*J Toxicol Pathol 2011; **24**: 49–62*

Original

Comparative Gene Expression Analysis in the Skeletal Muscles of Dysferlin-deficient SJL/J and A/J Mice

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Abstract: Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was conducted to determine whether or not there are interstrain or site-dependent differences in the gene expression profiles of skeletal muscles in SJL/J and A/J mice as dysferlinopathy models. Upon analysis by qRT-PCR, SJL/J mice showed a trend of increased gene expression level of uncoupling protein 2 in the rectus femoris and longissimus lumborum at 30 weeks of age when dystrophic lesions became histopathologically pronounced. Heme oxygenase 1 and S100 calcium binding protein A4 were upregulated in the rectus femoris, longissimus lumborum and abdominal muscles, in which dystrophic lesions occur more commonly in SJL mice. The gene expression levels of heat shock protein 70 in most muscles of A/J mice were lower than those of BALB/c mice as control. SJL/J mice exhibited a marked lowering of decay-accelerating factor 1/CD55 gene expression level in all studied muscles except for the heart at all ages compared with that of BALB/c mice. This study showed that there were some interstrain differences in the gene expression profiles of skeletal muscles between SJL/J and A/J mice. Further investigation is required to reveal whether these alterations of the expression levels are the cause of dystrophic changes or occur subsequent to muscle damage. (DOI: 10.1293/tox.24.49; J Toxicol Pathol 2011; **24**:49–62)

Key words: A/J mouse, decay-accelerating factor, dysferlin, heat shock protein, heme oxygenase-1, SJL/J mouse

Introduction

Limb-girdle muscular dystrophy type 2B (LGMD2B) and Miyoshi myopathy (MM) are both caused by recessively inherited mutations in the dysferlin gene[1]. LGMD2B is characterized by the progressive wasting and weakness of proximal lower limb-girdle muscles. Meanwhile, the distal muscle groups of the limbs and girdle are mostly affected in MM. Both disorders have been considered to be due to a loss of dysferlin protein at the plasma membrane in muscle fibers, which leads to abnormalities in vesicle traffic and membrane repair[2,3], and are collectively called 'dysferlinopathy'.

Two naturally occurring animal models for LGMD2B, SJL/J and A/J mice, have been identified to have mutations in the dysferlin gene associated with phenotypic features of progressive muscular dystrophy[4,5]. However, the type of dysferlin gene mutation differs between SJL/J and A/J mice. SJL/J mice have a splice site mutation that removes a part of the highly conserved C2E domain; the domain is known to bind to calcium, phospholipids or proteins to trigger signaling events and membrane trafficking[4,6,7]. On the other hand, A/J mice bear a unique ETn retrotransposon insertion near the 5' end (intron 4) of the dysferlin gene[5]. Interestingly, the two strains show phenotypic differences from each other: A/J mice display a later age of onset and a slower progression of the muscle disease than SJL/J mice[5]. Our previous study showed that there were differences in the progress and prevalent site of skeletal muscle lesions between SJL/J and A/J mice[8]. In particular, the difference in sensitivity to muscular dystrophic lesions between SJL/J and A/J mice was most apparent in the lumbar (longissimus and sublumbar) muscles. These findings support the hypothesis that additional enhancers or modifiers may be involved in the progression of skeletal muscle lesions in dysferlinopathy.

To shed some light on the molecular pathogenesis of dysferlinopathy, gene expression profiling studies have been undertaken in the skeletal muscles of SJL/J mice, C57BL/10. SJL-*Dysf* mice and LGMD2B patients[9–12]. However, gene expression profiles of A/J mice or gene expression comparison studies between SJL/J and A/J mice have not yet to be reported. In this study, toward the goal of discovering additional enhancers or modifiers associated with phenotypic divergence between SJL/J and A/J mice, the temporal or

Received: 21 September 2010, Accepted: 1 December 2010
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site-specific gene expression was analyzed by a quantitative real-time polymerase chain reaction (qRT-PCR) using TaqMan® Gene Expression Assays.

Materials and Methods

Animals

BALB/c mice were used as control because this strain does not show abnormality in the skeletal muscles by the age of 16 months and has been used as a control in the histopathological examination of skeletal muscles in SJL/J mice[13]. Male BALB/c mice and male SJL/J mice were obtained from Charles River Laboratories Japan Inc. (Kanagawa, Japan). Male A/J mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). All mice were brought to the test facility (Safety Research Laboratory (Kashima), Mitsubishi Tanabe Pharma Corporation, Osaka, Japan) at the age of 4, 7 or 8 weeks. These animals were housed in plastic cages in an animal room kept under controlled conditions (temperature of 23 ± 2 °C, humidity of 30 to 70%, ventilation of 12 times or more per hour, lighting for 12 hours between 06:30 to 18:30) in the test facility. The mice were given pelleted feed sterilized by 15-kGy gamma irradiation (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and industrial water *ad libitum* via an automatic water feeder.

All experimental procedures were approved by the Animal Ethics Committee of Mitsubishi Tanabe Pharma Corporation and were conducted in accordance with the Experimental Guide to the Care and Use of Laboratory Animals.

Histopathology

Three male mice from each strain were euthanized by exsanguination from the abdominal aorta under ether anesthesia at 10 and 30 weeks of age. The femoral, crural, brachial, forearm, abdominal and lumbar muscles were fixed in 10% neutral buffered formalin; skeletal muscles including bones were decalcified using 50% K-CX (Falma Co., Ltd., Tokyo, Japan) solution for decalcification, processed and embedded in paraffin. Tissue paraffin blocks were sectioned at a thickness of 2 μm, and each section was stained with hematoxylin and eosin (HE).

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

Genes that have been shown to change in the skeletal muscles of SJL/J mice, dystrophin-deficient mice or muscular dystrophy patients or to be linked to these gene expression changes were mainly selected for *q*RT-PCR analysis.

Lipid metabolism-associated genes: SJL/J mice have a mutation in the Tbc1d1 gene that results in a truncated protein lacking the TBC Rab–GTPase-activating protein domain[14] and showed increased fatty acid uptake/oxidation and reduced glucose uptake in isolated skeletal muscle. Uncoupling protein-3 and –2 (Ucp3, Ucp2) gene expressions as candidate genes in the regulation of lipids as metabolic fuels in skeletal muscle[15] were measured with that of peroxisome proliferative activated receptor-γ coactivator 1α (PGC-1α) regulating the expression of many genes involved with fatty acid oxidation[16].

Skeletal muscle atrophy-associated genes: Myostatin as an endogenous negative regulator of muscle growth[17] and follistatin as an endogenous antagonist of myostatin[18], which have attracted attention as therapeutic targets of muscular disorders[19], were quantified.

Ubiquitin-proteasome system-associated genes, ubiquitin-like protein gene and ER stress-associated genes: Normal dysferlin was degraded by an endoplasmic reticulum (ER)-associated degradation system (ERAD) composed of ubiquitin/proteasome. However, mutant dysferlin spontaneously aggregated in the ER and induced eukaryotic translation initiation factor 2 alpha phosphorylation and LC3 conversion, a key step for autophagosome formation, and, finally, ER stress cell death[20]. Because SJL/J mice produce incomplete dysferlin protein, ERAD and ER stress-associated genes were included in the examination of gene expression.

Heat shock protein genes: An *in vitro* study using mouse skeletal muscle myotubes indicated that an increased level of heat shock proteins (HSPs) may provide protection against the muscle damage that occurs by a pathological increase in intracellular calcium or uncoupling of the mitochondrial respiratory chain[21]. Some HSPs were selected as targets for evaluation of the protective system in the skeletal muscles of SJL/J and A/J mice.

ER-associated degradation of glycoprotein-associated genes: It is known that major histocompatibility complex (MHC) class I expression is markedly upregulated in myopathic muscles of dysferlin-deficient SJL/J mice[22]. The assembly and folding of MHC class I molecules are associated with calnexin, calreticulin and ERp57[23]. Transcriptional induction of the ER degradation enhancer mannosidase alpha-like 1 (Edem1) is required for degradation of misfolded glycoprotein substrates including MHC class I[24]. To confirm whether or not excess MHC class I causes the upregulation of glycoprotein ERAD, these gene expression profiles of SJL/J and A/J mice were determined.

Histone deacetylase genes and nitric oxide synthase genes: Histone deacetylase inhibitors and nitro oxide donors delay the progression of muscular dystrophy in dystrophin-deficient mice[25,26]. Because histone deacetylase and nitro oxide may be related to the development of dystrophic lesions in SJL/J and A/J mice, these gene expressions in SJL/J and A/J mice were compared with those of BALB/c mice.

Oxidative stress-associated genes: Absence of dystrophin appears to render muscle specifically more susceptible to oxidative stress[27]. Heme oxygenase 1 (Hmox1) is known to be induced by oxidative stress or other stress stimuli[28]. Thioredoxin reductase 1 (Txnrd1) is a key enzyme in the thioredoxin system as an anti-oxidation system[29]. To explore the relationship between oxidative stress and muscle damage in dysferlin-deficient mice, these gene expression levels were quantified in SJL/J and A/J mice.

Complement control factor genes: SJL/J mice were found to exhibit downregulation of the complement inhibitor, decay-accelerating factor 1 (Daf1)/CD55 antigen, in skeletal muscle only, and the absence of Daf1/CD55 increased susceptibility to complement attack in cultured human myotubes[30]. Daf1 and Daf2 were selected for quantification by qRT-PCR on the basis of these findings.

Three mice each at 10 and 30 weeks of age were prepared for qRT-PCR analysis separately from histopathological examination. The femoral (rectus femoris) and lumbar (longissimus lumborum) muscles were removed from mice, quickly cut into slices less than 0.5-cm thick and incubated overnight in RNA*later* RNA Stabilization Reagent (Qiagen, Valencia, CA, USA) at 2–8 °C. The rectus femoris and longissimus lumborum, in which there was shown to be a remarkable difference of severity of muscle lesions between SJL/J and A/J mice at the age of 35 weeks old in our previous report[8], were used as examined sites in the first qRT-PCR analysis. The tissue samples in the reagent were transferred to a freezer at –20 °C and stored until use. For qRT-PCR analysis, total RNA was isolated from each isolated muscle with an RNeasy® Fibrous Tissue Mini Kit (Qiagen) according to the instructions of the manufacturer. For each sample, pooled total RNA from 3 mice was reverse-transcribed using a High Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA, USA). The gene-specific primers and probes used for qRT-PCR analysis were available as TaqMan® Gene Expression Assays (Applied Biosystems; Table 1). The qRT-PCR reactions were performed on a 7500 Fast Real-Time PCR System (Applied Biosystems) in 20 µL of the reaction mixture containing 1x TaqMan Fast Universal PCR Master Mix, No AmpErase® UNG (Applied Biosystems), 1x Gene Expression Assay mix and 5 µL of diluted cDNA sample as a template. MicroAmp® Fast 96-Well Reaction Plates covered by optical adhesive covers (Applied Biosystems) were used. Amplification was conducted according to the following thermal profile: 1 cycle at 95 °C for 20 sec, and 40 cycles at 95 °C for 3 sec and 60 °C for 30 sec. Initial raw data analysis was performed using the Sequence Detection Software version 1.3.1. Relative mRNA levels were calculated by the comparative threshold cycle (Ct) method[31,32], as described in Applied Biosystems User Bulletin Number 2 (P/N 4303859).

