1   **Decreased aerobic capacity in ANO5-muscular dystrophy**

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23   **Running title:** Exercise in ANO5 dystrophy

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1 **ABSTRACT**

2 BACKGROUND: Anoctaminopathies are muscle diseases caused by recessive mutations

3 in the *ANO5* gene. The effects of anoctaminopathy on oxidative capacity have not

4 previously been studied in a controlled setting.

5 OBJECTIVE: To characterize oxidative capacity in a clinically and genetically well-

6 defined series of patients with anoctaminopathy.

7 METHODS: We sequenced the *ANO5* gene in 111 Finnish patients with suspected

8 LGMD2. Patients with positive findings underwent close clinical examination, including

9 electromyography, muscle MRI, and, in selected cases, muscle biopsy. Oxidative capacity

10 was analyzed using spiroergometry and compared to age-matched healthy controls.

11 RESULTS: We characterized 12 newly identified and 2 previously identified patients with

12 *ANO5* mutations from 11 families. Our material was genetically homogeneous with most

13 patients homozygous for the Finnish founder variant c.2272C>T (p.Arg758Cys). In one

14 family, we found a novel p.Met470Arg variant compound heterozygous with p.Arg758Cys.

15 Lower limb muscle MRI revealed progressive fatty degeneration of specific posterior

16 compartment muscles. Patients' spiroergometric profiles showed that anoctaminopathy

17 significantly impaired oxidative capacity with increasing ventilation.

18 CONCLUSIONS: Our findings support earlier reports that anoctaminopathy progresses

19 slowly and demonstrate that the disease impairs the capacity for aerobic exercise.

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1 **KEYWORDS**

2 Muscular dystrophies, limb-girdle; Muscular Diseases; Inborn Genetic Diseases; Aerobic

3 Exercise

4

5 **ABBREVIATIONS**

6 AT   anaerobic threshold

7 BE   base excess

8 CK   creatine kinase

9 EMG   electromyography

10 ExAC   Exome Aggregation Consortium

11 $FetCO_2$ fraction of end tidal CO_2

12 FVC   forced vital capacity

13 LGMD   limb-girdle muscular dystrophy

14 MMD3   Miyoshi myopathy

15 RPE   rate of perceived exertion

16 RQ   gas exchange ratio $V'CO_2/V'O_2$

17 SE   spin echo

18 TI   inversion time

19 $VECO_2$ ventilatory equivalent for CO_2 production

20 VEO_2 ventilatory equivalent for O_2 uptake

21 $V'O_2max$ maximal oxygen uptake

22 Wmax/3min   mean workload during the last 3 minutes of exercise

23 $Wmax/V'O_2max$ mechanical efficiency

1 INTRODUCTION

2 Anoctaminopathies are muscle diseases caused by recessively inherited *ANO5* mutations

3 first identified in proximal limb-girdle muscular dystrophy type 2L (LGMD2L) and distal

4 non-dysferlin Miyoshi myopathy (MMD3) [1, 2]. The phenotypic heterogeneity of

5 anoctaminopathy has since expanded, with the mildest cases presenting as the

6 asymptomatic elevation of creatine kinase ('hyperCK-emia') [1, 3]. The symptoms and

7 signs are restricted to skeletal muscle in most cases, but occasionally patients present with

8 a suspicion of heart involvement [4, 5]. Using muscle MRI, we and others have shown that

9 anoctaminopathy typically involves the posterior compartments of the thighs and calves

10 rather than the anterior compartments [6–8].

11

12 Vissing et al recently reported positive effects from a 10-week supervised aerobic exercise

13 regimen in patients with anoctaminopathy [9]. However, little controlled data exist

14 measuring the effects of the disease on baseline oxidative capacity. Spiroergometric testing

15 (breathing gas analysis during exercise) with the simultaneous analysis of blood lactate and

16 ammonia concentrations provides not only an objective means of evaluating pulmonary,

17 skeletal, and heart muscle involvement, but can also underline biochemical abnormalities

18 through the monitoring of blood metabolites [10].

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20 This study aimed to characterize a series of anoctaminopathy patients specifically

21 emphasizing oxidative performance, an aspect that has been little studied previously.

22 Patients were examined in detail, including by muscle MRI, changes in muscle pathology,

23 and spiroergometric testing.

