Correlation of insulin resistance and motor function in spinal and bulbar muscular atrophy

Hideaki Nakatsuji[1], Amane Araki[1,2], Atsushi Hashizume[1], Yasuhiro Hijikata[1], Shinichiro Yamada[1], Tomonori Inagaki[1], Keisuke Suzuki[1,3], Haruhiko Banno[1], Noriaki Suga[1,4], Yohei Okada[5,6], Manabu Ohyama[7], Tohru Nakagawa[8], Ken Kishida[9,10], Tohru Funahashi[9,11], Iichiro Shimomura[9], Hideyuki Okano[6], Masahisa Katsuno[1], Gen Sobue[1,12]

1. Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan

2. Department of Neurology, Kasugai Municipal Hospital, Kasugai, Japan

3. Department of Clinical Research, Innovation Center for Clinical Research, National Center for Geriatrics and Gerontology, Obu, Japan

4. Department of Neurology, Sarashina Rehabilitation Clinic, Ichihara, Japan

5. Department of Neurology, Aichi Medical University School of Medicine, Aichi, Japan.

6. Department of Physiology, Keio University School of Medicine, Tokyo, Japan

7. Department of Dermatology, Keio University School of Medicine, Tokyo, Japan

8. Hitachi, Ltd. Hitachi Health Care Center, Hitachi, Ibaraki, Japan

9. Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan

10. Kishida Clinic, Osaka, Japan

11. Department of Metabolism and Atherosclerosis, Graduate School of Medicine, Osaka University, Osaka, Japan

12. Research Division of Dementia and Neurodegenerative Disease, Nagoya University

Graduate School of Medicine, Nagoya, Japan

* **Reprint requests are to be sent to Dr Katsuno or Sobue:**

Masahisa Katsuno, MD

Department of Neurology, Nagoya University Graduate School of Medicine

65 Tsurumai-cho, Showa-ku, Nagoya 466-8550 Japan

TEL: +81-52-744-2385; FAX: +81-52-744-2384;

E-mail: ka2no@med.nagoya-u.ac.jp

Gen Sobue, MD

Research Division of Dementia and Neurodegenerative Disease, Nagoya University Graduate School of Medicine

65 Tsurumai-cho, Showa-ku, Nagoya 466-8550 Japan

TEL: +81-52-744-2943; FAX: +81-52-744-2967;

E-mail: sobueg@med.nagoya-u.ac.jp

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Abstract

Objectives: This study aimed to evaluate various metabolic parameters in patients with spinal and bulbar muscular atrophy (SBMA), to investigate the association between those indices and disease severity, and to explore the underlying molecular pathogenesis.

Methods: We compared the degree of obesity, metabolic parameters and blood pressure in 55 genetically confirmed SBMA patients against those in 483 age- and sex-matched healthy control. In SBMA patients, we investigated the correlation between these factors and motor functional indices.

Results: SBMA patients had lower body mass index, blood glucose, Hemoglobin A1c, but higher blood pressure, homeostasis model assessment of insulin resistance (HOMA-IR, a marker of insulin resistance), total cholesterol, and adiponectin levels than the control subjects. There were no differences in visceral fat areas, high density lipoprotein-cholesterol (HDL-C) or triglyceride levels in two groups. Revised amyotrophic lateral sclerosis functional rating scale (ALSFRS-R) correlated positively with HDL-C, but negatively with HOMA-IR. Through stepwise multiple regression analysis, we identified HOMA-IR as a significant metabolic determinant of ALSFRS-R. In biochemical analysis, we found that the decreased expressions of insulin receptors, insulin receptor substrate-1 and insulin receptor-β, in autopsied muscles and fibroblasts of SBMA patients.

Conclusions: The present study demonstrates that SBMA patients have insulin resistance, which is associated with the disease severity. The expressions of insulin receptors are attenuated in the skeletal muscle of SBMA, providing a possible pathomechanism of metabolic alterations. These findings suggested that insulin resistance is a metabolic index reflecting disease severity and pathogenesis as well as a potential therapeutic target for SBMA.

Keywords

spinal and bulbar muscular atrophy, SBMA, insulin resistance, insulin receptor

Introduction

Spinal and bulbar atrophy (SBMA) is a hereditary neuromuscular disease characterized by slowly progressive muscle atrophy and weakness [1, 2]. SBMA is caused by the expansion of a CAG trinucleotide repeat encoding a polyglutamine tract in the *androgen receptor* (*AR*) gene [3, 4]. Neuropathological hallmark is loss of lower motor neuron accompanied by nuclear accumulation of the polyglutamine-expanded AR protein, whereas muscle pathology shows both neurogenic and myopathic changes [5, 6]. In addition to neuromuscular symptoms, the patients with SBMA display signs of androgen insensitivity such as gynecomastia and reduced fertility, suggesting loss of native function of AR [7]. Furthermore, they often demonstrate metabolic alterations including abdominal obesity, glucose intolerance, and dyslipidemia [8 ,9].