The program calculates ΔCt and ΔΔCt with the following formula: ΔΔCt = ΔCt sample [Ct endogenous control gene (from BALB/c mice at 30 weeks of age, SJL/J or A/J mice) – Ct target gene (from BALB/c mice at 30 weeks of age, SJL/J or A/J mice)] – ΔCt control [Ct endogenous control gene (from BALB/c mice at 10 weeks of age) – Ct target gene (from BALB/c mice at 10 weeks of age)]. The relative gene expression was calculated using the expression $2^{-(\Delta\Delta Ct)}$. Glyceraldehyde-3-phosphate dehydrogenase (Gapdh) gene was selected as an endogenous control gene.

Quadruplicate measurements per gene were conducted and data are presented as the mean and standard deviation. Changes in gene expression are reported as fold changes relative to those of controls (the Ct values in BALB/c mice at the age of 10 weeks). We defined that the gene expression was regulated or downregulated in the present study if the relative gene expression was more than twice or less than one-half that of the control, respectively. Data were not statistically analyzed because qRT-PCR analysis was performed using pooled samples of equal quantities of total RNA obtained from three mice in each strain.

Additional qRT-PCR analysis

To clarify the site-specificity and temporal changes of gene expression, some genes that were upregulated more than twice or downregulated less than one-half that of the control in both rectus femoris and longissimus lumborum, in one of SJL/J and A/J mice on the first qRT-PCR analysis, and other genes associated with these genes (Table 2), were measured in eight sites of striated muscles (rectus femoris, gastrocnemius, triceps brachii, flexor carpi ulnaris, longissimus lumborum, abdominal muscle, diaphragm and heart) from three mice at the ages of 5, 15 and 30 weeks old by means of the above method. The eight striated muscles used for additional qRT-PCR analysis are separated into three groups based on the severity of muscle pathological lesions in SJL/J mice at an age of 35 weeks old[8]. The rectus femoris, longissimus lumborum and abdominal muscle are included in the severe lesion group, which exhibits slight to moderate degeneration/necrosis with moderate central nuclei in muscle fibers. The triceps brachii are referred to as the slight lesion group, which shows minimal degeneration/necrosis with moderate central nuclei in muscle fibers. Other muscles are classified in the minimal lesion group, with only minimal degeneration/necrosis with slight central nuclei or without central nuclei in muscle fibers.

Lipid metabolism-associated genes: As previously explained, SJL/J mice have a mutation in the Tbc1d1 gene. Checks were performed to ensure that the Tbc1d1 gene had not been expressed at any week of age or at any muscle site.

Apoptosis-associated gene: It was reported that Ucp2 increases the sensitivity of adult rat cardiomyocytes to hypoxia-reoxygenation by way of ATP depletion and acidosis, which in turn causes accumulation of proapoptotic protein Bcl-2 and 19-kDa interacting protein 3 (Bnip3)[33]. In the first qRT-PCR analysis, the gene expression levels of Ucp2 in the rectus femoris and longissimus lumborum of SJL/J mice at the age of 30 weeks old were higher than those of the control. Therefore, Bnip3 gene expression level was measured to explore the mechanism of degeneration/necrosis in skeletal muscle fibers.

Calcium binding protein gene: In muscular lesions in SJL/J mice, most infiltrating cells are F4/80 antigen-positive macrophages[8]. Recently, it was demonstrated that S100 calcium binding protein A4 (S100A4), a member of the S100 family of Ca^{2+} binding proteins, mediates macrophage recruitment and chemotaxis *in vivo*[34]. Therefore, to find an association between the severity of muscle lesions and S100A4 gene expression levels, qRT-PCR analysis was conducted

Table 1. TaqMan® Gene Expression Assays (Gene-specific Primers and Probes) used in qRT-PCR for the Rectus Femoris and Longissimus Lumborum from Mice at 10 and 30 Weeks of Age

Gene name [Synonym]	Gene symbol	Assay ID
Housekeeping genes		
Glyceraldehyde-3-phosphate dehydrogenase	Gapdh	Mm99999915_g1
Lipid metabolism-associated genes		
Peroxisome proliferative activated receptor, gamma, coactivator 1 alpha	Ppargc1a	Mm00731216_s1
Uncoupling protein 2 (mitochondrial, proton carrier)	Ucp2	Mm00627599_m1
Uncoupling protein 3 (mitochondrial, proton carrier)	Ucp3	Mm01163394_m1
Skeletal muscle atrophy-associated genes		
Myostatin	Mstn	Mm03024050_m1
Follistatin [1)]	Fst	Mm03023987_m1
Ubiquitin-proteasome system-associated genes		
F-box protein 32 [muscle atrophy F-box (MAFbx)/atrogin-1]	Fbxo32	Mm00499523_m1
Tripartite motif-containing 63 [muscle RING-finger protein (MuRF1)]	Trim63	Mm01185221_m1
Forkhead box O3	Foxo3	Mm01185722_m1
Ubiquitin-like protein gene		
Ubiquitin-fold modifier 1	Ufm1	Mm00787190_s1
ER stress-associated genes		
Heat shock protein 5 [immunoglobulin heavy-chain binding protein (Bip)/78 kDa glucose-regulated protein (Grp78)]	Hspa5	Mm00517691_m1
Activating transcription factor 6	Atf6	Mm01295319_m1
DNA-damage inducible transcript 3 [C/EBP homologous protein (Chop)/Gadd153]	Ddit3	Mm00492097_m1
Heat shock protein genes		
Heat shock protein 1 [Hsp25]	Hspb1	Mm00834384_g1
Heat shock protein 1B [Hsp70]	Hspa1b	Mm03038954_s1
Heat shock protein 90, beta (Grp94), member 1 [Grp94]	Hsp90b1	Mm00441926_m1
DnaJ (Hsp40) homolog, subfamily B, member 1 [Hsp40]	Dnajb1	Mm00444519_m1
ER-associated degradation of glycoproteins-associated genes		
ER degradation enhancer, mannosidase alpha-like 1	Edem1	Mm00551797_m1
Protein disulfide isomerase associated 3 [ERp57]	Pdia3	Mm00433130_m1
Calnexin	Canx	Mm00500330_m1
Calreticulin	Calr	Mm00482936_m1
Histone deacetylase genes		
Histone deacetylase 1 [1)]	Hdac1	Mm02745760_g1
Histone deacetylase 2 [1)]	Hdac2	Mm00515117_m1
Histone deacetylase 3 [1)]	Hdac3	Mm00515916_m1
Histone deacetylase 8 [1)]	Hdac8	Mm01224980_m1
Nitric oxide synthase genes		
Nitric oxide synthase 1, neuronal [nNOS] [1)]	Nos1	Mm00435175_m1
Nitric oxide synthase 2, inducible [iNOS] [1)]	Nos2	Mm01309898_m1
Nitric oxide synthase 3, endothelial cell [eNOS] [1)]	Nos3	Mm00435204_m1
Oxidative stress-associated genes		
Heme oxygenase (decycling) 1	Hmox1	Mm00516004_m1
Thioredoxin reductase 1	Txnrd1	Mm00443675_m1
Complement control factor genes		
CD55 antigen [Daf1]	Cd55	Mm00438377_m1
Decay accelerating factor 2	Daf2	Mm00432792_g1

1) These genes were profiled in only the rectus femoris.

using the eight striated muscles separated into three groups based on the severity of muscle lesions in SJL/J mice at an age of 35 weeks old[8].

Immunologically relevant gene: It is known that MHC class I (H2-K1) expression is markedly upregulated in myopathic muscles of dysferlin-deficient SJL/J mice[22]. Therefore, to ascertain whether or not H2-K1 gene expression was systemically upregulated, H2-K1 gene expression level was examined by qRT-PCR analysis.

Telomere-associated genes: Telomere shortening was found in tibialis anterior and diaphragm muscles from mdx mice in comparison with age-matched wild-type mice[35].

Table 2. TaqMan® Gene Expression Assays (Gene-specific Primers and Probes) used in qRT-PCR for the Skeletal Muscles from Mice at 5, 15 and 30 Weeks of Age

Gene name [Synonym]	Gene symbol	Assay ID
Housekeeping gene		
Glyceraldehyde-3-phosphate dehydrogenase	Gapdh	Mm99999915_g1
Lipid metabolism-associated genes		
Uncoupling protein 2 (mitochondrial, proton carrier)	Ucp2	Mm00627599_m1
TBC1 domain family, member 1 [TBC1D1][1)]	Tbc1d1	Mm00497989_m1
Heat shock protein genes		
Heat shock protein 1B [Hsp70]	Hspa1b	Mm03038954_s1
DnaJ (Hsp40) homolog, subfamily B, member 1 [Hsp40]	Dnajb1	Mm00444519_m1
ER-associated degradation of glycoproteins-associated genes		
ER degradation enhancer, mannosidase alpha-like 1	Edem1	Mm00551797_m1
Protein disulfide isomerase associated 3 [ERp57]	Pdia3	Mm00433130_m1
Oxidative stress-associated gene		
Heme oxygenase (decycling) 1	Hmox1	Mm00516004_m1
Complement control factor gene		
CD55 antigen [Daf1]	Cd55	Mm00438377_m1
Apoptosis-associated gene		
Bcl-2 and 19-kDa interacting protein 3[1)]	Bnip3	Mm01275601_g1
Calcium binding protein gene		
S100 calcium binding protein A4[1)]	S100a4	Mm00803372_g1
Immunologically-relevant gene		
Histocompatibility 2, K1, K region [H2-K1][1)]	H2-K1	Mm01612247_mH
Telomere-associated genes		
Telomeric repeat binding factor 1[1)]	Terf1	Mm00436923_m1
Poly (ADP-ribose) polymerase family, member 1[1)]	Parp1	Mm01321084_m1

1) Added TaqMan® Gene expression assays (gene-specific primers and probes).

In addition, it was documented that telomeric repeat binding factor-1 (Terf1) and poly (ADP-ribose) polymerase-1 (Parp1), which control telomere elongation, were overexpressed in the muscles of Duchenne muscular dystrophy[36]. It was ascertained whether or not two telomere elongation control factors were overexpressed in the skeletal muscles of two dysferlinopathy model mice by qRT-PCR.

Triplicate measurements for each gene were conducted and data are presented as the mean and standard deviation. Changes in gene expression are described as fold changes relative to that of controls (the Ct values in BALB/c mice at the age of 5 weeks).

Results

Histopathology

The results of histopathological examination corresponded to our published data[8]. Fig. 1 shows typical histopathological findings in the femoral muscles of BALB/c, SJL/J and A/J mice at 10 and 30 weeks of age.

In brief, at 10 weeks of age, no significant changes were observed in the skeletal muscle fibers of BALB/c and A/J mice, and some skeletal muscles (particularly femoral, brachial, abdominal and lumbar muscles) showed minimal degeneration and/or necrosis of muscle fibers in SJL/J mice. At 30 weeks of age, BALB/c mice did not exhibit histopathological changes in any skeletal muscles. However, the histopathological lesions of skeletal muscles in SJL/J mice progressed in severity and were increasingly frequent with age, and SJL/J mice revealed macrophage infiltration around degeneration and/or necrosis of muscle fibers. In contrast, histological lesions of these skeletal muscles in A/J mice showed a slow progression with age.

qRT-PCR

Tables 3 and 4 show the results of qRT-PCR analysis.

The first qRT-PCR analysis revealed genes that were upregulated more than twice or downregulated less than one-half that of the control in both rectus femoris and longissimus lumborum in one of SJL/J and A/J mice.

