

## **X-LINKED RECESSIVE BULBOSPINAL NEURONOPATHY (SBMA)**

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### **ABSTRACT**

X-linked recessive bulbospinal neuronopathy (SBMA) is an adult onset motor neuronopathy with androgen receptor (AR) gene mutation of expanded CAG repeat size in the first exon. The size of CAG repeats in the AR gene is one of the determinant factors of the severity and progression rate of SBMA phenotypes, but the meiotic and somatic instability of CAG repeats is far more stable as compared with other diseases caused by trinucleotide repeat expansions such as HD, DRPLA, MJD and SCA1. Several evidences suggest that aberrant transcriptional activity of androgen through mutant AR is related to the pathogenic mechanism of this disease.

### **INTRODUCTION**

X-linked recessive bulbospinal neuronopathy (SBMA) is characterized by adult onset proximal muscular atrophy, bulbar involvement, marked contraction fasciculation, and very slow progression.<sup>1-4)</sup> Historically, this disease was initially described in 1897 by Dr. H. Kawahara, a professor at Aichi Medical University. Subsequent reports have described clinical and genetic aspects of this disease.<sup>5)</sup>

In 1968, Kennedy et al.<sup>1)</sup> reported 11 males from two families and described this disease as a distinctive form of adult onset spinal muscular atrophy with X-linked recessive inheritance. Since then, many families, especially from Japan, have been described with a similar disease. Common features in all these reports were slowly progressive bulbar symptoms, proximal muscular atrophy with prominent fasciculation and tremors, gynaecomastia, hepatic involvement, a high incidence of diabetes mellitus, abnormalities in serum sex hormone levels, and testicular atrophy.<sup>3-7)</sup>

Androgen receptor gene mutation with an increased number of CAG repeats in the coding regions was found to be a specific abnormality in the SBMA gene.<sup>8)</sup> Since this discovery, in many disorders such as Huntington's disease (HD),<sup>9)</sup> dentatorubral-pallidoruysian atrophy (DRPLA),<sup>10)</sup> Machado-Joseph disease (MJD),<sup>11-14)</sup> and spinocerebellar ataxia type 1 (SCA1),<sup>15)</sup> trinucleotide repeat expansion of the responsible gene has been reported as the causative mutation. Characteristics of molecular genetics of these diseases are common to some extent; the number of CAG repeats varies considerably depending on the patient, and is correlated with the severity of the clinical manifestations; meiotic instability of CAG repeats is more prominent in paternal transmission than in maternal transmission; CAG repeats in the genes of these diseases are located in the coding region and translated into polyglutamine tracts; mutant genes with expanded CAG repeats are ubiquitously expressed through the neural and nonneural tissues, while pathological involvement only occurs in the restricted systems of the neural tissues. However,

the molecular mechanism of the pathogenic role of the increased CAG repeats in the responsible gene of each disease is unknown. Furthermore, the normal functions of the deduced proteins encoded at the loci for HD, DRPLA, SCA1 and MJD are unknown. Among these genes, only the function of the AR protein is known; we can therefore presume that the CAG repeat expansion in the AR gene for SBMA is associated with trinucleotide repeat expansion.

An AR gene deletion mutant lacking the 5'-terminal sequences, including the CAG repeat region, is known to reduce transcriptional activity;<sup>16)</sup> this suggests that the region of CAG repeat is required for transcriptional regulation. Furthermore, a study on co-transfected AR-free COS-1 cells with a cytomegalovirus-promoted hAR expression vector, and a reported plasmid composed of the human growth hormone gene connected to the long terminal repeat of the mammary tumor virus that contains several steroid-responsive elements (androgen), demonstrated that the transcriptional activity was reduced in correlation to the elongated CAG repeats in the AR gene.<sup>17,18)</sup> It can be concluded that a increased number of CAG repeats in the AR gene may affect the transduction signal of the androgen, and accordingly, the androgen actions in the target tissues.