Metabolic factors are known to play a role in the etiology and progression of various diseases, including neurodegenerative diseases. For example, diabetes is associated with an increased risk of Alzheimer’s disease and Parkinson's disease [10, 11]. With respect to lipid metabolism, hyperlipidemia accelerates memory impairment in Alzheimer's disease [10]. Hypermetabolism has been well characterized in amyotrophic lateral sclerosis (ALS), as the patients show elevated levels of glucose and lipids that negatively associate with disease progression in this disease [12, 13]. These findings

suggest that appropriate management of glucose and lipid metabolism leads to improvement of prognosis of neurodegenerative disorders. However, the relationship between metabolic disturbances and neurological deficits remains elusive in SBMA. The aims of the present study were to evaluate various metabolic parameters in patients with SBMA, to investigate the association between those indices and disease severity, and to explore the underlying molecular pathogenesis.

Methods

Standard protocol approvals, registrations, and patient consents.

This study adhered to the Ethics Guidelines for Human Genome/Gene Analysis Research and those for Medical and Health Research Involving Human Subjects endorsed by the Japanese government, and was approved by the Ethics Committees of Nagoya University Graduate School of Medicine and Hitachi Health Care Center. All participants provided written informed consent. The collection of autopsied human tissues and their use for this study were also approved by the Ethics Committee of Nagoya University Graduate School of Medicine, and written informed consent was obtained from the subjects' next of kin. Human skin fibroblasts were obtained via biopsy from SBMA patients with written informed consents. We also obtained control fibroblast

lines from the Japanese Collection of Research Bioresources Cell Bank (TIG114) [14] and Kurabo industries LTD. All of the experimental procedures associated with human fibroblasts were approved by the ethics committee of the Keio University School of Medicine (approval number 20080016), the ethics committee of the Aichi Medical University School of Medicine (approval number 14–004), and the ethics committee of the Nagoya University, School of Medicine (approval number 1226). Experimental procedures involving human subjects were conducted in conformance with the principles expressed in the Declaration of Helsinki.

Participants

We studied 55 consecutive male patients with SBMA undergoing no specific treatment and 483 age-matched healthy male subjects as controls (Table 1). We included genetically confirmed male Japanese SBMA subjects with more than one of the following symptoms: muscle weakness, muscle atrophy, or bulbar palsy. The subjects were excluded if they met any of the following criteria: (i) severe complications such as malignancy; (ii) other neurological complications; (iii) taken hormonal agents within 48 weeks before informed consent; or (iv) participated in any other clinical trials before informed consent. All subjects were outpatients and were followed in Nagoya University

Hospital. The data were collected between February 2012 and January 2013.

The healthy control subjects were employees who underwent a health checkup in year 2010 at Hitachi Ltd, Ibaraki Prefecture, Japan.

Evaluation of disease severity and serological parameters.

We evaluated the disease severity of SBMA using the following functional parameters in the present study: the revised amyotrophic lateral sclerosis functional rating scale (ALSFRS-R), modified Norris scale (Limb Norris score), and modified quantitative myasthenia gravis (QMG) score, all of which have been validated in SBMA patients [9, 15]. We defined the onset of disease as the time when muscle weakness began, but not when tremor of the fingers appeared.

Height (m) and weight (kg) were measured in the standing position and body mass index (BMI) was calculated [=weight (kg)/height $(m)^2$]. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in the sitting position to the nearest mmHg. Venous blood samples were collected for measurements of blood glucose, hemoglobin A1c (HbA1c), immune-reactive insulin (IRI), total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl

transpeptidase (γ-GTP), uric acid, and creatinine, while the subject was in the sitting position after more than 12 h of fasting. The serum levels of testosterone, luteinizing hormone (LH), and sex hormone binding globulin (SHBG) were measured with chemiluminescent immune assay. The serum levels of adiponectin were measured with a sandwich enzyme-linked immunosorbent assay system (Adiponectin Enzyme-Linked ImmunoSorbent Assay Kit; Otsuka Pharmaceutical, Tokushima, Japan). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR (milliunits per liter × milligrams per deciliter) = [(fasting IRI × fasting glucose)/405] [16, 17]. Androgen sensitivity index (ASI) was calculated using the following formula: ASI (IU × nmol/liter^2) = Testosterone × LH [18]. Visceral fat area (VFA) and subcutaneous fat area (SFA) were measured automatically using commercial software on CT scans taken at the umbilical level in a supine position (cm^2).

Immunoblotting.

