかった。これは遺伝学では創始者効果と呼ばれる現象で，あたかも昔存在した少数の先祖に，特有のハプロタイプを有する方々が存在し，代々子孫に伝わる過程で原因遺伝子から遠いマーカーでは組み換えなどで別のアレルを示すが，原因遺伝子から近いマーカー群でのアレルは保存される現象である。したがって，疾患の原因となる遺伝子は，この創始者効果がみられる16q22.1の600 kb領域に存在する可能性が高いということになる。

この最有力領域の600 kbの染色体領域には，21個の遺伝子がEnsembl (http://www.ensembl.org) などの遺伝子データベースに登録されていた。筆者らはこの遺伝子の全てのエクソンについて，患者と健常者を丹念に比較した。その結果，これまでにゲノム解読情報から蛋白機能の推測がなされて，データベースに登録されていただけの遺伝子*DKFZP434I216*の5'非翻訳領域(5'-UTR)に，一塩基の置換変化を発見した(図2)[4]。この変化は想定(predict)された翻訳開始点の，わずか16塩基5'-UTR側に遡ったところのcytosine (C) がthymine (T) に変化しているものであった。一方，この遺伝子変化は筆者らが有した健常者500名のDNAには一切見出さなかった。また，検索した他のいずれの遺伝子にも健常者と患者の間に違いはみられず，このCからTへの変化は，少なくとも本病型と密接に関わる唯一の遺伝子変化であると考えられた。

しかし，この遺伝子変化は翻訳領域の外にあるため，本疾患の「変異」と呼べるか否かは判然としなかった。また，特に注意したことは，この遺伝子変化が患者全員に共通したハプロタイプの中に存在するために，仮に日本人集団で極めて稀な多型性変化であった場合でも，あたかも患者にのみ共通する変化に見えてしまう。これは創始者効果が存在したための必然的現象とも言える。以上より，本遺伝子変化が原因であるかどうかは慎重に判断する必要がある。

図3 16q-ADCA患者の小脳皮質組織所見
小脳Purkinje細胞は細胞体が萎縮しており，その周囲にamorphousな構造物がみられる（ヘマトキシリン・エオジン染色，スケール：50μm）。

このようなことから筆者らは，*puratrophin-1*遺伝子でのC→T変化が，どのような生物学的意義を持つのか，その位置が5'-UTRであったため，まず転写活性に変化を起こすかの実験的検証をluciferase assayによって行うことにした。すると，この一塩基の置換によって，下流の遺伝子の発現が有意に低下することが裏付けられた[4]。このため筆者らはこの遺伝子変化のもつ意義，遺伝子の構造や発現などを決定することにし，この遺伝子を特徴的なPurkinje細胞萎縮が起きるという本疾患の神経病理所見から，*"puratrohin-1"*（Purkinje cell atrophy associated protein-1）と命名することにした。

Ⅲ. 16q-ADCAの神経病理所見

ここで本病型の神経病理像について触れたい。

筆者らは高齢発症の純粋小脳失調症の範疇に属する家系を多数集積し，経過を追跡しているうちに，ある家系の患者の病理学的検索を行える機会を得ることができた[12]。これがSCA4を含めて16番染色体長腕に連鎖する2病型の中での最初の病理報告となった。患者は70歳頃に歩行障害で発症し，緩徐に小脳失調が進行し，25年程度経過した96歳で老衰のために死亡した症例である。

肉眼的には小脳虫部上面が軽度萎縮していたほかは目立った変化はなかった。組織学的には，小脳皮質の変性が最も目立つ所見であったが，25年という経過のわりに変性の程度は強くなく，小脳皮質の3層ともが変性していたのは，肉眼的に萎縮の見られた小脳虫部上面にほぼ限られていた。病変の程度の軽い部位でも一貫してみられた所見は小脳Purkinje細胞の変化で，Purkinje細胞がこの疾患で最も障害されやすい疾患であることがわかった。すなわち，Purkinje細胞では，細胞が脱落していただけでなく，残存した細胞でもしばしば細胞体が萎縮し，その周囲にエオジン好性の構造物が取り巻いていた（図3）。この所見はこれまでの神経病理学で記載のない組織像であるが，エオジン好性構造物の本体は，ひとつにはPurkinje細胞の突起の遺残物であり，もうひとつは他の神経細胞体から及んだ神経前終末であることがわかっている。この病理所見を元に本病型の神経病理学的診断が可能である。なお，最近ドイツのSCA4家系[13]の病理所見が報告された[14]。その所見では，小脳皮質でも筆者らの症例のような特徴的Purkinje細胞変性の像はみられず，病変も脳幹部など広範囲に及んでおり，病理学的にもSCA4と16q-ADCAは異なっていることが判明した。