The aim of this review article, is to focus on the SBMA and androgen receptor mutation, and to give an overview of the findings so far reported, including our own.

## CLINICOPATHOLOGICAL FEATURES

### *Clinical features*

Our series consists of 95 patients (age range: 31 to 83 yrs).<sup>3,19)</sup> In most patients the initial symptom was fine finger tremor, which appeared in the middle of the third decade. Gynaecomastia was also seen. Contraction fasciculation was common in all patients and occurred in the facial, trunk and limb muscles. Dysphagia, which was present in all, was generally mild, but occasionally became troublesome later in the clinical course. Muscle atrophy and weakness were prominent in the tongue and proximal upper and lower limbs. The tendon reflexes were absent in most cases, but in some cases they were well preserved proximally in the limbs. An extensor plantar response was not present. Sensory involvement was largely restricted to vibration sense which was impaired distally in the legs in all cases in varying degree. In the upper limbs, impairment in vibration sense was minimal. Sensation for position and light touch was mostly preserved. Serum CK levels in most of the patients were up to 10 times above normal values. The cerebrospinal fluid was normal in all. Some patients had a mild to moderately impaired glucose tolerance, and also showed a mild elevation of serum glutamic-pyruvic transaminase (GPT) activity.

Motor nerve conduction was almost within the normal limits. Terminal motor latencies, however, were prolonged, even in the patients with normal motor conduction. Sural nerve action potentials were absent or reduced in amplitude. Electromyographic abnormalities with reduced motor unit recruitment patterns, and polyphasic units of long duration and increased amplitude (greater than 5 mV) were common in all patients. Positive sharp waves and fibrillations were not very frequent.<sup>20)</sup>

In the heterozygous female carrier, clinical symptoms are rarely seen. However, about half of the female carriers exhibit electromyographic abnormalities with giant spikes of long duration.<sup>21)</sup> In such female carriers, very mild fiber-type grouping can be seen in muscle biopsy specimens.

### *Pathological features*

In SBMA, a marked loss of ventral horn cells and large myelinated fibers are present through

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all the spinal segments.<sup>3,22)</sup> In terms of spinal segment, there is no apparent gradient in rostral-caudal distribution of fiber loss and motor neuron loss. Depletion of ventral horn cells is particularly prominent in the lateral nuclear groups through all spinal segment.<sup>23,24)</sup> Simple atrophy of neurons is observed, but central chromatolysis and neuronophagia are seldom seen. Reactive astrogliosis is also slight.<sup>3)</sup>

Myelin pallor in the fasciculus gracilis of the spinal cord is observed.<sup>3)</sup> This myelin pallor is more prominent in the rostral segment than in the caudal segment of the spinal cord, and corresponds to marked myelinated fiber loss. Dorsal spinal roots are normal under light microscopy. Teased fiber preparations, however, reveal a considerable increase in fibers with segmental demyelination and remyelination, and are frequently clustered along a single fiber. In the lumbar dorsal root ganglia, residual nodules of Nageotte,<sup>25)</sup> proliferation of capsule cells and neuronal-size atrophy are seen, but no substantial neuronal loss is observed. In the sural nerve, there is also a marked reduction of myelinated fibers mainly in those of a large diameter. Fiber size-frequency histograms in the sural nerve are nearly unimodal as a result of the loss of large myelinated fibers. Regeneration clusters are rarely seen. These observations suggest that central and peripheral distal axonopathy resulting from perikaryal dysfunction in the primary sensory neurons is part of the underlying pathological process.<sup>3,25)</sup>

Neurons in the intermediolateral columns and in Onuf's nuclei are well preserved. Neurons in Clarke's columns are also well populated. There is no apparent degeneration in the pyramidal tracts.<sup>3)</sup> Neurons in the hypoglossal, facial and trigeminal motor nuclei are severely depleted or atrophic, while those in the trochlear, abducens and oculomotor, gracile and cuneate nuclei are well populated. The CNS is otherwise normal under light microscopy.<sup>3,7,23,24)</sup>