We analyzed autopsied skeletal muscle specimens of 4 genetically diagnosed SBMA subjects and 4 sex- and age-matched control subjects (73.0 ± 4.8 and 71.0 ± 4.8 years old at the time of death, respectively). Pathologic diagnoses of the controls were dementia with Lewy bodies (n = 2), familial Parkinson's disease (n = 1), progressive

supranuclear palsy (n = 1). Dermal fibroblasts were collected at biopsy from three SBMA patients [14]. With regard to healthy control human skin fibroblasts, we obtained one line from the Japanese Collection of Research Bioresources Cell Bank and purchased two lines from Kurabo industries LTD. Fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum. Media was changed to DMEM containing 0.1% fatty acid free bovine serum albumin for 12 h and then collected for immunoblotting. These specimens were homogenized in CelLytic lysis buffer (Sigma-Aldrich, St. Louis, MO) containing with Halt Protease and Phosphatase Inhibitor Cocktails (Thermo Scientific, Waltham, MA, USA). Homogenates were then centrifuged at 2,500g for 20 minutes at 4°C. Equal amounts of protein were separated by 5–20% SDS–PAGE gels and transferred to Hybond-P membranes (GE Healthcare, Piscataway, NJ, USA) as described previously [19]. Immunoblot analysis was performed with the following primary antibodies: insulin receptor substrate-1 (IRS-1) (1:2000, Cell Signaling Technology, #2390); insulin receptor β (IRβ) (1:2000, Cell Signaling Technology, #3025); or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:5000, Cell Signaling Technology, #2118). This step was followed by incubation with secondary antibody conjugated with horseradish peroxidase at a 1:3000 dilution. The ECL Plus system (GE Healthcare) was used for detection of the protein

signal. An LAS-3000 imaging system (Fujifilm, Tokyo, Japan) was used to produce digital images. The signal intensities of the independent blots were quantified using IMAGE GAUGE software version 4.22 (Fuji) and expressed in arbitrary units as described previously [19].

Statistical analysis

The positively skewed variables, γGTP and HOMA-IR, were log-transformed for analysis. Differences between two groups were evaluated by unpaired Student's t-test. The relationship between two continuous variables was analyzed using Spearman's correlation coefficients. For multivariate analysis, stepwise multiple linear regression was performed. In all cases, p values <0.05 was considered statistically significant. All statistical analyses were performed with The Statistical Package for Social Sciences (version 23.0, SPSS Japan, Tokyo, Japan).

Result

Clinical and genetic feature of SBMA patients and control subjects.

The baseline characteristics of the SBMA patients and the healthy control subjects are summarized in Table 1. As for the SBMA patients, the mean duration of disease period

was 7.1 years, suggesting that they were mild to moderate grade cases,[8, 9]. The mean CAG repeat length of SBMA patients in the present study was similar to previous studies [9]. The SBMA patients had lower BMI, Glucose, HbA1c, γGTP, and creatinine levels than the controls. By contrast, blood pressures, an insulin resistance index HOMA-IR, TC, AST, ALT, and adiponectin levels were higher in SBMA. The HDL-C levels tended to be higher in the SBMA patients, although the difference from the controls was not statistically significant. There were no differences between the groups with regard to age, VFA, SFA, or TG levels.

Relationship between motor severity and metabolic features in SBMA patients.

To investigate the metabolic features that are associated with disease severity, we investigated the correlations between ALSFRS-R and various clinical parameters (Table 2). ALSFRS-R was positively correlated with HDL-C, and negatively with Log HOMA-IR, VFA, TG, AST, and ALT. Stepwise multiple regression analysis identified Log HOMA-IR as a significant determinant of ALSFRS-R (Table 2 and Fig.1). Log HOMA-IR also had correlations with Limb Norris Score and modified QMG Score (Fig. 1). These findings suggest that insulin resistance is associated with the disease severity of SBMA.

Pathomechanism for insulin resistance of SBMA

To clarify the molecular basis for insulin resistance in SBMA patients, we analyzed the relationship between HOMA-IR and anthropometric and serological variables (Table 3). The results showed that Log HOMA-IR positively correlated with BMI, SFA, VFA, TG, ALT, and Log γGTP, while it had negative correlations with HDL-C, testosterone, and adiponectin. Log HOMA-IR correlated with obesity-related indices (BMI, SFA, and VFA) in SBMA patients. Next, we performed a multivariate analysis of these variables (Table 3), in which we used analyzed BMI, SFA, and VFA separately, since they have strong correlation each other (Multivariate1, BMI; Multivariate2, SFA; and Multivariate3, VFA). The result identified BMI, SFA, VFA, and testosterone as significant determinants of Log HOMA-IR.

Loss-of-function of AR and insulin resistance in SBMA patients.

