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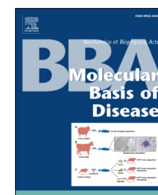

An impaired metabolism of nucleotides underpins a novel mechanism of cardiac remodeling leading to Huntington's disease related cardiomyopathy

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ABSTRACT

Huntington's disease (HD) is mainly thought of as a neurological disease, but multiple epidemiological studies have demonstrated a number of cardiovascular events leading to heart failure in HD patients. Our recent studies showed an increased risk of heart contractile dysfunction and dilated cardiomyopathy in HD pre-clinical models. This could potentially involve metabolic remodeling, that is a typical feature of the failing heart, with reduced activities of high energy phosphate generating pathways. In this study, we sought to identify metabolic abnormalities leading to HD-related cardiomyopathy in pre-clinical and clinical settings. We found that HD mouse models developed a profound deterioration in cardiac energy equilibrium, despite AMP-activated protein kinase hyperphosphorylation. This was accompanied by a reduced glucose usage and a significant deregulation of genes involved in *de novo* purine biosynthesis, in conversion of adenine nucleotides, and in adenosine metabolism. Consequently, we observed increased levels of nucleotide catabolites such as inosine, hypoxanthine, xanthine and uric acid, in murine and human HD serum. These effects may be caused locally by mutant HTT, *via* gain or loss of function effects, or distally by a lack of trophic signals from central nerve stimulation. Either may lead to energy equilibrium imbalances in cardiac cells, with activation of nucleotide catabolism plus an inhibition of re-synthesis. Our study suggests that future therapies should target cardiac mitochondrial dysfunction to ameliorate energetic dysfunction. Importantly, we describe the first set of biomarkers related to heart and skeletal muscle dysfunction in both pre-clinical and clinical HD settings.

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Abbreviations: HD, Huntington's disease; *HTT*, huntingtin gene; EDL, extensor digitorum longus muscle; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; Cr, creatine; PCr, phosphocreatine; PCr/Cr ratio, phosphocreatine/creatine ratio; NAD+, oxidized nicotinamide adenine dinucleotides; NADH, reduced nicotinamide adenine dinucleotides; AMPK, AMP-activated protein kinase; qPCR, quantitative polymerase chain reaction; *Ada*, Adenosine deaminase; *Adk*, Adenosine kinase; *Dpp4*, Dipeptydyl peptidase-4; *Nt5e*, Ecto-5′-nucelotidase; *Ampd3*, Adenosine deaminase 3; *Entpd2*, Ectonucleoside triphosphate diphosphohydrolase 2; *Nme1*, Nucleoside diphosphate kinase 1; *Nme2*, Nucleoside diphosphate kinase 2; *Nme3*, Nucleoside diphosphate kinase 3; *Gda*, Guanine deaminase inosine; *Pnp*, Purine nucleoside phosphorylase; *Xdh*, Xanthine dehydrogenase; *Hk2*, Hexokinase 2; *Ppargc1a*, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; *Ppara*, Peroxisome proliferator-activated receptor alpha; *Adsl*, Adenylosuccinate lyase; *Adlssl1*, Adenylosuccinate lyase 1; *Gart*, Phosphoribosylglycinamide formyltransferase; *Ppart*, Phosphoribosyl pyrophosphate amidotransferase; *Prpsap2*, Phosphoribosyl pyrophosphate synthetase-associated protein 2; *Aprt*, Adenine phosphoribosyltransferase; *Ak1*, Adenylate kinase 1; *Gmpr*, Guanosine monophosphate reductase; *Gmps*, Guanosine monophosphate synthetase; *Impdh2*, inosine monophosphate dehydrogenase 2.

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Summary

Huntington's disease (HD) is a fatal neurodegenerative disorder caused by a polyglutamine expansion in the huntingtin protein. HD-related cardiomyopathy has been widely described in different mouse models, however there is little known about the source of the pathological remodeling in HD hearts. We found that contractile dysfunction in HD settings might be caused by components of cellular energy imbalance, changes in catabolism of adenine nucleotides, steady-state internal redox derangements and an activation of AMPK, leading to a shift in the cardiac substrate preference. These changes were accompanied by increased concentrations of adenine nucleotide catabolites (inosine, hypoxanthine, xanthine and uric acid) and uridine in both HD mouse models and HD patients' plasma. These metabolites represent the first identified biomarkers related to striated muscle dysfunction in HD. Our study explores a mechanism that might lead to HD-related cardiomyopathy and opens new avenues for therapeutic treatments in HD.

1. Introduction

Huntington's disease (HD) is an inherited neurodegenerative disorder characterized by irrepressible motor dysfunction, cognitive decline and psychiatric disturbances that lead to progressive dementia and death; for a recent review see [1]. The source of HD is a CAG repeat expansion within the huntingtin gene (*HTT*) that translates into a polyglutamine stretch (polyQ) within the Exon-1 of HTT protein [1]. In the human population, the number of wild-type CAG repeats varies from 6 to 35, and the presence of 36 or more repeats defines the pathogenic HD allele [1]. HTT is a 348 kDa multi-domain protein that is normally expressed in various mammalian tissues, with the highest levels in the brain and testes [2]. It is believed that the polyQ expansion within the Exon-1 of HTT produces insoluble toxic aggregates, a hallmark of HD molecular pathology that can be detected in an early pre-symptomatic stage in pre-clinical and clinical settings [3]. These aggregates have been identified in HD brains, as well as in many non-central nervous system tissues [4], but not in hearts [5]. HTT protein is believed to act as a scaffolding protein since many interaction partners have been identified and, based on these findings, it is assumed that HTT is involved in gene transcription, intracellular signaling, trafficking, endocytosis and metabolism [6].