1 **MATERIALS AND METHODS**

2 **Patients**

3 Patients were examined at Helsinki University Hospital. All patients and healthy controls

4 subjects provided their informed consent to participate in the study, and investigations were

5 conducted in accordance with the 1975 Declaration of Helsinki. Furthermore, the ethics

6 committee of the Hospital District of Helsinki and Uusimaa approved the study protocol.

7 Total DNA was extracted from peripheral blood, and the coding exons and flanking intron

8 sequences of the *ANO5* gene (NM_213599.2) were sequenced. Primer sequences are

9 available upon request from the authors. Variants whose pathogenicity were previously

10 unknown were scored using PolyPhen-2 v.2.2.5 (http://genetics.bwh.harvard.edu/pph2/)

11 [11] and Mutation Taster 2 (http://www.mutationtaster.org/) [12]. The population

12 frequencies for variants were taken from the Exome Aggregation Consortium (ExAC,

13 http://exac.broadinstitute.org/, accessed August 2015). Novel variants were submitted to

14 the ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar/).

15

16 **Spiroergometry, control subjects, laboratory specimens, and statistical methods**

17 Spiroergometric exercise testing was completed for 12 *ANO5* variant–positive patients and

18 24 age- and gender-adjusted controls using follow-up venous lactate and ammonium

19 samples (Table 1).

20

21 Spiroergometry was performed as described earlier [13]. A cannula was inserted into the

22 left cubital vein, and the blood specimens for venous ammonia and lactate were drawn at

23 nine time points: at rest, low exercise, maximal exercise, and at 2, 4, 6, 10, 20, and 30 min

1   after exercise. Blood specimens were collected using the vacuum technique. Following a

2   waste specimen of 2 ml, the lactate and ammonia specimens were placed into fluoride

3   oxalate and EDTA syringes, respectively, centrifuged, and analyzed with a Cobas Integra

4   400+ analyzer (Roche Diagnostics, Mannheim, Germany). Lactate and ammonia were

5   assayed by enzymatic methods using lactate dehydrogenase and glutamate dehydrogenase,

6   respectively. Next, 3-ml samples for the analysis of the venous blood gases were placed in

7   Ca-titrated Lithium heparin syringes and analyzed using a Radiometer ABL800 analyzer

8   (Radiometer Medical, Bronshoj, Danmark). The test started with a 40-W workload

9   increased by 40 W in 3-min steps in women and increased by 50 W in 3-min steps in men.

10   If the patient's reported physical condition was low, we used 20-W increases in 2-min steps

11   or 30 W in 3-min steps. We attained a rate of perceived exertion (RPE) of 17 to 19 on the

12   Borg scale and a gas exchange ratio $V\acute{}CO_2/V\grave{}O_2$ (RQ) of more than 1.0 for all participants.

13

14   We used an unpaired t-test to analyze the spiroergometric results as well as the results of

15   the venous blood specimen between patients and controls; a nonparametric Mann-Whitney

16   U test was used for parameters not normally distributed. Because of the multiple

17   comparisons, we used a Bonferroni correction to the significance level applied. Due to the

18   slight difference in the weight and BMI between the groups, we controlled the results of

19   the spiroergometric variables for BMI.

20

21   The patient and control groups had slight differences in maximal level of exercise in the

22   spiroergometric test. To control for this difference in the comparison of spiroergometric

23   data, we normalized the data according to blood lactate levels. This is because the lactate

1 level increases with the level of anaerobic metabolism and is therefore a measure of the

2 level of exercise during the test.

3

4 Increase of lactate increases ventilation, which can be measured as increase of ventilatory

5 equivalent for O2 and CO2 (VE/O2% and VE/CO2) and decrease of fraction of end tidal

6 CO2 (FetCO2%). In the patients, VE/O2% and VE/CO2% were increased and FetCO2%

7 decreased. This could have been interpreted as increased anaerobic metabolism, suggesting

8 patients' exercise tests to be more maximal than those of the controls. By controlling

9 spiroergometric variables for maximal lactate levels in addition to BMI, we could see that

10 the level of exercise of the patients was not greater than in the controls. Before these

11 calculations, we had analyzed the behavior of lactate and ammonia associated with exercise,

12 and no specific findings were seen. The respiratory quotient, RQ, could also have been

13 used to control for the level of exercise. However, maximal lactate level serves here better

14 as adjustment because, in contrast to RQ, lactate is not a spiroergometric variable itself.