Ucp2 as a lipid metabolism-associated gene was upregulated in the rectus femoris and longissimus lumborum of SJL/J mice and downregulated in the longissimus lumborum of A/J mice at 30 weeks of age.

The gene expression level of heat shock protein 70 (Hsp70) in the rectus femoris and longissimus lumborum of BALB/c mice at 30 weeks of age was more than 30 times higher than that of the control. The gene expression level of heat shock protein 40 (Hsp40), which is known to be a co-chaperone regulating Hsp70, in the rectus femoris and longissimus lumborum of BALB/c mice at 30 weeks of age was also more than four times higher than that of the control. Hsp70 was upregulated in the rectus femoris and longissimus lumborum of SJL/J mice at 10 and 30 weeks of age

Table 3. Relative Expression Levels of mRNAs in The Rectus Femoris of BALB/c, SJL/J and A/J Mice

	BALB/c (30W)	SJL/J (10W)	SJL/J (30W)	A/J (10W)	A/J (30W)
	Ratio ± S.D.	Ratio ± S.D.	Ratio ± S.D.	Ratio ± S.D.	Ratio ± S.D.
Ppargc1a [PGC-1 α]	1.092 ± 0.048	0.829 ± 0.038	0.667 ± 0.028	0.988 ± 0.116	1.027 ± 0.029
Ucp2	0.941 ± 0.011	1.084 ± 0.069	2.493 ± 0.096	0.736 ± 0.249	0.685 ± 0.008
Ucp3	1.155 ± 0.038	0.860 ± 0.114	0.606 ± 0.056	0.880 ± 0.222	0.459 ± 0.033
Mstn [myostatin]	0.922 ± 0.056	0.949 ± 0.048	0.525 ± 0.049	1.394 ± 0.058	1.188 ± 0.044
Fst [follistatin]	1.939 ± 0.152	0.639 ± 0.039	1.948 ± 0.049	0.858 ± 0.050	1.529 ± 0.067
Fbxo32 [MAFbx]	2.254 ± 0.055	0.399 ± 0.014	1.492 ± 0.032	1.297 ± 0.134	1.386 ± 0.080
Trim63 [MuRF1]	1.173 ± 0.037	0.574 ± 0.065	0.753 ± 0.033	1.296 ± 0.071	0.555 ± 0.035
Foxo3	0.797 ± 0.026	0.851 ± 0.050	0.712 ± 0.025	1.194 ± 0.236	0.758 ± 0.030
Ufm1	0.817 ± 0.059	0.709 ± 0.029	0.526 ± 0.094	0.749 ± 0.043	0.918 ± 0.026
Hspa5 [Bip/Grp78]	0.894 ± 0.056	1.152 ± 0.142	1.356 ± 0.059	1.015 ± 0.075	0.630 ± 0.022
Atf6	0.882 ± 0.021	0.744 ± 0.085	0.683 ± 0.024	0.887 ± 0.073	0.554 ± 0.018
Ddit3 [Chop]	0.704 ± 0.041	1.569 ± 0.127	0.788 ± 0.030	1.044 ± 0.236	0.662 ± 0.055
Hspb1 [Hsp25]	1.956 ± 0.110	1.801 ± 0.062	1.846 ± 0.058	0.850 ± 0.172	0.868 ± 0.063
Hspa1b [Hsp70]	40.903 ± 2.059	21.411 ± 1.996	14.714 ± 0.729	1.496 ± 0.776	0.746 ± 0.015
Hsp90b1 [Grp94]	0.950 ± 0.053	1.068 ± 0.055	1.241 ± 0.080	0.915 ± 0.129	0.657 ± 0.062
Dnajb1 [Hsp40]	4.968 ± 0.164	4.118 ± 0.392	1.233 ± 0.091	1.387 ± 0.384	0.403 ± 0.009
Edem1	1.452 ± 0.090	1.301 ± 0.061	2.808 ± 0.134	0.957 ± 0.187	0.902 ± 0.060
Pdia3 [ERp57]	0.867 ± 0.036	1.112 ± 0.029	1.445 ± 0.100	1.175 ± 0.064	1.196 ± 0.037
Canx [calnexin]	0.974 ± 0.085	0.883 ± 0.063	1.103 ± 0.207	0.931 ± 0.065	1.018 ± 0.023
Calr [calreticulin]	0.916 ± 0.108	0.917 ± 0.103	1.199 ± 0.101	0.740 ± 0.048	0.756 ± 0.094
Hmox1	1.046 ± 0.029	1.035 ± 0.045	2.983 ± 0.050	1.023 ± 0.036	0.899 ± 0.025
Txnrd1	1.208 ± 0.034	0.851 ± 0.058	1.152 ± 0.023	1.062 ± 0.038	1.000 ± 0.036
Cd55 [Daf1]	0.840 ± 0.033	0.097 ± 0.004	0.127 ± 0.007	0.973 ± 0.036	0.973 ± 0.036
Daf2 (*)	(1.815 ± 0.702)	(Not detected)	(Not detected)	(2.109 ± 1.105)	(2.109 ± 1.105)
Nos1 [nNOS]	1.654 ± 0.091	0.455 ± 0.012	0.607 ± 0.023	1.353 ± 0.060	1.529 ± 0.047
Nos2 [iNOS]	0.618 ± 0.004	0.810 ± 0.022	0.476 ± 0.028	0.538 ± 0.037	0.352 ± 0.013
Nos3 [eNOS]	1.144 ± 0.053	1.207 ± 0.039	1.086 ± 0.008	1.098 ± 0.060	1.015 ± 0.080

Changes in gene expression are presented as fold changes relative to controls (the threshold cycle values in BALB/c mice at the age of 10 weeks old).

*: Because the threshold cycle values were over 30, these data were treated as informal data. Blue shading: The relative gene expression was less than one-half that of the control. Yellow shading: the relative gene expression was more than twice that of the control.

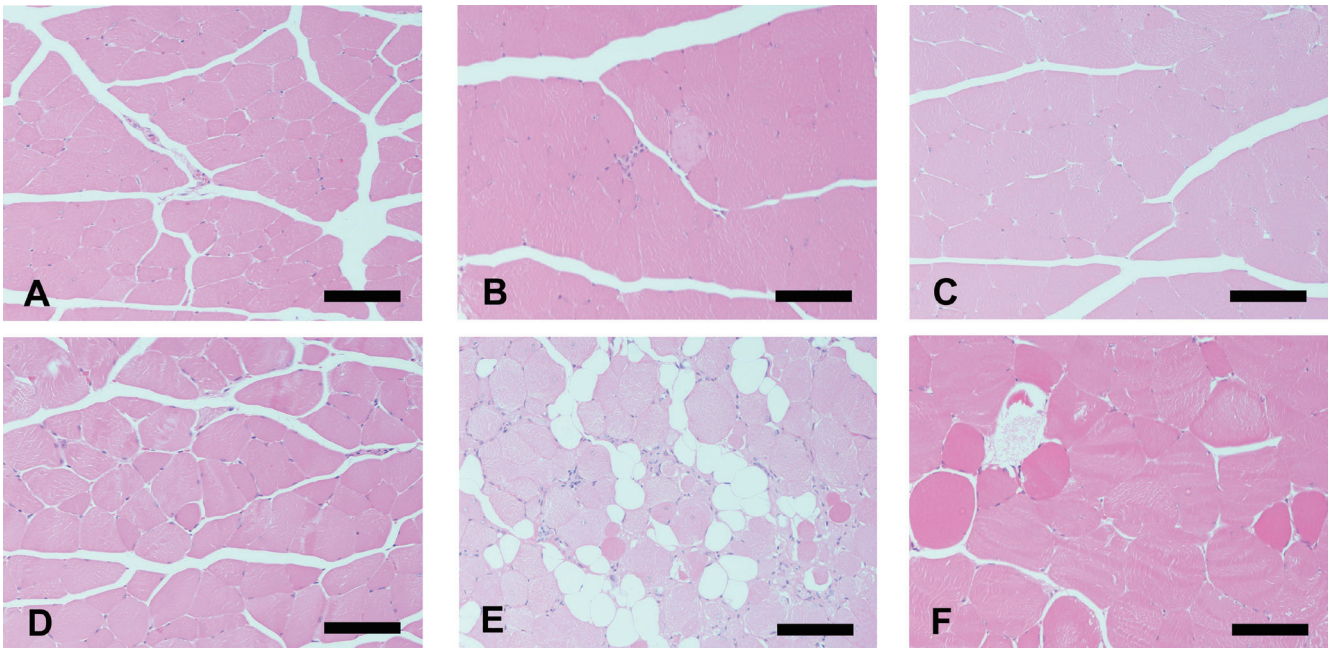

Fig. 1. Histopathology of the rectus femoris in BALB/c, SJL/J and A/J mice. At 10 weeks of age (upper figures), no significant changes are observed in the skeletal muscle fibers of BALB/c (A) and A/J mice (C), and a few muscle fibers show minimal degeneration with mononuclear cell infiltration in SJL/J mice (B). At 30 weeks of age (lower figures), BALB/c mice do not exhibit histopathological changes in any skeletal muscles (D). The histopathological lesions of skeletal muscles in SJL/J mice progress in severity with age and are characterized by the following findings: degenerative/necrotic muscle fibers, centronuclear muscle fibers, fatty infiltration and variation in size of muscle fibers (E). The muscle fibers in A/J mice show only degenerative/necrotic features and variation in size (F). HE staining. Bar: 100 μm.

more than 10 times higher than that of the control. Likewise, Hsp40 was upregulated in the rectus femoris and longissimus lumborum of SJL/J mice at 10 weeks of age more than three times higher than that of the control. However, the gene expression levels of Hsp70 and Hsp40 in SJL/J mice were lower than those of BALB/c mice at 30 weeks of age. Hsp70 and Hsp40 were downregulated in the longissimus lumborum or rectus femoris of A/J mice at 30 weeks of age compared with those of the control.

Edem1 mRNA in the rectus femoris and longissimus lumborum, and ERp57 mRNA in the longissimus lumborum showed high expression levels in SJL/J mice at 30 weeks of age compared with those of the control.

The expression level of Hmox1 mRNA in the rectus femoris and longissimus lumborum of SJL/J mice at 30 weeks of age was more than two times higher than that of the control.

The gene expression level of Daf1/CD55 in the rectus femoris and longissimus lumborum of SJL/J mice at 10 and 30 weeks of age was lower than that of the control.

Additional qRT-PCR

Tables 5 to 7 show the results of an additional qRT-PCR analysis. Fig. 2 indicates the changes of principal genes in the tested muscles.

Lipid metabolism-associated genes: Ucp2 gene expression showed a tendency to be upregulated with age in the rectus femoris and longissimus lumborum of SJL/J mice, but to be downregulated with age in other muscles of SJL/J mice and all muscles of BALB/c and A/J mice. Tbc1d1 gene expression level in SJL/J mice was lowered regardless of age and site.

Heat shock protein genes: The gene expression levels of Hsp70 and Hsp40 in BALB/c mice peaked at 15 weeks of age. Hsp70 gene in the rectus femoris and heart of BALB/c mice exhibited a persistently higher expression level than that of the control. On the other hand, SJL/J mice showed upregulation of Hsp70 gene expression in the rectus femoris, triceps brachii, longissimus lumborum and diaphragm at 30 weeks of age. The gene expression levels of Hsp70 and Hsp40 in most muscles of A/J mice were lower than those of the control.

