

found to be negatively associated with risk of Parkinson's disease ( $P=0.0009$ ). Consequently, they concluded that the *FGF20* gene was a susceptibility gene for Parkinson's disease [11].

Subsequently, Clarimon *et al.* sought to replicate the association of the *FGF20* gene with Parkinson's disease by performing a case-control association study with four SNPs [rs1989756, rs1989754, rs1721100, and ss20399075 (rs12720208)] using Finnish and Greek samples. They found a difference in allele frequency in only rs1989754, but the difference was not significant after the Bonferroni correction. They also found no significant difference in the distribution of haplotypes between patients and controls. They hence failed to replicate the association of the *FGF20* gene with Parkinson's disease [12]. Thus, it is still controversial as to whether the *FGF20* gene is a susceptibility gene for Parkinson's disease or not. We here conducted a case-control association study using a large number of Japanese samples in order to evaluate the association of the *FGF20* gene with risk of Parkinson's disease.

**Materials and methods**

We recruited 1388 unrelated Parkinson's disease patients (age,  $65.7 \pm 9.8$ ; male/female ratio, 0.84) and 1891 unrelated controls (age,  $48.5 \pm 17.6$ ; male/female ratio, 1.08). The diagnosis of Parkinson's disease was based on the presence of two or more of the cardinal features of Parkinson's disease (tremor, rigidity, bradykinesia, and postural instability), according to the criteria for Parkinson's disease [13]. Patients were evaluated by certified neurologists specializing in Parkinson's disease. The average age of onset was  $57.7 \pm 11.1$  years. All patients and controls were of Japanese ancestry. Informed consent was obtained from each individual, and approval for the study was obtained from the University Ethical Committees. Genomic DNA was extracted from venous blood using standard procedures.

The TaqMan SNP Genotyping Assay (Applied Biosystems, Foster, California, USA) was employed for five SNPs (rs12718379, rs1989756, rs1989754, rs1721100, and rs12720208). SNP information was obtained from the dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP/>) and the International HapMap Project database (<http://hapmap.org>) [14].

All statistical analyses were performed by using the software SNPalyze (Dynacom, Japan). Genotype deviation from Hardy-Weinberg equilibrium was assessed by the  $\chi^2$  test. The statistical significance of a case-control association was evaluated by the  $\chi^2$  test, and odds ratio and its 95% confidence intervals (CIs) were calculated by the Bootstrap method. Haplotype frequencies were estimated using an expectation-maximization algorithm [15]. We evaluated pair-wise linkage disequilibrium (LD) among SNPs by  $D'$  value, and  $r^2$  as standards for LD. Case-control haplotype analyses were carried out by calculating the permutation  $P$ -value on the basis of 10 000 replications [16].

**Results**

Two SNPs (rs1989756 and rs12720208) of the five SNPs, examined by van der Walt *et al.*, showed a monomorphism in 95 individuals drawn from the Japanese population, and therefore these two SNPs were excluded from further analysis. Three SNPs (rs1989754, rs1721100, and

Table 1 Summary of the association of three SNPs between Parkinson's disease patients and controls

SNP ID	Alleles		Genotype						Allele 1 versus Allele 2		Genotype II versus I2 + 22		Genotype II + I2 versus 22				
	1-2	Strand <sup>a</sup>	Patient			Control			Frequency of allele 1	Odds ratio (95% CI) <sup>b</sup>	P-value	Odds ratio (95% CI) <sup>b</sup>	P-value	Odds ratio (95% CI) <sup>b</sup>	P-value		
			I/I	I/2	2/2	Total	I/I	I/2								2/2	Total
rs12718379	A-G	Reverse	249	641	481	1371	375	902	597	1874	0.44	1.11 (1.01-1.23)	0.041	1.13 (0.95-1.34)	0.19	1.16 (1.00-1.34)	0.054
rs1989754	G-C	Forward	261	628	477	1366	381	895	586	1862	0.44	1.10 (1.00-1.22)	0.055	1.09 (0.92-1.28)	0.34	1.17 (1.01-1.35)	0.040
rs1721100	C-G	Forward	270	639	458	1367	407	925	542	1874	0.46	1.14 (1.03-1.26)	0.0089	1.13 (0.94-1.36)	0.17	1.24 (1.06-1.43)	0.0053

<sup>a</sup>Relative to the transcriptional direction.

<sup>b</sup>CI, confidence intervals; SNP, single nucleotide polymorphism.

rs12720208) are included in HapMap. rs12720208 also shows no polymorphism in JPT (Japanese in Tokyo) HapMap, consistent with the genotyping results of our samples. In the FGF20 gene region, 171 SNPs were observed in dbSNP. According to JPT HapMap, the Tagger method showed that two SNPs (rs1989754 and rs1721100) can represent the remainder of the HapMap SNPs of the FGF20 gene region, as tag SNPs with a criteria of  $r^2 > 0.8$  and a minor allele frequency  $> 0.1$ , although the number of tag SNPs differed between JPT and CEU [CEPH (Utah residents with ancestry from northern and western Europe)] [17]. Thus, we considered that a case-control association study using three SNPs [two tag SNPs (rs1989754 and rs1721100) plus rs12718379] was appropriate for assessing the association of the FGF20 gene with Parkinson's disease.

Table 1 shows the results of the SNP genotyping in the Parkinson's disease patients and controls. The association of rs1721100 was significant in allele 1 versus allele 2 [frequency of allele 1; 43% in patients and 46% in controls,  $P=0.0089$ , odds ratio 1.14 (95% CI, 1.03–1.26)] and in genotype 11+12 versus 22 [ $P=0.0053$ , odds ratio 1.24 (95% CI, 1.06–1.43)]. The association with rs1721100 was significant even after the Bonferroni correction (tests for three SNPs). As for rs12718379, a decrease in frequency of allele 1 was found in patients compared with controls [frequency of allele 1; 42% in patients and 44% in controls,  $P=0.041$ , odds ratio 1.11 (95% CI, 1.01–1.23)]. As for rs1989754, there was a difference in frequency of genotype 11+12 versus 22 between patients and controls [ $P=0.040$ , odds ratio 1.17 (95% CI, 1.01–1.35)]. Neither rs12718379 nor rs1989754, however, showed a significant association with Parkinson's disease after the Bonferroni correction. The genotype frequencies of all three SNPs were not significantly different from the values expected from the Hardy-Weinberg equilibrium.

We calculated the LD among the three SNPs in patients and controls.  $D'$  values (absolute value) and  $r^2$  for pair-wise LD of controls are shown in Table 2. A high LD was detected between each pair of SNPs, and the same trend was observed in patients and in the JPT samples of the HapMap database (data not shown). These findings suggested that the three SNPs were in single LD, and we therefore performed haplotype association analysis. Haplotype fre-

quencies of the three SNPs were estimated in patients and controls (Table 3). Two common haplotypes (haplotypes 1 and 2) covered  $> 90\%$  of the population haplotypes in both patients and controls. The frequency of haplotype 2 (A-G-C) was significantly less in patients than controls (38% in patients and 41% in controls, permutation- $P=0.0075$ ). This indicates that haplotype 2 is a protective haplotype for Parkinson's disease in the Japanese population. Taken together, our genetic analyses support the FGF20 gene being a susceptibility gene for Parkinson's disease in the Japanese population.

## Discussion

Our results are consistent with the report by van der Walt *et al.* [11], which showed an association of the FGF20 gene with risk of Parkinson's disease. The significance of the FGF20 gene for Parkinson's disease susceptibility in our study, however, was not so strong as that shown by van der Walt *et al.* This discrepancy may result from: (i) the ethnic differences between the Japanese samples and samples from the United States; the association in the Japanese population might be smaller than in the United States, or (ii) the difference in epidemiological approaches; we performed a case-control association study by the  $\chi^2$  test in unrelated samples, while they analysed family-based samples by the PDT. rs12720208, the strongly associated SNP in the report by van der Walt *et al.*, was excluded from our study because we were not able to find polymorphism of this SNP in the Japanese samples. It is interesting that rs1721100, the most strongly associated SNP in our study, and rs12720208, however, are both located in the 3' UTR region of the FGF20 gene. LD indices between rs12720208 and rs1721100 showed that these two SNPs are in a single LD block ( $D'=1$ ) and that the correlation was not strong ( $r^2=0.28$ ) (on the basis of CEU HapMap).

On the other hand, the case-control association study by Clarimon *et al.* [12] failed to replicate the association of the FGF20 gene with risk of Parkinson's disease, although the rs1989754 G allele frequency was higher in patients than controls in the Finnish samples (52% in patients and 42% in controls,  $P=0.03$  before Bonferroni correction). However, as their sample size was not large enough, their study does not disprove the association of the FGF20 gene with Parkinson's disease convincingly if the influence for Parkinson's disease in the Greek and Finnish population is to the same extent as in our Japanese sample. The sample size of their study was considerably smaller than ours (Finnish series, 144 patients and 135 controls; Greek series, 151 patients and 186 controls in their study, compared with 1388 patients and 1891 controls in our study). As mentioned in their report, their experiment had 80% power to detect

**Table 2** Linkage disequilibrium between SNPs in the FGF20 gene

SNP ID	rs12718379	rs1989754	rs1721100
rs12718379	—		
rs1989754	0.94 (0.98)	—	
rs1721100	0.68 (0.86)	0.72 (0.88)	—

$r^2$  ( $D'$ ) values of controls are shown for each pair of single nucleotide polymorphisms (SNPs).