15

16 **Muscle MRI**

17 MRI was performed using a 1.5 T Siemens system. Axial images were acquired for the

18 pelvic, thigh, and leg muscles. The pulse sequences were T1-weighted spin echo (SE) with

19 TR of 600 to 700 ms, TE of 15 ms, and a STIR fat suppression sequence with TR of 3200

20 to 4300 ms, TE of 33 ms, and an inversion time (TI) of 160 ms. Slices of 7 mm were used.

21 The involvement of each muscle was scored on a scale of 0 to 3, where 0 indicated normal,

22 1 indicated minor involvement, 2 indicated moderate involvement, and 3 indicated

23 maximal involvement. Aggregate scores were computed for the muscles of the thigh

1 (quadriceps femoris, adductors, hamstrings, sartorius, and gracilis) and lower leg (tibialis

2 posterior, peroneus, deep posterior compartment, soleus, and gastrocnemius). Based on

3 five leg and thigh muscles, the maximum aggregated score was 15. Follow-up scans were

4 performed using the same scanner and identical parameters for patients 1 (2 years between

5 scans), 2 (4 years between scans), 4 (2 years between scans), 7 (2 years between scans), 9

6 (2 years between scans), and 13 (12 years between scans).

7

8 **RESULTS**

9 **Screening of the *ANO5* gene**

10 We sequenced the *ANO5* coding regions and flanking intron sequences in 111 patients with

11 suspected myopathy of an undetermined cause based on symptoms and clinical findings,

12 an elevated CK, myopathic electromyography (EMG), muscle biopsy, or muscle MRI. We

13 identified 12 patients from 10 families as positive for *ANO5* variants (Table 2). Clinical

14 and genetic data from patients 13 and 14 were previously reported [1, 2, 8].

15

16 The most common genetic finding was homozygous *ANO5* c.2272C>T (p.Arg758Cys).

17 Eight patients were homozygous for this variant, while the remaining four were compound

18 heterozygous for p.Arg758Cys in combination with either c.191dupA (p.Asn62LysfsX15),

19 c.1409T>G (p.Met470Arg), or c.1664G>T (p.Ser555Ile) (Table 2). The latter two variants

20 have not previously been confirmed as pathogenic in anoctaminopathy. Both were

21 predicted as 'disease causing' by Mutation Taster, whereas PolyPhen-2 rated p.Met470Arg

22 as 'possibly damaging' and p.Ser555Ile as 'probably damaging'. The population frequency

1 of p.Ser555Ile was 1.2×10^{-4} in European populations, whereas p.Met470Arg was not found

2 in the ExAC database.

3

4 **Clinical, neurophysiologic, and pathologic findings**

5 Despite being relatively genetically homogeneous, our anoctaminopathy series showed

6 some variability in clinical features (Table 2). Six of our 14 patients were female. The age

7 of reported symptom onset ranged from 12 to 62 years, with an average age of 33 for female

8 patients (SD 5.9 years) and 31 for male patients (SD 16 years). The disease duration ranged

9 from 6 to 58 years. At onset, three patients complained of muscle pain, unpleasant muscle

10 sensations in the calves, and cramps. Six patients initially experienced difficulty walking

11 on their tiptoes, two patients complained of difficulty climbing stairs, and two patients

12 complained of difficulty running and climbing stairs. One patient reported no muscle

13 symptoms and an increased CK was found incidentally. During the course of the disease,

14 muscle pain, stiffness, or cramps were common among all patients. Clinical examination

15 showed distal lower limb muscle involvement in six patients, proximal involvement in two

16 patients, and both distal and proximal involvement in six patients.

17 Plasma CK ranged from 240 to 12,290 IU/l. EMG findings were myopathic in 7 out of 12

18 patients tested. Four patients exhibited both neurogenic and myopathic features, whereas

19 one patient had a normal EMG (Table 2). Muscle biopsies were obtained from 13 patients.

20 The findings ranged from necrotizing myopathy or strong dystrophic changes to mild

21 atrophy of type 2 fibers. Three out of nine patients who underwent cardiac ultrasound had

22 slightly abnormal findings primarily attributed to hypertension (Table 2). ECGs showed

23 normal findings for all patients (Table 4).

1 **Skeletal muscle imaging**

2 Table 3 summarizes the muscle MRI findings. Muscle T1-weighted MRI scans showed

3 fatty replacement predominantly in the adductors, hamstrings, gastrocnemius, and soleus.

4 Muscle MRI STIR-weighted scans showed hyperintensities suggesting myoedema

5 primarily in the hamstrings, quadriceps, adductors, gastrocnemius, and soleus. These

6 findings were more evident at the early stages of disease.

