



# The metabolic and endocrine characteristics in spinal and bulbar muscular atrophy

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## Abstract

**Objective** Spinal and bulbar muscular atrophy (SBMA) is caused by an abnormal expansion of the CAG repeat in the androgen receptor gene. This study aimed to systematically phenotype a German SBMA cohort ( $n = 80$ ) based on laboratory markers for neuromuscular, metabolic, and endocrine status, and thus provide a basis for the selection of biomarkers for future therapeutic trials.

**Methods** We assessed a panel of 28 laboratory parameters. The clinical course and blood biomarkers were correlated with disease duration and CAG repeat length. A subset of 11 patients was evaluated with body fat MRI.

**Results** Almost all patients reported muscle weakness (99%), followed by dysphagia (77%), tremor (76%), and gynecomastia (75%) as major complaints. Creatine kinase was the most consistently elevated (94%) serum marker, which, however, did not relate with either the disease duration or the CAG repeat length. Paresis duration and CAG repeat length correlated with dehydroepiandrosterone sulfate after correction for body mass index and age. The androgen insensitivity index was elevated in nearly half of the participants (48%).

**Conclusions** Metabolic alterations in glucose homeostasis (diabetes) and fat metabolism (combined hyperlipidemia), and sex hormone abnormalities (androgen insensitivity) could be observed among SBMA patients without association with the neuromuscular phenotype. Dehydroepiandrosterone sulfate was the only biomarker that correlated strongly with both weakness duration and the CAG repeat length after adjusting for age and BMI, indicating its potential as a biomarker for both disease severity and duration and, therefore, its possible use as a reliable outcome measure in future therapeutic studies.

**Keywords** Kennedy's disease · Spinobulbar muscular atrophy · Motor neuron disease · Diabetes · Biomarker · MRI

## Introduction

Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is an adult-onset lower motor neuron disorder with an X-linked inheritance pattern. SBMA is characterized by late-onset, slowly progressive weakness, atrophy and fasciculations of the bulbar and limb skeletal musculature. SBMA is caused by the abnormal expansion of an unstable CAG repeat in the coding region of the androgen receptor gene (AR) [1]; more than  $> 38$  repeats produce the fully penetrant disease phenotype [2]. Clinical features,

especially muscle strength, and testosterone levels show an association with disease severity [3]. Insulin resistance has also been postulated to serve as potential therapeutic target because of its association with disease severity [4]. Establishing the causality of the SBMA mutation and its molecular pathophysiology helped to identify a range of potential therapeutic targets (for reviews, see [5–7]). Encouraging results from animal trials based on androgen reduction led to independent clinical trials focused on hormonal interventions in SBMA [8, 9]. Katsuno and colleagues tested the therapeutic potential of leuprorelin, a gonadotropin-lowering drug that reduces testicular testosterone production [9]. Fernandes–Rhodes investigated the effect of the anti-androgen dutasteride [8]. While both trials failed to demonstrate a clear therapeutic benefit for androgen-lowering therapies, an

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urgent need for objective outcome measures became apparent [10].

The CAG repeat encodes a polyglutamine tract in the androgen receptor, and its size correlates with age of onset but not disease progression rate [11–15]. The effects of the mutation fall into two categories: loss of (androgen receptor) function causes androgen insensitivity with gynecomastia and decreased fertility, while (androgen-dependent) toxic gain of function is thought to causally underlie motor neuron degeneration [16]. Metabolic abnormalities are frequently reported, albeit without any current consensus on whether they rely on gain or loss of androgen receptor function. Hormonal and biochemical studies have reported creatine kinase (CK) elevation, dyslipidemia, glucose intolerance, and partial androgen insensitivity with increased testosterone and luteinizing hormone (LH) [17–22]. Partial androgen resistance is present in more than 80% of patients [15]. Hypertriglyceridemia may be related to mitochondrial disturbances [23] and androgen receptor insensitivity. CK elevation is considered to be due to myopathic disease secondary to motor neuron degeneration [21, 24, 25]. The present study aimed to characterize a large SBMA cohort with regard to laboratory markers for neuromuscular disease, metabolic and hormonal status, thereby providing a rational basis for the selection of potential biomarkers in future therapeutic trials.

## Methods

Inclusion criteria were genetically confirmed SBMA and consent to the study; exclusion criteria were inability to provide detailed information on personal history, symptoms, and co-medication. Patients opting to participate in the imaging arm of the study had to be able to undergo MRI studies. Eighty genetically confirmed SBMA patients were recruited through the German neuromuscular referral centers in the Universities of Berlin, Bochum, Dresden, Halle, Hannover, Rostock, Ulm, and Würzburg. Blood samples and information about their disease history and characteristics, comorbidities, medication, neurological, metabolic, and endocrine symptoms were obtained in structured personal interviews. The patients completed a standardized questionnaire addressing the presentation of specific aspects: muscle weakness (onset), muscle cramps, body mass index (BMI), myalgia, family history, tremor, sensory deficits, comorbidities, dysphagia, dysarthria, gynecomastia, and respiratory symptoms. Laboratory parameters were divided into hormones and lipid and glucose metabolism markers. Parameters were considered “elevated” if they fell outside the established reference range. Disease onset was defined age at the first (any) symptom. Fasting blood samples were collected under standardized conditions between 8 and 10

AM and analyzed for glucose, HbA1c, insulin, C peptide, proinsulin, aspartate transaminase (AST), alanine transaminase (ALT), CK, cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, 17-OH progesterone, androstendione, cortisol, dehydroepiandrosterone (DHEA), DHEA sulfate, follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, estradiol, progesterone, prolactin, SHBG, plasminogen activator inhibitor (PAI), growth hormone, insulin like growth factor 1 (IGF-1), and insulin like growth factor 1 binding protein 3 (IGF1BP3).

