Expression levels of MEF transcription factors in skeletal muscles of Huntington's disease mouse model

Poziom ekspresji czynników transkrypcyjnych MEF w mięśniach szkieletowych mysiego modelu choroby Huntingtona

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Wprowadzenie. Rodzina czynników transkrypcyjnych MEF2 składa się z 4 białek MEF2A, MEF2B, MEF2C and MEF2D i odgrywa kluczową rolę w różnicowaniu i rozwoju mięśni szkieletowych. Nadal nie ustalono czy atrofia mięśni w chorobie Huntingtona (Huntington's disease – HD) związana jest ze zmienionym profilem transkryptów Mef2.

Cel. Oszacowanie poziomów transkryptów Mef2 w mięśniach czworogłowym uda (skeletal muscles Quadriceps – Q) oraz prostowniku długim palców (Extensor Digitorum Longus – EDL) w mysim modelu HD – R6/2.

Materiały i metody. Mięsnie Q oraz EDL pobrano z mysiego modelu R6/2 celem wykonania łańcuchowej reakcji z użyciem odwrotnej transkryptazy służącej ilościowej ocenie transkryptu genu Mef2.

Wyniki. Uzyskane wyniki wskazują na statystycznie istotne podwyższenie poziomu transkryptów Mef2a w mięśniach czworogłowym uda oraz prostowniku długim palców mysiego modelu R6/2.

Wnioski. Gen Mef2a jest głównym regulatorem transkrypcyjnym mięśni szkieletowych. Poziom transkryptów Mef2a był podwyższony w mięśniach szkieletowych myszy R6/2, zarówno u osobników objawowych, jak i tych w stadiach zaawansowanej HD. Nasze wyniki wyskazują, iż białko MEF2A może być zaangażowane w patologiczną przebudowę mięśni szkieletowych w HD.

Słowa kluczowe: *choroba Huntingtona, zanik mięśni szkieletowych, MEF2, modele mysie*

Introduction. The MEF2 (Myocyte Enhancer Factor 2) family consists of 4 proteins MEF2A, MEF2B, MEF2C and MEF2D and plays a central role in skeletal muscle differentiation and development. It remains to be established whether Mef2 transcripts are altered in the Huntington's disease (HD) skeletal muscles.

Aim. To validate the transcriptional level of Mef2 transcription factors in two types of skeletal muscles Quadriceps (Q) and Extensor Digitorum Longus (EDL) of the R6/2 mouse model.

Material & methods. Q and EDL muscles were harvested from R6/2 mice for a quantitative reverse transcriptase-polymerase chain reaction analysis of Mef2 transcripts.

Results. We found transcript levels of Mef2a to be significantly up-regulated in fully symptomatic R6/2 mouse model.

Conclusions. Mef2a gene is a key transcriptional regulator of skeletal muscles. Mef2a transcript levels were up-regulated in skeletal muscles of symptomatic and end-stage R6/2 mice. Our findings show that MEF2A might play a role in HD skeletal muscle pathological remodelling.

Key words: *Huntington's disease, skeletal muscle atrophy, MEF2, mouse models*

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Introduction

Huntington's disease (HD) is a progressive monogenic neurodegenerative disorder for which there is no effective therapy [1]. Huntington's disease is predominantly characterised by wide-spread central nervous system (CNS) degeneration with a significant peripheral pathology including skeletal muscles [2, 3]. The CAG expansion over 35 repeats within the Exon-1 of Huntingtin gene (*HTT*) is the source of HD [1]. Consequently, this mutation leads to a number of pathological events within CNS as well in the skeletal muscles [4] and heart [5, 6]. Skeletal muscle malfunction in HD is manifested by a pronounce wasting [4], energy imbalance and altered nucleotide

metabolism [4, 7, 8] leading to the contractile dysfunction [4]. It has been shown that HD related skeletal muscle atrophy might be caused by an intrinsic effect of mutant HTT that alters expression levels of muscle chloride channel *Clc-1* [9, 10] in HD mouse models. MEF2 transcriptional factors play a key role during embryonic myogenesis as well in skeletal muscle growth and regeneration [11]. There are 4 members of MEF2 family of proteins MEF2A, MEF2B, MEF2C, MEF2D but MEF2B is not present in skeletal muscles [11]. There is strong evidence that MEF2 proteins play a pivotal role in energy homeostasis [12], cell proliferation and differentiation [13, 14].

In the current study we explored a potential mechanism by which MEF2s might be involved in HD-related skeletal muscle wasting. We have examined whether there are alterations in the transcriptional profiles of MEF2s in the skeletal muscles of pre-symptomatic (8 week), symptomatic (12 week) and end-stage 15 week-old R6/2 mice.