The liver in most of the patients, is involved in forming a fatty liver. Testicular atrophy with marked reduction of spermatogenesis is common.<sup>7)</sup>

Hypertrophic fibers, often containing internal nuclei, fiber splitting, groups of atrophic fibers and clumping of sarcolemmal nuclei are common findings in all samples. Muscle fibers are frequently replaced by adipose tissue. Histochemical staining (ATPase) shows fiber-type grouping with large groups of type 1 and type 2a fibers.<sup>3)</sup>

## ANDROGEN RECEPTOR GENE MUTATION AND PHENOTYPES

*Phenotype-genotype correlation*

We determined the CAG repeat size in the androgen receptor gene by direct cycle sequencing or by radiolabelling PCR of the CAG repeat region,<sup>26-29)</sup> and semiquantified the clinical phenotypes such as muscular weakness, activity of daily life, presence or absence of gynaecomastia and diabetes mellitus, bulbar involvement, and plasma CK levels. When obtained phenotypes and genotypes are correlated, there is a significant correlation between the age at onset and the number of CAG repeats ( $r=0.608$ ,  $p<0.0001$ , Fig. 1).<sup>26,27,29,30)</sup> The presence or absence of gynaecomastia also correlates fairly well with the number of CAG repeats ( $t=2.03$ ,  $p<0.05$ ),<sup>25)</sup> indicating that the patients, young at onset and with gynaecomastia, have a more elongated CAG repeat, and vice versa. Muscular weakness, ADL score, presence or absence of glucose intolerance, disturbance of swallowing, and plasma CK and GPT levels are not correlated with the number of CAG repeats. However, when muscular weakness and ADL scores are adjusted with age by dividing the age at examination, there are significant correlations with the CAG repeats (Fig. 2,  $r=0.409$ ,  $p<0.002$ ;  $r=0.386$ ,  $p<0.002$ ).<sup>19,26)</sup> There are, however, no correlations when those phenotypes are adjusted by the duration of illness.<sup>19,26)</sup>

Why there is a strong correlation between the age-adjusted phenotypes and CAG repeat size,