Although our analysis demonstrated that obesity-related variables correlated with the index of insulin resistance in SBMA patients, they did not show overt obesity in the present study: BMI was lower in SBMA than in control subjects, and VFA, SFA, and TG were equivalent between the groups (Table 1). These results suggested that there might be another primary mechanism for insulin resistance in SBMA. As reduction of AR

function is shown to contribute to insulin resistance and metabolic disorders [20], we hypothesized that loss-of-function of AR could contribute to insulin resistance in SBMA. To verify this, we examined the relationship between the hormonal parameters including androgen sensitivity index ASI, the product of LH and testosterone, which is shown to be elevated by negative feedback on the hypothalamic-pituitary-testicular axis when the subject has androgen insensitivity [18]. Although the frequency of androgen insensitivity was similar to that in another SBMA cohort [18] (Supplementary Table 1), SHBG, testosterone and ASI were inversely, rather than positively, correlated with Log HOMA-IR (Supplementary Table 2). The indices of androgen insensitivity also correlated with the increase of serum levels of adiponectin, an adipocytokine suppressing insulin resistance [21]. Therefore, our results do not indicate that androgen insensitivity is a primary cause of insulin resistance in SBMA, although we cannot exclude the possibility that loss-of-function of AR underlies the metabolic alteration.

Insulin receptor protein levels in the skeletal muscle of SBMA patients.

Recent basic and clinical studies have indicated primary involvement of skeletal muscle in the pathogenesis of SBMA [15, 19, 22, 23]. Although muscle atrophy induces insulin resistance [24], we did not find a correlation of Log HOMA-IR with serum creatinine, a

marker of muscle mass [9, 15], in our cohort of SBMA patients (Table 3). Next, we focused on the insulin receptor pathway in skeletal muscles of the patients. The decrease of the insulin receptor levels in the insulin responsive tissues is known to result in insulin resistance [25]. Therefore, to clarify the causes of insulin resistance in SBMA, we analyzed the protein expression of molecules that are involved in insulin receptor pathway, in autopsied skeletal muscles from SBMA patients and disease controls. The protein levels of Insulin Receptor β subunit (IRβ) and Insulin receptor substrate 1 (IRS-1) in the autopsied specimens of SBMA patients were lower than those of control patients (Fig. 2A-C). Moreover, we observed similar alterations of IRβ and IRS-1 protein expression in fibroblasts of SBMA patients (Fig. 2D-F). We also investigated other molecules such as glucose transporter type 4 in autopsied specimens, but the expression levels of them were equivalent to the controls (data not shown). Taken together, these findings suggest that the decrease of the insulin receptor levels and eventual inhibition of insulin signaling in the skeletal muscle may contribute to insulin resistance in SBMA.

Discussion

In the present study, we showed that insulin resistance is intensified in SBMA patients.

Moreover, the degree of insulin resistance was strongly correlated with the severity of motor dysfunction in SBMA. Decreased expression of insulin receptors in muscle appears to underlie the insulin resistance in SBMA.

Despite unequivocal resistance to insulin, serum glucose and HbA1c levels were not elevated in the patients with SBMA in the present study. Normoglycemia with insulin resistance is often observed in pre stage of type 2 diabetes mellitus as well as in abdominal obesity and heart failure [16, 26]. In subjects with insulin resistance, hyperinsulinemia due to compensated insulin secretion from the pancreas often suppresses the elevation of serum glucose and HbA1c levels. Additionally, adiponectin may also play a role for attenuation of glucose dysregulation in SBMA. Adiponectin is an adipocytokine which possesses anti-diabetic, anti-atherosclerotic, and anti-inflammatory properties [27]. In the present study, compared with controls, SBMA patients had higher adiponectin levels, which was associated with increased androgen insensitivity. Given that adiponectin levels are higher in women than in men and that androgens decrease adiponectin secretion from adipose tissue [28], it is conceivable that loss-of-function of AR may increase serum adiponectin levels, which further leads to attenuate glucose dysmetabolism, in SBMA.

Clinical and basic studies demonstrate tight relationships between insulin resistance

and neurodegeneration. Alzheimer's disease and Parkinson's disease often accompany insulin resistance and glucose intolerance, which exacerbate the neurodegenerative process of the diseases [29, 30]. In accord with our results, insulin resistance was also shown in other polyglutamine diseases. For instance, HOMA-IR was substantially elevated in patients with Huntington's disease and spinocerebellar ataxia type 1 [31, 32]. Though the molecular mechanism by which insulin resistance modulates neurodegeneration has not been fully elucidated, several possible pathways have been proposed. The neurodegenerative diseases including SBMA are often associated with mitochondrial dysfunction, endoplasmic reticulum stress, inflammation, and metabolic alterations [19, 33], all of which are also known to be enhanced by insulin resistance [30]. Decreased androgen signal is potent inducers of insulin resistance [20, 34]. Although it is possible that loss of AR function affects insulin sensitivity in SBMA, we could not find valid association between HOMA-IR and indices of androgen insensitivity in the SBMA patients we examined. Muscle wasting is another possible cause of insulin resistance [24], particularly given that myopathic pathogenesis has been suggested in SBMA [15, 19, 22, 23]. However we could not find correlation between HOMA-IR and serum creatinine, which reflects muscle mass [15], in the SBMA patients. Instead, our analysis using fibroblasts and autopsied muscles demonstrated the decreased

expression of insulin receptors, IRβ and IRS-1, in SBMA. Given that the skeletal muscle-specific loss of insulin receptors results in insulin resistance in mice [35], our results suggest that insulin resistance in SBMA may stem from the down-regulation of insulin receptors. As muscle disuse atrophy is shown to decrease muscular IRβ and IRS-1 protein levels with concomitant c-Jun-NH2-terminal kinase (JNK) activation [36], decreased physical activity due to the disease progression may enhance insulin resistance in SBMA, the pathogenesis of which is also linked to hyperactivity of JNK pathway [37].