**Table 3** Haplotype association analysis using three SNPs in the FGF20 gene

Haplotype ID	Base at SNP			Haplotype frequency		P-value
	rs12718379	rs1989754	rs1721100	Patient	Control	
Haplotype 1	G	C	G	0.53	0.50	0.054
Haplotype 2	A	G	C	0.38	0.41	0.0075
Haplotype 3	G	C	C	0.045	0.047	0.75
Haplotype 4	A	G	G	0.035	0.028	0.11

risks from 1.7 to 3.6 in the Finnish samples and from 1.6 to 2.1 in the Greek samples, whereas the odds ratio of the *FGF20* gene in our data was 1.14. The possibility of type 2 errors in their study could not be excluded as an explanation for this negative finding. Another explanation for lack of replication could be genetic heterogeneity; there might not be an association between the *FGF20* gene and Parkinson's disease in the Greek and Finnish populations, whereas there might be in the Japanese and the United States-based population.

In this study, the three SNPs (rs12718379, rs1989754, and rs1721100) showed a difference between patients and controls to some degree. After Bonferroni correction, however, a significant association was detected only in rs1721100. The correlations between rs1721100 and the other two SNPs were not strong ( $r^2=0.68$  with rs12718379 and 0.72 with rs1989754), which might explain the different extents of significance among the three SNPs.

The *EFHA2* gene is located 25 kb upstream of the 5' UTR, and the *MSR1* gene is located 800 kb downstream of the *FGF20* gene. The entire region of the *FGF20* gene is within a single LD block of 20.8 kb (on the basis of JPT HapMap). Moreover, no other known genes reside within this LD block. Therefore, we concluded that our positive finding results from the association between the *FGF20* gene and Parkinson's disease.

### Conclusion

We performed a case-control association study using a large number of samples (1388 Parkinson's disease patients and 1891 controls) in the Japanese population, and found a significant association of Parkinson's disease with rs1721100 and haplotype 2 (A-G-C) in the *FGF20* gene. Our results, together with those of van der Walt *et al.*, demonstrate an association of the *FGF20* gene with Parkinson's disease in two different ethnic groups. This evidence suggests the involvement of the *FGF20* gene in the pathogenesis of Parkinson's disease.

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## Redefining the disease locus of 16q22.1-linked autosomal dominant cerebellar ataxia

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**Abstract** The 16q22.1-linked autosomal dominant cerebellar ataxia (16q-ADCA; Online Mendelian Inheritance in Man [OMIM] #117210) is one of the most common ADCAs in Japan. Previously, we had reported that the patients share a common haplotype by founder effect and that a C-to-T substitution (–16C>T) in the *puratrophin-1* gene was strongly associated with the disease. However, recently, an exceptional patient without the substitution was reported, indicating that a true pathogenic mutation might be present elsewhere. In this study, we clarified the disease locus more definitely by the haplotype analysis of families showing pure cerebellar ataxia. In addition to microsatellite markers, the

single nucleotide polymorphisms (SNPs) that we identified on the disease chromosome were examined to confirm the borders of the disease locus. The analysis of 64 families with the –16C>T substitution in the *puratrophin-1* gene revealed one family showing an ancestral recombination event between SNP04 and SNP05 on the disease chromosome. The analysis of 22 families without identifiable genetic mutations revealed another family carrying the common haplotype centromeric to the *puratrophin-1* gene, but lacking the –16C>T substitution in this gene. We concluded that the disease locus of 16q-ADCA was definitely confined to a 900-kb genomic region between the SNP04 and the –16C>T substitution in the *puratrophin-1* gene in 16q22.1.

**Keywords** 16q-ADCA · Pure cerebellar ataxia · Haplotype · SNP · Founder effect · SCA4

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### Introduction

Autosomal dominant cerebellar ataxia (ADCA) is a clinical entity of heterogeneous neurodegenerative diseases that show dominantly inherited, progressive cerebellar ataxia that can be variably associated with other neurological and systemic features (Harding 1982). ADCA is now classified by the responsible mutations or gene loci. Subtypes of ADCA of which causative genes or gene loci have been identified are known as spinocerebellar ataxia type (SCA) 1, 2, 3 (or Machado-Joseph disease), 4–8, 10–19, 21–23, 25, 26, 28, dentatorubral and pallidolulsian atrophy (DRPLA), and ADCA with mutation in the fibroblast growth factor (FGF) 14 gene (Schöls et al. 2004; Yu et al. 2005; Cagnoli et al. 2006).

Among these, mutations in SCA1, SCA2, SCA3/MJD, SCA6, SCA7, SCA17, and DRPLA have been identified as

the expansions of a trinucleotide (CAG) repeat that encodes the polyglutamine tract, uniformly causing the aggregation of polyglutamine-containing causative protein (Ross and Poirier 2004). The expansion of noncoding trinucleotide (CAG or CTG) or pentanucleotide (ATTCT) repeats are involved in SCA8, SCA10, and SCA12 (Holmes et al. 1999; Koob et al. 1999; Matsuura et al. 2000). Very few families are affected by missense mutations in beta-III spectrin (*SPTBN2*) (SCA5 (see Ikeda et al. 2006)), voltage-gated potassium channel KCNC3 (SCA13 (see Waters et al. 2006)), protein kinase C gamma (PKC gamma) (SCA14 (see Chen et al. 2003)), and *FGF14* genes (ADCA with *FGF14* mutation (see van Swieten et al. 2003). However, genes or even loci remain unidentified for 20–40% of families with ADCA (Sasaki et al. 2003).

We had previously found that Japanese families with ADCA map to the human chromosome 16q22.1 (16q-ADCA), the gene locus of SCA4 (Flanigan et al. 1996; Hellenbroich et al. 2005; Nagaoka et al. 2000). However, our families show clinically pure cerebellar ataxia without other neurological signs, such as sensory neuropathy or pyramidal tract signs seen in SCA4. All 16q-ADCA patients shared a common haplotype, presumably due to inheritance from a disease chromosome of a founder (Takashima et al. 2001). Our haplotype analysis of 52 families with DNA polymorphic microsatellite markers revealed that they all share a common haplotype for the 400-kb genomic region in 16q22.1 (Ishikawa et al. 2005). Within this region, we found that a heterozygous single nucleotide C-to-T substitution (–16C>T) in the untranslated region of the *puratrophin-1* gene was entirely segregated with all patients, suggesting a strong association with the disease. This substitution was also found in other cohorts of Japanese families with ataxia (Ouyang et al. 2006; Onodera et al. 2006), while it was not found in Caucasian patients in Europe (Wieczorek et al. 2006). The frequency of 16q-ADCA is considered to be relatively high in Japan, counted as the third or fourth major subtype of ADCA after MJD, SCA6, and DRPLA (Takano et al. 1998; Sasaki et al. 2003; Ohata et al. 2006).

However, one group recently reported an exceptional patient without the –16C>T substitution in the *puratrophin-1* gene, in a family in which all of the other affected subjects carried the substitution (Ohata et al. 2006). This patient shared the common haplotype in a region centromeric to the substitution in the *puratrophin-1* gene, suggesting that a true pathogenic mutation may be present in a different gene lying centromeric to the –16C>T substitution in the *puratrophin-1* gene. Moreover, other patients sharing the common haplotype centromeric to the substitution in the *puratrophin-1* gene without the substitution might exist.

In this study, we re-examined the haplotype of families showing ataxia in order to clarify a common genomic re-

gion shared in all 16q-ADCA patients. Because slippage mutation might cause minor deviations in repeat size for microsatellite markers (Ikeda et al. 2004), single nucleotide polymorphisms (SNPs) detected by ourselves on the disease chromosome were used in the analysis to confirm recombinant regions that are not conserved among families.

## Materials and methods

### Haplotype analysis

DNA samples from patients showing ataxia referred to our department were examined. After informed consent was obtained, genomic DNA was extracted from peripheral blood lymphocytes or lymphoblastoid cell lines by the use of methods described elsewhere (Ishikawa et al. 1997). All families were excluded for SCA1, SCA2, SCA3/ MJD, SCA6, SCA7, SCA8, SCA12, SCA14, SCA17, and DRPLA by testing for mutations in the disease genes.

Firstly, common haplotypes of the 16q-ADCA families with the –16C>T substitution in the *puratrophin-1* gene were analyzed. Genotypes were determined for 19 microsatellite markers (D16S3043, D16S3031, D16S3019, CTATT01, TAGA02, GGAA05, D16S397, GGAA10, GATA01, D16S421, TA001, GA001, 17 msm, D16S3107, GGAA01, CTTT01, GT01, D16S3095, D16S512) in 16q22.1 by the use of methods described elsewhere (Ishikawa et al. 2005). Compared to our previous study (Ishikawa et al. 2005), several new markers with high specificity to the 16q-ADCA chromosome were added and the region analyzed was expanded to beyond the previous critical region spanning GATA01 and 17 msm (Ishikawa et al. 2005) in order to determine the maximum genomic region conserved in all of the affected individuals from all of the families. Although the phase of the markers were not confidently determined in families that have only a few examined members, the possibility that they carried the haplotype was indicated in those cases.

Secondly, haplotypes of families without the –16C>T substitution in the *puratrophin-1* gene were also analyzed to see if they had the common haplotype centromeric to the substitution in the *puratrophin-1* gene. Their genotypes were determined for 14 markers (D16S3043, D16S3019, CTATT01, TAGA02, GGAA05, D16S397, D16S3086, GATA01, GA001, 17 msm, CTTT01, GT01, D16S3095, D16S512), which are relatively highly specific to the common haplotype in 16q-ADCA.

