CrossMark

## ORIGINAL COMMUNICATION

# 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]

**Abstract** 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. 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. SBMA patients had lower body mass index, blood glucose, and 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 decreased expressions of insulin receptors, insulin receptor substrate-1 and insulin receptor-β, in autopsied muscles and fibroblasts of SBMA patients. This 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

✉ Masahisa Katsuno
ka2no@med.nagoya-u.ac.jp

✉ Gen Sobue
sobueg@med.nagoya-u.ac.jp

1   Department of Neurology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, 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, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

disease severity and pathogenesis as well as a potential therapeutic target for SBMA.

## Introduction

Spinal and bulbar atrophy (SBMA) is a hereditary neuro-muscular 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 this 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 was 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 No. 20080016), the ethics committee of the Aichi Medical University School of Medicine (approval No. 14-004), and the ethics committee of the Nagoya University, School of Medicine (approval No. 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: (1) severe complications, such as malignancy; (2) other neurological complications; (3) taken hormonal agents within 48 weeks before informed consent; or (4) 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 this 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

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

| | SBMA ($n = 55$) | Control ($n = 483$) | $p$ value |
|---|---|---|---|
| Age (years) | $51.1 \pm 9.6$ | $53.2 \pm 8.8$ | 0.100 |
| Disease duration (years) | $7.1 \pm 3.8$ | – | – |
| CAG repeat length in AR gene | $48.1 \pm 3.2$ | – | – |
| BMI (kg/m$^2$) | **$22.7 \pm 3.6$** | **$24.2 \pm 3.0$** | **0.005** |
| Subcutaneous fat area (SFA) (cm$^2$) | $144.4 \pm 58.0$ | $137.6 \pm 55.4$ | 0.391 |
| Visceral fat area (VFA) (cm$^2$) | $122.1 \pm 61.4$ | $128.2 \pm 52.0$ | 0.414 |
| SBP (mmHg) | **$134.4 \pm 15.3$** | **$122.7 \pm 11.5$** | **<0.001** |
| DBP (mmHg) | **$84.0 \pm 9.7$** | **$78.2 \pm 7.5$** | **<0.001** |
| Glucose (mg/dL) | **$103.7 \pm 14.7$** | **$109.7 \pm 18.2$** | **0.018** |
| HbA1c (%) | **$5.6 \pm 0.4$** | **$5.8 \pm 0.6$** | **0.042** |
| IRI (mIU/L) | **$9.9 \pm 9.3$** | **$6.7 \pm 4.5$** | **<0.001** |
| HOMA-IR | **$2.6 \pm 2.7$** | **$1.8 \pm 1.3$** | **<0.001** |
| TC (mg/dL) | **$214.9 \pm 32.7$** | **$204.9 \pm 32.3$** | **0.032** |
| TG (mg/dL) | $145.3 \pm 8.4$ | $141.5 \pm 95.8$ | 0.775 |
| HDL-C (mg/dL) | $59.6 \pm 16.5$ | $56.1 \pm 12.8$ | 0.067 |
| AST (IU/L) | **$43.6 \pm 15.3$** | **$24.6 \pm 9.9$** | **<0.001** |
| ALT (IU/L) | **$51.4 \pm 25.7$** | **$28.1 \pm 18.3$** | **<0.001** |
| γGTP (IU/L) | **$38.1 \pm 36.5$** | **$52.4 \pm 49.9$** | **0.041** |
| Uric acid (mg/dL) | $6.0 \pm 1.3$ | $6.1 \pm 1.2$ | 0.743 |
| Creatinine (mg/dL) | **$0.45 \pm 0.11$** | **$0.88 \pm 0.13$** | **<0.001** |
| Adiponectin (μg/mL) | **$9.4 \pm 3.6$** | **$6.6 \pm 3.4$** | **<0.001** |

Data are shown as mean ± SD

Data with stastistical significance are shown in bold

*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

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/l$^2$) = 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 four genetically diagnosed SBMA subjects and four sex- and age-matched control subjects ($73.0 \pm 4.8$ and $71.0 \pm 4.8$ years old at the time of death, respectively). Pathologic diagnoses of the controls were dementia with Lewy bodies ($n = 2$), familial Parkinson's disease ($n = 1$), and 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, USA) containing with Halt Protease and Phosphatase Inhibitor Cocktails (Thermo Scientific, Waltham, MA, USA). Homogenates were then centrifuged at 2500 g for 20 min 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 the 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 $<0.05$ were 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 features 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 this study was similar to the 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; Fig. 1). Log HOMA-IR also had correlations with Limb Norris Score and modified QMG Score (Fig. 1).