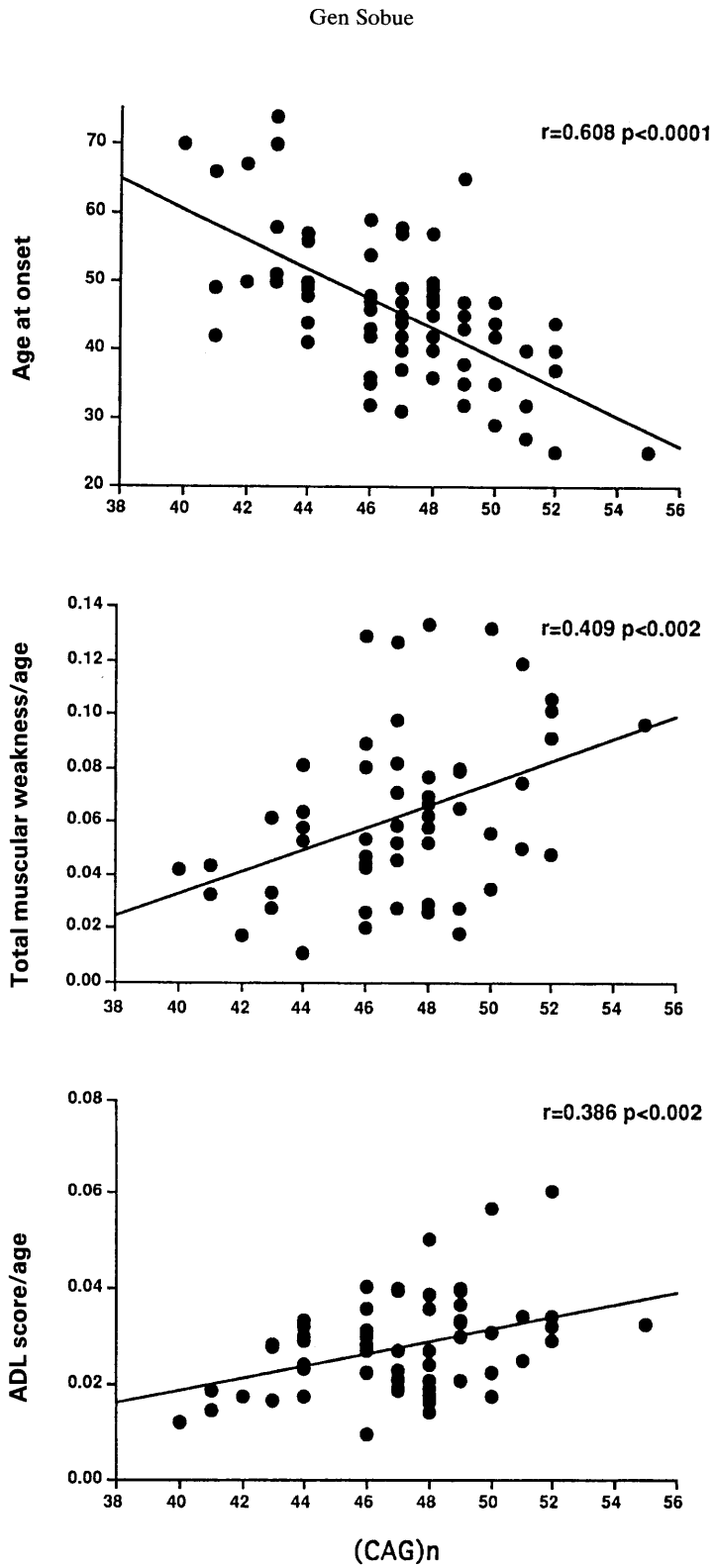


Fig. 1. Correlation between the phenotypes and CAG repeat size in the androgen receptor gene.

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but not between duration-adjusted phenotypes and CAG repeat size, remains to be explained. One interpretation is that the degree of CAG repeat size is one of the factors determining the progression of SBMA phenotypes, and that the disease process may start before the clinical symptoms manifest. The disease process, particularly the loss of motor neurons, may progress even in the preclinical stage, and once the involvement level reaches the threshold of the clinical onset, the clinical phenotypes would emerge. An expanded CAG repeat of a 10-year-old boy expressed giant spikes in the electromyogram. Although this was clinically normal (data not shown), it would support the speculation that the pathological process is in progress in the preclinical stage. Further studies in assessing the symptoms in preclinical patients with CAG repeat expansion are required to elucidate the disease process.

When examining the sibling-to-sibling variation of the phenotypes and the number of the CAG repeats in the mutant allele, there is a significant intrafamilial variation in the clinical phenotype, whereas the variation of CAG repeat size among the SBMA siblings of a family is less than three (Table 1).<sup>19)</sup> These variations in the phenotypes among the affected patients with small variation of the mutation size suggest that the CAG repeat size is one of the phenotypic determinants, but other unknown factors, such as nongenetic environmental factors or polymorphism in the flanking region to the androgen receptor gene, would influence the phenotypic manifestation.

*Phenotype-genotype in female carriers*

By PCR amplification of CAG repeat of the AR gene, we can accurately diagnose the female heterozygous carrier.<sup>21)</sup> About half of definite female heterozygous carriers exhibit high amplitude motor unit potentials in the examined muscles (Table 2),<sup>21)</sup> although all of them are neurologically intact and have normal serum levels for muscle enzyme, muscle structural proteins and glycolytic metabolites. The underlying pathological background of the EMG abnormality observed in the carriers is still obscure, but type 2 fiber preponderance and very mild fiber-type grouping seen in carriers with an EMG abnormality may suggest that very mild and subclinical denervation and reinnervation is present in these carriers.<sup>21)</sup> Interestingly, carriers which belong to the same family exhibit different EMG and muscle biopsy findings.<sup>21)</sup> These results indicate that a subset of heterozygous female carriers exhibit phenotypic manifestations, although they are subclinical.