### Single nucleotide polymorphisms

We searched for single nucleotide polymorphisms (SNPs) on the disease chromosome by ourselves because most of

**Table 1** The haplotype analysis of 16q22.1-linked autosomal dominant cerebellar ataxia (16q-ADCA) families with the -16CT substitution of the *puratrophin-1* gene. The gray squares indicate that the alleles are one repeat-unit different from the common allele of 16q-ADCA and the black squares indicate alleles with two or more repeat-unit differences. One repeat-unit difference was seen for

markers D16S397, GGAA10, GATA01, and TA001, close to the *puratrophin-1* gene in several families, and greater repeat-units differences were observed for GGAA05 and other centromeric markers. Similarly, greater repeat-units differences were observed for 17msm and markers lying telomeric to 17msm. n.e.=not examined

Marker	most common haplotype	family No. frequency in control (%)	P2	P4	P14	T2	T3	T4	T5	T6	T7	T12	T15	T19	T21	T25	T26	T28	T30	T37	T42	T43	T44	T46	
D16S3043	1	25.0	1	1/6	7	5	8	1	1/8	1	1/5	5	1/7	n.e.	1/8	n.e.	n.e.	1	n.e.	1	n.e.	1/5	1/5	1	
D16S3031	9	68.1	9	9	9	9	9	9	10	1	10	9	9/10	9	9	9	9	1/9	9	9	9	9	9	9	
D16S3019	4	41.4	4	4	4	4	4	4/5	3/4	3/4	3/4	1/4	4	n.e.	4	n.e.	n.e.	4/7	3/4	2/4	n.e.	3/4	3/4	1	
CTATT01	1	32.4	1	2/4	1	1	1	1/4	1	1	1	1/3	n.e.	1/3	n.e.	n.e.	1/3	n.e.	1	n.e.	1/2	1	0/3		
TAGA02	4	10.3	4	6	4	4	4	4/6	4/6	4/5	4	2/4	4/5	n.e.	4/5	n.e.	n.e.	4/5	4	3/4	n.e.	5/6	4/5	2/6	
GGAA05	1	1.4	1	6	1	1	5	1	1	1	2	1	2/4	1/3	1/5	5	1/2	1/7	1/5	1/3	1/3	1/5	2/4	3/6	
D16S397	1	47.1	n.e.	1/2	1	1	1	0/4	1/2	1/3	1/3	2/3	1	n.e.	1	n.e.	n.e.	1/4	-3/0	-3/1	n.e.	-3/1	1	-3/1	
GGAA10	3	13.2	3	3	3	3	4	3/5	3	3	3	3	3	3	3/6	2/3	2/4	3/7	3/5	3/8	4/6	3	3/6	3/5	3/7
GATA01	2	44.1	2	2/3	2	2	2	3	3	3	2	2	1/2	2/3	1/3	3/4	1/3	1/3	1	2	3	1/3	2/3	2/3	
D16S421	3	75.7	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
<i>puratrophin-1</i> (CT)	T	0.0	T	T	T	T	T	T	T	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	
TA001	1	23.8	1	1	1	1	1	1	1	1	1	1	1	1/9	1/7	2/8	1/6	1/10	1/9	1/9	1	1/9	1/9	1/5	
GA001	4	0.1	4	4	4	4	4	4	4	4/7	4/7	4	4	1/4	4/8	4/5	4/7	4/7	4/6	4/11	4/5	4/7	4/5	4/7	
17msm	2	8.3	2	2	2	2	2	2	2	2	2	5	2	2/5	2/5	2/4	2/4	2/4	2/4	2/5	2/4	2/4	4/6	2/4	
D16S3107	7	13.9	7	7	7	7	7	6	7	7	7	7	5	6	5/6	3/7	6/10	6	7	5/7	3/7	6/7	4/7	6/7	
GGAA01	6	18.8	6	6	6	6	6	6	6	6	6	6	1/6	3/6	2/6	2/6	4/6	6/7	6	6	6	6/7	6/7	5/6	
CTTT01	8	28.2	8	8	8	8	9/10	8	5	9	8	10	8	9/10	3/9	9/10	8/10	8	8/11	6/9	8/15	9/10	1/7	3/8	
GT01	6	15.8	6	6	6	6	6	6	7	6	6	4	6	2/6	1/6	4/6	4/6	2/6	5/6	4/6	6	3/6	3/4	4/6	
D16S3095	1	9.7	2	3	1	0	1	1	2	3	2	1/2	1	1	1/2	1/2	1/2	1/3	1	1	1/2	1/3	2	1	
D16S512	1	32.3	n.e.	2/4	1	4	1/5	4	2/4	1/5	1/5	1/5	4	n.e.	4/5	n.e.	n.e.	5	1	1/2	n.e.	2/4	4	4/5	

the SNPs obtained from public databases were not present on the disease allele or did not have enough specificity to the disease chromosome. SNPs were revealed by direct sequencing of the genomic DNA from a homozygous patient who carries the common haplotypes between D16S3031 and GT01 in both of the chromosomes. Primers were designed to amplify about 800 bp from genomic DNA (primer sequences are available on request), and polymerase chain reaction (PCR) and sequencing were performed with the same methods as previously described (Ishikawa et al. 2005). Comparing the sequenced data and the annotated databases with use of DNASIS (Hitachi) software revealed many SNPs. With the sequenced data of the control genomic DNA, SNPs with high specificity to the 16q-ADCAs were chosen. With these SNPs, 16q-ADCA families were analyzed to reveal the borders of the maximally conserved genomic region.

**Results**

Haplotype analysis of 16q-ADCA with the -16C>T substitution in the *puratrophin-1* gene

One hundred and twenty-five patients from 64 families were diagnosed as 16q-ADCA based on the clinical features and the presence of the -16C>T substitution in the *puratrophin-1* gene. The families included 52 families that we had pre-

viously reported (Ishikawa et al. 2005) and 12 new families that had not been reported elsewhere. They all share similar haplotypes around the *puratrophin-1* gene. The most common haplotype among these families are shown in the left column in Table 1. Twenty-two families out of the 64 families showed different alleles at least for one of the DNA markers as shown in Table 1. The remaining 42 families, which are not listed in Table 1, harbored or had the possibility to harbor the common haplotype.

There was one repeat-unit difference from the common alleles for D16S397, GGAA10, GATA01, and TA001 close to the *puratrophin-1* gene in 13 out of 22 families. For centromeric DNA markers from the *puratrophin-1* gene, such as GGAA05, TAGA02, D16S3031, and D16S3043, eight families (P4, P14, T2, T3, T6, T12, T25, T46) harbored alleles with greater differences in repeat number (more than two repeat-units). Furthermore, families P4 and T46 carried different alleles in three consecutive markers, GGAA05, TAGA02, and CTATT01.

Similarly, for telomeric DNA markers such as 17 msm, D16S3107, CTTT01, and GT01, greater differences were seen in three families (T12, T15, T44). Especially, families T12 and T44 harbored different alleles for markers 17 msm, CTTT01, and GT01, which were highly specific to the common haplotype.

The presence of large differences in repeat number and successively different alleles would indicate that the families were sharing the common chromosomal region,

inherited from a founder, between markers GGAA05 and 17 msm.

Haplotype analysis of families without identifiable genetic mutations

Twenty-three patients from 22 families presenting pure cerebellar ataxia did not carry identifiable genetic mutations. Nine families showed autosomal dominant inheritance, and the other families had no apparent family history. Their haplotypes are shown in Table 2. Although no family carried entirely identical alleles to the common haplotype consecutively for the markers telomeric to the *puratrophin-1* gene, one family (U09) harbored the identical alleles for the markers between D16S3043 and GATA01 centromeric to the *puratrophin-1* gene. It suggested the possibility that the U09 family have the common haplotype of 16q-ADCA in the region centromeric to the -16C>T substitution in the *puratrophin-1* gene.

Haplotype analysis with SNPs

Four markers, GGAA05, D16S397, GGAA10, and GATA01 centromeric to the *puratrophin-1* gene, showing different alleles in Table 1 suggested that ancestral chromosomal recombination might have occurred around the markers. Family U09 and the family reported by Ohata et al. (2006) also suggested ancestral chromosomal recombination around the substitution in the *puratrophin-1* gene. Therefore, we searched the SNPs around these four markers and the *puratrophin-1* gene. Five SNPs were

identified around the marker GGAA05, one SNP around D16S397, four SNPs around GGAA10, one SNP around GATA01, and two SNPs around the *puratrophin-1* gene (Table 3). SNP05 and SNP06 showed high specificity to the disease chromosome because they were absent in 200 control chromosomes.

Eighteen families showed different alleles for GGAA05, D16S397, GGAA10, or GATA01 (Table 1). Among them, sufficient amounts of DNA samples were not available in four families (T25, T26, T30, T42). The remaining 14 families were analyzed as shown in Table 4. While 13 out of the 14 families carried all of the same SNPs, family T46 did not carry SNP01, SNP02, SNP03, and SNP04. This confirmed that the genomic region between SNP01 and SNP04 of family T46 was a recombinant region, which was not conserved in all families.