Ⅳ. *Puratrophin-1*遺伝子変化と神経細胞障害の関連

次に筆者らは，この遺伝子の発現状況をmRNAと蛋白について調べた。小脳皮質に発現するメッセンジャーRNAレベルでは，本来複雑なalternative transcriptionがあることがわかったが，主な転写産物は2種類で，いずれも8,561～8,728塩基長の長いmRNAであると想定された。このうち1つは，exon 1（最初のエクソン）からexon 21（最後のエクソン）にかけての3,576塩基にわたる長い翻訳領域を有する全長型転写産物（full-length mRNA）であった。もう1つはexon1とexon2の間にイントロンが挿入され，その結果frame-shiftを起こして短いopen reading frame（ORF）を有する転写産物であった（図4）。

この全長型転写産物から，puratrophin-1蛋白の機能を予測したところ，データベース通りspectrin repeatと*Rho* guanine-nucleotide exchange factor（RhoGEF）の2つの重要なドメインがあることが予想された。このことは，puratrophin-1蛋白が，Golgi装置などの膜型細胞小器官において発現し，細胞の分裂や分化などの際にactin分子と結合することによって，その細胞内小器官の形態をダイナミックに制御して，細胞骨格を担う役目を果たしていることを示唆している[14,15]。

次にpuratrophin-1蛋白に対する抗体を作製し，免疫組織化学により臓器での発現を確認するとともに，実際に予想通りGolgi装置などと関連するかを調べることにした。まず対照ヒト小脳ではPurkinje細胞が均一に染色され，確かに神経細胞で発現していることが

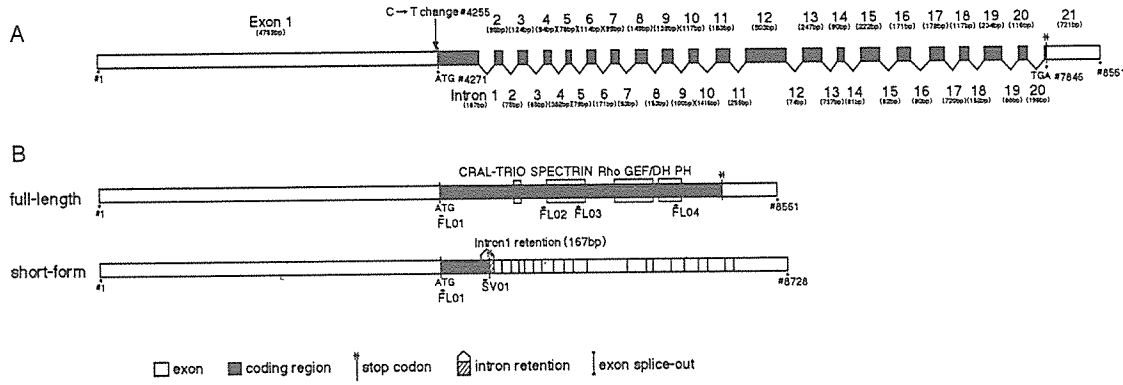

図 4 *puratrophin-1* 遺伝子 mRNA と蛋白モチーフ（文献 4 より改変）
A：*puratrophin-1* ゲノム構造。21 個のエクソンがあり，第 1 エクソンの翻訳開始点直前 16 塩基の部位に C→T 変化がある。B：大きく分けて full-length（全長型）と short-form（短い型）の 2 種類の mRNA が存在する。