A heterozygous carrier of SBMA with manifested clinical symptoms (manifesting carrier) might be present, as is observed in Duchenne muscular dystrophy and other X-chromosome-linked neurological disorders, but the clinical implication of the present findings of a subclinical EMG abnormality in the SBMA carrier may be less significant. However, this observation may provide another example for molecular genetic survey of X-chromosome inactivation and partial gene expressions. According to Lyon's theory,<sup>31)</sup> the heteropyknotic X-chromosome is genetically inactivated; the inactivated X-chromosome could be either of maternal or paternal origin and is randomly distributed through different cells. The molecular mechanism of X-chromosome inactivation is still unclear, but the methylation pattern of cytosine residues is known to be different in some genes, and methylation is considered to play a role in the maintenance of X-chromosome inactivation. The underlying mechanism as to why some carriers exhibit partial expressions of abnormal genes and some carriers do not is unclear at present, but heterozygous females who express subclinical abnormalities may have a greater number of active mutant X-chromosomes than normal ones, as is shown in Duchenne muscular dystrophy.

*Gametic and somatic instability of CAG repeat size in the AR gene*

The CAG repeat size of the mutant allele in paternal-offspring and maternal-offspring pairs

Table 1. (CAG)n and phenotypic variation in the siblings of a family.

Family	Generation	Age	Disease condition	(CAG)n			
				Abnormal allele	Age at onset	TMW	ADL
A	I3	64	Carrier	45			
	I4	63	SBMA	44	56	4.00	2.00
	I5	61	Carrier	48			
	III10	11	Carrier	44			
B	I1	55	Carrier	48			
	I4	51	SBMA	47	42	5.00	2.00
	I6	43	SBMA	44	41	3.50	1.00
	II1	34	Carrier	48			
	II3	31	SBMA	47	31	0.50	0.50
	II9	24	Carrier	50			
	III11	17	Carrier	52			
C	II2	56	SBMA	46	48	3.00	1.50
	II6	46	SBMA	46	46	0.50	0.50
D	II3	59	SBMA	47	58	3.50	0.00
	II5	56	SBMA	47			
E	I6	59	SBMA	41	49	1.75	1.00
	II7	21	Carrier	41			
F	II1	60	SBMA	49	35	4.75	2.00
	II4	50	SBMA	49	32	4.00	2.00
	II6	47	SBMA	50	44	2.75	1.50
G	I1	56	SBMA	47	47	4.00	1.50
	I2	48	SBMA	46	47	1.25	2.00
	I4	36	SBMA	48	36	2.50	0.50
I	II1	62	Carrier	52			
	I2	59	Carrier	52			
	I3	52	SBMA	52	44	4.75	1.50
	II1	40	SBMA	51	40	3.00	1.00
J	II6	30	Carrier	52			
	II8	23	Carrier	48			

TMW: total muscular weakness; ADL: activity of the daily life determined by the mobility score described in the text.

was assessed (Table 3).<sup>19)</sup> There was a significant difference in the size of repeat variation in maternal and paternal transmissions. Five of nine paternal meioses showed an increase in the number of CAG repeats with an average increase of +1.44. By contrast, six maternal transmissions showed no change or a decrease in the number of CAG repeats with an average change of -0.33. This difference in intergenerational change between paternal and maternal transmission is statistically significant when the CAG repeat number is tabulated ( $p < 0.01$ ).<sup>19)</sup>

Biased paternal origin of repeat expansion has been reported in all the diseases in which expansion of the CAG repeat is the responsible gene deficit in HD, SCA1, DRPLA and MJD.