These SNPs were also analyzed for the U09 family suspected of having the common haplotype of 16q-ADCA (Table 4). The family had all 13 SNPs, including SNP05 and SNP06, which are highly specific to the disease chromosome. This strongly suggested that family U09 shared the 16q-ADCA common haplotype centromeric to the -16C>T substitution in the *puratrophin-1* gene.

Discussion

16q-ADCA is one of the most common ataxic diseases in Japan. We previously showed that 52 families shared the common haplotype in the genomic 400-kb region between the markers GATA01 and 17 msm by analysis with

**Table 2** The haplotype analysis of families without identifiable genetic mutation. The black squares indicate that the families carry the identical alleles to the common alleles of 16q-ADCA, and the gray squares indicate alleles with one repeat-unit difference. Only

family U09 harbored the identical alleles consecutively for the markers from D16S3043 to GATA01, suggesting that this family may harbor the common haplotype of 16q-ADCA. n.c.=notclear. A.D.=autosomal dominant inheritance was suspected

Marker	Family No.	Family history frequency in control (%)																						
		U01	U02	U03	U04	U05	U06	U07	U08	U09	U10	U11	U12	U13	U14	U15	U16	U17	U18	U19	U20	U21	U22	
		n.c.	n.c.	n.c.	A.D.	n.c.	n.c.	n.c.	A.D.	n.c.	n.c.	A.D.	A.D.	A.D.	n.c.	A.D.	A.D.	A.D.	A.D.	n.c.	n.c.	n.c.	n.c.	
D16S3043	1	25.0	1/6	4/8	1/2	1/7	1	4/6	5	5	1/7	1/7	4/5	4/5	1/6	1/5	5	1/6	5/7	1/6	1	1	1/5	1/8
D16S3019	4	41.4		1/4	4	1	3/4	5	3/4	4	4	3/4	1	2	4	4/5	4/5	3/4	1	2	1	1	1/3	1/2
CTATT01	1	32.4	3/4	1/4	1/3	3	2/4	1/2	3	3	1/4	1/3	1/3	1/3	4	1	1/3	2/5	1	2/3	1/3	1/2	1	1/3
TAGA02	4	10.3	3/5	6	2/4	4/5	5/6	4/6	5/6	5/6	4/5	5	5	6/7	5	2/6	3/5	4/5	6	4/5	4/6	3/6	2/6	5/6
GGAA05	1	1.4	3	4/5	4/5	4	5	3/4	4/6	4	1/3	3	2/4	4	4	5	4/5	2/6	4	4/5	4/6	5/6	4/6	4/5
D16S397	1	47.1	-1/1	1/3	2/3	4/6	4	1	3	4	1	3	1/4	1	1	1/2	1/4	3/5	1/4	2/6	1	-3/3	1/3	-3/6
D16S3086	2	65.7	2	2/3	3/4	3/4	3	2	3	3	2	3	2/4	2	2	2/3	2/3					3	2/3	3/4
GATA01	2	44.1	2	1/2	1/3	2/4	3	3	1/3	3	1/2	2/3	2	2/3	2	2/3	2/3	2	1	0/2	3/4	2	1	0/2
<i>puratrophin-1</i> (CT)	T	0.0	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
GA001	4	0.1	1/8	6/8	8	8/9	8	7/11	8/9	1	5/7	7/10	6/7	5/8	5	5/8	5/9	1/7	6/7	5/7	6/9	6/8	7/9	7/8
17msm	2	8.3	3/4	4	4/5	4/5	5	2/6	5	2	5	3/4	4	3/4	4	1/3	2/4	7	5	2/6	4	2/4	4/5	2/5
CTTT01	8	28.2	5/7	8/10	6/10	6/9	4/5	7/10	5/6	9	5/10	6/7	9	7/9	5/6	7/8	7/10	5/11	5/7	7/10	8/10	4/7	6/8	7/9
GT01	6	15.8	2/4	4/6	2/6	2/4	2/5	1/2	2/3	7	2/4	2/6	1/4	4/6	3/4	4	2/6	3/4	4/7	2/6	3/6	4/6	5/8	2
D16S3095	1	9.7	2	1/3	2/3	2/5	1/3	2/3	2/4	6	2	2/3	2/4	1/2	3	2/3	1/2	1/3	3/4	3/4	1/2	2/6	2/4	2/4
D16S512	1	32.3	2/4	1/5	4/5	2/4	2/4	4	2/4	1	2	2/4	1/4	4/5	4	2/4	4	2/3	1/4	2	4/5	4/5	4/5	4

**Table 3** Single nucleotide polymorphisms (SNPs) on the disease chromosome of 16q22.1-linked autosomal dominant cerebellar ataxia (16q-ADCA). We identified thirteen SNPs by ourselves. SNP05 and SNP06 were absent in control chromosomes ( $n=200$ ) and are thought to be highly specific to the disease chromosome

SNP/marker	Position on Chr 16	SNP change on 16q-ADCA	Frequency in control (%)
	GGAA05 64,938,933		
SNP01	64,972,150	A → G	27.8
SNP02	64,977,170	A → C	22.2
SNP03	64,977,733	T → C	30.0
SNP04	64,982,678	C → T	27.8
SNP05	65,049,292	G → A	0.0
	D16S397 65,295,770		
SNP06	65,337,827	A → G	0.0
SNP07	65,449,825	C → T	56.3
SNP08	65,451,833	T → A	45.5
	GGAA10 65,452,426		
SNP09	65,457,741	T → A	42.4
SNP10	65,458,302	T → C	45.5
SNP11	65,669,454	T → C	30.3
	GATA01 65,700,022		
SNP12	65,771,917	G → A	18.2
SNP13	65,793,152	C → T	8.7
<i>puratrophin-1</i> (C/T)	65,871,434	C → T	0.0

microsatellite markers. Within this region, we had found that the single nucleotide -16C>T substitution in the *puratrophin-1* gene was strongly associated with the disease (Ishikawa et al. 2005). Since then, a number of patients with the substitution and the common haplotype were reported in various areas of Japan. However, a report of the one exceptional patient without the substitution in the family in which all other affected subjects carried the substitution (Ohata et al. 2006) raised the possibility that a true pathogenic mutation may be present in a different gene. This exceptional patient indicated that the mutation might be lying centromeric to the substitution in the *puratrophin-1* gene, where the patient shared the common haplotype with other affected individuals in the family.

Here, we re-examined the 16q-ADCA families with the -16C>T substitution in the *puratrophin-1* gene with microsatellite markers and found four possible centromeric borders of the disease locus (GATA01, D16S397, GGAA10, GGAA05), based on the difference of alleles. We searched for informative SNPs around the markers capable of distinguishing the chromosomes derived from a founder and analyzed haplotypes with the SNPs. Because all of the examined families carried SNPs around the markers GATA01, D16S397, and GGAA10, ancestral chromosomal recombination around the markers was not confirmed. The differences in alleles for these markers was only one repeat-unit, suggesting that the allele differences

**Table 4** The haplotype analysis with single nucleotide polymorphisms (SNPs). Fourteen families of 16q-ADCA with different alleles for microsatellite markers and family U09 are shown. The gray squares indicate that the family carried the SNPs common to 16q-ADCA. Family T46 did not carry the common SNPs from SNP01 to SNP04. This is consistent with the finding microsatellite markers

(Table 1), further suggesting that the centromeric border of the disease locus is SNP04. Family U09 carried all of the 13 SNPs. This would also support the theory that family U09 shares the 16q-ADCA common haplotype centromeric to the substitution in the *puratrophin-1* gene

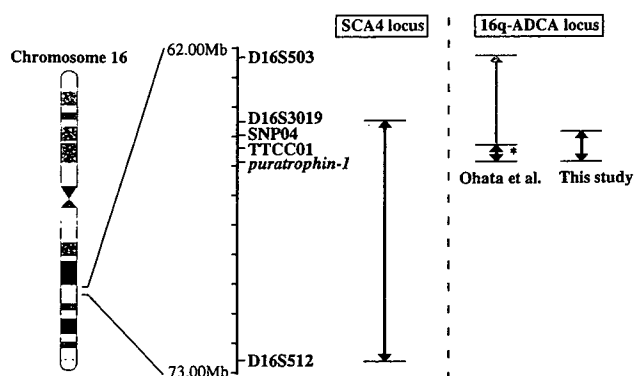
SNP	SNP change on 16q-ADCA	frequency in control (%)	family No.														
			P4	T3	T4	T5	T6	T7	T12	T15	T21	T28	T37	T43	T44	T46	U09
SNP01	A → G	27.8	G/A	G/A	G/A	G	G	G/A	G	G/A	G/A	G/A	G	G/A	G	A	G/A
SNP02	A → C	22.2	C/A	C/A	C/A	C	C	C/A	C	C/A	C/A	C/A	C	C/A	C/A	A	C/A
SNP03	T → C	30.0	C/T	C/T	C/T	C	C	C/T	C	C/T	C/T	C/T	C	C/T	C	T	C/T
SNP04	C → T	27.8	T/C	T	T/C	T	T	T/C	T	T/C	T/C	T	T	T/C	T	C	C/T
SNP05	G → A	0.0	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G
SNP06	A → G	0.0	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A
SNP07	C → T	56.3	T	T/C	T/C	T	T	T/C	T/C	T	T	T/C	T/C	T/C	T	T/C	T/C
SNP08	T → A	45.5	A	A/T	A/T	A	A	A/T	A/T	A	A	A/T	A/T	A/T	A	A/T	A
SNP09	T → A	42.4	A	A/T	A/T	A	A	A/T	A/T	A	A	A/T	A/T	A/T	A	A/T	A
SNP10	T → C	45.5	C	C/T	C/T	C	C	C/T	C/T	C	C	C/T	C/T	C/T	C/T	C/T	C
SNP11	T → C	30.3	C	C/T	C/T	C/T	C	C/T	C	C	C/T	C/T	C/T	C/T	C	C/T	C/T
SNP12	G → A	18.2	A	A/G	A/G	A/G	A/G	A/G	A/G	A	A/G	A/G	A/G	A/G	A	A/G	A/G
SNP13	C → T	8.7	T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	T	C/T	C/T
<i>puratrophin-1</i> (C/T)	C → T	0.0	T	T	T	T	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	C



in GATA01, D16S397, and GGAA10 might have resulted not from recombination events, but from the microsatellite slippage mutation (Ikeda et al. 2004). On the other hand, four families (P4, T3, T25, T46) showed great allele differences in GGAA05 and one family (T46) did not carry four SNPs, confirming that family T46 did not share the genomic region centromeric to GGAA05 with the other 16q-ADCA families. This strongly indicates that the centromeric border of the disease locus of 16q-ADCA could be placed at SNP04.