ER-associated degradation of glycoprotein-associated genes: The rectus femoris and longissimus lumborum of SJL/J mice showed a tendency toward an increase in the gene expression levels of Edem1 and ERp57 at 30 weeks of age.

Oxidative stress-associated genes: Hmox1 gene expression level was increased with age in the longissimus lumborum of SJL/J mice, and there was a trend for an increase in its level in the rectus femoris of SJL/J mice at 30 weeks of age.

Complement control factor genes: Daf1/CD55 gene expression level in BALB/c mice showed a trend to increase by 15 weeks of age. In contrast, SJL/J mice exhibited a marked lowering of Daf1/CD55 gene expression level in the limb, lumbar and abdominal muscles at all ages. There was no abnormality in the gene expression levels of Daf1/CD55 in all muscles of A/J mice and in the heart of SJL/J mice in comparison to that of the control.

Table 4. Relative Expression Levels of MRNAs in The Longissimus Lumborum of BALB/c, SJL/J and A/J Mice

	BALB/c (30W)	SJL/J (10W)	SJL/J (30W)	A/J (10W)	A/J (30W)
	Ratio ± S.D.	Ratio ± S.D.	Ratio ± S.D.	Ratio ± S.D.	Ratio ± S.D.
Ppargc1a [PGC-1 α]	1.284 ± 0.066	0.964 ± 0.024	0.543 ± 0.046	1.094 ± 0.078	0.775 ± 0.013
Ucp2	0.501 ± 0.023	1.419 ± 0.173	2.203 ± 0.048	1.246 ± 0.255	0.481 ± 0.016
Ucp3	0.747 ± 0.035	0.797 ± 0.022	0.187 ± 0.011	0.811 ± 0.124	0.289 ± 0.018
Mstn [myostatin]	1.002 ± 0.049	0.711 ± 0.036	0.171 ± 0.012	1.681 ± 0.170	1.276 ± 0.046
Fbxo32 [MAFbx]	1.825 ± 0.030	0.306 ± 0.010	0.781 ± 0.041	1.256 ± 0.046	1.336 ± 0.033
Trim63 [MuRF1]	0.977 ± 0.036	0.421 ± 0.015	0.552 ± 0.037	1.136 ± 0.088	0.605 ± 0.063
Foxo3	0.963 ± 0.043	0.844 ± 0.021	0.860 ± 0.050	1.338 ± 0.126	0.940 ± 0.028
Ufm1	0.925 ± 0.190	0.696 ± 0.048	0.624 ± 0.130	1.052 ± 0.034	0.765 ± 0.161
Hspa5 [Bip/Grp78]	0.975 ± 0.132	1.329 ± 0.066	1.600 ± 0.282	1.204 ± 0.062	0.674 ± 0.120
Atf6	1.032 ± 0.064	0.750 ± 0.029	0.876 ± 0.119	0.848 ± 0.029	0.626 ± 0.049
Ddit3 [Chop]	1.103 ± 0.018	1.669 ± 0.056	1.157 ± 0.076	1.254 ± 0.061	0.986 ± 0.050
Hspb1 [Hsp25]	1.552 ± 0.036	1.384 ± 0.042	1.934 ± 0.077	0.941 ± 0.072	0.788 ± 0.027
Hspa1b [Hsp70]	32.007 ± 1.253	13.463 ± 1.143	10.095 ± 0.462	0.911 ± 0.111	0.443 ± 0.020
Hsp90b1 [Grp94]	1.114 ± 0.078	1.385 ± 0.090	2.175 ± 0.100	1.023 ± 0.042	0.921 ± 0.039
Dnajb1 [Hsp40]	4.161 ± 0.854	3.103 ± 0.298	1.141 ± 0.240	1.476 ± 0.119	0.478 ± 0.118
Edem1	0.941 ± 0.048	1.546 ± 0.055	2.521 ± 0.099	1.132 ± 0.122	0.626 ± 0.023
Pdia3 [ERp57]	0.864 ± 0.085	1.386 ± 0.113	2.361 ± 0.393	0.887 ± 0.023	0.690 ± 0.066
Canx [calnexin]	1.053 ± 0.105	0.986 ± 0.034	1.368 ± 0.138	1.106 ± 0.047	0.822 ± 0.098
Calr [calreticulin]	1.010 ± 0.014	1.382 ± 0.080	1.873 ± 0.065	1.235 ± 0.089	0.946 ± 0.035
Hmox1	0.866 ± 0.015	1.676 ± 0.048	2.667 ± 0.074	1.066 ± 0.031	0.578 ± 0.013
Txnrd1	1.082 ± 0.048	0.666 ± 0.022	0.444 ± 0.021	1.014 ± 0.040	0.609 ± 0.012
Cd55 [Daf1]	1.000 ± 0.039	0.109 ± 0.003	0.229 ± 0.010	1.072 ± 0.034	0.953 ± 0.092
Daf2 (*)	(1.908 ± 0.259)	(Not detected)	(Not detected)	(1.870 ± 0.619)	(1.100 ± 0.675)

Changes in gene expression are presented as fold changes relative to controls (the threshold cycle values in BALB/c mice at the age of 10 weeks old).

*: Because the threshold cycle values were over 30, these data were treated as informal data. Blue shading: The relative gene expression was less than one-half that of the control. Yellow shading: the relative gene expression was more than twice that of the control.

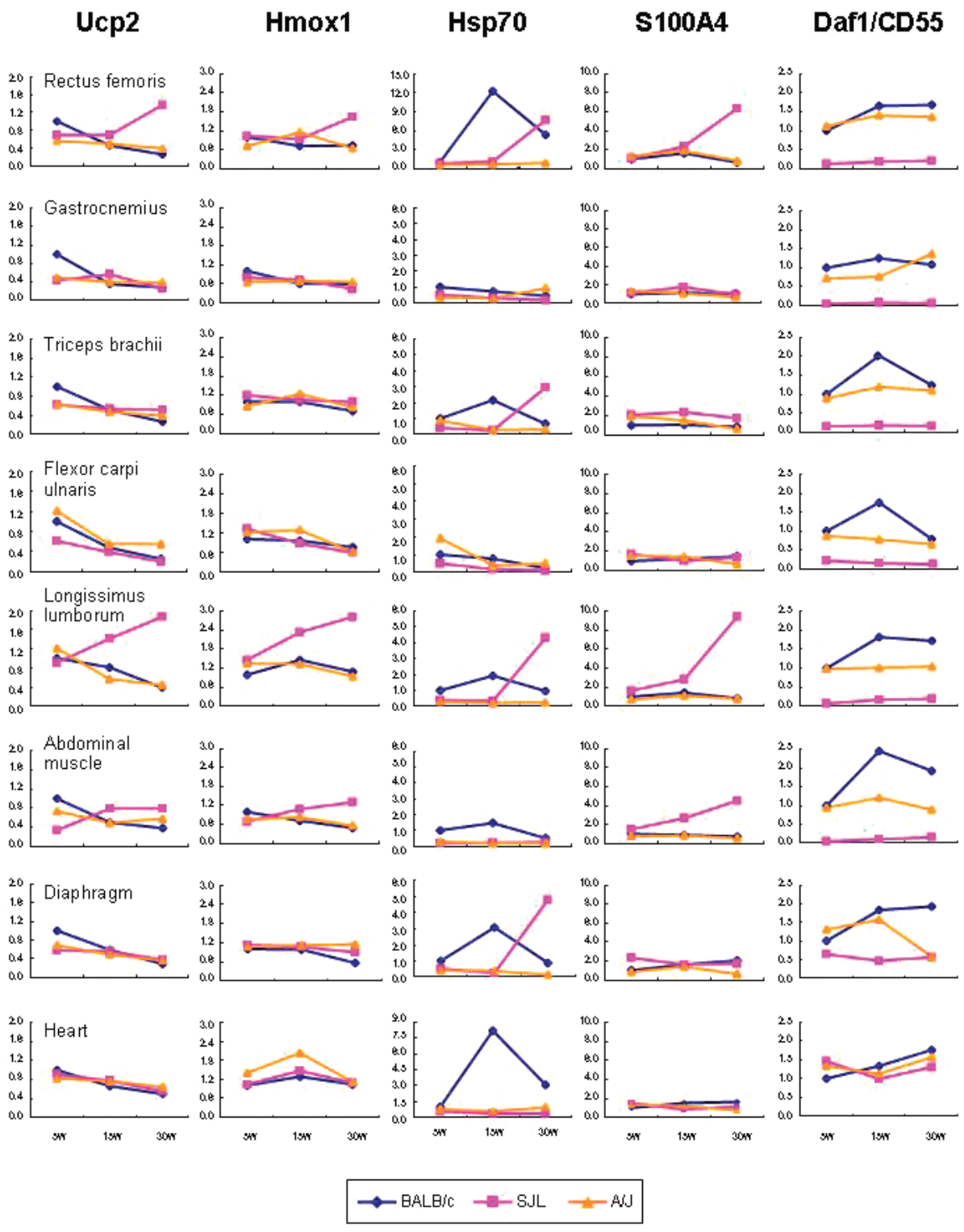

Fig. 2. The changes of principal genes in each muscle site of BALB/c, SJL/J and A/J mice. The presented genes (Ucp2, Hmox1, Hsp70, S100A4 and Daf1/CD55) are those for which the changes are suspected of involvement in muscular lesions observed in SJL/J and A/J mice. The severity of muscular lesions is as follows: lumbar muscle (longissimus lumborum) > femoral muscle (rectus femoris) > abdominal muscle > brachial muscle (triceps brachii) > crural muscle (gastrocnemius) > forearm muscle (flexor carpi ulnaris) > diaphragm. Ucp2 gene expression in the rectus femoris and longissimus lumborum of SJL/J mice shows a tendency to be upregulated with age. Hmox1 gene expression in the rectus femoris and longissimus lumborum of SJL/J mice is upregulated at 30 weeks of age. Hsp70 gene expression levels in most muscles of A/J mice are lower at all ages. S100A4 gene expression in the rectus femoris, longissimus lumborum and abdominal muscles of SJL/J mice is upregulated at 30 weeks of age. Daf1/CD55 gene expression in all studied muscles except for the heart of SJL/J mice shows a marked downregulation at all ages.

Apoptosis-associated genes: Bnip3 mRNA level was sporadically increased in BALB/c mice and was temporally increased in the heart in A/J mice with age.

Calcium binding protein genes: The rectus femoris, longissimus lumborum and abdominal muscles of SJL/J mice showed an increase in S100A4 gene expression level with age.

Immunologically relevant genes: H2-K1 gene was significantly expressed in SJL/J and A/J mice regardless of age and site.

Telomere-associated genes: Terf1 and Parp1 gene expression levels in SJL/J and A/J mice showed no alteration in any site or age compared with those of the control.

Discussion

This study showed that there were interstrain and site-dependent differences in the gene expression profiles of skeletal muscles in dysferlinopathy model mice, SJL/J and A/J mice.