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Table 2. ELECTROMYOGRAPHIC FINDINGS IN THE SIBLINGS OF X-BSNP FAMILIES

Family	Sibling age (yrs)	Patient, carrier or normal	Denervation potentials <sup>a</sup>		High amplitude motor unit potentials							
			fibrillation	positive sharp	deltoid	biceps	brachio-radialis	quadriceps femoris	tibialis anterior	gastrocnemius		
A	II-3/52	x-BSNP	0	+	0	+	+	+	+	+	+	+
	III-2/26	carrier	0	0	0	+	+	+	+	+	+	0
	II-2/59	carrier	0	0	0	+	+	+	+	+	+	+
B	II-2/50	x-BSNP	0	+	+	+	+	+	+	+	+	+
	II-3/43	x-BSNP	+	+	+	+	+	+	---	+	+	---
	II-1/59	carrier	0	0	0	0	0	0	0	0	0	0
	III-1/36	carrier	0	0	0 <sup>b</sup>	0 <sup>b</sup>	+	+	+	+	+	+
	III-3/25	carrier	0	0	0 <sup>b</sup>	0 <sup>b</sup>	+	+	+	+	+	0
	III-5/16	carrier	0	0	0	0	0	0	0	0	0	0
	III-2/25	normal	0	0	0	0	+	+	+	+	+	0
	III-4/21	normal	0	0	0	0	0	0	0	0	+	0
C	II-1/72	x-BSNP	+	+	0	+	+	+	+	+	+	+
	III-2/43	carrier	0	0	0	0	0	0	0	0	0	0
D	II-1/58	x-BSNP	+	+	0	+	+	+	+	+	+	+
	III-1/26	carrier	0	0	0	0	0	0	+	+	0	0

+: high amplitude motor unit potentials (more than 6 mV) or denervation potentials are present; 0: not detected; ---: not examined.

<sup>a</sup> When denervation potentials are observed even in one of 6 examined muscles, it is designated.

<sup>b</sup> High amplitude motor unit potentials with more than 4 mV but less than 6 mV.

Table 3. (CAG)<sub>n</sub> meiotic transmission

Paternal transmission				Maternal transmission			
Generation	Parental	Offspring	Difference	Generation	Parental	Offspring	Difference
A-I4-II7*	44	44	0	A-II7-III10	44	44	0
B-I4-II9	47	50	+3	B-I1-III1	48	48	0
B-I4-II11	47	52	+5	B-I1-II3	48	47	-1
E-I5-II7	41	41	0	C-I2-II2	46	46	0
F-II6-III6	50	51	+1	C-I2-II6	46	46	0
H-I1-II4	48	49	+1	I-I1-II1	52	51	-1
I-I3-II7	52	51	-1				
J-I4-II6	48	52	+4				-0.33
J-I4-II8	48	48	0				
			+1.44				

\*Generation indicates paternal transmission from father (I4) to daughter (II7) in family A. In the case of female carrier, CAG repeat size of mutant allele was described.

However, the magnitude of the average gain in paternal transmission is larger in HD, SCA1, DRPLA and MJD; an increase of 9 repeat units in HD, 3.3 repeat units in SCA1, 4.2 repeat units in DRPLA and 3.4 repeat units in MJD, as compared with 1.4 repeat units in SBMA. Moreover, in HD, SCA1 and DRPLA, genetic anticipation is apparent and the juvenile onset cases of HD and DRPLA with severe phenotypes are transmitted by paternal meiosis. In contrast, genetic anticipation is not apparent and the spectrum of clinical phenotypes are rather narrow in SBMA, which may be consequent to the X-linked recessive trait and the small size of paternal expansion of the CAG repeat units.<sup>19,32)</sup>