Aim

To validate the transcriptional level of *Mef2* transcription factors in two types of skeletal muscles Quadriceps (Q) and Extensor Digitorum Longus (EDL) of the R6/2 mouse model.

Materials and methods

Mouse maintenance

The R6/2 HD mouse line was bred and genotyped as previously described [4]. All experimental procedures were conducted under a project license from the Home Office, UK, and approved by the Animal Welfare and Ethical Review Body of Imperial College London. Experimental groups included the R6/2 mice at 8, 12 and 15 weeks of age (n>6) and their littermates (n>6). All animals had unlimited access to water and breeding chow (Special Diet Services, Witham, UK), and housing conditions and environmental enrichment were as described previously [4].

RNA extraction and Taqman real-time PCR expression analysis

Total RNA from skeletal muscles (Q and EDL) was extracted with a mini-RNA kit (Qiagen), according to the manufacturer's instructions. The reverse transcription reaction (RT) was performed using MMLV superscript reverse transcriptase (Invitrogen) and random hexamers (Invitrogen) [15]. All Taqman qPCR reactions were performed using the LightCycler® 480 Instrument (Roche). Estimation of mRNA copy number was determined in triplicate for each RNA sample by comparison to the geometric mean of three endogenous housekeeping genes (Primer Design). Primer and probe sets for genes of interest were purchased from Primer Design or Sigma. Following primers and probes were used:

- *Mef2a* Fw: ACATGGACAAAGTCCTTCTCAAA, Rw: CAACCATTAAGGCCTTTCTTTCT, Taqman probe: AGTATAACGAGCCTCATGAAAGCAG
- *Mef2c*: Fw: GAGAGAAGAAACACGGGGACTAT, Rw: TCAATCCAAATTTCCTCTTCGTA, Taqman probe: AAGATTCAGATTACGAGGATAATGGATG
- *Mef2d*: Fw: GCCGCACCAATGCTGACATCATCG, Rw: CTGCTCCAGTGAGTCCTCCCCATC, Taqman probe: TGAGGAAGAAGGGTTTCAACGGCTG.

Statistical analysis

All Taqman qPCR values were normalized to the geometric mean of three housekeeping genes. Error bars are ±SEM (n>6). One-way ANOVA with Bonferroni *post-hoc* test: *p<0.05, **p<0.01; ***p<0.001.

Results

We previously identified a number of molecular and physiological events leading to the HD-skeletal muscle malfunction [4, 7]. In the present study we sought to establish transcriptional profile of 3 members of MEF2 family of transcription factors in the commonly used R6/2 mouse model of HD. We performed a quantitative analysis of *Mef2a*, *Mef2c* and *Mef2d* mRNA on the cDNA library isolated from two types of skeletal muscle quadriceps and EDL of pre-symptomatic (8 weeks), symptomatic (12 weeks) and end-stage of disease (15 weeks) of age R6/2 mice.

We found a significant up-regulation of *Mef2a* mRNA only in the Quadriceps of symptomatic (12 weeks) and end-stage of disease (15 weeks) of age R6/2 mice (Fig. 1). However, *Mef2a* transcripts were already significantly up-regulated in the EDL muscles of pre-symptomatic (8 weeks) and end-stage of disease (15 weeks) of age R6/2 mice (Fig. 2). *Mef2c* transcripts were transiently up-regulated in quadriceps of symptomatic R6/2 mice (Fig. 1), while they remained unchanged in EDL muscles of pre-symptomatic and symptomatic R6/2 mice (Fig. 2). Interestingly, *Mef2c* transcripts were found to be down-regulated in quadriceps of end-stage R6/2 animals (Fig. 1). Similarly, *Mef2d* transcripts were significantly up-regulated in quadriceps of symptomatic R6/2 mice followed by a significant down-regulation in quadriceps of end-stage R6/2 mice (Fig. 1). *Mef2d* mRNA were unchanged in EDL muscles of pre-symptomatic and symptomatic R6/2 mice (Fig. 2).

Overall, we identified MEF2A to be significantly and consistently up-regulated in the two types of examined skeletal muscles of symptomatic and end-stage R6/2 animals.