Upon analysis by qRT-PCR, Tbc1d1 gene expression level in SJL/J mice was lowered regardless of age by a mutation in the Tbc1d1 gene responsible for increased fatty acid uptake/oxidation and decreased glucose uptake[14]. In quantitative analysis of the gene expression levels of PGC-1α, Ucp2 and Ucp3 as lipid metabolism-associated genes, mRNA expression level of Ucp2 showed a trend to be upregulated in the rectus femoris and longissimus lumborum of SJL/J mice at 30 weeks of age in contrast to those of BALB/c and A/J mice. Forced expression of Ucp2 in pancreatic islets was found to result in decreased ATP content, and the islet cells of UCP2 knockout mice showed increased ATP level[37]. Overexpression of UCP2 in primary cardiomyocytes led to a significant decline in ATP level and enhanced sensitivity to hypoxia-reoxygenation[33]. Ucp2-mediated energy loss may be related to muscle degeneration/necrosis in SJL/J mice. Tbc1d gene-deficient cells exhibited inhibited trafficking of glucose transporter GLUT4 from intracellular vesicles to plasma membrane[38], which is suggested to show a decrease in intracellular glucose level and a subsequent enhancement of fatty acid oxidation. As a result, the skeletal muscles in SJL/J mice are likely to have uncoupling.

Most upregulation of Edem1, ERp57, Hmox1 and S100A4 was observed in the rectus femoris, longissimus lumborum or abdominal muscles, in which dystrophic lesions occur more commonly in SJL mice. These upregulations approximately coincide with the occurrence of dystrophic changes in these sites.

Edem1 is needed for degradation of misfolded glycoprotein substrates including MHC class I[24]. ERp57 contributes to the formation of native disulfide bonds in nascent MHC class I heavy chains[24]. SJL/J mice show marked upregulation of MHC class I expression in myopathic muscles[22]. In fact, the expression level of MHC class I (H2-K1) gene in the rectus femoris and longissimus lumborum of SJL/J mice was significantly higher regardless of age and site compared

Table 5. Expression Levels of MRNAs in BALB/c Mice at 15 and 30 Weeks of Age Relative to the Control (BALB/c Mice at 5 Weeks of Age)

		Rectus femoris		Gastrocnemius		Triceps brachii		Flexor carpi ulnaris		Longissimus lumborum		Abdominal muscle		Diaphragm		Heart	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BALB/c: 15W	**Ucp2**	0.453	± 0.014	0.355	± 0.020	0.507	± 0.002	0.478	± 0.003	0.814	± 0.026	0.498	± 0.029	0.586	± 0.012	0.666	± 0.025
	Tbc1d1	0.986	± 0.068	0.446	± 0.047	0.666	± 0.150	0.693	± 0.062	1.139	± 0.043	0.540	± 0.064	0.532	± 0.021	0.809	± 0.106
	Hsp70	12.279	± 0.226	0.720	± 0.033	2.164	± 0.087	0.766	± 0.062	1.919	± 0.043	1.471	± 0.036	3.067	± 0.080	8.188	± 0.283
	Hsp40	1.412	± 0.086	0.414	± 0.010	0.744	± 0.012	0.558	± 0.022	0.821	± 0.020	0.661	± 0.014	0.786	± 0.011	1.099	± 0.028
	Edem1	0.799	± 0.026	0.598	± 0.038	0.762	± 0.081	0.600	± 0.048	0.974	± 0.070	0.708	± 0.022	1.248	± 0.033	0.937	± 0.011
	ERp57	0.980	± 0.038	0.754	± 0.008	0.914	± 0.006	0.906	± 0.025	1.200	± 0.052	0.847	± 0.037	1.257	± 0.044	1.391	± 0.025
	Hmox1	0.717	± 0.027	0.599	± 0.026	1.002	± 0.050	0.944	± 0.024	1.474	± 0.036	0.712	± 0.024	0.991	± 0.018	1.289	± 0.045
	Daf1	1.660	± 0.054	1.245	± 0.022	2.014	± 0.043	1.744	± 0.032	1.823	± 0.019	2.462	± 0.089	1.832	± 0.007	1.332	± 0.065
	Bnip3	1.951	± 0.039	1.519	± 0.006	1.544	± 0.168	1.849	± 0.007	2.004	± 0.167	2.350	± 0.047	1.794	± 0.021	1.555	± 0.137
	S100a4	1.608	± 0.047	1.126	± 0.043	1.063	± 0.065	1.221	± 0.064	1.445	± 0.167	0.861	± 0.078	1.614	± 0.089	1.494	± 0.181
	H2-K1	1.679	± 0.273	0.978	± 0.099	2.301	± 0.425	1.323	± 0.216	2.890	± 0.383	1.226	± 0.240	1.029	± 0.044	1.747	± 0.244
	Terf1	1.643	± 0.177	0.599	± 0.048	0.669	± 0.047	0.902	± 0.070	1.012	± 0.134	0.884	± 0.198	0.672	± 0.045	0.674	± 0.043
	Parp1	0.661	± 0.221	0.465	± 0.020	0.521	± 0.037	0.573	± 0.072	0.648	± 0.060	0.806	± 0.059	0.490	± 0.105	0.556	± 0.043
BALB/c: 30W	**Ucp2**	0.254	± 0.009	0.281	± 0.008	0.266	± 0.009	0.252	± 0.036	0.387	± 0.036	0.374	± 0.012	0.291	± 0.010	0.500	± 0.017
	Tbc1d1	1.053	± 0.008	0.413	± 0.017	0.543	± 0.023	0.736	± 0.031	1.007	± 0.012	0.343	± 0.012	0.335	± 0.016	0.660	± 0.021
	Hsp70	5.371	± 0.205	0.454	± 0.046	0.657	± 0.011	0.217	± 0.034	0.973	± 0.050	0.484	± 0.009	0.851	± 0.030	3.103	± 0.108
	Hsp40	0.881	± 0.037	0.322	± 0.015	0.435	± 0.011	0.522	± 0.015	0.738	± 0.003	0.347	± 0.006	0.316	± 0.007	0.779	± 0.014
	Edem1	0.612	± 0.022	0.542	± 0.016	0.520	± 0.046	0.473	± 0.017	0.783	± 0.038	0.520	± 0.031	0.482	± 0.008	0.473	± 0.008
	ERp57	0.759	± 0.035	0.669	± 0.003	0.624	± 0.011	0.663	± 0.078	0.931	± 0.050	0.710	± 0.032	0.855	± 0.025	1.338	± 0.042
	Hmox1	0.731	± 0.012	0.592	± 0.010	0.724	± 0.041	0.748	± 0.037	1.105	± 0.036	0.479	± 0.021	0.564	± 0.016	1.028	± 0.039
	Daf1	1.679	± 0.064	1.090	± 0.071	1.232	± 0.047	0.791	± 0.090	1.717	± 0.007	1.935	± 0.015	1.937	± 0.063	1.765	± 0.019
	Bnip3	1.855	± 0.071	1.632	± 0.063	1.143	± 0.064	2.273	± 0.121	1.977	± 0.064	1.733	± 0.068	2.271	± 0.031	2.499	± 0.130
	S100a4	0.678	± 0.047	1.043	± 0.124	0.821	± 0.093	1.480	± 0.163	0.828	± 0.098	0.740	± 0.031	2.067	± 0.107	1.623	± 0.013
	H2-K1	0.910	± 0.164	0.424	± 0.131	0.910	± 0.144	0.170	±	1.542	± 0.176	0.907	± 0.235	0.259	± 0.017	1.182	± 0.075
	Terf1	2.035	± 0.204	1.559	± 0.161	1.019	± 0.115	0.684	± 0.189	2.335	± 0.221	1.546	± 0.113	1.646	± 0.098	1.766	± 0.178
	Parp1	0.929	± 0.013	0.597	± 0.243	0.638	± 0.043	0.574	± 0.103	0.964	± 0.274	0.986	± 0.138	0.759	± 0.029	1.008	± 0.260

Changes in gene expression are presented as fold changes relative to controls (the threshold cycle values in BALB/c mice at 5 weeks of age).
Blue shading: The relative gene expression was less than one-half that of the control. Yellow shading: the relative gene expression was more than twice that of the control.

Table 6. Expression Levels of MRNAs in SJL/J Mice at 5, 15 and 30 Weeks of Age Relative to the Control (BALB/c Mice at 5 Weeks of Age)