Ethical approval of the Ulm University Hospital ethics board was obtained for all participants (protocol no. 73/10 and 92/10). Written informed consent was obtained from all participants.

A subgroup of 11 patients ( $58 \pm 12$  years, range 40–79 years) from the Ulm center received a total body fat MRI. For comparison, total body fat MRI data from a group of 29 male healthy controls ( $65 \pm 12$  years, range 53–81 years) were selected from our database.

Genetic testing was performed by PCR-based fragment analysis according to the standard procedures [1, 26]. CAG repeat length  $> 38$  was considered diagnostic for SBMA.

## MRI acquisitions

Adipose tissue volumes were quantified by MRI performed on a 1.5-T whole-body imager (Symphony, Siemens Medical, Erlangen, Germany), as described previously [27–29]. Briefly, measures of visceral (VFT) and subcutaneous (SFT) abdominal fat tissue were determined as the sum of VFT voxels from the top of the liver to the femoral heads inside the abdominal muscular wall, and as the sum of fat tissue voxels underneath the skin layer surrounding the abdomen from the top of the liver to the femur heads. VFT, SFT, and total fat tissue (TFT = VFT + SFT) volumes were expressed in  $\text{dm}^3$  and normalized to the body height.

## Statistics

Statistical analyses were performed using GraphPad Prism 6 for Windows (GraphPad Software, Inc., La Jolla, CA, USA) and SAS, version 9.4 (SAS Institute, Chicago, IL, USA). Relations between CAG repeat length and age-at-onset of weakness as well as age-at-onset of any first symptom were analyzed using linear regression and Pearson’s correlation coefficient “*r*” and associated *p* values were calculated. The positively skewed homeostasis model assessment of insulin resistance (HOMA-IR) was log-transformed for analysis. A multiple linear regression analysis was conducted to adjust for age and BMI. Statistical significance was set at  $p < 0.05$ . Results from all tests were considered exploratory, in keeping with the study design, and therefore, no adjustment for multiple testing was done [30].

## Results

### Cohort characteristics

The reported mean age-at-onset (any symptom) was  $39.1 \pm 15.4$  years (10.0–75.0 years). The mean disease duration at inclusion was  $17.0 \pm 11.6$  years (range 2.0–47.0 years, age at inclusion  $55.9 \pm 10.5$  years, range 25.0–80.0 years). The mean age-at-weakness-onset was  $44.4 \pm 12.0$  years (25.0–75.0) and the mean length of the CAG expansion in AR was  $46.2 \pm 3.3$  (range 39.0–55.0). The most common symptoms reported were muscle weakness (99%), followed by dysphagia (77%), tremor (76%), gynecomastia (75%), muscle cramps (71%), dysarthria (64%), sensory deficits/tingling (64%), myalgia (57%), and respiratory symptoms (34%). The mean BMI was  $26.1 \pm 4.4$  kg/m<sup>2</sup> (17.5–40.2). Dot plots of samples in relation with reference range for all biomarkers are summarized in Fig. 1.

CK was the laboratory value most consistently elevated above the reference range (mean  $1053 \pm 900$  U/l, range 124–6140 U/l) and was elevated in 94% of participants. However, no correlations with CAG repeat length, disease duration, or onset of weakness were noted.

### Relation with CAG repeat length and disease duration

#### Biomarker correlation with CAG repeat length

Given that polyglutamine diseases and SBMA in particular show dependence on CAG repeat length, we probed the correlation between repeat expansion length and laboratory parameters. The age-at-weakness-onset was inversely correlated with the CAG repeat expansion length ( $r = -0.72$ ;  $p < 0.0001$ ) (Fig. 2a). The age-of-gynecomastia-onset (mean  $30.0 \pm 15.4$  years, range 12.0–70.0) and the age-at-first-disease-symptom were also related to the CAG repeat length ( $r = -0.59$ ;  $p < 0.0001$  in Fig. 2b;  $r = -0.66$ ;  $p < 0.0001$  in Fig. 2c). No such correlation was observed for BMI ( $r = -0.18$ ;  $p = 0.1204$ ).

With regard to hormones, CAG repeat length correlated with androstendion ( $r = 0.27$ ;  $p = 0.017$ ), DHEA ( $r = 0.36$ ;  $p = 0.005$ ), DHEA sulfate ( $r = 0.49$ ;  $p < 0.0001$ ), and estradiol ( $r = 0.27$ ;  $p = 0.017$ ). HbA1c was inversely correlated with CAG repeat length but not with paresis or disease duration. IGF1BP3 also significantly correlated with CAG repeat length ( $r = 0.38$ ;  $p = 0.0004$ ). Figure 3 details the results for selected correlations.

After correction for age and BMI in a multiple linear regression model, only DHEA sulfate significantly

correlated with CAG repeat length ( $r = 0.59$ ;  $p = 0.0022$ ); no significant correlations were observed for androstendion, DHEA, and estradiol. However, IGF1BP3 tended towards a significant correlation with CAG repeat length ( $r = 0.55$ ;  $p = 0.066$ ).