The U09 family had the identical alleles for all markers and SNPs in the region centromeric to the  $-16C>T$  substitution in the *puratrophin-1* gene. It is impossible to conclude that the family has the common haplotype of 16q-ADCA because only one examined family member was available for the present genetic analysis. However, carrying the rare alleles for GGAA05 and infrequent SNPs, both highly specific to the disease chromosome, strongly suggests that the U09 family shares a part of the 16q-ADCA common haplotype. The patient in the U09 family developed pure cerebellar ataxia later in life without apparent family history. Because 16q-ADCA patients were found among sporadic cases (Ouyang et al. 2006), these clinical features of the U09 family are consistent with those of 16q-ADCA. Importantly, this family had not been reported previously and, therefore, would be the second case of 16q-ADCA without the substitution in the *puratrophin-1* gene following the family reported by Ohata et al. (2006). These cases indicate that the telomeric end of the disease locus could be placed at the  $-16C>T$  substitution in the *puratrophin-1* gene.

Haplotype analysis of a number of 16q-ADCA families with microsatellite markers and SNPs in this study suggests



**Fig. 1** A summary of critical intervals for 16q-ADCA and SCA4. Our study could define the disease locus of 16q-ADCA to a 900-kb genomic region between SNP04 and the  $-16C>T$  substitution in the *puratrophin-1* gene. This region is completely inside the candidate locus of SCA4 (Flanigan et al. 1996). The haplotype region (asterisk) between TTCC01 and the *puratrophin-1* gene shown by Ohata et al. (2006) is also shown, together with an alternative critical region between D16S503 and the *puratrophin-1* gene (see text for details)

that the gene locus of 16q-ADCA could be re-assigned to a 900-kb genomic region between SNP04 and the substitution in the *puratrophin-1* gene (Fig. 1). This region partly overlaps with, but is not the same as, the candidate region previously set by Ohata et al. (2006). They showed that three large 16q-ADCA families shared a common haplotype between D16S3086 and D16S412, and suggested the possibility that real pathogenic mutation would exist in the region between TTCC01 and the  $-16C>T$  substitution in the *puratrophin-1* gene. However, the allele difference for TTCC01 in their families was only one repeat-unit, and all of their patients shared identical allele for TAGA02, lying centromeric to TTCC01. Since the possibility of slippage mutation remains as an explanation for the allele difference seen in TTCC01, as we observed for GATA01, D16S397, and GGAA10, it would be cautious to place the centromeric border at the marker TTCC01. Given that the allele differences in TTCC01 is due to slippage mutation, the centromeric border in their families would be alternatively set at D16S503, since an obligate recombination was seen between D16S503 and TAGA02. It would be, thus, important to analyze GGAA05 and specific SNPs in their families to see to what extent their patients harbor conserved haplotypes.

Although we found a patient without the  $-16C>T$  substitution in the *puratrophin-1* gene, the substitution was present in all patients except the one in the U09 family (i.e., 125/126=99.2% sensitivity; 100% specificity) and, thus, the *puratrophin-1* genetic change still remains to be a useful marker. Molecular diagnosis with multiple microsatellite markers and SNPs will help to identify 16q-ADCA patients more accurately. Through the present study, we showed that the truly pathogenic mutation would lie in a 900-kb genomic region between SNP04 and the  $-16C>T$  substitution in the *puratrophin-1* gene. Further investigations for finding a genetic mutation within the critical region are needed to elucidate the molecular pathogenesis of 16q-ADCA.

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## 短 報

## 嚥下造影検査が重症筋無力症増悪の評価に有効であった1例

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要旨：重症筋無力症（MG）の嚥下障害の評価に、固形物の嚥下造影検査（VF）による塩化エドロフォニウム（EC）試験が有用であった64歳の女性例を報告した。62歳時に、頸部筋力低下、複視、左眼瞼下垂でMGを発症し、拡大胸腺摘除をおこなった。64歳時、ブレドニゾロン（PSL）の内服で症状は改善していたが、嚥下困難感と開鼻声が出現した。EC試験では嚥下困難感の自覚的な改善に乏しく、開鼻声、僧帽筋の神経反復刺激試験も改善しなかった。VFではEC静注後の固形物の咀嚼嚥下が改善し、MGの増悪と診断できた。PSL増量後、嚥下困難感は消失し、VFで嚥下機能の改善が確認された。

（臨床神経，47：669—671，2007）

Key words：重症筋無力症，嚥下障害，嚥下造影検査，エドロフォニウム試験，咀嚼嚥下

## はじめに

重症筋無力症（myasthenia gravis：MG）患者の嚥下性肺炎の合併はクリーゼの危険因子とされるため嚥下障害の評価は重要である<sup>1)</sup>。嚥下機能の評価には塩化エドロフォニウム（edrophonium chloride：EC）静注前後の嚥下造影検査（videofluorography：VF）の比較が有用であるが、VFで使用する造影剤加模擬食品についての検討は少ない<sup>2)~4)</sup>。EC静注前後の固形物のVFがMGの嚥下機能の評価に有用であった症例を報告する。

## 症 例

患者：64歳 女性。

主訴：飲み込みづらい。

既往歴・生活歴・家族歴：いずれも特記事項なし。

現病歴：62歳時、臥位での頭部挙上困難、左眼瞼下垂、複視が出現した。血清抗アセチルコリン受容体抗体（抗AChR抗体）63nmol/L、左僧帽筋の神経反復刺激試験は減衰率25%で、MGと診断された。拡大胸腺摘除術を受けたところ、胸腺過形成であった。ピリドスチグミン180mgを内服し、寛解した。63歳時、呼吸不全、左眼瞼下垂、複視が出現し、3日間、非侵襲的陽圧換気で管理された。経口ブレドニゾロン（PSL）を開始し、70mgまで漸増して症状は改善した。その後、ピリドスチグミン120mg連日とPSL20mg隔日の内服で寛解した。64歳時、頸部の筋力低下が増悪し、左眼瞼下垂、複視が出現した。PSL20mg隔日内服から25mg連日内服に増量し、

ピリドスチグミン120mgを維持し、症状は軽快した。寛解してその半年後、食事で喉にひっかかる感じやむせこみ、開鼻声が出現したため、入院した。

現症：身長158cm、体重68kg、血圧168/88mmHg、脈拍82/分整、呼吸回数17回/分であった。意識障害や心気傾向はなかった。開鼻声、複視、左眼瞼下垂をみとめ、これらの症状は夕方になると増悪した。通常の食事は経口摂取可能で、咀嚼中の疲労の訴えはなかったが、喉に詰まる感を訴えた。液体の嚥下ではときにむせ込んだ。咽頭反射、軟口蓋反射、舌運動は正常であった。筋力は寛解時と変化なく、徒手筋力テストで頸部前屈筋2、肩外転筋は右4、左2、肩挙上筋は両側4で、その他の筋力は正常であった。

検査：血算・血液生化学は正常で、CK64IU/L、抗AChR抗体25nmol/Lと寛解時と著変なかった。座位での動脈血液ガスは室内気でpH7.42、PaCO<sub>2</sub>43.5mmHg、PaO<sub>2</sub>75.2mmHg、SaO<sub>2</sub>94%、肺活量は1.75L（予測値の71.6%）、ピークフローは5.47L/sであった。胸部X線写真、胸部CTでは肺野と縦隔に異常なかった。頭部MRIは正常であった。EC10mg静注後、開鼻声、複視、眼瞼下垂、食物の飲み込みづらさは改善せず、僧帽筋での反復刺激試験でも減衰率56%でEC静注前と変化なかった。

VF：「嚥下造影の標準的手順」<sup>5)</sup>に準拠し、書面による同意をえて検査した。EC10mg静注前後でバリウム水溶液10mlを検者の合図で嚥下する命令嚥下とバリウム加コンビーフ8gを自由に咀嚼し、任意のタイミングで嚥下する咀嚼嚥下を30フレーム/秒で記録した。照射時間を減らすためプラセボの評価はしなかった。それぞれの嚥下について、1)嚥下回数、2)咽頭での残留の有無、3)バリウムが下顎下縁を越え輪状咽

Table 1 Comparison of videofluorography results before and after edrophonium chloride (EC) injection

	Liquid (10 ml barium)			Solid food (8 g of corned beef hash)		
	At the time of admission		3 months after admission	At the time of admission		3 months after admission
	Before EC	After EC		Before EC	After EC	
1) Number of swallows	3	1	1	5	1	2
2) Residue in VAL and in piriform fossa	++ +	+ +	- -	++ +	- -	+ -
3) Pharyngeal transit duration (s)	0.84	0.66	0.59	2.86	1.99	2.17
4) Aspiration	None	None	None	None	None	None

EC: edrophonium chloride. VAL: valleculae. The initiation of swallowing is defined as the starting point of the sudden and rapid superior and anterior motion of the hyoid bone. A3 point scale was used; - corresponds to no residue; +, to a coating of residue (a line of barium on a structure); and ++, to pooling of barium. There was no evidence of aspiration in any of the recordings. The first videofluorography was performed at the time of admission. After the EC injection, the number of swallows decreased, and the residue disappeared, particularly with solid food. The patient was treated with 70 mg of PSL for 4 weeks, and the second videofluorography was performed 3 months after the first videofluorography. The number of swallows, the residue, and pharyngeal transit duration improved.