		Rectus femoris	Gastrocnemius	Triceps brachii	Flexor carpi ulnaris	Longissimus lumborum	Abdominal muscle	Diaphragm	Heart
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean SD	Mean SD	Mean SD
SJL/J: 5W	**Ucp2**	0.685 ± 0.041	0.428 ± 0.006	0.624 ± 0.009	0.613 ± 0.030	0.909 ± 0.038	0.338 ± 0.015	0.573 ± 0.014	0.916 ± 0.031
	Tbc1d1	0.037 ± 0.000	0.024 ± 0.001	0.034 ± 0.002	0.073 ± 0.002	0.024 ± 0.002	0.012 ± 0.001	0.098 ± 0.005	0.184 ± 0.005
	Hsp70	0.756 ± 0.039	0.495 ± 0.043	0.400 ± 0.013	0.481 ± 0.049	0.353 ± 0.009	0.180 ± 0.007	0.499 ± 0.017	0.522 ± 0.014
	Hsp40	0.506 ± 0.023	0.350 ± 0.021	0.396 ± 0.007	0.597 ± 0.004	0.495 ± 0.006	0.272 ± 0.002	0.458 ± 0.014	0.584 ± 0.009
	Edem1	0.972 ± 0.040	0.745 ± 0.056	0.978 ± 0.112	0.739 ± 0.033	1.059 ± 0.030	0.847 ± 0.033	1.080 ± 0.062	1.022 ± 0.052
	ERp57	1.140 ± 0.077	0.825 ± 0.003	1.095 ± 0.040	1.149 ± 0.056	1.118 ± 0.066	0.648 ± 0.030	1.636 ± 0.013	1.569 ± 0.023
	Hmox1	1.028 ± 0.060	0.794 ± 0.025	1.195 ± 0.068	1.314 ± 0.041	1.471 ± 0.054	0.685 ± 0.036	1.141 ± 0.032	1.035 ± 0.027
	Daf1	0.121 ± 0.003	0.056 ± 0.004	0.155 ± 0.005	0.217 ± 0.014	0.075 ± 0.000	0.037 ± 0.000	0.644 ± 0.043	1.454 ± 0.038
	Bnip3	1.208 ± 0.161	1.307 ± 0.036	0.956 ± 0.018	0.998 ± 0.050	0.638 ± 0.039	0.125 ± 0.007	0.883 ± 0.013	1.005 ± 0.021
	S100a4	1.113 ± 0.016	1.124 ± 0.062	2.069 ± 0.143	1.710 ± 0.052	1.693 ± 0.111	1.476 ± 0.062	2.330 ± 0.102	1.477 ± 0.073
	H2-K1	14094.680 ± 838.619	10196.288 ± 742.223	25660.767 ± 3392.968	15049.757 ± 1360.745	33107.335 ± 2549.355	13470.626 ± 730.871	18386.032 ± 3923.541	39504.601 ± 1587.689
	Terf1	1.126 ± 0.213	0.648 ± 0.145	0.784 ± 0.100	0.690 ± 0.093	0.767 ± 0.031	0.326 ± 0.025	1.023 ± 0.329	1.159 ± 0.037
	Parp1	0.785 ± 0.036	0.491 ± 0.067	0.559 ± 0.028	0.663 ± 0.171	0.609 ± 0.045	0.393 ± 0.069	0.696 ± 0.097	1.036 ± 0.022
SJL/J: 15W	**Ucp2**	0.686 ± 0.060	0.561 ± 0.015	0.535 ± 0.004	0.386 ± 0.014	1.408 ± 0.080	0.794 ± 0.039	0.552 ± 0.013	0.777 ± 0.024
	Tbc1d1	0.079 ± 0.004	0.051 ± 0.003	0.063 ± 0.002	0.091 ± 0.007	0.085 ± 0.010	0.064 ± 0.008	0.128 ± 0.021	0.210 ± 0.013
	Hsp70	1.085 ± 0.095	0.277 ± 0.015	0.248 ± 0.030	0.172 ± 0.011	0.326 ± 0.010	0.199 ± 0.010	0.244 ± 0.006	0.359 ± 0.016
	Hsp40	0.442 ± 0.046	0.316 ± 0.006	0.302 ± 0.017	0.416 ± 0.015	0.689 ± 0.003	0.298 ± 0.011	0.402 ± 0.022	0.533 ± 0.009
	Edem1	0.827 ± 0.046	0.599 ± 0.027	0.620 ± 0.031	0.429 ± 0.012	1.215 ± 0.076	0.723 ± 0.051	0.739 ± 0.010	0.753 ± 0.018
	ERp57	0.930 ± 0.052	0.688 ± 0.010	0.709 ± 0.003	0.511 ± 0.029	1.014 ± 0.024	0.590 ± 0.064	0.771 ± 0.032	1.045 ± 0.042
	Hmox1	0.935 ± 0.032	0.714 ± 0.026	1.071 ± 0.030	0.865 ± 0.048	2.331 ± 0.118	1.081 ± 0.013	1.062 ± 0.027	1.465 ± 0.141
	Daf1	0.183 ± 0.003	0.085 ± 0.006	0.178 ± 0.004	0.150 ± 0.002	0.163 ± 0.014	0.097 ± 0.004	0.459 ± 0.012	0.986 ± 0.028
	Bnip3	1.894 ± 0.179	1.816 ± 0.099	1.152 ± 0.132	0.976 ± 0.048	1.228 ± 0.112	0.906 ± 0.063	1.245 ± 0.149	1.498 ± 0.130
	S100a4	2.340 ± 0.164	1.757 ± 0.182	2.322 ± 0.254	1.018 ± 0.115	2.816 ± 0.213	2.669 ± 0.180	1.595 ± 0.143	0.858 ± 0.033
	H2-K1	67940.349 ± 14310.272	32770.360 ± 4903.434	45394.548 ± 2830.483	17422.364 ± 3098.475	116282.673 ± 7615.842	38721.276 ± 7707.629	19529.714 ± 1720.340	38993.776 ± 2541.751
	Terf1	1.553 ± 0.283	0.913 ± 0.082	0.882 ± 0.320	0.575 ± 0.016	0.872 ± 0.066	0.414 ± 0.006	0.768 ± 0.108	0.884 ± 0.034
	Parp1	1.005 ± 0.126	0.743 ± 0.047	0.780 ± 0.132	0.778 ± 0.049	1.019 ± 0.056	0.898 ± 0.187	0.921 ± 0.110	0.958 ± 0.014
SJL/J: 30W	**Ucp2**	1.372 ± 0.016	0.252 ± 0.003	0.507 ± 0.009	0.205 ± 0.023	1.872 ± 0.124	0.786 ± 0.031	0.382 ± 0.007	0.559 ± 0.013
	Tbc1d1	0.038 ± 0.001	0.016 ± 0.000	0.040 ± 0.002	0.051 ± 0.003	0.036 ± 0.001	0.018 ± 0.001	0.066 ± 0.003	0.156 ± 0.002
	Hsp70	7.691 ± 0.489	0.157 ± 0.013	2.941 ± 0.054	0.093 ± 0.027	4.298 ± 0.102	0.242 ± 0.008	4.802 ± 0.153	0.357 ± 0.009
	Hsp40	0.634 ± 0.020	0.199 ± 0.011	0.442 ± 0.015	0.308 ± 0.005	0.691 ± 0.013	0.274 ± 0.008	0.428 ± 0.002	0.424 ± 0.028
	Edem1	1.565 ± 0.142	0.417 ± 0.022	0.822 ± 0.046	0.431 ± 0.023	1.859 ± 0.136	0.869 ± 0.051	1.082 ± 0.037	0.839 ± 0.104
	ERp57	1.578 ± 0.076	0.431 ± 0.016	0.768 ± 0.009	0.544 ± 0.053	1.845 ± 0.091	0.849 ± 0.045	1.292 ± 0.014	1.192 ± 0.026
	Hmox1	1.635 ± 0.057	0.427 ± 0.017	1.007 ± 0.048	0.589 ± 0.015	2.817 ± 0.127	1.295 ± 0.039	0.897 ± 0.019	1.089 ± 0.034
	Daf1	0.209 ± 0.006	0.067 ± 0.003	0.165 ± 0.002	0.128 ± 0.018	0.196 ± 0.002	0.154 ± 0.005	0.559 ± 0.004	1.299 ± 0.059
	Bnip3	0.850 ± 0.111	1.220 ± 0.047	1.194 ± 0.050	1.792 ± 0.113	0.955 ± 0.079	0.754 ± 0.013	1.167 ± 0.092	1.369 ± 0.024
	S100a4	6.311 ± 0.110	0.932 ± 0.049	1.703 ± 0.086	1.411 ± 0.049	9.448 ± 0.628	4.476 ± 0.231	1.717 ± 0.032	1.127 ± 0.031
	H2-K1	73038.969 ± 4547.809	15429.124 ± 306.924	53929.274 ± 4826.590	12486.706 ± 1562.356	140693.694 ± 12160.936	60039.000 ± 2525.374	19289.769 ± 2158.350	42522.582 ± 1107.967
	Terf1	1.478 ± 0.240	0.799 ± 0.098	0.865 ± 0.340	0.719 ± 0.078	1.237 ± 0.051	0.740 ± 0.210	1.015 ± 0.049	1.014 ± 0.102
	Parp1	0.653 ± 0.121	0.638 ± 0.031	0.696 ± 0.207	0.703 ± 0.012	0.554 ± 0.037	0.599 ± 0.210	0.855 ± 0.320	0.795 ± 0.082

Changes in gene expression are presented as fold changes relative to controls (the threshold cycle values in BALB/c mice at 5 weeks of age).
Blue shading: The relative gene expression was less than one-half that of the control. Yellow shading: the relative gene expression was more than twice that of the control.

Table 7. Expression Levels of MRNAs in A/J Mice at 5, 15 and 30 Weeks of Age Relative to the Control (BALB/c Mice at 5 Weeks of Age)

		Rectus femoris	Gastrocnemius	Triceps brachii	Flexor carpi ulnaris	Longissimus lumborum	Abdominal muscle	Diaphragm	Heart
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean SD	Mean SD	Mean SD
A/J: 5W	**Ucp2**	0.558 ± 0.025	0.494 ± 0.009	0.634 ± 0.015	1.216 ± 0.018	1.214 ± 0.047	0.733 ± 0.024	0.694 ± 0.054	0.820 ± 0.018
	Tbc1d1	0.801 ± 0.055	0.430 ± 0.051	0.613 ± 0.115	0.941 ± 0.133	0.732 ± 0.028	0.290 ± 0.010	0.793 ± 0.049	1.161 ± 0.090
	Hsp70	0.585 ± 0.030	0.337 ± 0.026	0.865 ± 0.046	1.934 ± 0.142	0.305 ± 0.011	0.258 ± 0.008	0.397 ± 0.023	0.734 ± 0.034
	Hsp40	0.344 ± 0.012	0.328 ± 0.013	0.334 ± 0.024	0.652 ± 0.014	0.496 ± 0.002	0.254 ± 0.002	0.398 ± 0.004	0.532 ± 0.040
	Edem1	0.615 ± 0.015	0.669 ± 0.023	0.662 ± 0.060	0.676 ± 0.073	0.910 ± 0.054	0.557 ± 0.022	1.197 ± 0.013	0.895 ± 0.037
	ERp57	0.776 ± 0.019	0.767 ± 0.030	0.809 ± 0.017	0.919 ± 0.045	0.946 ± 0.045	0.699 ± 0.042	1.131 ± 0.048	1.458 ± 0.049
	Hmox1	0.712 ± 0.067	0.649 ± 0.035	0.861 ± 0.053	1.209 ± 0.026	1.357 ± 0.038	0.772 ± 0.024	1.084 ± 0.027	1.401 ± 0.039
	Daf1	1.126 ± 0.055	0.716 ± 0.041	0.885 ± 0.038	0.880 ± 0.013	0.989 ± 0.012	0.948 ± 0.028	1.308 ± 0.042	1.328 ± 0.103
	Bnip3	1.285 ± 0.044	0.716 ± 0.051	0.811 ± 0.012	1.032 ± 0.111	1.059 ± 0.008	0.979 ± 0.122	1.021 ± 0.034	1.488 ± 0.053
	S100a4	1.312 ± 0.041	1.329 ± 0.150	1.946 ± 0.067	1.571 ± 0.250	0.714 ± 0.078	0.764 ± 0.064	0.816 ± 0.029	1.324 ± 0.023
	H2-K1	7962.612 ± 1029.356	9258.174 ± 781.698	17191.121 ± 2114.664	13125.964 ± 4386.809	19238.929 ± 1578.524	7231.714 ± 276.500	10761.827 ± 371.363	17123.302 ± 912.345
	Terf1	1.160 ± 0.203	0.778 ± 0.066	0.694 ± 0.145	1.001 ± 0.240	1.470 ± 0.212	0.705 ± 0.135	1.366 ± 0.232	1.152 ± 0.067
	Parp1	0.880 ± 0.122	0.740 ± 0.016	0.632 ± 0.126	0.842 ± 0.026	0.880 ± 0.064	0.873 ± 0.078	0.774 ± 0.140	0.854 ± 0.027
A/J: 15W	**Ucp2**	0.484 ± 0.014	0.399 ± 0.006	0.462 ± 0.069	0.545 ± 0.011	0.561 ± 0.069	0.492 ± 0.022	0.486 ± 0.029	0.765 ± 0.011
	Tbc1d1	1.159 ± 0.052	0.653 ± 0.032	1.114 ± 0.124	1.722 ± 0.090	1.096 ± 0.081	1.531 ± 0.325	1.039 ± 0.184	1.241 ± 0.086
	Hsp70	0.639 ± 0.052	0.278 ± 0.012	0.254 ± 0.017	0.388 ± 0.034	0.192 ± 0.010	0.159 ± 0.020	0.371 ± 0.020	0.560 ± 0.025
	Hsp40	0.383 ± 0.004	0.317 ± 0.022	0.355 ± 0.021	0.470 ± 0.005	0.426 ± 0.023	0.276 ± 0.007	0.435 ± 0.017	0.655 ± 0.048
	Edem1	0.535 ± 0.007	0.419 ± 0.016	0.434 ± 0.047	0.428 ± 0.038	0.488 ± 0.027	0.468 ± 0.033	0.659 ± 0.007	0.757 ± 0.060
	ERp57	0.625 ± 0.077	0.501 ± 0.014	0.502 ± 0.024	0.582 ± 0.052	0.581 ± 0.016	0.474 ± 0.059	0.815 ± 0.036	1.026 ± 0.044
	Hmox1	1.145 ± 0.028	0.687 ± 0.038	1.252 ± 0.092	1.276 ± 0.087	1.336 ± 0.130	0.836 ± 0.058	1.123 ± 0.063	2.032 ± 0.029
	Daf1	1.405 ± 0.037	0.763 ± 0.023	1.198 ± 0.046	0.772 ± 0.024	1.007 ± 0.021	1.211 ± 0.109	1.574 ± 0.083	1.118 ± 0.071
	Bnip3	2.230 ± 0.015	1.577 ± 0.057	1.675 ± 0.128	1.812 ± 0.082	1.945 ± 0.177	1.828 ± 0.071	1.191 ± 0.075	1.591 ± 0.036
	S100a4	1.894 ± 0.060	1.041 ± 0.201	1.491 ± 0.111	1.420 ± 0.101	1.094 ± 0.054	0.857 ± 0.063	1.370 ± 0.065	1.145 ± 0.292
	H2-K1	12448.979 ± 1347.142	11786.526 ± 2121.294	73175.562 ± 2809.291	82781.939 ± 1511.291	48883.924 ± 11310.439	43866.874 ± 5814.681	15532.230 ± 1286.573	64223.901 ± 4057.869
	Terf1	1.220 ± 0.142	0.978 ± 0.106	1.054 ± 0.163	0.984 ± 0.194	1.252 ± 0.061	0.994 ± 0.059	0.881 ± 0.072	1.004 ± 0.082
	Parp1	1.038 ± 0.126	0.798 ± 0.218	1.023 ± 0.273	0.983 ± 0.168	1.291 ± 0.089	1.518 ± 0.090	1.039 ± 0.208	0.949 ± 0.031
A/J: 30W	**Ucp2**	0.385 ± 0.015	0.418 ± 0.008	0.393 ± 0.019	0.541 ± 0.090	0.439 ± 0.019	0.577 ± 0.062	0.365 ± 0.048	0.645 ± 0.031
	Tbc1d1	0.786 ± 0.047	0.640 ± 0.042	0.783 ± 0.076	1.290 ± 0.121	0.946 ± 0.072	1.137 ± 0.032	1.015 ± 0.064	0.932 ± 0.070
	Hsp70	0.929 ± 0.107	0.246 ± 0.008	0.286 ± 0.017	0.485 ± 0.071	0.247 ± 0.024	0.156 ± 0.003	0.145 ± 0.018	0.952 ± 0.061
	Hsp40	0.352 ± 0.005	0.349 ± 0.026	0.329 ± 0.024	0.390 ± 0.033	0.430 ± 0.021	0.246 ± 0.018	0.580 ± 0.039	0.319 ± 0.003
	Edem1	0.570 ± 0.024	0.437 ± 0.009	0.517 ± 0.031	0.347 ± 0.035	0.445 ± 0.022	0.574 ± 0.049	0.679 ± 0.068	0.924 ± 0.006
	ERp57	0.663 ± 0.028	0.451 ± 0.027	0.472 ± 0.005	0.398 ± 0.059	0.564 ± 0.049	0.457 ± 0.063	1.128 ± 0.097	0.610 ± 0.061
	Hmox1	0.655 ± 0.034	0.546 ± 0.006	0.837 ± 0.033	0.604 ± 0.067	0.947 ± 0.092	0.574 ± 0.044	1.147 ± 0.062	1.109 ± 0.019
	Daf1	1.361 ± 0.173	0.684 ± 0.028	1.095 ± 0.049	0.657 ± 0.112	1.048 ± 0.104	0.888 ± 0.099	0.566 ± 0.247	1.567 ± 0.096
	Bnip3	1.520 ± 0.006	1.454 ± 0.133	1.479 ± 0.045	1.758 ± 0.309	1.855 ± 0.193	1.878 ± 0.013	1.180 ± 0.164	2.860 ± 0.234
	S100a4	0.843 ± 0.116	0.661 ± 0.065	0.625 ± 0.031	0.678 ± 0.057	0.797 ± 0.077	0.575 ± 0.037	0.606 ± 0.021	0.725 ± 0.065
	H2-K1	12182.773 ± 1181.766	9144.340 ± 1240.724	21876.887 ± 2937.865	15482.863 ± 2620.605	41758.456 ± 5398.266	40037.724 ± 5770.926	26991.288 ± 7564.804	26883.006 ± 1809.722
	Terf1	1.298 ± 0.142	1.165 ± 0.078	1.022 ± 0.153	1.138 ± 0.141	1.748 ± 0.311	0.837 ± 0.240	1.389 ± 0.058	0.937 ± 0.280
	Parp1	0.724 ± 0.066	1.087 ± 0.054	1.038 ± 0.099	0.919 ± 0.127	1.632 ± 0.159	1.217 ± 0.457	1.298 ± 0.182	0.945 ± 0.134