Nondiabetic older men with insulin resistance also have greater muscle mass loss than insulin-sensitive older men [38], suggesting that insulin resistance may play a role in the development of muscle atrophy. These findings imply that the restoration of insulin resistance may lead to amelioration of neurodegeneration. This view is supported by the observations that pioglitazone, a peroxisome proliferator-activated receptor-γ agonist which increases insulin sensitivity, is known to ameliorate neurodegeneration in animal models of various diseases including Huntington's disease, Parkinson's disease, ALS, and Alzheimer's disease [39-41]. Furthermore, pioglitazone has beneficial effects on the motor function and histopathological findings of an SBMA model mouse [19], suggesting the potential of this drug as a disease-modifying therapy for SBMA, though clinical trials

are needed to verify the results of animal studies.

In conclusion, the present study demonstrates that SBMA patients have insulin resistance, which is associated with the disease severity. The expressions of insulin receptors are attenuated in the skeletal muscle of SBMA, providing a possible pathomechanism of metabolic alterations. These findings suggested that insulin resistance is a metabolic index reflecting disease severity and pathogenesis as well as a potential therapeutic target for SBMA.

Contributors

H.N.: drafting the manuscript, analysis/interpretation of the data, acquisition of data, statistical analysis, research project execution, study design and concept.

A.A.: analysis/interpretation of the data, statistical analysis, acquisition of data.

A.H.: analysis/interpretation of the data, acquisition of data.

Y.H.: analysis/interpretation of the data, acquisition of data.

S.Y.: analysis/interpretation of the data, acquisition of data.

T.I.: analysis/interpretation of the data, acquisition of data.

K.S.: analysis/interpretation of the data, acquisition of data.

H.B.: analysis/interpretation of the data, acquisition of data.

N.S.: analysis/interpretation of the data, acquisition of data.

Y.O.: analysis/interpretation of the data, acquisition of data

M.O.: research project execution

T.N.: research project execution, acquisition of data

K.K.: research project execution, revising the manuscript

T.F.: research project execution, revising the manuscript

I.S.: research project execution, revising the manuscript

H.O.: research project execution

M.K.: research project organization, research project execution, revising the manuscript, interpretation of the data, study design and concept.

G.S.: research project organization, research project execution, revising the manuscript, interpretation of the data, study design and concept.

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Conflicts of interest

Drs. Nakatsuji, Araki, Hashizume, Hijikata, Yamada, Inagaki, Suzuki, Banno, Suga, Ohyama, Nakagawa and Shimomura report no disclosures.

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Ethical standards

This study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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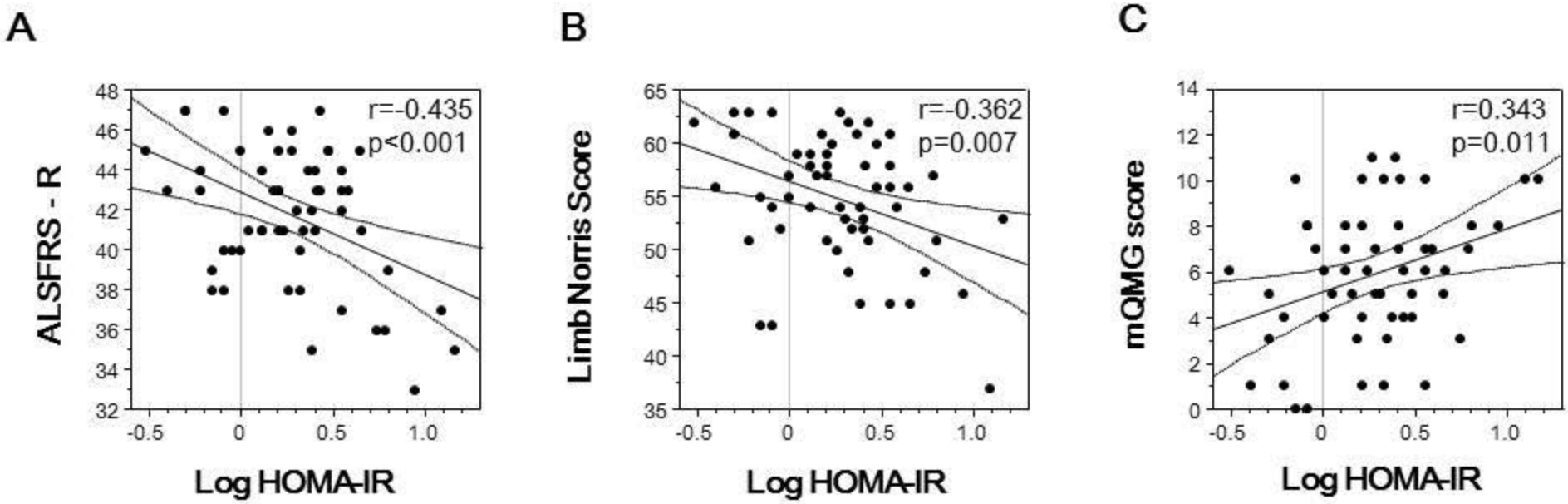
Figure legends