7

8 In six patients who completed two follow-up MRI scans, the total T1 score increased by

9 an average of 0.6 (SD 0.7) points per year in the thighs and 0.6 (SD 0.3) points per year in

10 the legs based on the 0 to 15 scoring scale. The STIR score does not show any significant

11 change over time. Thus, the progression of the disease is reflected in any sequential

12 increases in fatty degeneration observable in T1 imaging. Representative images from one

13 patient are shown in Figures 1A–F.

14

15 **Spiroergometric testing**

16 Table 1 shows the anthropometric and spirometric characteristics for patients and controls.

17 During the exercise test, patients with anoctaminopathy exhibited a significantly lower

18 maximal working capacity (Wmax/3min), maximum oxygen uptake (VO2max), oxygen

19 pulse (V'O2/HR), and mechanical efficiency (Wmax/V'O2max) than those for controls

20 (Table 4). However, the respiratory quotient ($V'CO_2/V'O_2$) tended to be higher in patients,

21 and signs of increased ventilation demonstrated by a significantly increased ventilatory

22 equivalent for O_2 (VE/V'O$_2$) and CO_2 (VE/V'CO$_2$) in addition to a decreased fraction of

23 end-tidal CO_2 (FetCO$_2$) were seen in patients compared to controls.

1   In the blood gas analyses, patients exhibited the lowest blood pH of 7.3 at 2 to 6 min and

2   the lowest base excess (BE) -5.5 mmol/l at 6 min after exercise, while the corresponding

3   values in controls were pH of 7.26 at 2 min and BE -7.77 mmol/l at 4 min after exercise.

4   For the BE values, we found a significant difference between patients and controls at 2 and

5   4 min after exercise ($p = 0.039$ and 0.013, respectively), and a significant difference in the

6   pH values at 2 min after exercise ($p = 0.048$). In addition, we found significantly lower

7   lactate readings in patients compared to controls ($p = 0.049$) at 2 and 4 min after exercise.

8   However, we found no difference between patients and controls in the levels of ammonia

9   (Figure 2).

10

11   **DISCUSSION**

12   We studied a cohort of patients with *ANO5*-related dystrophy, providing a detailed clinical

13   analysis. Anoctaminopathy has been reported in several countries, including Canada [2],

14   the UK and Germany [6, 14], Finland [1, 3], the Netherlands [2, 15], Denmark [5], the

15   Czech Republic [16], and Italy and other countries in Europe and worldwide [17–19]. We

16   found that 11% of our suspected LGMD patients had *ANO5* mutations, supporting that they

17   are a common cause of myopathy in northern Europe.

18

19   We found the variant c.2272C>T (p.Arg758Cys) in all of our patients, appearing as

20   homozygous in 10 patients from 8 families and as compound heterozygous with another

21   variant in 4 patients from 3 families. This finding is not surprising since c.2272C>T has a

22   carrier frequency of 0.35% in Finland, thus explaining most known Finnish cases [3].

23   Among the compound heterozygous patients, one had the c.191dupA (p.Asn62Lysfs15X)

variant, which is common in other Northern European populations [14]. One patient had the c.1664G>T (p.Ser555Ile) variant, which was recently found in the heterozygous state in a patient sequenced as part of a cohort of patients with LGMD or unspecified myopathy [17]. Bioinformatic predictions support the pathogenicity of this variant. However, since carriers are found in European populations, it is likely that a second hit is necessary on the other allele for this variant to cause disease, as was the case in our patient. Finally, two siblings (Family J, patients 11 and 12) presented with previously unknown variant c.1409T>G (p.Met470Arg). This variant received high scores for pathogenicity using bioinformatics tools, and was not found in public exome databases.

The clinical findings in our anoctaminopathy patients correspond to previous descriptions, featuring asymmetric proximal or distal muscle involvement and myalgia [1–3, 5, 6, 14–20]. We found a tendency towards asymmetric muscle involvement, a feature of anoctaminopathy not typically reported for other forms of LGMD [2, 21] with the exception of facioscapulohumeral muscle dystrophy. Echocardiographic abnormalities were documented in only three patients, all associated with hypertension.

Our findings provide further confirmation of the variable age of onset of anoctaminopathy. Even in the presence of the homozygous p.Arg758Cys variant, initial symptoms were reported between the ages of 12 and 62 years. The variable age of onset suggests the presence of genetic or environmental disease modifiers. Previous reports have well-documented an association between being male and anoctaminopathy [3, 14, 15, 22].