Fig. 1 Videofluorography

Videofluorography performed while consumption of 8 g of corned beef hash with barium. On the left: Before edrophonium chloride (EC) injection. The residue is prominent in the valleculae (large arrow) and the hypopharynx (small arrows). On the right: After 10 mg EC injection. No residue is observed in the valleculae and the piriform fossa following the ingestion of corned beef hash. Dysphagia was improved by EC injection. The ingestion of corned beef hash was useful in evaluating the swallowing disturbance. The round objects are 24 mm in diameter (lower right arrow).

頭筋を通過するまでの時間 (pharyngeal transit duration : PTD)<sup>9)</sup>, 4) 誤嚥の有無をコンピューターで解析した。

VF 結果: 液体の命令嚥下では, EC 静注後, 嚥下回数は 3 回から 1 回に減り, 喉頭蓋谷の残留は軽度減少し, PTD は 0.18 秒短縮したが, 鼻腔へのバリウム逆流と, 梨状陥凹の残留は不変だった。咀嚼嚥下では, EC 静注後, 嚥下回数は 5 回から 1 回に減り, PTD は 1.87 秒短縮したが, 喉頭蓋谷と梨状陥凹の残留は消失した。いずれの検査でも, 誤嚥はなかった (Table 1, Fig. 1)。EC 静注後, 自覚症状の改善はなかった。

経過: PSL 70mg 連日を 4 週間内服後, 嚥下困難感は消失した。VF では, 液体の命令嚥下と咀嚼嚥下ともに, 嚥下回数と咽頭の残留は減少し, PTD は短縮した (Table 1)。

## 考 察

寛解にみえた MG 患者が嚥下困難感を訴え, EC 試験では固形物の VF 所見が改善したことから MG による嚥下障害と診断した。食事中のむせ込みや咀嚼での疲労はなく, 液体嚥下ではむせ込みはまれで, 評価の指標にはならなかった。EC 10mg 静注後の嚥下においても他覚所見と自覚症状に乖離があり, MG では VF による客観的な評価が必要であることを示した。なお, 複視や眼瞼下垂, 開鼻声は EC 10mg では改善なく, MG では筋の部位によって EC に対する反応性が異なることが示唆された。

MGによる嚥下障害は、嚥下の反復で増悪する嚥下動作の遅延、咽頭での食物残留などを特徴とし、コリン作動性クラーゼによる嚥下障害との鑑別には、EC静注後の嚥下機能の評価が有用である<sup>2)~4)</sup>。EC試験と同時に固形物のVFをおこなった報告として、経口摂取可能なMG患者に20分間食事をさせた後にVFをおこない、EC静注後に喉頭蓋谷の食物の残留が消失した症例がある<sup>7)</sup>。本例では、バリウム加コンビーフを選択し、過剰な負荷をかけてVF中の誤嚥の危険性を高めることなく、客観的に嚥下機能の改善を評価できることを示した。評価には、嚥下回数、咽頭のバリウム残留、咽頭でのバリウム通過時間が有用であった。

一般に液体の命令嚥下では重力による受動的輸送の要素が強く、固形物の咀嚼嚥下では咽頭筋群の収縮によって食物を送り込む能動的輸送と重力の両方が関与している<sup>8)</sup>。MGによる嚥下障害では重力による受動的輸送は悪化せず、EC静注で舌筋や咽頭筋群による能動的輸送が改善すると考えられ、病態の評価には液体よりも固形物のVFが良いと考えた。また、固形物のVFは液体のVFよりも喉頭蓋谷や梨状陥凹の残留を確認しやすいため、ECの効果を検査室で視認しやすい点でも有用であると考えた。

嚥下障害があるMG患者のVFの適応には十分な検討が必要であるが、造影剤加模擬食品として固形物の選択が有用であった例を報告した。

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## Abstract

### Videofluorographic evaluation of dysphagia in a patient with myasthenia gravis

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A 64-year-old woman with myasthenia gravis (MG) presented with isolated bulbar symptoms. Two years earlier, she had developed neck weakness, diplopia, and ptosis and was diagnosed with MG. Extensive thymectomy was performed, and she was treated with prednisolone (PSL). The neck weakness, diplopia, and ptosis improved over a 2-year period. However, dysphagia developed, and her voice took on a nasal tone that did not improve subjectively even after administration of 10 mg of edrophonium chloride (EC). We then performed videofluorography (VF). After consumption of 10 ml of liquid barium and 8 g of corned beef hash, she attempted to swallow, but the residue remained in the valleculae and the piriform fossa. After the EC injection, her dysphagia on ingestion of corned beef hash improved; however, there was slight subjective improvement in swallowing. Drinking of liquid barium resulted in some residue with slight improvement of dysphagia. After treatment with 70 mg of PSL for 4 weeks, VF showed improvement of dysphagia. Thus, VF, particularly during consumption of solid food, with EC administration is helpful in evaluating bulbar symptoms in patients with MG.

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**Key words:** Myasthenia gravis, dysphagia, videofluorography, edrophonium test, chew and swallow

パーキンソン病の治療  
薬物療法

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# 薬物療法

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## はじめに

今回は「Parkinson 病 What's new?」という特集であることから、パーキンソン病治療の一般論は成書にゆずり、ここでは、現在使用可能な抗パーキンソン病薬についての最近の話題、現在申請中の薬剤、および治験段階にある薬剤について述べたい。なお、治験段階の薬剤のうち、アデノシン A<sub>2A</sub> 受容体拮抗薬については別項に詳細に述べられているのでそちらを参照されたい。

## パーキンソン病治療についての最近の話題

### 1. L-dopa 製剤の位置づけ

治療ガイドラインが相次いで発表された 2000 年前後は、パーキンソン病治療においてはドパミン受容体刺激薬の有用性が非常に強調され、L-dopa はむしろ患者にされていた感がある。しかし、この 2 年ぐらいで国際的にも L-dopa の有用性が再評価されてきているように思われる。ターニングポイントは ELLDOPA study であろう。これは 2002 年マイアミでの Movement Disorder Society の年次総会の最終日に発表されたが、内容の重大性からか意外性からか論文としての発表は 2004 年になった<sup>1)</sup>。

ELLDOPA study とは 1998~2001 年に北米でなされた臨床研究で、パーキンソン病の診断後 2 年以内の初期例について、偽薬、L-dopa 合剤 150 mg, 300 mg, 600 mg/日の 4 群(各群約 90 名)の二重盲検にて、40 週間投与、その後薬物を中止し 2 週間まで UPDRS III で評価したものである。投与前に比較した改善度は投与中止 2 週間後でも L-dopa 投与群では偽薬群より有意によかった。半減期が 1 時間程度である L-dopa の直接効果が 2 週間も持続するはずもなく、L-dopa が神経保護作用をもつ、あるいは少なくとも L-dopa により神経回路網あるいは四肢の運動能力をより正常化することがよりよい状態を維持することにつながることが示唆され、少なくとも L-dopa は毒ではないこと

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を示した。しかも、投与前後で行った  $\beta$ -CIT SCAN (ドパミントランスポーターを評価する SPECT) では L-dopa 投与群では量依存性に取り込みが低下していた。この結果は、それまでの PET, SPECT を用いたドパミン受容体刺激薬の神経保護作用を示唆するかもしれない研究<sup>2,3)</sup>(画像による神経終末の取り込み低下はドパミン受容体刺激薬のほうがよいが、臨床症状は L-dopa のほうがよい)の説得力をかなり弱める結果となった。

加えて、COMT 阻害薬である entacapone が北米、欧州で使用され、さらにドパ合剤+entacapone の合剤が北米で認可されたこと、新たな MAOB 阻害薬である rasagiline の登場、rasagiline による「delayed start」<sup>4)</sup>という新たな神経保護作用の評価方法による話題性などが加わり、最近では国際的にも「やはり L-dopa がパーキンソン病治療の golden standard である」というところに戻ってきている感がある。