Fig. 1 Correlation between HOMA-IR and motor function in SBMA patients

A-C. Simple correlations of log HOMA-IR with ALSFRS-R (A), Limb Norris Score (B), and modified QMG score (C). Solid lines, regression lines; Dotted lines, 95% confidence interval for the mean values. ALSFRS-R, revised amyotrophic lateral sclerosis functional rating scale; mQMG, modified quantitative myasthenia gravis score.

Fig. 2 Insulin receptor protein levels in the skeletal muscle and the skin fibroblasts of SBMA patients

A-C. Western blots showing the IRS-1 and IRβ protein levels in human skeletal muscle (A) and quantitation of the expression levels of the proteins (n = 4) (B, C). D-F. Western blots showing the IRS-1 and IRβ protein levels in human skin fibroblast cells (D) and quantitation of the expression levels (n = 3) (E, F). Data are shown as Mean ± SD.


A
ALSFRS - R
r=-0.435
p<0.001
Log HOMA-IR
B
Limb Norris Score
r=-0.362
p=0.007
Log HOMA-IR
C
mQMG score
r=0.343
p=0.011
Log HOMA-IR

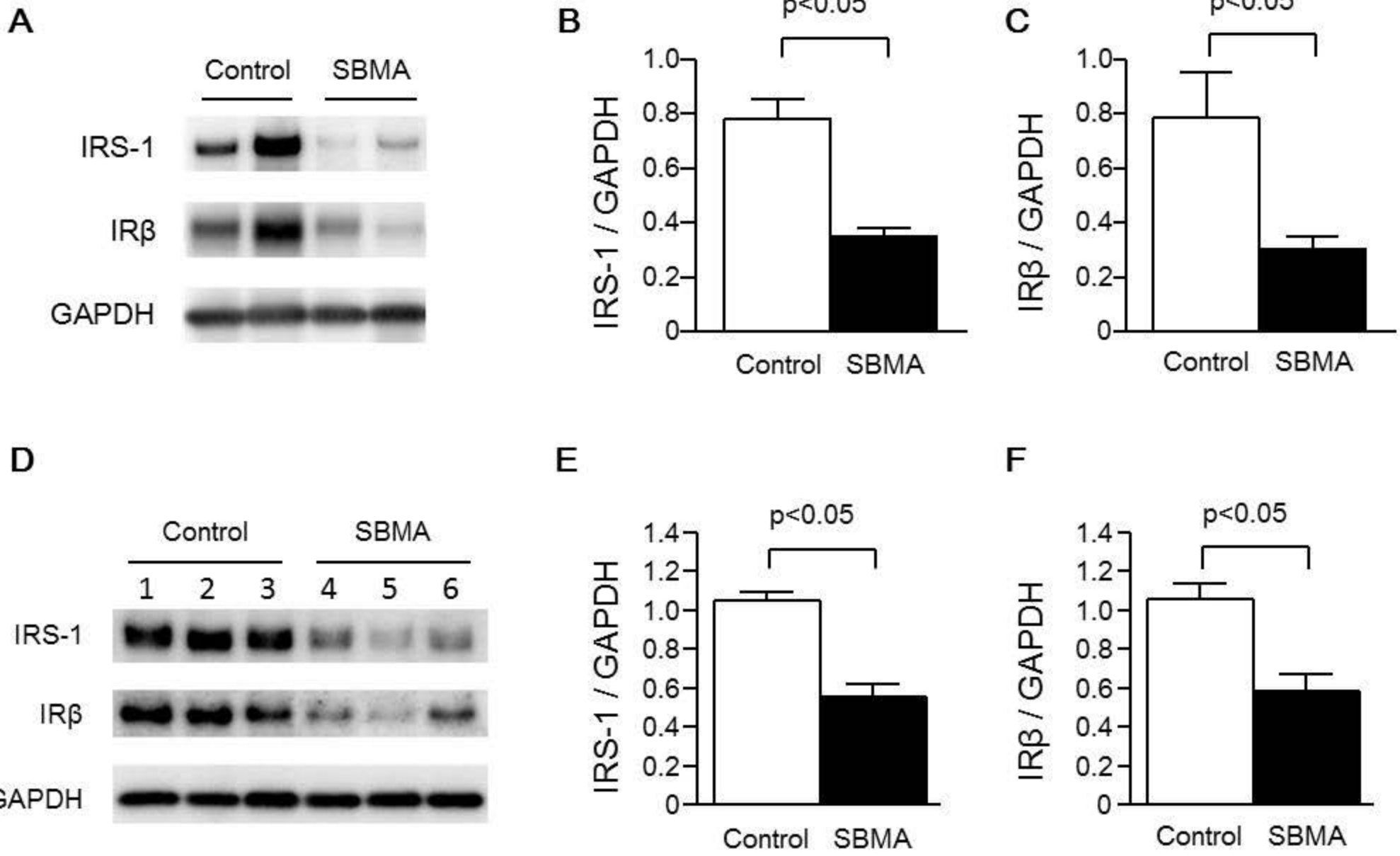

A
Control
SBMA
IRS-1
IRβ
GAPDH
B
p<0.05
IRS-1 / GAPDH
1.0
0.8
0.6
0.4
0.2
0
Control
SBMA
C
p<0.05
IRβ / GAPDH
1.0
0.8
0.6
0.4
0.2
0
Control
SBMA
D
Control
SBMA
1
2
3
4
5
6
IRS-1
IRβ
GAPDH
E
p<0.05
IRS-1 / GAPDH
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Control
SBMA
F
p<0.05
IRβ / GAPDH
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Control
SBMA

Table 1. Clinical and genetic features of patients with SBMA and control subjects.

	SBMA (n=55)	Control (n=483)	*p* value
Age, years	51.1 ± 9.6	53.2 ± 8.8	0.100
Disease duration, years	7.1 ± 3.8	----------	----------
CAG repeat length in AR gene	48.1 ± 3.2	----------	----------
BMI, kg/m²	**22.7 ± 3.6**	**24.2 ± 3.0**	**0.005**
Subcutaneous fat area (SFA), cm²	144.4 ± 58.0	137.6 ± 55.4	0.391
Visceral fat area (VFA), cm²	122.1 ± 61.4	128.2 ± 52.0	0.414