Despite the lack of HTT toxic aggregates in HD hearts, there is strong evidence that heart malfunction can be a potent contributor of HD progression. In fact, several epidemiological studies have indicated that heart failure is the second most common cause of death in HD patients; for a recent review see [7]. Although there is not enough molecular data underpinning such HD-related cardiomyopathy in humans, a recent clinical study published by Stephen and colleagues revealed a significant contractile heart dysfunction [8]. The study was performed on a large cohort of 598 patients with early symptoms of HD, participating in a clinical trial using standard 12-lead electrocardiograms (ECGs). It was found that abnormal ECGs were typical for 25.3% of early symptomatic patients and were manifested by rates of bradycardia, prolonged intra-ventricular conduction, as well as QTC prolongation, likely leading to arrhythmia and aggravated cardiac failure [8]. Importantly, these findings in human HD patients are in line with our previously published study in preclinical settings, where we noticed a contractile heart dysfunction in two symptomatic HD mouse models, namely R6/2 and *Hdh*Q150 [5]. We showed that heart contractile dysfunction was accompanied by a re-expression of foetal genes, apoptotic cardiomyocyte loss, and a moderate degree of interstitial fibrosis [5]. Moreover, we showed that R6/2 HD murine hearts are not able to respond to chronic isoproterenol treatment to the same degree as wild type hearts, and some of the hypertrophic signals are likely to be attenuated in symptomatic HD animals [9].

It is well established that myocardial contraction depends strongly on the mitochondrial energy supply [10]. This is supported by the fact that 25–35% of the myocardial volume is occupied by mitochondria [11]. Furthermore, proteomic analysis of cardiac mitochondria in patients with dilated cardiomyopathy showed alterations in substrate utilization (glucose, pyruvate and fatty acids) and in energy production [12]. One of the typical features of cardiomyopathy is a reduction in the activity of ATP-producing pathways, which could be intensified by a deficiency of cardiac energy substrates [13]. Therefore, we hypothesized that cardiac nucleotides and energy metabolism might contribute to a novel mechanism leading to HD heart pathology.

In order to identify these new pathological mechanisms, we performed our current studies using the same HD mouse models in which we previously described contractile abnormalities leading to heart failure, namely R6/2 and *Hdh*Q150 [5]. Interestingly, our study underlines the fact that HD-related cardiomyopathy includes components of cellular energy imbalance, changes in catabolism of adenine nucleotides, steady state internal redox derangements and an activation of AMPK, leading to a shift in the cardiac substrate preference. Moreover, cardiac energy metabolism impairment results in increased concentrations of adenine nucleotide catabolites (inosine, hypoxanthine, xanthine and uric acid) and uridine in both HD mouse models and HD patients' plasma. These metabolites represent the first identified biomarkers related to striated muscle dysfunction in HD.

2. Materials and methods

2.1. Mouse maintenance and genotyping

Mouse HD lines were maintained and genotyped as previously described [14], and all experimental procedures performed on mice were conducted under a project license from the Home Office, UK, and approved by ethical committee at Imperial College London, and by the Medical University of Gdansk Ethics Committee for Animal Experiments. Experimental groups included the R6/2 mouse model at 12 weeks of age ($n = 5$), their C57BL/6 J congenic lines littermates ($n = 5$) and the *Hdh*Q150 HD mouse model at 22 months of age ($n = 5$), compared to their C57BL/6 J littermates ($n = 5$).

2.2. HD patients and control subjects

Plasma samples from HD patients and control patients ($n = 5$ per group) were obtained from the Polish centre of the European Huntington's Disease Network in Poznan, and approved by the local bioethical board. Written informed consent was obtained from all subjects according to the International Conference on Harmonisation – Good Clinical Practice (ICH-GCP) guidelines (http://www.ich.org/LOB/media/MEDIA482.pdf). HD patients and healthy control subjects that were matched for age, sex, and body mass index (BMI) were enrolled in the presented study. Details about HD patients and control subjects are provided in Supplementary Table 2.

2.3. Measurement of nucleotides and corresponding levels of catabolites in murine heart and serum

Prior to extraction, heart samples were placed for 24 h in a freeze dryer (Modulyo, Thermo Electron Corporation, USA), at −55 °C. Freeze-dried fragments of hearts were extracted with 0.4 M perchloric acid in 1:10 ratio, followed by neutralization with 2 M KOH. Murine blood samples were collected from IVC (lat. *Inferior vena cava*) and centrifuged at 2000 × *g* for 5 min. The obtained volumes of serum varied between 20 and 50 μL. Next, serum components were extracted with 1.3 M perchloric acid (1:1 ratio). Levels of nucleotides were measured by a reverse phase-high pressure liquid chromatography (RP-HPLC) method using the LC system (Agilent Technologies 1100 series, USA), as described previously [15]. Results are presented as nmol/mg of dry tissue for heart samples and as μM for mouse serum.

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