#### Correlation of biomarkers with disease duration

Significant correlations for all laboratory markers were tested with disease duration. A multiple linear regression model was used to rule out correlations that might manifest as a result of ageing, BMI or metabolic syndromes; subsequently, none of the tested laboratory values significantly correlated with disease duration (age-at-onset of any first symptom).

With respect to the duration from paresis onset, the following biomarkers were related significantly to duration of weakness: IGF1BP3 ( $r = 0.24$ ;  $p = 0.03$ ), FSH ( $r = 0.29$ ;  $p = 0.01$ ) and DHEA sulfate ( $r = 0.26$ ;  $p = 0.02$ ). In the multiple linear regression model, only DHEA sulfate ( $r = 0.3$ ;  $p = 0.0478$ ) related with duration after weakness-onset.

#### Endocrine parameters

We screened for a panel of endocrinological parameters that may be altered by SBMA-associated androgen insensitivity to test their potential as disease markers.

Gynecomastia was observed in 75% of participants. SHBG and androstendione were elevated in 48 and 38% of patients, respectively, while fasting morning cortisol level was elevated in 24%. The Androgen Sensitivity Index (ASI) (which reflects androgen resistance) was increased in 48% of patients (detailed in Table 1).

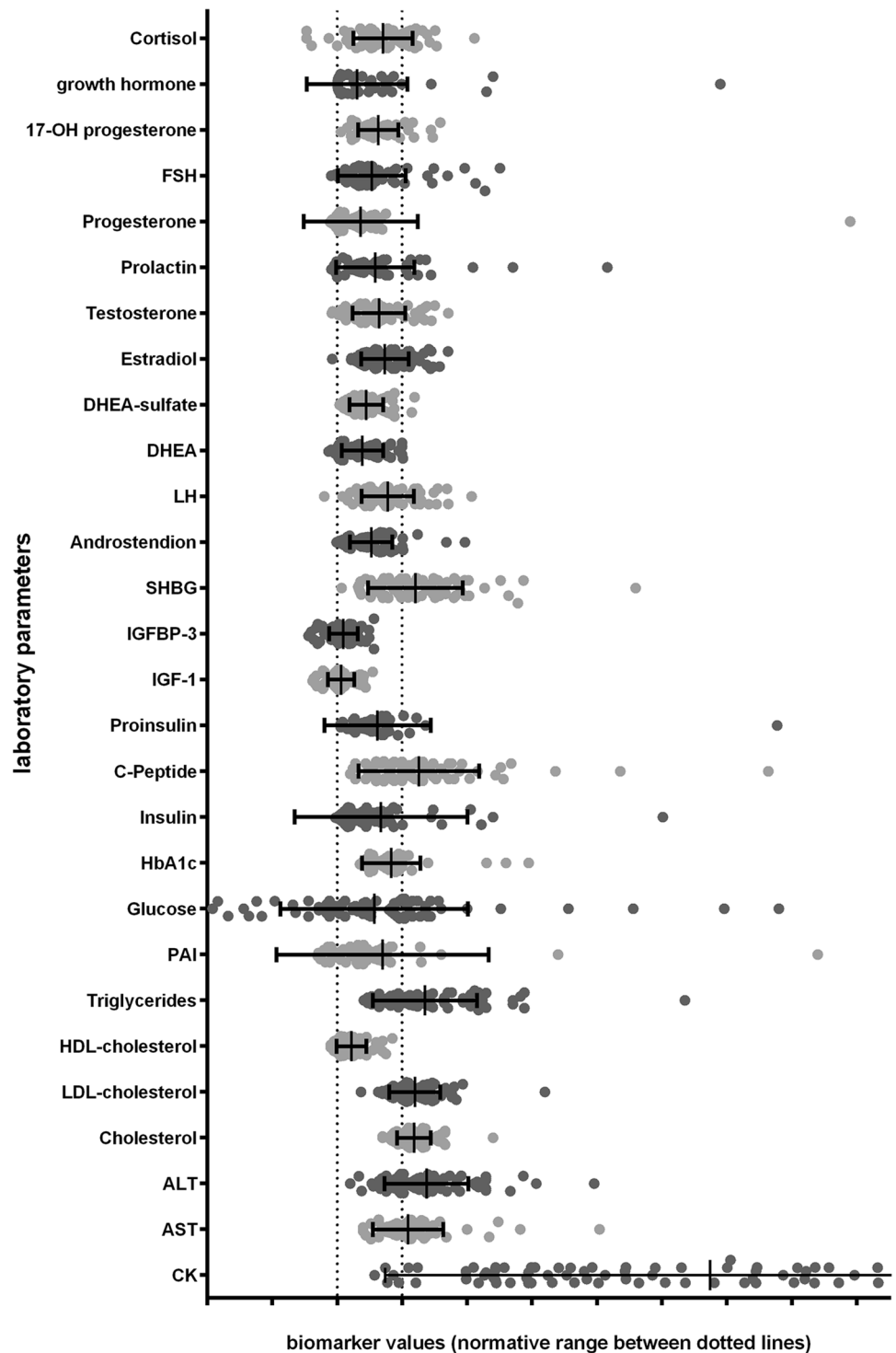
DHEA sulfate levels correlated significantly with duration of paresis in the multiple linear regression model with adjustment for age and BMI.