SBP, mmHg	**134.4 ± 15.3**	**122.7 ± 11.5**	**<0.001**
DBP, mmHg	**84.0 ± 9.7**	**78.2 ± 7.5**	**<0.001**
Glucose, mg/dL	**103.7 ± 14.7**	**109.7 ± 18.2**	**0.018**
HbA1c, %	**5.6 ± 0.4**	**5.8 ± 0.6**	**0.042**
IRI, mIU/L	**9.9 ± 9.3**	**6.7 ± 4.5**	**<0.001**
HOMA-IR	**2.6 ± 2.7**	**1.8 ± 1.3**	**<0.001**
TC, mg/dL	**214.9 ± 32.7**	**204.9 ± 32.3**	**0.032**
TG, mg/dL	145.3 ± 8.4	141.5 ± 95.8	0.775
HDL-C, mg/dL	59.6 ± 16.5	56.1 ± 12.8	0.067
AST, IU/L	**43.6 ± 15.3**	**24.6 ± 9.9**	**<0.001**
ALT, IU/L	**51.4 ± 25.7**	**28.1 ± 18.3**	**<0.001**
γGTP, IU/L	**38.1 ± 36.5**	**52.4 ± 49.9**	**0.041**
Uric acid, mg/dL	6.0 ± 1.3	6.1 ± 1.2	0.743
Creatinine, mg/dL	**0.45 ± 0.11**	**0.88 ± 0.13**	**<0.001**
Adiponectin, μg/mL	**9.4 ± 3.6**	**6.6 ± 3.4**	**<0.001**

Data are shown as Mean ± SD.

AR, androgen receptor; BMI; Body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, IRI; immuno-reactive insulin, HOMA-IR; the homeostasis model assessment of insulin resistance, TC; total cholesterol, TG; triglyceride, HDL-C; high-density lipoprotein cholesterol, AST; aspartate aminotransferase, ALT; alanine aminotransferase, γ-GTP; γ-glutamyltranspeptidase.

Table2. Correlation between ALSFRS-R and metabolic features in SBMA patients

ALSFRS-R	*Univariate*		*Multivariate*	
	r	*p*	*β*	*P*
BMI	-0.133	0.334		
SFA	-0.199	0.145		
VFA	**-0.304**	**0.024**		
SBP	-0.009	0.947		
DBP	-0.018	0.898		
Glucose	-0.248	0.068		
HbA1c	-0.205	0.133		
Log HOMA-IR	**-0.435**	**0.001**	**-0.435**	**<0.001**
TC	0.131	0.339		
TG	**-0.291**	**0.031**	-0.169	0.199
HDL-C	**0.362**	**0.007**	0.201	0.153
AST	**-0.310**	**0.021**		
ALT	**-0.328**	**0.014**	-0.174	0.207
Log γGTP	0.075	0.584		
Total adiponectin	0.169	0.216		
Testosterone	0.156	0.254		
SHBG	0.186	0.174		
ASI	0.162	0.237		
			Adjusted *r*2 = 0.174	

Significant level was set at $p < 0.05$ (bold typeface).