There is also tissue-specific variation in the CAG repeat size in the neural and nonneural tissues (tissue-specific somatic mosaicism) of trinucleotide diseases.<sup>33,34)</sup> However, the spatial pattern of tissue-specific somatic mosaicism in the CAG repeat size is significantly different among DRPLA, MJD and SBMA (Table 4).<sup>33,34)</sup> The major bands of the mutant CAG repeat allele are significantly smaller in the cerebellar cortex in HD, DRPLA and MJD patients by 2 to 6 repeats, and are larger in the colon and liver.<sup>33)</sup> There is also 1 to 2 repeat-sized small variations of the major bands among the neural tissues of HD and DRPLA. In contrast, there is no tissue-specific variation of the major bands of CAG repeats and diversity of extra bands among the tissues including the cerebellum in the SBMA patients. Lack of significant tissue-specific somatic mosaicism in SBMA including the cerebellar cortex may suggest that CAG repeat expansion in the mutant AR gene is far more stable as compared with that in HD, DRPLA and MJD. At onset, HD, DRPLA and MJD exhibit a wide range of phenotypic heterogeneity with age and the degree of severity. The instability of CAG repeat size in these regions may explain these characteristic variations in severity and age at onset of the disease. Whereas, in SBMA, juvenile onset is rare and the clinical phenotypic diversity is relatively narrow as compared to these diseases. The CAG repeat in the mutant AR gene is far more stable as compared with those in the gene responsible for HD, DRPLA, and MJD, which also correlates to the stability in somatic CAG repeat size.<sup>33,35)</sup>



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Table 4. Somatic mosaicism of (CAG)n and histopathological findings

	Histopathological Findings	(CAG)n		
		Mutant allele		Normal allele
		Major band size	Range	
<b>DRPLA</b>				
Frontal cortex	+	59	55-63	15
white matter	+	59	55-63	15
Temporal cortex	+	59	55-63	15
white matter	-	59	55-63	15
Parietal cortex	-	59	54-63	15
white matter	-			
Occipital cortex	-	59	55-64	15
white matter	-	59	54-64	15
Cerebellum cortex	-	53	50-56	15
white matter	++	59	53-64	15
Basal ganglia				
Thalamus	+	59	54-64	15
Putamen	++	59	54-63	15
Brain stem				
midbrain	+			
red nucleus	+++	59	54-63	15
pons	++	59	54-65	15
medulla oblongata	-	59	54-62	15
<b>MJD</b>				
Frontal cortex	-	80	76-83	14
white matter	-	80	76-83	14
Temporal cortex	-	80	76-83	14
Occipital cortex	-	80	76-83	14
white matter	-	80	76-83	14
Corpus callosum	-	80	76-83	14
Caudate nucleus	-	80	76-83	14
Putamen	-	80	76-83	14
Globus pallidus	+++	80	76-83	14
Thalamus	-	80	76-83	14
Hippocampus	-	80	76-83	14
Cerebellar cortex	++	78	74-81	14
Thoracic spinal cord	+	80	76-83	14
Lumbar spinal cord	+	80	76-83	14
Sympathetic ganglion	-	80	76-83	14
Dorsal root ganglion	-	80	76-83	14
Femoral nerve	+	80	76-83	14
Ulnar nerve	+	80	76-83	14
Liver	-	85	79-91	14
Spleen	-	80	76-83	14
Kidney	-	81	76-89	14
Colon	-	81	76-89	14
Skeletal muscle	-	80	76-83	14
Heart muscle	-	80	76-83	14
<b>SBMA</b>				
Temporal cortex	-	47	43-48	
white matter	-	47	43-48	
Cerebellar cortex	-	47	43-48	
Thoracic spinal cord	+++	47	43-48	
Lumbar spinal cord	+++	47	43-48	
Dorsal root ganglion	+	47	43-48	
Sciatic nerve	+	47	43-48	
Sympathetic ganglion	-	47	43-48	
Femoral nerve	+	47	43-48	
Skeletal muscle	+++	47	43-48	
Heart muscle	-	47	43-48	
Mammary gland	+	47	43-48	
Liver	+	47	43-48	
Spleen	-	47	43-48	
Adrenal	-	47	43-48	
Kidney	-	47	43-48	
Testis	++	47	43-48	
Scrotal skin	+	47	43-48	
Skin	+	47	43-48	