#### Lipid and glucose metabolism

Assessed lipid metabolism parameters revealed hypercholesterolemia, increased triglycerides, and increased LDL-cholesterol levels in 78, 56, and 66% respectively. Details are shown in Table 2.

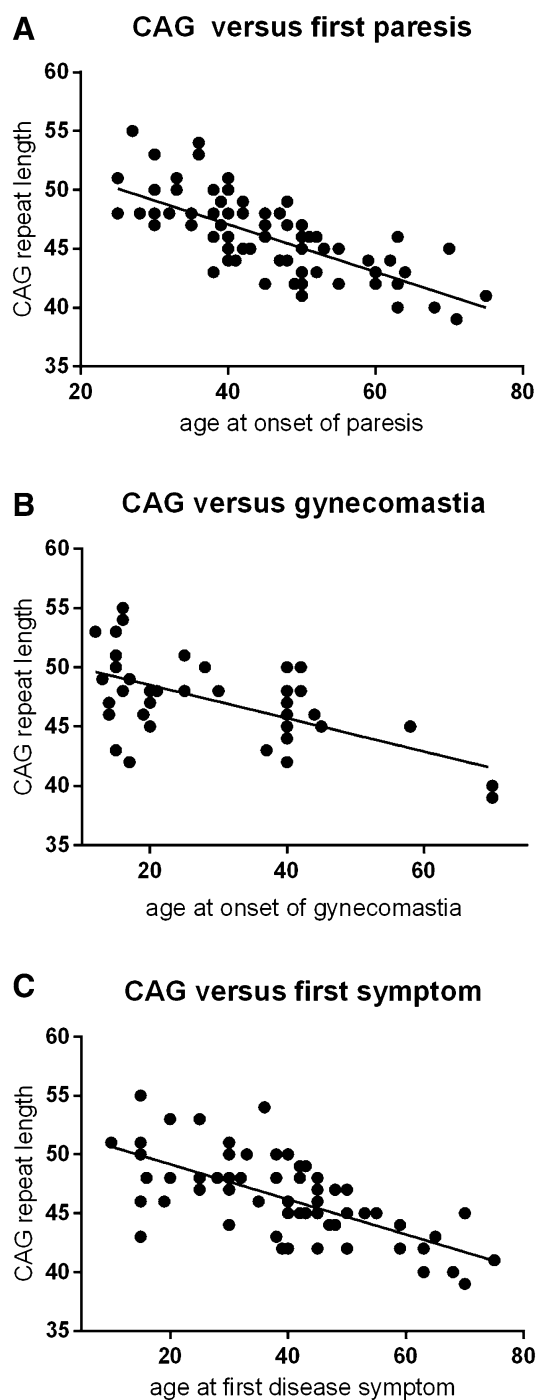
An analysis of glucose metabolism-related parameters revealed increased C peptide, indicating the production of high levels of endogenous insulin with concomitant insulin resistance. Of the 42 patients with increased C peptide, only 4 showed increased HbA1c. A history of diabetes mellitus was reported in 9/80 patients (11.3%). Of these diabetic, six had elevated C peptide, four had increased insulin levels, and five patients had increased HbA1c. Eight of these nine diabetic patients took oral antidiabetics. With regard to the entire patient cohort, C peptide

**Fig. 1** Dot plots with mean and standard deviation of samples in relation with reference range for all biomarkers. Reference range for each laboratory parameter was plotted between dotted lines



was increased in 53%, insulin levels in 11% and glucose in 29% of patients. A similar percentage of patients also had reduced fasting glucose levels (28%). HbA1c was increased without a history of diabetes in three patients. No correlation between HbA1c and weakness duration was observed; however, HbA1c was inversely correlated with CAG repeat length, even after correcting for age

and BMI (Table 3). We calculated the insulin resistance index HOMA-IR, which was elevated in 35 patients of our cohort (44%). Log HOMA-IR did not correlate with disease duration and duration of weakness or CAG repeat length ( $r = -0.19$ ,  $p = 0.09$ ). Log HOMA-IR was significantly related to other laboratory markers, i.e., triglycerides ( $r = 0.44$ ,  $p < 0.01$ ), HDL ( $r = -0.45$ ,  $p < 0.01$ ),



**Fig. 2** Correlations of CAG repeat expansion length with age-at-weakness-onset (a), age-at-gynecomastia-onset (b), and age-at-first-disease-symptom (c)

growth factor ( $r = 0.23$ ,  $p = 0.04$ ), IGF1 ( $r = 0.25$ ,  $p = 0.02$ ), estradiol ( $r = -0.36$ ,  $p < 0.01$ ), testosterone ( $r = -0.44$ ,  $p < 0.01$ ), SHBG ( $r = -0.39$ ,  $p < 0.01$ ), C-peptide ( $r = 0.82$ ,  $p < 0.01$ ), insulin ( $r = 0.70$ ,  $p < 0.01$ ), proinsulin ( $r = 0.58$ ,  $p < 0.01$ ), and HbA1c ( $r = 0.27$ ,  $p = 0.03$ ). Log HOMA-IR also correlated with

BMI ( $r = 0.48$ ,  $p < 0.01$ ). After correction for age and BMI in a multiple linear regression model, all laboratory markers with exception of growth factor still significantly correlated with HOMA-IR (Table 3).