SHBG, sex hormone binding globulin; ASI; Androgen sensitivity index. It was calculated using the following formula: ASI (IU × nmol/liter^2) = Testosterone × LH.

Table 3. Correlation between Log HOMA-IR and clinical features in SBMA patients

Log HOMA-IR	*Univariate*		*Multivariate 1 (BMI)*		*Multivariate 2 (SFA)*		*Multivariate 3 (VFA)*	
	r	*p*	*B*	*P*	*β*	*p*	*β*	*p*
BMI	**0.680**	**<0.001**	**0.521**	**<0.001**	--------	--------	--------	--------
SFA	**0.634**	**<0.001**	--------	--------	**0.526**	**<0.001**	--------	--------
VFA	**0.718**	**<0.001**	--------	--------	--------	--------	**0.624**	**<0.001**
TG	**0.321**	**0.017**	0.132	0.220	0.195	0.056	0.009	0.931
HDL-C	**-0.474**	**<0.001**	**-0.253**	**0.013**	-0.192	0.093	-0.122	0.251
ALT	**0.429**	**0.001**	0.161	0.101	0.208	0.053	0.175	0.072
Log γGTP	**0.267**	**0.049**	-0.025	0.816	0.023	0.841	0.038	0.709
Testosterone	**-0.487**	**<0.001**	**-0.253**	**0.013**	**-0.294**	**0.009**	**-0.259**	**0.010**
Creatinine	0.149	0.279	0.125	0.176	0.188	0.061	0.128	0.163
Adiponectin	**-0.352**	**0.008**	-0.167	0.089	-0.138	0.209	-0.079	0.432
			Adjusted *r*2 = 0.557		Adjusted *r*2 = 0.457		Adjusted *r*2 = 0.558	

Supplementary Data

Correlation of insulin resistance and motor function in spinal and bulbar muscular atrophy

Hideaki Nakatsuji[1], Amane Araki[1,2], Atsushi Hashizume[1], Yasuhiro Hijikata[1], Shinichiro Yamada[1], Tomonori Inagaki[1], Keisuke Suzuki[1,3], Haruhiko Banno[1], Noriaki Suga[1,4], Yohei Okada[5,6], Manabu Ohyama[7], Tohru Nakagawa[8], Ken Kishida[9,10], Tohru Funahashi[9,11], Iichiro Shimomura[9], Hideyuki Okano[6], Masahisa Katsuno[1], Gen Sobue[1,12]

1. Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan

2. Department of Neurology, Kasugai Municipal Hospital, Kasugai, Japan

3. Department of Clinical Research, Innovation Center for Clinical Research, National Center for Geriatrics and Gerontology, Obu, Japan

4. Department of Neurology, Sarashina Rehabilitation Clinic, Ichihara, Japan

5. Department of Neurology, Aichi Medical University School of Medicine, Aichi, Japan.

6. Department of Physiology, Keio University School of Medicine, Tokyo, Japan

7. Department of Dermatology, Keio University School of Medicine, Tokyo, Japan

8. Hitachi, Ltd. Hitachi Health Care Center, Hitachi, Ibaraki, Japan

9. Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan

10. Kishida Clinic, Osaka, Japan

11. Department of Metabolism and Atherosclerosis, Graduate School of Medicine, Osaka University, Osaka, Japan

12. Research Division of Dementia and Neurodegenerative Disease, Nagoya University Graduate School of Medicine, Nagoya, Japan

Contents

Supplementary Table 1. Indices of androgen insensitivity in SBMA patients

	Mean±SD (range)	Reference range	Out of reference range, n (%)
SHBG, nmol/L	50.0 ± 23.4 (19.0-131.0)	10-57	20/55 (36%)
Testosterone, nmol/L	28.6 ± 10.9 (14.2-70.4)	7.8-36.1	13/55 (24%)
LH, IU/L	4.9 ± 1.9 (2.0-9.7)	0.79-5.72	16/55 (29%)
ASI, IU nmol/L^2	123.2 ± 76.3 (34.7-346.3)	6.7–138.7	22/55 (40%)

SHBG, sex hormone binding globulin; LH, luteinizing hormone; ASI, Androgen sensitivity index. ASI was calculated using the following formula: ASI (IU × nmol/liter^2) = Testosterone × LH.

Supplementary Table 2. Correlation of HOMA-IR and adiponectin with indices of androgen insensitivity in SBMA patients

	Log HOMA-IR		Serum adiponectin	
	r	p	r	p
SHBG	**-0.484**	**<0.001**	**0.550**	**<0.001**
Testosterone	**-0.487**	**<0.001**	**0.386**	**0.004**
LH	-0.144	0.293	0.087	0.527
ASI	**-0.418**	**0.002**	**0.330**	**0.014**

Strong correlation level was set at r > 0.30 (bold typeface). HOMA-IR, homeostasis model assessment of insulin resistance; SHBG, sex hormone binding globulin.