However, we found no significant difference in the average age of disease onset based on gender, although the variance for the age of onset was greater among the men in our study.

Earlier radiologic studies by us and others demonstrated that anoctaminopathy affects lower leg muscles, suggesting predominantly posterior compartment involvement in both the thighs and the legs. The tibialis anterior, gracilis, and sartorius are better spared, similar to other LGMDs, indicating that MRI may not be the best diagnostic tool [1, 6–8]. The results presented here further demonstrate that fatty degeneration tends to increase as the disease progresses. Therefore, T1-weighted MRI may be useful as a follow-up tool or as an assessment tool for potential treatment. The STIR technique is a sensitive tool in early diagnostics, but not in the later stages of disease. Furthermore, EMG results remain variable, featuring both myopathic and neurogenic findings.

To our knowledge, this is the first study to explore the oxidative capacity of anoctaminopathy patients compared to an anthropometrically matched control population. Patients with anoctaminopathy showed significantly lower oxygen uptake than controls, which may serve as a primary feature of the disease or secondary to deconditioning provoked by muscle weakness and pains. Additionally, the patients' oxygen pulse (V'O2/HR) was lower than controls, most likely associated with the diminished capacity of the peripheral muscles [23, 24]. The level of maximal oxygen uptake reported here corresponds to that measured by Vissing et al [9] before regular aerobic training.

1   In patients, we found an excess of ventilation accompanied an increase in the ventilatory

2   equivalents for O_2 and CO_2. In addition, more than half (7/12 patients) had $FetCO_2$ lower

3   than 4.5% during maximal exercise suggesting slight hyperventilation. By contrast,

4   controls showed normoventilation, although we expected them to display a stronger

5   respiratory compensation due to stronger metabolic acidosis associated with exercise

6   compared to patients. The spirometric findings did not explain this inconsistency. The

7   forced vital capacity (FVC) was slightly lower in patients than in healthy controls, but only

8   one patient (patient 9) developed restrictive ventilatory impairment (FVC < 80% of the

9   predicted value), suggesting that ventilatory function was not the cause of the increased

10 ventilation. The increased ventilation might be explained by the lowered aerobic exercise

11 capacity of the muscles leading to secondary hyperventilation during exercise. In addition,

12 the reduced mechanical efficiency (Wmax/V'O_2max) most likely reflected this finding,

13 indicating an increased need for oxygen uptake related to the attained workload.

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15 Patients' lactate and ammonia levels tended to be lower than those among controls. This

16 might be explained by the lower exercise capacity due to muscle disease. However, the RQ

17 levels of patients tended to be higher than those among controls indicating at least the same

18 level of aerobic capacity among both patients and healthy controls. We should note that an

19 increase in ventilation may partly explain the elevated RQ levels we observed in patients.

20 We found no specific findings or indications of metabolic muscular myopathy.

21

22 In conclusion, our report confirms the importance of the recessive *ANO5* variants as causes

23 of muscle disease with significant variability in clinical severity. In addition, we found a

1 new disease-associated variant, and provide an initial and detailed case–control

2 comparison suggesting a decreased oxidative capacity. Our results support aerobic training

3 as a useful intervention to mitigate the symptoms of anoctaminopathy. Further studies

4 featuring a larger sample of patients are needed to confirm our findings.

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12 **CONFLICTS OF INTEREST**

13 The authors have no conflicts of interest to report.

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	Patients with an *ANO5* deficiency (*N* = 12)	Healthy controls (*N* = 24)
Sex (m/f)	6/6	12/12
Weight (kg)	81.7 (18.8)	75.1 (14.8)
Height (cm)	169.8 (10.9)	165.3 (36.4)
BMI	28.2 (5.4)	23.9 (5.8)
Age	50.6 (10.5)	45.7 (10.6)
FVC (l)	4.05 (0.87)	4.6 (1.3)
FVC % of pred.	94.3 (10.4)	102.5 (12.3)
FEV1 (l)	3.33 (0.73)	3.63 (0.21)
FEV1 % of pred.	95.2 (11.6)	98.9 (11.4)

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2 **Table 1. Anthropometric characteristics of patients and controls.** FVC = forced vital

3 capacity, FEV1 = forced expiratory volume in 1 s, pred. = predicted value. Means and

4 standard deviations (in parenthesis) are presented.