**Table 2** Correlation between ALSFRS-R and metabolic features in SBMA patients

| ALSFRS-R | Univariate | | Multivariate | |
|---|---|---|---|---|
| | $r$ | $p$ | $\beta$ | $p$ |
| 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 × n-mol/l²) = Testosterone × LH
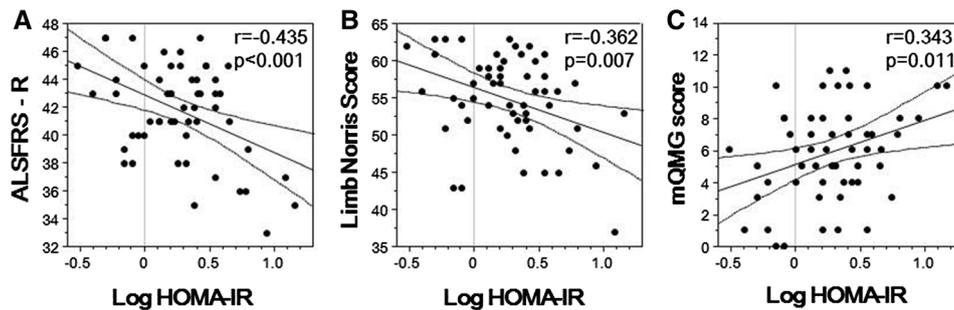
**Fig. 1** Correlation between HOMA-IR and motor function in SBMA patients. Simple correlations of log HOMA-IR with ALSFRS-R (**a**), Limb Norris score (**b**), and modified QMG score (**c**). *Solid lines*, *regression lines*; *Dotted lines*, 95% confidence interval for the mean values. *ALSFRS-R* revised amyotrophic lateral sclerosis functional rating scale, *mQMG* modified quantitative myasthenia gravis score

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 this 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

**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 $r^2 = 0.558$ | |

Data with statistical significance are shown in bold

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 $\beta$ subunit (IR$\beta$) 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 IR$\beta$ 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 this 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 this study. Normoglycemia with insulin resistance is often observed in prestage of type 2 diabetes mellitus as well as in abdominal obesity and heart failure
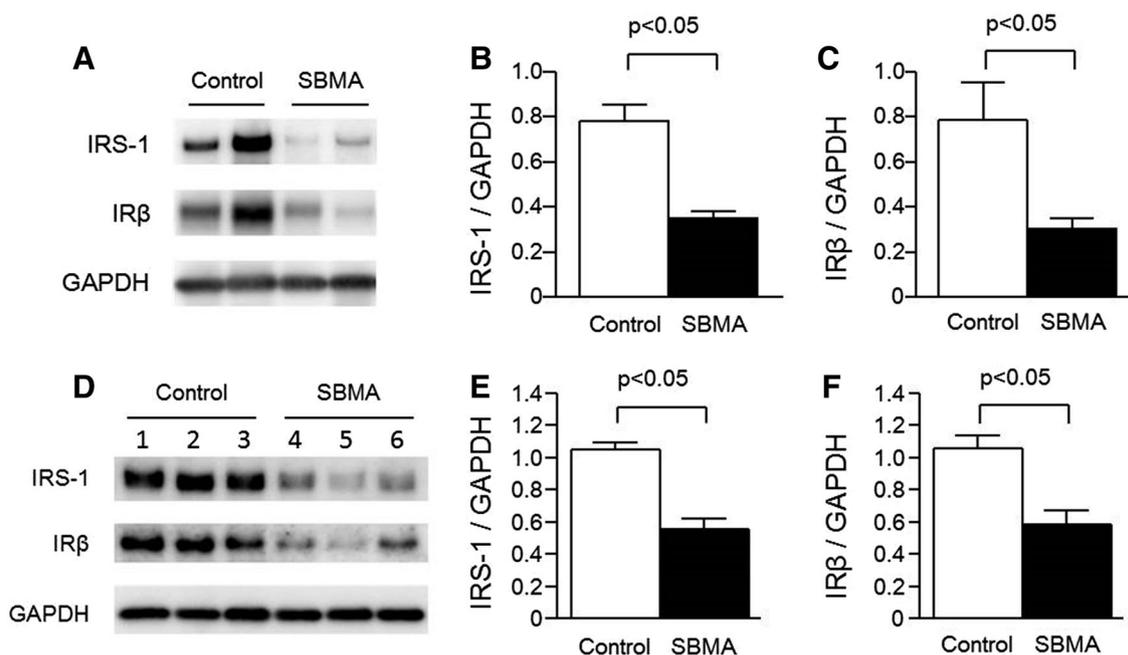