### Skeletal muscle

Although CK was elevated in 94% of the patients, it did not correlate with any of the aforementioned markers. AST and ALT were concomitantly elevated in 47% (mean  $54.59 \pm 27.09$  U/l, range 20.00–202.00) and 68% (mean  $61.82 \pm 29.13$  U/l, range 9.00–178.00) of cases, respectively. None of the patients displayed any elevation in either IGF1 or IGF1BP-3 values; values below normal are detailed in Table 2. IGF1BP3 significantly correlated with duration of weakness, but this correlation did not reach statistical significance after correcting for age and BMI ( $p = 0.16$ ).

### Body fat Imaging

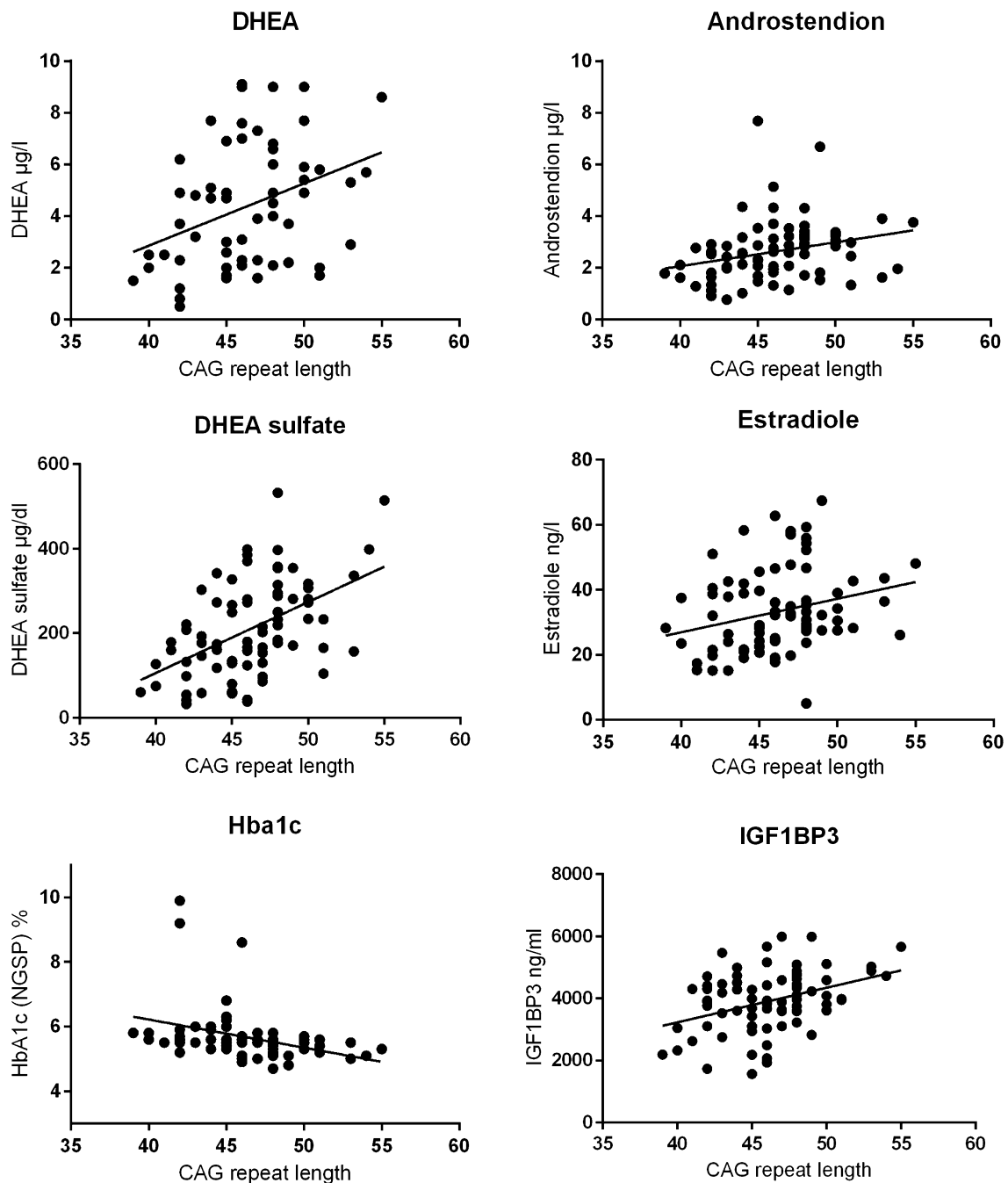
#### Between-group comparisons of body fat composition

This cross-sectional MRI study included 11 SBMA patients and 29 appropriately matched controls. Body fat distribution was evaluated at the group level (Fig. 4); no significant differences were observed for either TFT ( $18.7 \pm 7.9$  l/m for SBMA vs.  $18.3 \pm 6.4$  l/m for controls) or SFT ( $12.9 \pm 6.0$  l/m for SBMA vs.  $15.2 \pm 5.9$  l/m for controls). However, patients had significantly higher VFT ( $5.8 \pm 2.6$  l/m for SBMA vs.  $3.1 \pm 1.7$  l/m for controls,  $p < 0.007$ ) (Fig. 4); SBMA patients had a significantly higher VFT/SFT ratio ( $0.51 \pm 0.23$  l/m for SBMA vs.  $0.22 \pm 0.1$  l/m for controls,  $p < 0.001$ ).