--: No pathological involvement, +: mild, ++: moderate and +++: severe pathological involvement

## ANDROGEN RECEPTOR GENE EXPRESSION AND POSSIBLE MECHANISM OF DISEASE PROCESS

### *Androgen receptor gene expression*

When AR mRNA expression in various tissues is analyzed by RT-PCR and Northern blot analysis, the mutant AR mRNAs are expressed in a wide variety of tissues of SBMA patients including the testis, scrotal skin, liver, skeletal and cardiac muscles, sciatic nerve, spinal cord, and sympathetic and dorsal root ganglia, and all are abnormally elongated in the size of CAG repeat.<sup>36,37)</sup> Thus, the mutant AR gene with increased size of the tandem CAG repeat is directly transcribed in various tissues. The pathological involvement in the neural and nonneural tissues are, however, highly restricted to the lower motor neurons, skeletal muscles, dorsal root ganglion neurons, testis and liver.<sup>37)</sup> The sympathetic neurons, brain, kidney, spleen etc., express the mutant AR mRNAs with expanded CAG repeats, while these tissues are pathologically spared. This discrepancy between pathological involvement and spatial distribution of the mutant gene expression is also commonly observed in HD, DRPLA, MJD and SCA1. The regional specificity of neuropathology in SBMA may also be attributable to the tissue-specific events such as aberrant tissue-specific transcriptional regulation through the universally expressed mutant AR gene.

### *Aberrant transcriptional activity through mutant AR*

When the fibroblasts from the human scrotal skin are cultured and treated with dihydrotestosterone for up to 48 hours, AR and amphiregulin mRNA levels are increased by three- to fourfold. In the flanking promoter region of the human AR gene, there is a sequence of the androgen responsive element; thus an increase of AR gene expression may be directly regulated by androgen itself. The AR mRNA levels in the scrotal fibroblasts treated with dihydrotestosterone are significantly higher in patients with SBMA than the controls, whereas the amphiregulin mRNA levels are far less in the fibroblasts from SBMA. Since the binding kinetics of dihydrotestosterone to the fibroblasts from SBMA patients does not differ from those of the control,<sup>38)</sup> the differential expression levels of AR mRNA and amphiregulin mRNA between the SBMA and the control may be consequent to the difference in the transcriptional activity through mutant AR with different CAG repeat size.<sup>39,40)</sup>

AR-free COS-1 cells are co-transfected with a cytomegalovirus-promoted hAR (with various sized CAG repeats) expression vector and a receptor plasmid composed of the growth hormone gene connected to the long terminal repeat of the mouse tumor virus, which contains androgen responsive elements, and are treated with androgen. In this COS-1 system, human AR transactivity and CAG length are related inversely.<sup>17,18)</sup> These results indicate that a normal-sized CAG repeat expresses the transactivational competence of a CAG free AR, and that CAG repeat expansion increases the negative effect.

These two observations on the scrotal fibroblasts and artificial COS-1 system would suggest that the disease process of SBMA is related to the aberrated transcriptional activity of the mutant AR.

### *Transgenic mice with AR gene with expanded CAG repeat size*

In recently tests, the transgenic mice carrying either the normal or expanded repeat human AR gene was generated.<sup>41)</sup> Unlike the disease allele in humans, the AR cDNA containing the expanded repeat in the transgenic mice showed no change in repeat length with transmission. SBMA AR was detected in the transgenic mice, but at a level lower than that of normal

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endogenous expression; there were no pathological alterations nor a physiological pattern of disease expression.

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