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Patient (family)	Sex	Age at onset	Current age	CK, IU/l, range	ECG and cardiac US	Spiro-ergo-metry	Clinical phenotype	MRI thighs	MRI legs	Biopsy	EMG	ANO5 genotype
1 (A)	M	17	65	1,337–3,279	Normal	+	Distal weakness of right UL, later LL fatigue, myalgia, cramps	5	3	Chronic necrotizing myopathy with dystrophic features	Myopathic	p.R758C + p.R758C
2 (A)	M	62	68	650–4,542	Mildly hypertrophic left ventricle	+	Mild distal LL weakness	6	3	Chronic necrotizing myopathy with dystrophic features	Myopathic	p.R758C + p.R758C
3 (B)	F	31	43	272–2,023	Normal	+	LL myalgia, cramps and weakness	0	2	No diagnostic changes, mild fiber type 2 atrophy	Myopathic	p.R758C + p.R758C
4 (C)	M	12	52	1,027–60,000	Mild diastolic relaxation abnormality	+	Distal LL cramps, exercise intolerance, later UL weakness	3	4	Nonspecific myopathy	Myopathic	p.R758C + p.R758C
5 (D)	F	34	53	1,367–3,363	Normal	+	Distal LL weakness, stiffness, myalgia, UL weakness (marginal)	6	6	No diagnostic changes, mild fiber type 2 atrophy	Myopathic	p.R758C + p.R758C
6 (E)	M	40	60	766–2,517	ECG normal, US ND	-	Progressive proximal LL weakness, later proximal right UL weakness	9	6	Dystrophic and secondary inflammatory changes	Myopathic, LL neuropathy	p.R758C + p.N62Kfs 15X
7 (F)	F	38	51	921–5,119	Slight apical septal thickening	+	Restless legs, CK elevation, later LL myalgia	0	3	Mild chronic myopathy	Myopathic	p.R758C + p.R758C
8 (G)	M	38	69	1,300	Normal	+	Proximal LL and UL weakness, myalgia	14	3	Dystrophic features	Myopathic	p.R758C + p.R758C
9 (H)	F	29	56	2,000–3,000	ECG normal, US ND	+	LL myalgia and mild weakness	6	3	Mild necrotizing myopathy with dystrophic features	Myopathic and neurogenic features	p.R758C + p.R758C
10 (I)	F	25	31	240–2,973	Normal	+	LL myalgia, fatigue	0	0	Myopathic changes	Normal	p.R758C + p.S555I

Patient (family)	Sex	Age at onset	Current age	CK, IU/l, range	ECG and cardiac US	Spiro-ergo-metry	Clinical phenotype	MRI thighs	MRI legs	Biopsy	EMG	ANO5 genotype
11 (J)	M	37	56	730–3,500	ECG normal, US ND	-	LL predominant weakness and cramps, cerebral white matter hyperintensities	0	1	Mild type 2 fiber atrophy	Myopathic and neurogenic features	p.R758C + p.M70R
12 (J)	F	41	Deceased age 52	271–11,743	Normal	+	Left predominant LL weakness, myalgia, recurrent myoglobinuria, central pontine hyperintensity	0	0	Myopathic changes	Myopathic and neurogenic features	p.R758C + p.M470R
13 (K)	M	20	56	2,750–12,290	ECG normal, US ND	+	Asymptomatic, CK elevation by chance	4	5	Mild dystrophic features	Myopathic	p.R758C + p.R758C
14 (K)	M	25	49	3,822–10,471	ECG normal, US ND	+	LL muscle weakness, myalgia	7	5	Dystrophic features	Myopathic	p.R758C + p.R758C

Table 2. Summary of clinical and genetic findings. MRI shows total scores of muscle involvement on T1-weighted imaging for the thighs and the legs (muscles in the legs and the thighs divided into five groups and scored 0 to 3 such that a total score of 15 indicates maximal involvement and 0 indicates no involvement). For patients who had two scans, the results from the latest scan are shown. CK = creatine kinase, LL = lower limbs, ND = not done, UL = upper limbs, US = ultrasound.

	Muscle group	T1	STIR
Thighs	Adductors	1.7	0.5
	Hamstrings	1.5	1.0
	Quadriceps	0.7	0.7
	Gracilis	0.2	0.2
	Sartorius	0.1	0.2
Legs	Gastrocnemius	2.1	1.1
	Soleus	0.7	1.1
	Anterior compartment	0.2	0.1
	Peroneal compartment	0	0
	Deep posterior compartment	0	0

Table 3. Muscle imaging. Muscle involvement on MRI imaging is scored 0 to 3, where 0 indicates no involvement and 3 indicates maximal involvement. Average scores are shown for T1 and STIR images.