### 2. 各種ドパミン受容体刺激薬の差異

現在わが国で使用可能なドパミン受容体刺激薬は bromocriptine, pergolide, talipexole, cabergoline, pramipexole の 5 剤で、さらにごく最近 ropinirole が認可された。多数のドパミン受容体刺激薬のうちどれが一番よいかということになると、エビデンスレベルについてはその薬剤が開発された時代背景があるので多少の違いはあるが、集団として統計学的な有意差が出るほどの差はない<sup>5,6)</sup>。たとえば、pramipexole のわが国での治験は偽薬および bromocriptine を対象として行われたが、実薬群はいずれも偽薬より優位に UPDRS III のスコアを改善したが、pramipexole と bromocriptine の間には有意差は認められなかった<sup>7)</sup>。ただし、5 剤のドパミン受容体刺激薬は麦角剤、非麦角剤という構造の差異、半減期の差異などそれぞれ特徴があり、患者によりある薬剤では副作用が出現しやすいが他剤では出にくいなどの違いもあるため、選択肢が増えることはきわめて歓迎すべきことといえる。

### 3. 神経保護作用の評価について

2002年、2003年に相次いで pramipexole<sup>2)</sup>, ropinirole<sup>3)</sup>を用いたドパミン受容体刺激薬で治療を開始した群と L-dopa で治療を開始した群について、臨床症状と  $\beta$ -CIT SPECT あるいは fluoro-DOPA PET によりドパミン神経終末を評価することにより、神経保護作用を評価するための大規模研究が発表された。これらはいずれも非常にきれいなデータでドパミン受容体刺激薬の神経保護作用が示されたかにみえたが、画像上明らかな差があるにもかかわらず、臨床効果には差がない、あるいは L-dopa 開始群のほうがよいという結果で評価が難しくなった。さらに上記の ELLDOPA study<sup>1)</sup>が報告され、 $\beta$ -CIT SPECT や fluoro-DOPA PET では薬物の神経保護作用を立証するのは困難という結論に至った。In vitro では多くの薬剤の神経保護作用が報告され、臨床的に意味があるかどうかの評価は極めて重要である。その評価方法について、次に出てきたのが delayed start という方法である。rasagiline 1 mg/日、2 mg/日、偽薬の3群で6ヵ月投与したのち、偽薬群は 2 mg/日投与し、3群ともさらに6ヵ月間治療が継続され、UPDRS IIIで評価された。研究開始より12ヵ月後の時点で6ヵ月遅れて rasagiline が投与された delayed start 群は、early start 群にキャッチアップできなかったことから、rasagiline に神経保護作用がある可能性が示唆されると報告されている<sup>4)</sup>。この方法が果たして本当に薬剤の神経保護作用を評価できるかはまだ議論のあるところで、今後も検討が必要である。

#### 現在申請中の薬剤

##### 1. ropinirole

わが国でも近日発売される予定の経口の非麦角系ドパミン受容体刺激薬である。pramipexole にきわめて類似しているが、以下のようなちがいがあ。すなわち、受容体親和性については、ドパミン受容体亜型への親和性はドパミン同様 D2>D3 で、pramipexole ほど D3 への親和性は突出していない。セロトニン系など他の受容体への親和性がきわめて低いのは pramipexole と同様である。半減期は約6時間で、肝臓でグルクロン酸抱合などを受けた後、腎臓から排泄されるために、腎障害の影響は受けにくい<sup>8)</sup>。臨床効果については多数のエビデンスがあり、L-dopa 併用

での効果は、insufficient data で possibly useful であるものの、単独使用、および運動合併症の予防、治療とも pramipexole と同様、efficacious, clinically useful と評価されている<sup>5,6)</sup>。副作用の内容は、pramipexole によく似ているが、これまでの偽薬対照試験での結果の解析では、ropinirole は pramipexole に比較して、幻覚の発現頻度は低いが、低血圧や眠気の出現頻度はやや高いという報告がある<sup>9)</sup>。

##### 2. entacapone

2007年にはわが国での承認が予想される COMT (catechol-o-methyltransferase) 阻害剤である。もともとドパの主な代謝経路はドパ脱炭酸酵素 (DDC) によりドパミンに変換されるものであるが、carbidopa, benserazide などの DDC 阻害剤との合剤が広く使われるようになり、COMT により 3-o-methyldopa (3 OMD) となる経路が活性化されるようになった。3 OMD は半減期が16時間と長いために、長期ドパ服用により血中濃度はむしろドパより3 OMD のほうが高くなる。3 OMD は腸管からの取り込みおよび血液脳関門の通過には、ドパと同じ LNAA (Large Neutral Amino Acid) system と呼ばれる能動輸送システムを使用するため、大量になりすぎるとドパの吸収を阻害する可能性もある。末梢でのドパの3 OMD への代謝を阻害し、ドパの半減期を延長することを期待して開発された薬剤である。COMT 阻害剤としては tolcapone が先行し高い効果をあげていたが、稀ではあるが重篤な肝障害が報告されたため、現在は北米で厳重な肝機能観察下のみで使用されている。entacapone はすでに欧米で広く使用されているが重篤な肝障害は報告されていない。

entacapone はその作用機序からドパと DDC 阻害剤との合剤との併用で初めて効果を示す。単回投与ではドパの最高血中濃度 (Cmax) は上昇せずに半減期が延長し、wearing-off の改善効果が期待できる<sup>10)</sup>。なお、entacapone は半減期がドパと同じく1時間程度と短いことから服用はドパと同時に服用するため、1日に3~4回多ければ8回の服用もありうる。単回投与で Cmax の上昇がないことから不随意運動は増加しないことが期待されるが、ドパの血中濃度があまり低くならないうちに次のドパを服用することから当然累積による血中濃度の上昇は起こり、off 時間の減少とともに不随意運動も起こりうることになり、症状のコン



トロールにはこれまで以上にドパの服用量の調節が重要になるといえる。それに伴い 50 mg のドパ合剤が使用できることが望まれる。

これまでの海外の大規模試験では、ドパ服用と同時に entacapone 100~200 mg ずつ投与し(最高 1 日 8 回まで) off 時間は 1.6 時間(偽薬は 0.9 時間)短縮した<sup>11)</sup>。また、わが国では開発の予定は今のところないが新しい MAOB 阻害薬の rasagiline と、entacapone、偽薬との並行群間比較試験(LARGO study)<sup>12)</sup>では、rasagiline 1.0 mg/日と entacapone 200 mg/回(ドパと同時投与)にてほぼ同程度で偽薬に比較して有意な改善である、off 時間の短縮(1.2 時間)を認めた。いずれの試験においても不随意運動は多少増えても日常生活に問題になるほどではなく、ドパの減量で対応可能であるとされている。海外ではすでにドパ/carbidopa/entacapone の合剤も発売されている。

### 3. zonisamide

zonisamide は抗てんかん薬としてわが国で開発された薬剤で、わが国では 1980 年代から、近年は欧米でも、難治性てんかんを対象に広く使用されている。われわれは偶然の臨床知見から zonisamide の抗パーキンソン作用を発見した<sup>13)</sup>。すなわち、経過 10 年のパーキンソン病患者がたまたまてんかん発作をおこし、これに対する治療のために zonisamide 300 mg を投与したところ、てんかん発作の消失とともにパーキンソニズムも著明に改善した。これをきっかけに小規模の臨床研究を行い、オープン試験でパーキンソン病の運動症状および wearing-off 現象の改善を認めた。また、この時点で 1 日 50 mg 程度の少量でも効果が明らかであること、半減期が長い薬剤(約 63 時間)であるので、1 日 1 回投与で十分パーキンソニズムの改善を期待できることがわかった。その後、国内で L-dopa 併用の進行期パーキンソン病患者(現在わが国で使用可能な抗パーキンソン病薬はすべて併用可能)を対象に製薬会社による大規模二重盲検試験を行い、50 mg/日(1 日 1 回投与)で UPDRS III および wearing-off の改善を確認し<sup>14)</sup>、現在申請中である。現在わが国で使用可能な抗パーキンソン病薬を使用して、なお治療効果が不十分なパーキンソン病患者を対象とした二重盲検試験では、罹患期間平均約 9 年で、UPDRS III で平均約 6 点の改善、1 日の off 時間は 1.5 時間程度延長した。off 時間の改善は COMT 阻害剤の

entacapone と同程度である。また、長期投与試験では、投与後 4~16 週にかけて著明に改善し、その改善度は経過により、むしろより改善する傾向にあり、1 年間持続した。平均罹患期間 10 年の患者で投与初期の改善のみならず、1 年間にわたってより症状が改善する傾向にあることは重要な所見と思われる。

zonisamide は抗てんかん薬としては 300~600 mg/日が常用量であるが、抗パーキンソン効果はそれよりもかなり低い濃度で効果を呈する。抗てんかん薬としての作用機序は、Na チャンネル阻害と T 型 Ca チャンネル阻害が報告されており、GABA 系への直接作用はないとされている。抗パーキンソン効果の作用機序としては、基礎実験から zonisamide がドパミン合成の亢進、中等度の MAOB 阻害作用を持つことが明らかになっている<sup>15)</sup>。ただし、大規模二重盲検において、より強力な MAOB 阻害剤である selegiline との併用の有無で zonisamide の効果に変化しなかったことから、MAOB 阻害作用が本剤の効果の中心ではないと考えられる。

zonisamide は抗てんかん薬としての開発当初から radical scavenger として神経保護作用がいわれていた。最近、浅沼らはパーキンソンモデルラットにドパを投与した際に惹起されるキノン体生成増加を zonisamide がほぼ完全に抑制することを報告しており、長期試験での効果改善作用と考え合わせて、神経保護という面でも期待できる<sup>16)</sup>。