Changes in gene expression are presented as fold changes relative to controls (the threshold cycle values in BALB/c mice at 5 weeks of age).
Blue shading: The relative gene expression was less than one-half that of the control. Yellow shading: the relative gene expression was more than twice that of the control.

with those of A/J and BALB/c mice in our study. Therefore, gene changes of Edem1 and ERp57 in SJL/J mice may be associated with a very marked upregulation of H2-K1 expression in the rectus femoris and longissimus lumborum.

Hmox1 provides the first line of defense against oxidative stress because it rapidly responds to oxidants[39]. However, Txnrd1, which is a part of the anti-oxidation system as well as Hmox1, was not upregulated in any muscles of SJL/J mice. Recently, calcium-dependent upregulation of Hsp70 and Hmox-1 in skeletal muscle cells or hepatocytes was reported[40,41]. Because dysferlin null muscle fibers are defective in Ca^{2+}-dependent resealing of sarcolemma disruptions[42], these muscle fibers may cause persistent calcium influx into the cytoplasm after membrane injury. The gene expression levels of Hmox1 were correlated with the severity of histopathological lesions in femoral (rectus femoris), lumbar (longissimus lumborum) and abdominal muscles; calcium influx into the cytoplasm following muscle injury may induce Hmox1 gene expression. However, Hsp70 was also upregulated in the diaphragm of SJL/J mice, in where there were few histopathological changes at all examined times. In addition, change of Hsp70 gene expression level was not observed in the abdominal muscles of SJL/J mice, in which histopathological changes were found at 30 weeks of age. The above muscles and hearts without histopathological abnormalities in BALB/c mice exhibited upregulation of this gene expression from 15 weeks of age. It was reported that significant increases in Hsp70 were observed at 12 weeks postpartum in normal rats[43]. The physiological gene expression mechanism of Hsp70 may develop somewhat later in these muscles excluding the heart of SJL/J mice. Therefore, an unknown factor other than persistent calcium influx may also cause Hmox1 induction in these muscles of SJL/J mice.

In contrast, the gene expression levels of Hsp70 in most muscles of A/J mice were lower than that of the control. Loss of *fer-1*, dysferlin homolog, in *C. elegans* causes downregulation of *hsp-70*[44]. It is possible that the downregulation of Hsp70 gene expression in the skeletal muscles of A/J mice is caused by the functional loss of dysferlin.

In muscle lesions in SJL/J mice, most infiltrating cells are F4/80 antigen-positive macrophages[8]. Recently, it was demonstrated that S100A4, a member of the S100 family of Ca^{2+}-binding proteins, mediates macrophage recruitment and chemotaxis *in vivo*[34]. S100A4 upregulation in the rectus femoris and longissimus lumborum of SJL/J mice may be linked to muscle pathological characteristics in SJL/J mice.

SJL/J mice exhibited a marked lowering of Daf1/CD55 gene expression level in all studied muscles except for the heart at all ages compared with that of BALB/c mice. In contrast, there was no predominant difference in the Daf1/CD55 gene expression levels of A/J mice compared with that of BALB/c mice. It was reported that the gene expression of Daf1/CD55 as a complement inhibitor was downregulated in the skeletal muscles of LGMD2B patients or SJL/J mice[30]. Moreover, the serum concentration of the fifth component of complement (C5) in SJL/J mice is known to be significantly greater than that of other strains[45]. On the other hand, A/J mice are genetically deficient in C5[46]. These results show the possibility that the difference in sensitivity to complement-dependent cytotoxicity causes the difference in phenotype between the two dysferlin-deficient mice. However, the downregulation of Daf1/CD55 alone cannot explain site-specificity of muscle lesions.

In LGMD2B patients or SJL mice, it is shown that MHC class I is overexpressed on muscle fibers[22,47]. Meanwhile, the results of study using SJL.129P2(B6)-B2m^{tm1Unc} mice developed by targeted gene mutation of β-2-microglobulin, which is required for proper assembly of MHC class I proteins on the cell surface, revealed that MHC class I is not required for the appearance of spontaneous myopathy in SJL/J mice[22]. Despite prolonged overexpression of MHC class I gene, there were few histopathological changes in the heart and diaphragm of SJL/J mice or A/J mice in our previous study[8]. Accordingly, it was suggested that upregulation of MHC class I in SJL/J and A/J mice was not directly associated with the progression of dystrophic lesions.

This study showed that there were some interstrain differences in the gene expression profiles of skeletal muscles between SJL/J and A/J mice (both dysferlinopathy model mice). The genes, the changes of which correlate with the severity of muscular lesion, were Ucp2, Hmox1 and S100A4 in SJL/J mice. SJL/J mice showed a marked downregulation of Daf1/CD55 gene expression in all studied muscles except for the heart at all ages. The downregulation of Hsp70 gene expression was observed in the examined skeletal muscles of A/J mice. Further investigation is required to reveal whether alterations of their expression levels are the cause of dystrophic changes or occur subsequent to muscle damage.

Acknowledgments: The authors are grateful to all the staff of Tanabe R&D Service Co., Ltd. for their help with maintenance of animals.

References

1. Cohn RD, and Campbell KP. Molecular basis of muscular dystrophies. Muscle Nerve. **23**: 1456–1471. 2000.[Medline] [CrossRef]
2. Han R, Bansal D, Miyake K, Muniz VP, Weiss RM, McNeil PL, and Campbell KP. Dysferlin-mediated membrane repair protects the heart from stress-induced left ventricular injury. J Clin Invest. **117**: 1805–1813. 2007.[Medline] [CrossRef]
3. Glover L, and Brown Jr RH. Dysferlin in membrane trafficking and patch repair. Traffic. **8**: 785–794. 2007.[Medline] [CrossRef]
4. Bittner RE, Anderson LV, Burkhardt E, Bashir R, Vafiadaki E, Ivanova S, Raffelsberger T, Maerk I, Hoger H, Jung M, Karbasiyan M, Storch M, Lassmann H, Moss JA, Davison K, Harrison R, Bushby KM, and Reis A. Dysferlin deletion in SJL mice (SJL-*Dysf*) defines a natural model for limb