CAG repeat length positively correlated with the amount of subcutaneous fat. The proportion of subcutaneous fat relative to both visceral and whole-body fat increased with disease duration and progression rate. Of the assessed serum markers, body fat was associated with increased triglycerides, insulin, C peptide, HbA1c, plasminogen activator inhibitor (PAI), and low HDL-cholesterol. With respect to glucose metabolism markers (insulin, C peptide, HbA1c, PAI), the proportion of visceral fat increases in the entirely enlarged fat mass (Fig. 4).

### Discussion

The recent negative results reported by SBMA clinical trials have highlighted the urgent need for rationally selected and objective outcome measures [10]. Given the multifaceted nature of the disease and clinical presentation that encompasses neuromuscular, metabolic, and endocrinological



**Fig. 3** Statistically significant CAG repeat length correlations with laboratory parameters

abnormalities, we attempted to fully characterize a large German cohort of genetically confirmed SBMA patients.

CK, a well-established indicator of muscle integrity, was by far the most consistently elevated disease marker, potentially indicating skeletal muscle involvement. Although not novel, this finding reaffirms and underscores skeletal muscle's centrality as a site of SBMA disease pathology, thereby also emphasizing its significance as a potential therapeutic target [31–33]. Previous biopsy studies have reported

a combination of denervation and myopathy [34, 35]. The elevation of CK also corroborated the self-reported clinical status of the patients, for whom weakness, cramping, and myalgia were the most common complaints. Surprisingly, no correlation of CK with either CAG repeat length or weakness duration was observed.

We must also point out the possibility of recollection bias within the clinical data reported, as there were collected via patient interviews. Another limitation of our study is

**Table 1** Hormonal profile of patients with SBMA

Hormone	Outliers in %	Reference range	Mean + SD	Range
17-OH-progesteron µg/l	10.8 ↑	0.05–1.60 µg/l	1.03 ± 0.48	0.14–2.51
Androstendion µg/l	37.5 ↑	1.30–4.30 µg/l > 20 years 1.30–3.80 µg/l > 40 years 1.20–3.20 µg/l > 50 years 1.10–2.70 µg/l > 60 years 0.80–2.90 µg/l > 70 years	2.63 ± 1.14	0.77–7.69
Cortisol µg/dl	23.1 ↑	6.2–19.4 µg/dl	15.9 ± 5.5	1.0–34.1
DHEA µg/l	5 ↓	1.5–9.0 µg/l	4.40 ± 2.39	0.50–9.10
DHEA_sulfate µg/dl	21.8 ↑	160.0–449.0 µg/dl > 24 years 88.9–427 µg/dl > 34 years 44.3–331 µg/dl > 44 years 51.7–295 µg/dl > 54 years 33.6–249 µg/dl > 64 years 16.2–123 µg/dl > 74 years	209.6 ± 112.7	32.4–532.3
FSH IU/l	12.5 ↑	1.50–12.40 IU/l	7.3 ± 5.7	0.5–28.8
Growth hormone µg/l	17.5 ↑	< 5 µg/l	1.54 ± 3.87	0.05–29.50
LH IU/l	22.5 ↑	1.70–8.60 IU/l	7.07 ± 2.78	0.32–15.98
Estradiol ng/l	21.3 ↑	7.6–42.6 ng/l	33.28 ± 12.83	5.00–67.40
Progesterone µg/l	12.5 ↓	0.20–1.40 µg/l	0.63 ± 1.05	0.07–9.68
Prolactin µg/l	13.9 ↑	4.0–15.2 µg/l	10.55 ± 6.72	3.00–50.60
SHBG nmol/l	49.4 ↑	14.50–48.40 nmol/l	55.24 ± 24.71	16.78–170.30
Testosterone µg/l	20.3 ↑	2.490–8.360 µg/l < 50 years 1.930–7.400 µg/l > 50 years	6.06 ± 2.60	1.41–12.94
Androgen sensitivity index (ASI) (LH in IU/l × Testosterone in nmol/l)	48.1 ↑	< 138	147.7 ± 94.3	12.5–501.6