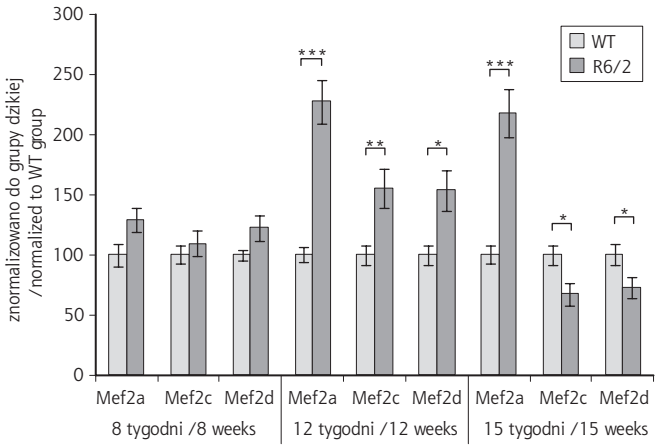

Fig. 1. Transcriptional profiles of *Mef2* family members in the Quadriceps muscles of pre-symptomatic, symptomatic and end-stage R6/2 mice

Ryc. 1. Profil transkrypcyjny białek z rodziny *Mef2* w mięśniu czworogłowym uda myszy R6/2 z różnym zaawansowaniem choroby: przedobjawowe, objawowe, końcowa faza

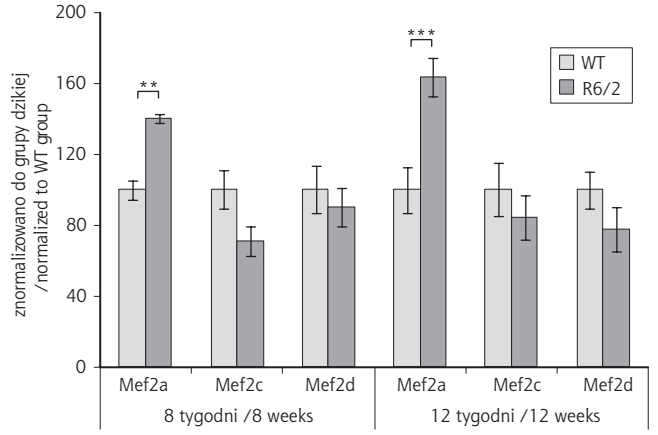

Fig. 2. Transcriptional profiles of *Mef2* family members in the EDL muscles of pre-symptomatic, symptomatic and end-stage R6/2 mice

Ryc. 2. Profil transkrypcyjny białek z rodziny *Mef2* w mięśniu prostowniku długim palców myszy R6/2 z różnym zaawansowaniem choroby: przedobjawowe, objawowe, końcowa faza

Discussion

Our current studies aimed to find out whether there were any changes in the transcriptional signatures of *Mef2s* that consists of 3 family members in atrophied skeletal muscles, using well-established HD R6/2 mouse model. We sought to establish transcriptional profile of *Mef2a*, *Mef2c* and *Mef2d* in line with disease progression in two distinct types of skeletal muscle Quadriceps (mix of slow and fast type fibers) and EDL (an example of fast type fibers) of pre- (8 weeks of age), symptomatic (12 weeks of age) and end-stage of disease (15 weeks of age) R6/2 mouse model. For the first time, we can report that *Mef2a* transcripts were significantly up-regulated in Quadricep muscles at symptomatic and end-stage of disease. More importantly *Mef2a* mRNA was already up-regulated in EDL of pre-symptomatic R6/2 mice. In fact, it has been shown that MEF2A deficiency resulted in impaired differentiation of myoblasts isolated from injured skeletal muscle [16, 17]. Hence, one might conclude that MEF2A could be potentially involved in the pathological remodelling of atrophied HD skeletal muscles. In addition, we found that *Mef2c* and *Mef2d* transcripts were transiently up-regulated in Quadriceps but not EDL muscles at the symptomatic stage of R6/2 mice, followed by their significant down-regulation at the end-stage of disease. MEF2C has been already showed to be responsible for metabolic homeostasis and control of overall body size [12], which are affected in HD mouse models [4].

Our study implies that altered expression of MEF2 family members including MEF2A might play a pivotal role in skeletal muscles pathological remodelling leading to HD-muscle atrophy. Interestingly MEF2 family members (MEF2A and C) were already showed to be altered in myotonic dystrophy and other neuromuscular disorders [18]. These transcriptional changes in key transcriptional regulators are potentially molecular markers of skeletal muscle pathological remodelling and disease progression, as well they are potential targets for therapeutic intervention, to reduce HD toxicity.

Conclusion

Mef2a gene is a key transcriptional regulator of skeletal muscles. *Mef2a* transcript levels were up-regulated in skeletal muscles of symptomatic and end-stage R6/2 mice. Our findings show that MEF2A might play a role in HD skeletal muscle pathological remodelling.

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Conflict of interest: The authors declare no competing financial interests.

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