girdle muscular dystrophy 2B. Nat Genet. **23**: 141–142. 1999.[Medline] [CrossRef]
5. Ho M, Post CM, Donahue LR, Lidov HG, Bronson RT, Goolsby H, Watkins SC, Cox GA, and Brown Jr RH. Disruption of muscle membrane and phenotype divergence in two novel mouse models of dysferlin deficiency. Hum Mol Genet. **13**: 1999–2010. 2004.[Medline] [CrossRef]
6. Vafiadaki E, Reis A, Keers S, Harrison R, Anderson LV, Raffelsberger T, Ivanova S, Hoger H, Bittner RE, Bushby K, and Bashir R. Cloning of the mouse dysferlin gene and genomic characterization of the SJL-Dysf mutation. Neuroreport. **12**: 625–629. 2001.[Medline] [CrossRef]
7. Rizo J, and Sudhof TC. C_2-domains, structure and function of a universal Ca^{2+}-binding domain. J Biol Chem. **273**: 15879–15882. 1998.[Medline] [CrossRef]
8. Kobayashi K, Izawa T, Kuwamura M, and Yamate J. The distribution and characterization of skeletal muscle lesions in dysferlin-deficient SJL and A/J mice. Exp Toxicol Pathol. **62**: 509–517. 2010.[Medline] [CrossRef]
9. Campanaro S, Romualdi C, Fanin M, Celegato B, Pacchioni B, Trevisan S, Laveder P, De Pittà C, Pegoraro E, Hayashi YK, Valle G, Angelini C, and Lanfranchi G. Gene expression profiling in dysferlinopathies using a dedicated muscle microarray. Hum Mol Genet. **11**: 3283–3298. 2002.[Medline] [CrossRef]
10. von der Hagen M, Laval SH, Cree LM, Haldane F, Pocock M, Wappler I, Peters H, Reitsamer HA, Hoger H, Wiedner M, Oberndorfer F, Anderson LV, Straub V, Bittner RE, and Bushby KM. The differential gene expression profiles of proximal and distal muscle groups are altered in pre-pathological dysferlin-deficient mice. Neuromuscul Disord. **15**: 863–877. 2005.[Medline] [CrossRef]
11. Suzuki N, Aoki M, Hinuma Y, Takahashi T, Onodera Y, Ishigaki A, Kato M, Warita H, Tateyama M, and Itoyama Y. Expression profiling with progression of dystrophic change in dysferlin-deficient mice (SJL). Neurosci Res. **52**: 47–60. 2005.[Medline] [CrossRef]
12. Turk R, Sterrenburg E, van der Wees CG, de Meijer EJ, de Menezes RX, Groh S, Campbell KP, Noguchi S, van Ommen GJ, den Dunnen JT, and 't Hoen PA. Common pathological mechanisms in mouse models for muscular dystrophies. FASEB J. **20**: 127–129. 2006.[Medline]
13. Weller AH, Magliato SA, Bell KP, and Rosenberg NL. Spontaneous myopathy in the SJL/J mouse: pathology and strength loss. Muscle Nerve. **20**: 72–82. 1997.[Medline] [CrossRef]
14. Chadt A, Leicht K, Deshmukh A, Jiang LQ, Scherneck S, Bernhardt U, Dreja T, Vogel H, Schmolz K, Kluge R, Zierath JR, Hultschig C, Hoeben RC, Schürmann A, Joost HG, and Al-Hasani H. *Tbc1d1* mutation in lean mouse strain confers leanness and protects from diet-induced obesity. Nat Genet. **40**: 1354–1359. 2008.[Medline] [CrossRef]
15. Samec S, Seydoux J, and Dulloo AG. Skeletal muscle UCP3 and UCP2 gene expression in response to inhibition of free fatty acid flux through mitochondrial beta-oxidation. Pflugers Arch. **438**: 452–457. 1999.[Medline] [CrossRef]
16. Bonen A. PGC-1alpha-induced improvements in skeletal muscle metabolism and insulin sensitivity. Appl Physiol Nutr Metab. **34**: 307–314. 2009.[Medline] [CrossRef]
17. Carnac G, Vernus B, and Bonnieu A. Myostatin in the pathophysiology of skeletal muscle. Curr Genomics. **8**: 415–422. 2007.[Medline] [CrossRef]
18. Amthor H, Nicholas G, McKinnell I, Kemp CF, Sharma M, Kambadur R, and Patel K. Follistatin complexes Myostatin and antagonises Myostatin-mediated inhibition of myogenesis. Dev Biol. **270**: 19–30. 2004.[Medline] [CrossRef]
19. Rodino-Klapac LR, Haidet AM, Kota J, Handy C, Kaspar BK, and Mendell JR. Inhibition of myostatin with emphasis on follistatin as a therapy for muscle disease. Muscle Nerve. **39**: 283–296. 2009.[Medline] [CrossRef]
20. Fujita E, Kouroku Y, Isoai A, Kumagai H, Misutani A, Matsuda C, Hayashi YK, and Momoi T. Two endoplasmic reticulum-associated degradation (ERAD) systems for the novel variant of the mutant dysferlin: ubiquitin/proteasome ERAD(I) and autophagy/lysosome ERAD(II). Hum Mol Genet. **16**: 618–629. 2007.[Medline] [CrossRef]
21. Maglara AA, Vasilaki A, Jackson MJ, and McArdle A. Damage to developing mouse skeletal muscle myotubes in culture: protective effect of heat shock proteins. J Physiol. **548**: 837–846. 2003.[Medline] [CrossRef]
22. Kostek CA, Dominov JA, and Miller JB. Upregulation of MHC class I expression accompanies but is not required for spontaneous myopathy in dysferlin-deficient SJL/J mice. Am J Pathol. **160**: 833–839. 2002.[Medline]
23. Zhang Y, Baig E, and Williams DB. Functions of ERp57 in the folding and assembly of major histocompatibility complex class I molecules. J Biol Chem. **281**: 14622–14631. 2006.[Medline] [CrossRef]
24. Yoshida H, Matsui T, Hosokawa N, Kaufman RJ, Nagata K, and Mori K. A time-dependent phase shift in the mammalian unfolded protein response. Dev Cell. **4**: 265–271. 2003.[Medline] [CrossRef]
25. Minetti GC, Colussi C, Adami R, Serra C, Mozzetta C, Parente V, Fortuni S, Straino S, Sampaolesi M, Di Padova M, Illi B, Gallinari P, Steinkühler C, Capogrossi MC, Sartorelli V, Bottinelli R, Gaetano C, and Puri PL. Functional and morphological recovery of dystrophic muscles in mice treated with deacetylase inhibitors. Nat Med. **12**: 1147–1150. 2006.[Medline] [CrossRef]
26. Brunelli S, Sciorati C, D'Antona G, Innocenzi A, Covarello D, Galvez BG, Perrotta C, Monopoli A, Sanvito F, Bottinelli R, Ongini E, Cossu G, and Clementi E. Nitric oxide release combined with nonsteroidal antiinflammatory activity prevents muscular dystrophy pathology and enhances stem cell therapy. Proc Natl Acad Sci USA. **104**: 264–269. 2007.[Medline] [CrossRef]
27. Rando TA, Disatnik MH, Yu Y, and Franco A. Muscle cells from mdx mice have an increased susceptibility to oxidative stress. Neuromuscul Disord. **8**: 14–21. 1998.[Medline] [CrossRef]
28. Soares MP, and Bach FH. Heme oxygenase-1: from biology to therapeutic potential. Trends Mol Med. **15**: 50–58. 2009.[Medline] [CrossRef]
29. Arnér ES, and Holmgren A. Physiological functions of thio-

redoxin and thioredoxin reductase. Eur J Biochem. **267**: 6102–6109. 2000.[Medline] [CrossRef]

30. Wenzel K, Zabojszcza J, Carl M, Taubert S, Lass A, Harris CL, Ho M, Schulz H, Hummel O, Hubner N, Osterziel KJ, and Spuler S. Increased susceptibility to complement attack due to downregulation of decay-accelerating factor/CD55 in dysferlin-deficient muscular dystrophy. J Immunol. **175**: 6219–6225. 2005.[Medline]
31. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. **29**: e45. 2001.[Medline] [CrossRef]
32. Pfaffl MW, Horgan GW, and Dempfle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res. **30**: e36. 2002.[Medline] [CrossRef]
33. Bodyak N, Rigor DL, Chen YS, Han Y, Bisping E, Pu WT, and Kang PM. Uncoupling protein 2 modulates cell viability in adult rat cardiomyocytes. Am J Physiol Heart Circ Physiol. **293**: H829–H835. 2007.[Medline] [CrossRef]
34. Li ZH, Dulyaninova NG, House RP, Almo SC, and Bresnick AR. S100A4 regulates macrophage chemotaxis. Mol Biol Cell. **21**: 2598–2610. 2010.[Medline] [CrossRef]
35. Lund TC, Grange RW, and Lowe DA. Telomere shortening in diaphragm and tibialis anterior muscles of aged mdx mice. Muscle Nerve. **36**: 387–390. 2007.[Medline] [CrossRef]
36. Aguennouz M, Vita GL, Messina S, Cama A, Lanzano N, Ciranni A, Rodolico C, Di Giorgio RM, and Vita G. Telomere shortening is associated to TRF1 and PARP1 overexpression in Duchenne muscular dystrophy. Neurobiol Aging. 2010 (in press). [Medline] [CrossRef]
37. Langin D. The role of uncoupling protein 2 in the development of type 2 diabetes. Drugs Today (Barc). **39**: 287–295. 2003.[Medline] [CrossRef]
38. Roach WG, Chavez JA, Mîinea CP, and Lienhard GE. Substrate specificity and effect on GLUT4 translocation of the Rab GTPase-activating protein Tbc1d1. Biochem J. **15**(403): 353–358. 2007.[Medline]
39. Islam T, McConnell R, Gauderman WJ, Avol E, Peters JM, and Gilliland FD. Ozone, oxidant defense genes, and risk of asthma during adolescence. Am J Respir Crit Care Med. **177**: 388–395. 2008.[Medline] [CrossRef]
40. Jorquera G, Juretić N, Jaimovich E, and Riveros N. Membrane depolarization induces calcium-dependentupregulation of Hsp70 and Hmox-1 in skeletal muscle cells. Am J Physiol Cell Physiol. **297**: C581–C590. 2009.[Medline] [CrossRef]
41. Silomon M, Bauer I, Bauer M, Nolting J, Paxian M, and Rensing H. Induction of heme oxygenase-1 and heat shock protein 70 in rat hepatocytes: the role of calcium signaling. Cell Mol Biol Lett. **12**: 25–38. 2007.[Medline] [CrossRef]
42. Bansal D, Miyake K, Vogel SS, Groh S, Chen CC, Williamson R, McNeil PL, and Campbell KP. Defective membrane repair in dysferlin-deficient muscular dystrophy. Nature. **423**: 168–172. 2003.[Medline] [CrossRef]
43. O'Neill DE, and Noble EG. Constitutive expression of inducible Hsp70 is linked to natural shifts in skeletal muscle phenotype. Acta Physiol Scand. **181**: 35–41. 2004.[Medline] [CrossRef]
44. Krajacic P, Hermanowski J, Lozynska O, Khurana TS, and Lamitina T. The *C. elegans* Dysferlin homolog *fer-1* is expressed in muscle and *fer-1* mutations initiate altered gene expression of muscle enriched genes. Physiol Genomics. **40**: 8–14. 2009.[Medline] [CrossRef]
45. Lynch DM, and Kay PH. Studies on the polymorphism of the fifth component of complement in laboratory mice. Exp Clin Immunogenet. **12**: 253–620. 1995.[Medline]
46. Rhodes JC, Wicker LS, and Urba WJ. Genetic control of susceptibility to Cryptococcus neoformans in mice. Infect Immun. **29**: 494–499. 1980.[Medline]
47. Confalonieri P, Oliva L, Andreetta F, Lorenzoni R, Dassi P, Mariani E, Morandi L, Mora M, Cornelio F, and Mantegazza R. Muscle inflammation and MHC class I upregulation in muscular dystrophy with lack of dysferlin: an immunopathological study. J Neuroimmunol. **142**: 130–136. 2003.[Medline] [CrossRef]