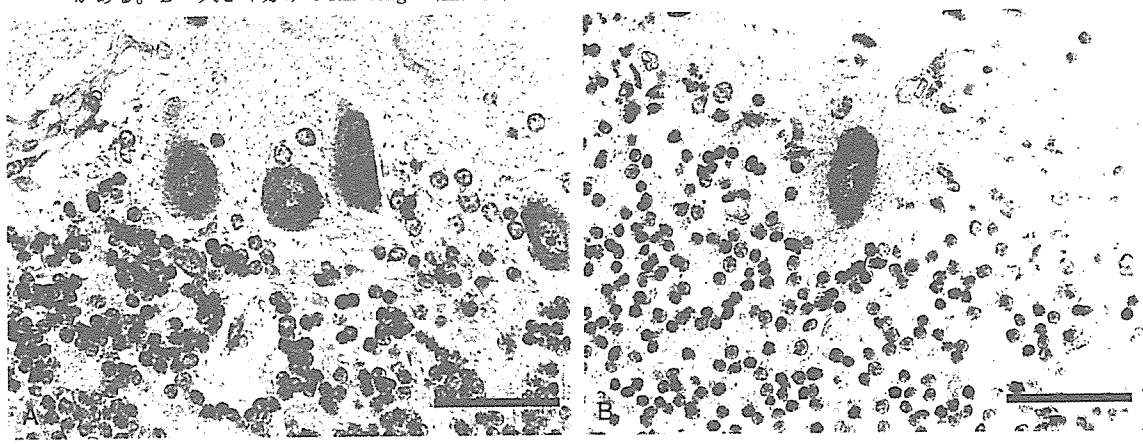

図 5 puratrophin-1 蛋白の免疫組織化学
A：コントロール小脳皮質。Purkinje 細胞の細胞体が均一に染色される。B：16q-ADCA 患者の小脳皮質。Purkinje 細胞内に強い免疫反応を示す構造が見える（A, B いずれも diaminobenzidine を用いた免疫組織化学，スケール：50 μm）。

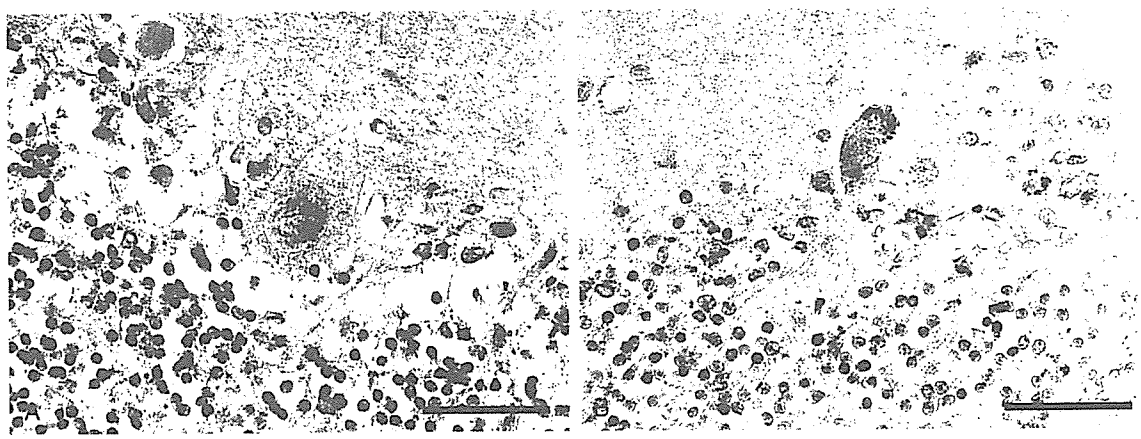

図 6 16q-ADCA 患者小脳皮質の Golgi 関連膜蛋白（G58K）と spectrin に対する免疫組織化学（文献 4 より）
A：G58k に対する抗体を用いた免疫組織化学。細胞内に異常な凝集体形成が見られる。B：α&β spectrin に対する抗体を用いた免疫組織化学。同様に Purkinje 細胞内に強い免疫反応を示す構造が見える（A, B いずれも diaminobenzidine を用いた免疫組織化学，スケール：50 μm）。

表 16q-ADCAと米国およびドイツSCA 4家系，およびSCA1，SCA6，MJDとの臨床徴候の比較

	16q22.1	SCA6	SCA4	SCA4	MJD	SCA1
引用文献	自験例	22)	10)	14)	23)	24)
人種	日本人	日本人	アメリカ人	ドイツ人	日本人	日本人
患者数（人）	120	140	20	14	75	35
発症年齢（歳）						
運動失調症状	61.2	46.2	39.3	38.3	38.4	36.0
聴力障害	66.4					
重要臨床徴候の出現頻度						
歩行失調	100%	100%	95%	100%	92.0%	100%
四肢失調	92.6%	91.9%	95%	100%	92.0%	100%
小脳性言語	92.6%	94.6%	50.0%	100%	92.0%	100%
注視眼振	55%	62%		57%	94.6%	37.0%
筋トーヌス						
正常	42.9%					
低下	57.1%					
亢進	0%				86.6%	23.0%
腱反射						
正常	71.4%					
亢進	0%					
低下	28.6%	15.2%				
消失	0%		100%			
Babinski徴候	0%	1%	20%	7%		83.0%
振戦	14.3%	8.6%				9.0%
振動覚低下	5.0%	5.0%	100%	100%		40.0%
感覚神経活動電位消失	0%		92.3%	100%		
認知症	0%	1%				
聴力低下	42.7%	10.0%				