Fig. 2 Insulin receptor protein levels in the skeletal muscle and the skin fibroblasts of SBMA patients. Western blots showing the IRS-1 and IR$\beta$ protein levels in human skeletal muscle (a) and quantitation of the expression levels of the proteins ($n = 4$) (b, c). Western blots showing the IRS-1 and IR$\beta$ protein levels in human skin fibroblast cells (d) and quantitation of the expression levels ($n = 3$) (e, f). Data are shown as mean $\pm$ SD

[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. In addition, 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 this 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 downregulation 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, this 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.

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

# References

1. Katsuno M, Tanaka F, Adachi H et al (2012) Pathogenesis and therapy of spinal and bulbar muscular atrophy (SBMA). Prog Neurobiol 99:246–256
2. Giorgetti E, Lieberman AP (2016) Polyglutamine androgen receptor-mediated neuromuscular disease. Cell Mol Life Sci 73(21):3991–3999
3. La Spada AR, Wilson EM, Lubahn DB et al (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 352:77–79
4. Katsuno M, Adachi H, Kume A et al (2002) Testosterone reduction prevents phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. Neuron 35:843–854
5. Adachi H, Katsuno M, Minamiyama M et al (2005) Widespread nuclear and cytoplasmic accumulation of mutant androgen receptor in SBMA patients. Brain 128:659–670
6. Sorarù G, D'Ascenzo C, Polo A et al (2008) Spinal and bulbar muscular atrophy: skeletal muscle pathology in male patients and heterozygous females. J Neurol Sci 264:100–105
7. Dejager S, Bry-Gauillard H, Bruckert E et al (2002) A comprehensive endocrine description of Kennedy's disease revealing androgen insensitivity linked to CAG repeat length. J Clin Endocrinol Metab 87:3893–3901
8. Rhodes LE, Freeman BK, Auh S et al (2009) Clinical features of spinal and bulbar muscular atrophy. Brain 132:3242–3251
9. Hashizume A, Katsuno M, Banno H et al (2012) Longitudinal changes of outcome measures in spinal and bulbar muscular atrophy. Brain 135:2838–2848
10. Vagelatos NT, Eslick GD (2013) Type 2 diabetes as a risk factor for Alzheimer's disease: the confounders, interactions, and neuropathology associated with this relationship. Epidemiol Rev 35:152–160
11. Xu Q, Park Y, Huang X et al (2011) Diabetes and risk of Parkinson's disease. Diabetes Care 34:910–915
12. Dupuis L, Corcia P, Fergani A et al (2008) Dyslipidemia is a protective factor in amyotrophic lateral sclerosis. Neurology 70:1004–1009
13. Kioumourtzoglou MA, Rotem RS, Seals RM et al (2015) Diabetes mellitus, obesity, and diagnosis of amyotrophic lateral sclerosis: a population-based study. JAMA Neurol 72:905–911
14. Shimojo D, Onodera K, Doi-Torii Y et al (2015) Rapid, efficient, and simple motor neuron differentiation from human pluripotent stem cells. Mol Brain 8:79
15. Hijikata H, Katsuno M, Suzuki K et al (2016) Impaired muscle uptake of creatine in spinal and bulbar muscular atrophy. Ann Clin Transl Neurol 3:537–546
16. Nakatsuji H, Kishida K, Kitamura T et al (2010) Dysregulation of glucose, insulin, triglyceride, blood pressure, and oxidative stress after an oral glucose tolerance test in men with abdominal obesity. Metabolism 59:520–526
17. Matthews DR, Hosker JP, Rudenski AS et al (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
18. Querin G, Bertolin C, Da Re E et al (2016) Non-neural phenotype of spinal and bulbar muscular atrophy: results from a large cohort of Italian patients. J Neurol Neurosurg Psychiatry 87:810–816
19. Iida M, Katsuno M, Nakatsuji H et al (2015) Pioglitazone suppresses neuronal and muscular degeneration caused by polyglutamine-expanded androgen receptors. Hum Mol Genet 24:314–329
20. Yu IC, Lin HY, Sparks JD et al (2014) Androgen receptor roles in insulin resistance and obesity in males: the linkage of androgen-deprivation therapy to metabolic syndrome. Diabetes 63:3180–3188
21. Maeda N, Shimomura I, Kishida K et al (2002) Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 8:731–737
22. Cortes CJ, Ling SC, Guo LT et al (2014) Muscle expression of mutant androgen receptor accounts for systemic and motor neuron disease phenotypes in spinal and bulbar muscular atrophy. Neuron 82:295–307
23. Palazzolo I, Stack C, Kong L et al (2009) Overexpression of IGF-1 in muscle attenuates disease in a mouse model of spinal and bulbar muscular atrophy. Neuron 63:316–328
24. Srikanthan P, Hevener AL, Karlamangla AS (2010) Sarcopenia exacerbates obesity-associated insulin resistance and dysglycemia: findings from the National Health and Nutrition Examination Survey III. PLoS One 5:e10805
25. Wijngaarden MA, van der Zon GC, van Dijk KW et al (2013) Effects of prolonged fasting on AMPK signaling, gene expression, and mitochondrial respiratory chain content in skeletal muscle from lean and obese individuals. Am J Physiol Endocrinol Metab 304:E1012–E1021
26. Swan JW, Anker SD, Walton C et al (1997) Insulin resistance in chronic heart failure: relation to severity and etiology of heart failure. J Am Coll Cardiol 30:527–532
27. Ouchi N, Parker JL, Lugus JJ et al (2011) Adipokines in inflammation and metabolic disease. Nat Rev Immunol 11:85–97
28. Nishizawa H, Shimomura I, Kishida K et al (2002) Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. Diabetes 51:2734–2741
29. Craft S, Watson GS (2004) Insulin and neurodegenerative disease: shared and specific mechanisms. Lancet Neurol 3:169–178
30. Santiago JA, Potashkin JA (2013) Shared dysregulated pathways lead to Parkinson's disease and diabetes. Trends Mol Med 19:176–186
31. Lalić NM, Marić J, Svetel M et al (2008) Glucose homeostasis in Huntington disease: abnormalities in insulin sensitivity and early-phase insulin secretion. Arch Neurol 65:476–480
32. Lalić NM, Dragasević N, Stefanova E et al (2010) Impaired insulin sensitivity and secretion in normoglycemic patients with spinocerebellar ataxia type 1. Mov Disord 25:1976–1980
33. Yu Z, Wang AM, Adachi H et al (2011) Macroautophagy is regulated by the UPR-mediator CHOP and accentuates the phenotype of SBMA mice. PLoS Genet 7:e1002321