↑ = above normal range; ↓ = below normal range

**Table 2** Lipid and glucose metabolism and muscle-associated parameters in SBMA

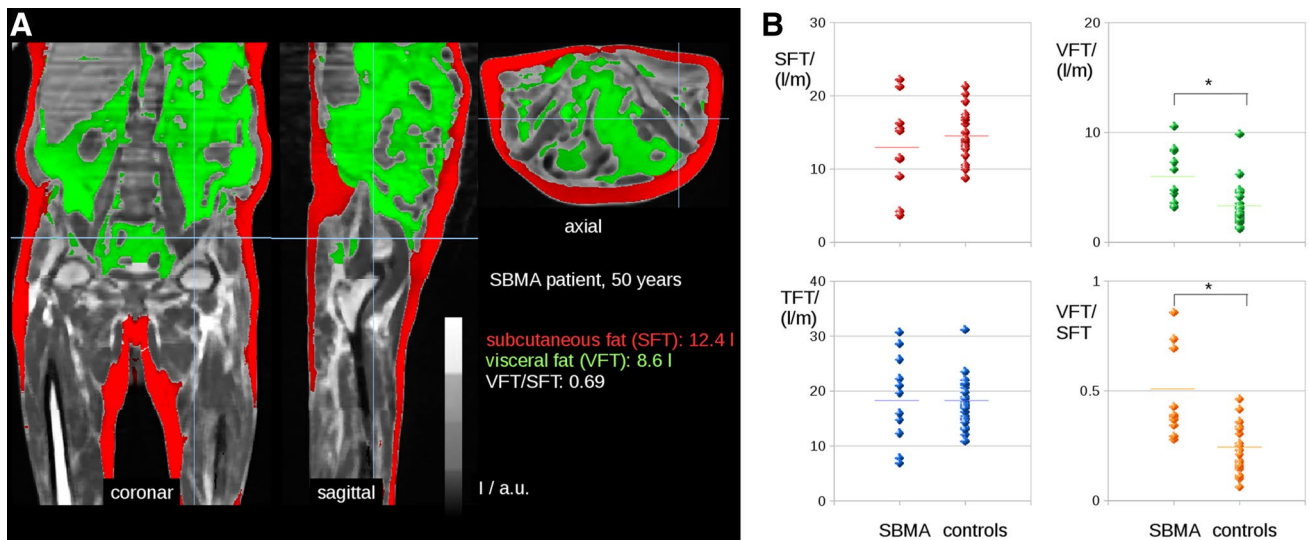
Parameter	Outliers in %	Reference range	Mean ± SD	Range
Cholesterine mmol/l	78 ↑	< 5.0 mmol/l	5.9 ± 1.3 mmol/l	3.5–12.0
Triglycerides mmol/l	56 ↑	< 1.7 mmol/l	2.3 ± 1.4 mmol/l	0.7–9.1
HDL-cholesterine mmol/l	15 ↓	> 1.0 mmol/l	1.4 ± 0.5 mmol/l	0.8–2.7
LDL cholesterine mmol/l	66 ↑	< 3.0 mmol/l	3.6 ± 1.2 mmol/l	1.1–9.6
Fasting glucose mg/dl	28 ↑, 29 ↓	74–99 mg/dl	89 ± 35 mg/dl	26–244
C-Peptide µg/l	52.5 ↑	0.5–3.0 µg/l	3.6 ± 2.3 µg/l	1.0–17.1
Insulin mU/l	11.25 ↑	2.6–24.9 mU/l	17.6 ± 29.6 mU/l	2.0–241.2
Proinsulin pmol/l	7.7 ↑	0.0–9.4 pmol/l	5.8 ± 7.7 pmol/l	0.5–63.7
Plasminogene activator inhibitor (PAI) U/l	25 ↓	2.00–7.00 U/l	5.5 ± 8.2	0.5–53.0
HbA1c % (NGSP)	9.5 ↑	4.0–6.0%	5.7 ± 0.9%	4.7–9.9
HOMA-IR (fasting immune-reactive insulin in mU/l × fasting glucose in mg/dl)/405	44 ↑	< 2.5	4.7 ± 9.8	0.4–67.9
Creatine kinase	94 ↑	20–200 U/l	1053 ± 900	124–6140
AST	47 ↑	< 50 U/l	55 ± 27	20–202
ALT	68 ↑	< 45 U/l	62 ± 29	9–178
IGF1	15 ↓	116–341 ng/ml	129.3 ± 46.1 ng/ml	31.0–239.0
IGF1BP3	20 ↓	3500–7900 ng/ml	3915.0 ± 959.3 ng/ml	1560.0–5990.0

↑ = above normal range; ↓ = below normal range

**Table 3** Results of multiple linear regression analysis, where significant associations of CAG repeat length, paresis duration, or log HOMA-IR are shown with the selected variables in the left column together with age and BMI

	CAG repeat length	Age at bloodtest	BMI
Androstendion		- 4.26; $p < 0.001$	
DHEA		- 2.78; $p = 0.006$	- 1.98; $p = 0.053$
DHEA sulfate	3.18; $p = 0.002$	- 3.20; $p = .0002$	
Estradiole		- 4.13; $p < 0.001$	
Hba1c	- 2.01; $p = 0.049$	- 3.51; $p < 0.001$	
	Paresis duration	Age at bloodtest	BMI
DHEA sulfate	2.01; $p = 0.048$		
IGF1BP3			
FSH			
	Log HOMA-IR	Age at bloodtest	BMI
Triglycerides	4.55; $p < 0.001$		4.66; $p < 0.001$
HDL	- 2.78; $p = 0.007$		3.56; $p < 0.001$
IGF1	2.07; $p = 0.042$		4.62; $p < 0.001$
Testosterone	- 3.27; $p = 0.002$		2.88; $p = 0.005$
SHBG	- 2.26; $p = 0.027$		3.61; $p < 0.001$
C peptide	10.64; $p < 0.001$		2.42; $p = 0.018$
Proinsulin	4.94; $p < 0.001$		3.66; $p < 0.001$
IGF1BP3			4.70; $p < 0.001$

Each cell contains the test statistics with the corresponding  $p$  value and was adjusted for the other two variables. Only associations are shown that were found to be significant ( $p < 0.05$ ) in the multiple regression analysis



**Fig. 4 a** Individual example of an MRI subset after post-processing. Sagittal, coronal, and axial slices. Visceral fat tissue volume (VFT): green; subcutaneous fat tissue volume (SFT): red. **b** Comparison of fat volumes between 11 SBMA patients and 29 matched controls. No

significant between-group differences were observed for subcutaneous and total fat tissue volume but were observed for visceral fat tissue volume and the quotient between SFT and VFT volume

the absence of an age-matched control group with either healthy subjects or patients with other muscular atrophies, as some of the laboratory values may be influenced by age and

muscle mass. No longitudinal observation of the mentioned biomarkers is so far available which may be important for the validation of the correlations.