示唆された。一方，本疾患患者の小脳では，Purkinje細胞でpuratrophin-1蛋白の凝集体が認められた（図5）。常染色体優性遺伝性変性疾患では原因蛋白の凝集が認められることが多いことから[16]，本遺伝子変化が確かに16q-ADCAの病態に関与することを示唆すると考えられた。

さらに興味深いことに，puratrophin-1蛋白が関連すると予想されたGolgi装置関連膜蛋白G58kに対する抗体や，spectrin蛋白に対する抗体を用いて免疫組織化学的検索を行うと，いずれの抗体でも対照者小脳Purkinje細胞が均一に染色されたのに対して，患者小脳Purkinje細胞では凝集構造物がみられた（図6）。このような所見は，他の脊髄小脳変性症患者でもみられず，単にPurkinje細胞の変性機序に関連したものである可能性があげられる。

以上のことより，遺伝子レベルでは*puratrophin-1*遺伝子5'-UTRでの一塩基置換が，ただ単に患者群で認められただけではなく，蛋白レベルでは患者で最も強く障害されるPurkinje細胞で，蛋白の封入体を形成し，かつ関連する別の蛋白も凝集していることがわかった。

*puratrophin-1*遺伝子の発現量低下がどのように蛋白の凝集を起こして，細胞障害を起こすのかは不明であり，今後解決してゆくべき重要な課題である。一方，puratrophin-1蛋白の機能という観点に着目すると，RhoGEF蛋白はsmall G-protein GTPaseであるRhoGTPaseを活性化することによって，細胞内小器官の膜輸送（membrane trafficking）やアクチンのダイナミックスに関与し，最終的には細胞の増殖や分化，移動など，実に様々な細胞の反応を制御していることが知られている[17]。本病型で注目されたpuratrophin-1蛋白は，このRhoGEFドメイン以外にspectrin repeatドメインを有しており，特にGolgi装置などにおいて，アクチン分子のダイナミックスを制御することによって，細胞内小器官での膜の安定化，ひいては細胞骨格の安定化などに関与することが想定される[17,18,19]。したがって，puratrophin-1蛋白の異常が，何らかの形でアクチンを介して神経細胞に障害を与えていると仮説を

立てることは可能かもしれない。実際，患者小脳 Purkinje 細胞においては，microtubules と結合する Golgi 装置膜蛋白である G58k 蛋白が凝集しており，puratrophin-1 蛋白の異常が Golgi 装置での膜蛋白異常に関連している可能性があげられる[4]。

V. 16q-ADCA の臨床像と疾患頻度

puratrophin-1 遺伝子の C→T 変化を用いて遺伝子診断を行ったところ，本疾患の頻度は筆者らの施設では，SCA6 や MJD に次いで頻度の多い疾患であることが判明した。筆者らのほかにも，長野県や鹿児島県でも報告があり，おそらくほぼ全国的にこの病型が存在すると思われる[20,21]。この病型の臨床症候を SCA4 や他の失調症と比較した（表）[10,14,22-24]。本病型は最も高齢発症で，純粋小脳失調を呈し，中には難聴を認めることが特徴であると考える。逆にこのような特徴を有する患者では，16q-ADCA を疑う必要がある。

おわりに

筆者らは脊髄小脳変性症の一病型の原因を探索した結果，特異的な遺伝子変化をもつ新しい遺伝子，*puratrophin-1* を見出した。この *puratrophin-1* 遺伝子の変化あるいは puratrophin-1 蛋白の機能異常が小脳変性症だけでなく，実は聴覚神経障害にも関連している可能性が示唆されている。今後この遺伝子異常の意義を含めて，正確に病態を解明していく必要がある。

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Abstract

Molecular genetics and clinical aspects of 16q-ADCA

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We describe clinical features and specific genetic change in 16q22.1-linked form of autosomal dominant cerebellar ataxia (16q-ADCA). Through extensive positional cloning strategy, we identified that all affected individuals with 16q-ADCA harbor heterozygous C-to-T, single nucleotide change in the 5'-untranslated region of the gene *DKFZP434I216*, which we renamed "*puratrophin-1*" (Purkinje cell atrophy associated protein-1). Using this genetic change, we found that 16q-ADCA is a common subtype among ADCAs in Japan. Clinically, this form is characterized as pure cerebellar ataxia with latest age of onset. Further analysis is needed to elucidate how this genetic change contributes to the pathogenic mechanism of this disease.

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悪性腫瘍に関連した神経障害

小脳障害

石川 欽也

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