Parameter	ANO5, $N = 12$	Healthy controls, $N = 24$	P-Value
Maximum heart rate	172 (12.5)	162.3 (30.3)	NS
Maximum heart rate %	89.1 (15.8)	93.1 (8.7)	NS
AT% of expected maximal V'O$_2$	51.6 (16.8)	66.7 (20.2)	NS
Wmax/3 min (W)	108.92 (46.9)	208.54 (74.74)	0.00
Wmax/3 min % of predicted	67.8 (28.1)	114.36 (26.2)	<0.001
V'O$_2$ max (maximum oxygen uptake) (l/min)	1.86 (0.61)	3.7 (4.04)	NS
V'O$_2$max % of predicted	86.1 (29.5)	122.1 (26.8)	0.001
V'O$_2$max (ml/min/kg)	22.9 (5.7)	38.4 (11.9)	0.002
V'O$_2$max/kg of predicted (%)	76.5 (22.7)	120.0 (27.0)	0.002
V'O$_2$/HR % of predicted (%)	101.8 (33.1)	136.3 (25.6)	0.001
Respiratory quotient (V'CO$_2$max/V'O$_2$max, RQ)	1.22 (0.08)	1.13 (0.64)	0.001
VEO$_2$ % of predicted (%)	136.7 (16.6)	110.8 (15.5)	<0.001
VECO$_2$ % of predicted (%)	124.1 (14.3)	109.7 (14.0)	0.002
Fraction of end-tidal CO$_2$ (FetCO$_2$)(%)	4.65 (0.48)	5.3 (0.51)	<0.001
Wmax/VO$_2$max (%)	16.3 (4.8)	20.8 (1.8)	0.004
Breathing frequency (1/min)	37.92 (10.7)	37.45 (8.9)	NS
Tidal volume % or predicted (%)	93.4 (28.6)	112.1 (18.2)	NS
Subjective maximum strength perceived	18.8 (0.87)	18.39 (0.90)	NS

Table 4. Spiroergometric data for patients with ANO5 and healthy controls. AT = anaerobic threshold, Wmax/3 min = mean workload during the last 3 min of exercise, maximal V'O$_2$max = maximal oxygen uptake, VECO2 = ventilatory equivalent for CO2 production, VEO2 = ventilatory equivalent for O2 uptake, Wmax/V'O$_2$ max = mechanical efficiency. Comparisons were controlled for the maximal lactate levels and BMI. Level of significance after Bonferroni correction is $P < 0.003$.

FIGURE LEGENDS

Figure 1. Muscle imaging. Lower limb MRI from patient 9 shows the disease progression over 2 years. The first images for the thighs (**A–B**) are compared to later images (**D–E**), illustrating that the most prominent changes can be found in the progressive fatty infiltration symmetrically in the adductors, biceps femoris, quadriceps (vastus lateralis and intermedius, and rectus femoris), and left semimembranosus muscles. In the legs (**C** and **F**), selective and progressive involvement of the medial head of the gastrocnemius muscle can be found, with more prominent changes visible on the left-hand side**.**