## 現在治験中の薬剤

### 1. rotigotine

非麦角系ドパミン受容体刺激薬の貼付薬で、現在後期第 2 相を施行中である。1 日 1 回容量に応じて 10 cm<sup>2</sup>(4.5 mg)~30 cm<sup>2</sup>(13.5 mg)のパッチを貼付する。貼付剤であることから安定した血中濃度および効果が期待される。ただし、興味あることに貼付後 4 時間までは濃度上昇が鈍く T<sub>max</sub> は 8 時間である。また剝離後約 3 時間で 50%の濃度まで低下することから、反復投与でも投与(貼付)後 1~2 時間まで濃度が低下し、4~8 時間後まで徐々に濃度が上昇し、その後は 24 時間後まで濃度はほぼ一定である。受容体への親和性は D 3 が最も高く、pramipexole と類似しており、pramipexole 1.5 mg と rotigotine 13.5 mg がほぼ同等とされている。代謝は硫酸抱合、グルクロン酸抱合が主

体で、不活性化体に変換されたのち腎臓から排泄されるため、ropiniroleと同様に腎機能低下の影響は受けにくい。海外での治験ではパーキンソン病の初期、進行期ともに運動症状の改善が報告されている<sup>17,18)</sup>。貼付剤ということで、嚥下困難な患者や、消化管手術前後など経口摂取不能の場合は、他剤には変えがたい有用性がある。一方、当然貼付による局所反応が心配されるが、悪心などと同程度あるいはそれ以下の頻度で認容性は高いようである。

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## む す び

パーキンソン病の治療薬は次々に開発されている。時に新たな薬剤への期待が大きすぎて従来の薬剤の効果を過小評価してしまうこともあり、薬剤の評価には時代背景も大きく関与することを思い知らされている。臨床家としてはそれぞれの薬剤の薬理的性質を十分理解したうえで、治験など大規模研究の結果とともに、目の前の患者さんでの効果をよく見極め、個々の患者さんに最適な薬剤を選択していく必要がある。

# 多発性硬化症が疑われた、 頸髄海綿状血管腫の 49 歳女性例

発表

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第 33 回 Spinal Cord Club (2006 年 9 月 8 日) 於：エーザイ別館 (東京) 収録

## 症例提示

### [臨床経過]

青木：症例は 49 歳の女性です。

主訴は、左前腕尺側に触るとビリビリと痛むです。

現病歴は以下のとおりです。

45 歳：4 年前の 2 月 23 日の起床後、左前腕尺側に冷たいものが触れると、同部に焼けるような痛みを自覚した。2 カ月で自然軽快したが、寒風があたると左 III-V 指の先端部がビリビリと痛むことがあった。

49 歳：3 月 4 日、左手に水が触れた時、左 III-V 指にビリビリする激痛を自覚した。数日後、左手掌が腫脹し、左肩と左上腕の筋が張って痛み、左手が肩より上にあがらなかった。20 日、前医の MRI で第 6 椎体レベルの頸髄腫脹と、同レベルの後角と後索に T1 強調像で低信号と高信号の混在と、T2 強調像で高信号を認め、同部は淡く造影された。3 月末、左手掌全体、左手指と左前腕の尺側に、末梢に向けてビリッとした痛みが走るがあった。4 月中旬頃、左前腕尺側に触れるとゾクゾクする不快感が生じ、その 2~3 秒後に同部の皮膚が毛羽立つような激痛が生じ、家事ができなくなった。NSAIDs を内服したが、左手の痛みは改善しなかった。5 月初め頃から、左手に電気が走るような痛みは消失した。多発性硬化症 (multiple

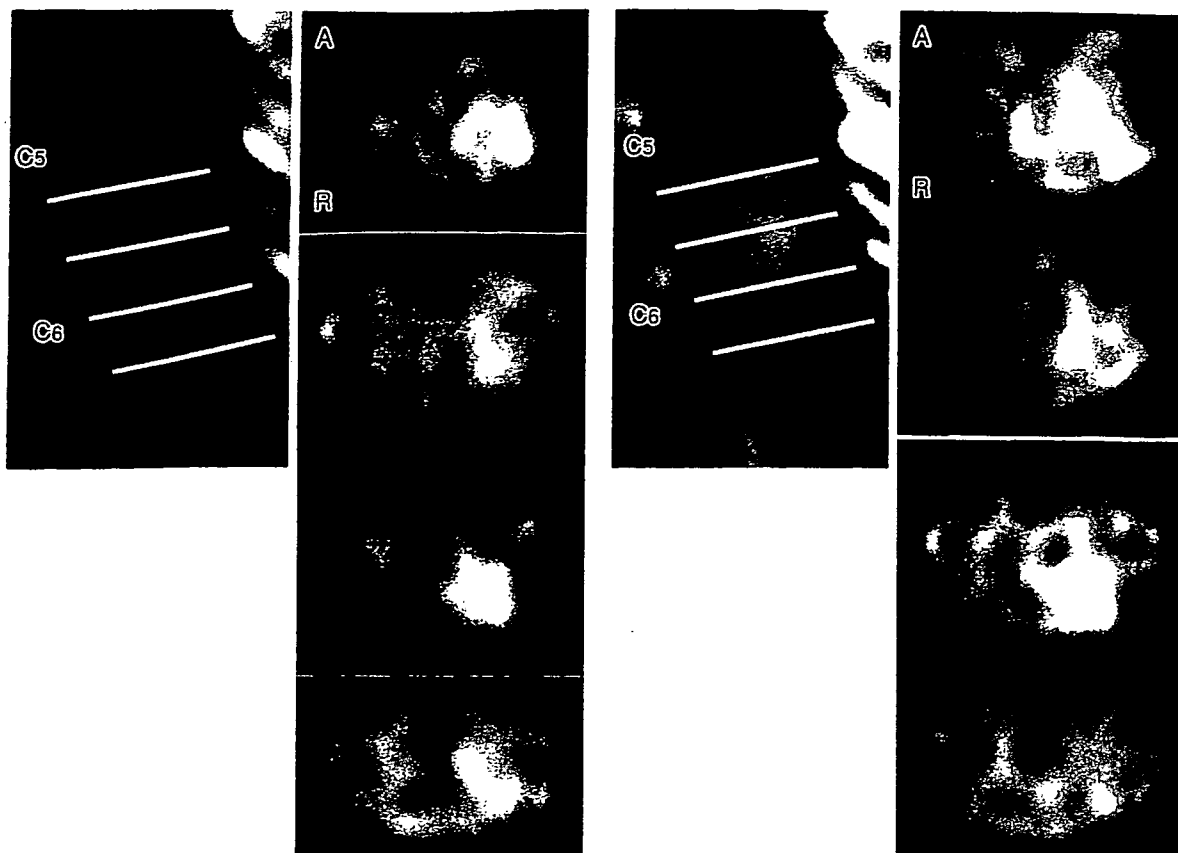


図 1 疼痛の分布  
黒く塗った部分が疼痛  
のある部位を示す。

sclerosis ; MS) を疑われ、5 月に当院紹介受診した。

現症は、身長 154 cm、体重 52 kg、血圧 152/107 mmHg、脈拍 78/分、整でした。左前腕尺側、左手掌と手背の尺側半分と、左 III-V 指の皮膚に痛みを伴う dysesthesia を認めました (安静時に棘が刺さったようなチクチクした痛みがあり、触れると毛羽立つような不快感が生じ、2~3 秒後、同部にビリビリとした激痛が生じた) (図 1)。そのほかの表在覚と深部覚は正常でした。左手指の軽度伸展制限を認めましたが、疼痛が原因と判断しました。筋力は、徒手筋力テストで手関節の背屈と掌屈は左 4、手指ピンチは軽度低下していました。腱反射は正常でした。

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a : T1 強調像.

b : 造影像.

図 2 前医で撮影した MRI (3月の再発から16日後)

検査所見は、血液検査、髄液検査ともに異常ありません。電気生理はNCS, SSEP, VEPを行いました。異常ありませんでした。画像は、頭部と胸・腰髄MRIは異常ありませんでした。

画像所見として、前医での頸髄MRIをお示しします(図2)。3月の再発から2週間頃のT1強調像と造影像です。矢状断像に入っている線は横断像に対応しています。T1強調像では、第6椎体レベルの頸髄で腫大を認め、少し見づらいのですがT1の高信号、低信号が入り混じっています。それを横断像で見ますと、左の後索から後角にあたる部分に高信号を認めました。それに対応した造影像では、その範囲がやや広くなる形で造影されています。

図3は、3月と6月の頸髄MRI所見を経時的に並べてみたものです。T2強調像で、高信号は3カ月後には低信号になり、T1強調像で、腫大していた頸髄が縮小し、高信号と低信号の混在は低信号

に変わっています。それから淡く造影されていたものが、3カ月後には造影がわからなくなっています。6月のT2\*像では、左後索から後角に対応する部分に低信号があつて髄内出血が疑われることと、T2\*像頸髄前方正中に線状の高信号があつて、それが造影でも線状に造影されることから、cavernous hemangioma(海綿状血管腫)の合併があるだろうと考えています。

疼痛の治療経過(図4)ですが、初発時45歳です。今回が49歳で、疼痛が生じまして、visual analog scaleで9くらいの激しい疼痛がありました。それでベタメタゾン、塩酸イミプラミン、カルバマゼピン、バルプロ酸、塩酸メキシチレンなどを投与しましたが、疼痛の改善はわずかでした。その後、クロナゼパムを使用した頃からようやく痛みが自覚的に2~3割改善しました。

その後、脳神経外科に転科しまして星状神経節ブロックが6回行われましたが、効果がなく、ジ