34. Navarro G, Allard C, Xu W et al (2015) The role of androgens in metabolism, obesity, and diabetes in males and females. Obesity (Silver Spring) 23:713–719

35. Chang PY, Benecke H, Le Marchand-Brustel Y et al (1994) Expression of a dominant-negative mutant human insulin receptor in the muscle of transgenic mice. J Biol Chem 269:16034–16040

36. Hilder TL, Tou JC, Grindeland RE et al (2003) Phosphorylation of insulin receptor substrate-1 serine 307 correlates with JNK activity in atrophic skeletal muscle. FEBS Lett 553:63–67

37. Minamiyama M, Katsuno M, Adachi H et al (2012) Naratriptan mitigates CGRP1-associated motor neuron degeneration caused by an expanded polyglutamine repeat tract. Nat Med 18:1531–1538

38. Lee CG, Boyko EJ, Strotmeyer ES et al (2011) Association between insulin resistance and lean mass loss and fat mass gain in older men without diabetes mellitus. J Am Geriatr Soc 59:1217–1224

39. Kalonia H, Kumar P, Kumar A (2010) Pioglitazone ameliorates behavioral, biochemical and cellular alterations in quinolinic acid induced neurotoxicity: possible role of peroxisome proliferator activated receptor-Upsilon (PPARUpsilon) in Huntington's disease. Pharmacol Biochem Behav 96:115–124

40. Dehmer T, Heneka MT, Sastre M et al (2004) Protection by pioglitazone in the MPTP model of Parkinson's disease correlates with I kappa B alpha induction and block of NF kappa B and iNOS activation. J Neurochem 88:494–501

41. Kiaei M, Kipiani K, Chen J et al (2005) Peroxisome proliferator-activated receptor-gamma agonist extends survival in transgenic mouse model of amyotrophic lateral sclerosis. Exp Neurol 191:331–336