Figure 2. Results from exercise testing. Spiroergometric results showing the levels of ammonia and lactate in 12 patients and 24 healthy controls. Significantly lower lactate levels are visible in patients compared to controls ($P = 0.049$) at 2 and 4 min after exercise. No differences exist in the levels of ammonia between patients and controls. Mean and standard deviation, $*P < 0.05$.
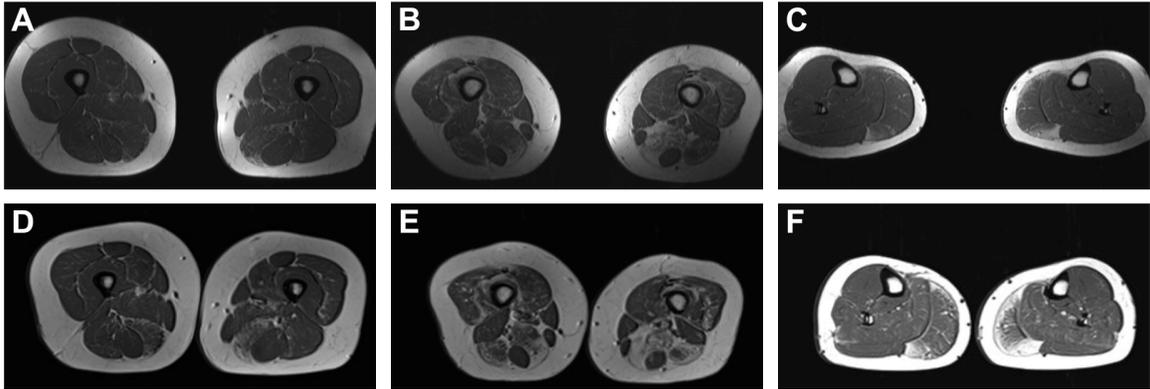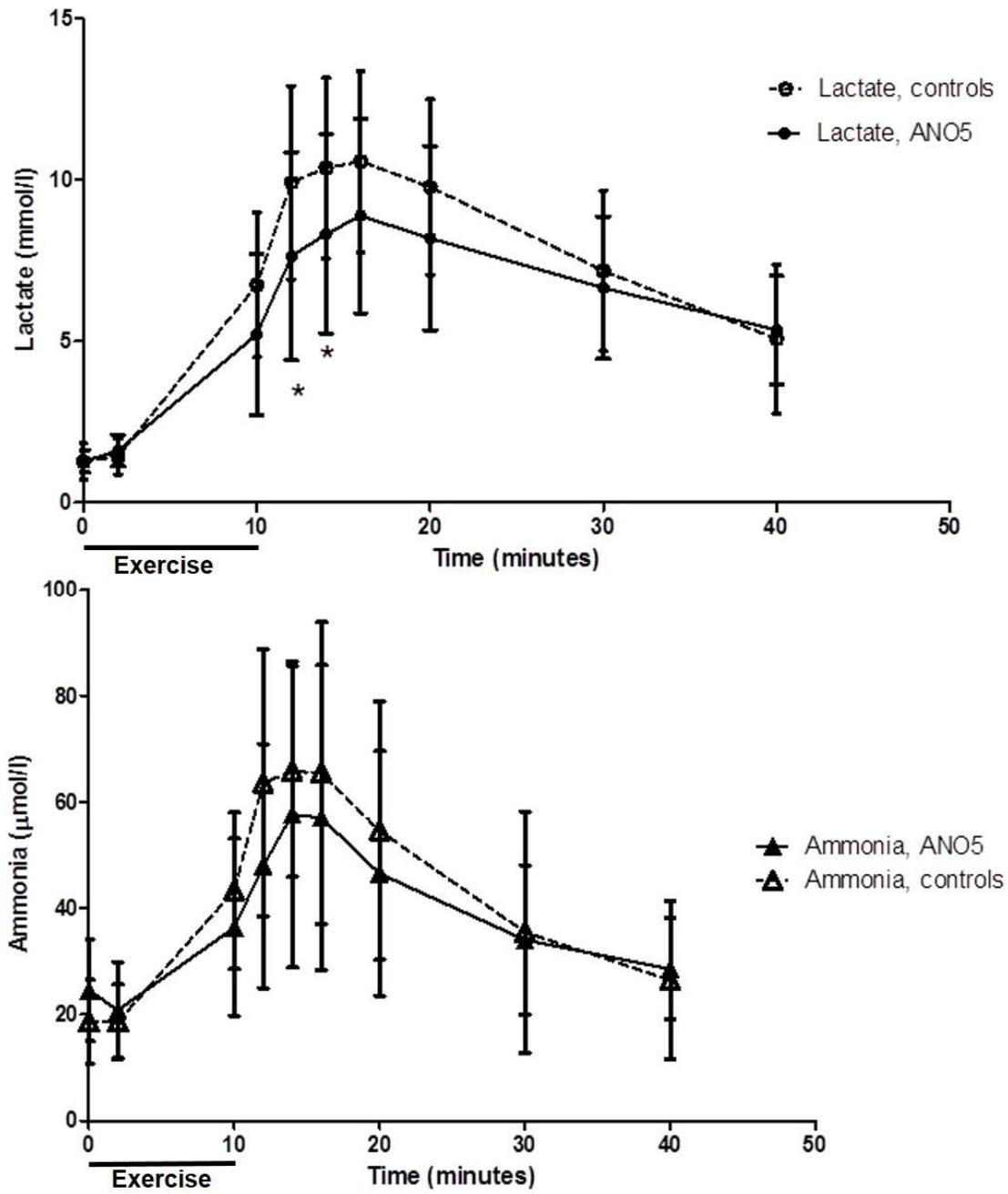
Figure 1.

Figure 2.

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