On one hand, we hypothesize that the mutated androgen receptor may correlate with the disease and/or paresis duration with a concomitant longitudinal worsening of symptoms. Indeed, DHEA sulfate, FSH, and IGF1BP3 positively correlated with the duration of muscle weakness, albeit with only the DHEA sulfate correlation reaching significance after correction for age and BMI. DHEA sulfate is an indicator of adrenal androgen production and its upregulation in our patients likely reflects an increased androgen insensitivity. The clinical signs of androgen insensitivity include gynecomastia, testicular atrophy, in addition to decreased fertility. Abdominal obesity is common [36]. Therefore, DHEA sulfate might reflect androgen insensitivity, which might become more pronounced during the progression of the disease. Conversely, no correlation of disease duration with the ASI (product of LH and testosterone) was observed.

This phenomenon has also been reported by previous studies, where ASI was elevated in 36% [17] of the cohort with testosterone, LH and FSH also elevated (10, 27, and 32%, respectively), but no correlations between testosterone/ASI values and CAG repeat length or age-at-onset were observed. A recent Chinese study with 155 patients reported similar laboratory values for testosterone but much higher estradiol levels [36] relative to our cohort. On the other hand, a correlation of disease symptoms or biomarkers may also correlate with CAG repeat length. Androstenedion, DHEA, DHEA sulfate, estradiol, and IGF1BP3 were the only parameters positively correlating with CAG repeat length, but as before, only DHEA sulfate reached significance after correction for age and BMI. No correlation with CAG repeat length for either the ASI or testosterone values was observed.

Most notably, lipid markers (cholesterol and LDL cholesterol) were elevated in 78 and 66%, respectively, indicating dysregulated lipid metabolism in SBMA. Ni and colleagues reported similarly elevated triglycerides (66%), although elevated cholesterol was noted in only 22% of their Chinese cohort [22]. These discrepancies may be attributed to a variety of factors ranging from nutritional influences to ethnic background. Body fat MRI, which has not been reported elsewhere for SBMA patients, revealed higher visceral fat volumes compared to controls and a higher proportion of visceral fat compared to subcutaneous fat. This may reflect an effect of hyperlipidemia; curiously, this has also been reported in patients with amyotrophic lateral sclerosis, the most frequently occurring motor neuron disease [37].

The high percentage of C-peptide elevation points to a higher proportion of patients with insulin resistance, albeit without any manifestation of diabetes. HbA1c values and diabetes did not correlate with either symptom or weakness duration, but were inversely correlated with CAG repeat length. Since manifest diabetic patients were also included, reduced HbA1c with higher CAG repeats may reflect

adequately managed diabetes treatment. Hypercholesterinemia and insulin resistance are core features of the metabolic syndrome, but in SBMA, these changes do not appear to be a function of disease duration. Log HOMA-IR is described to correlate with BMI, obesity-related indices, HDL, and testosterone [4]. We could reproduce these laboratory findings and expand to other insulin resistance associated biomarkers, namely, triglycerides, IGF1,

SHBG and C peptide as well as proinsulin. Any association of log HOMA-IR to CAG repeat length or disease/weakness duration was missing. The binding protein 3 of insulin like growth factor 1 (IGF1BP3) positively correlated with CAG repeat length, but did not reach significance when corrected for age and BMI. IGF1 has been reported to block toxicity of the androgen receptor at its binding position and to attenuate neuromuscular symptoms when it is overexpressed in a mouse model of SBMA [20, 31]. In SBMA mouse models, muscle-specific overexpression of the anabolic hormone IGF-1 improves both functional outcomes and overall survival [20, 38]. IGF1 elevation may, therefore, be interpreted as an organism's rescue mechanism and is reported to be increased with muscle exercise [39]. In our study, it was not identified as a biomarker for disease duration or polyglutamine expansion.

Inverse associations between CAG repeat length and age of onset, muscle strength, and testosterone levels have been previously reported in SBMA [2, 12]. Similar to other trinucleotide repeat diseases, the longer the CAG repeat, the earlier the onset and the more pronounced the disease severity are reported. Evidence for an association between several tested laboratory parameters and either disease duration or repeat length remains limited. It is possible that reported hormonal and metabolic disturbances may also be influenced by factors like physical activity and/or nutrition.

The present study identified DHEA sulfate as a potential biomarker, given that it correlated well with both CAG repeat length and paresis duration. We recommend the re-evaluation of IGF1BP3 within a larger cohort, given that it tended towards a positive albeit insignificant correlation with both repeat length and weakness duration.

## Conclusions

Here, we report a range of laboratory parameters that are altered in SBMA patients. Most common were high CK values, followed by elevated AST and ALT, diabetic or pre-diabetic glucose metabolism and hyperlipidemia. Sex hormone levels were compatible with a picture of mild androgen insensitivity as shown by high levels of androgens and estradiol. Although many laboratory parameters were consistently elevated in this cohort, these did not correlate with any included clinical symptoms or phenotypical

SBMA features (disease severity and duration). In a multiple linear regression analysis with correction for age and BMI, only DHEA sulfate correlated with the paresis duration and CAG repeat length. Therefore, DHEA sulfate may serve as an informative laboratory marker for disease severity.

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## Compliance with ethical standards

**Conflicts of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All persons gave their informed consent prior to their inclusion in the study. The study was approved by the local ethics committee of the University of Ulm (73/10 and 92/10).

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