BIOCHEMICAL MARKERS OF MUSCULAR DYSTROPHY

БІОХІМІЧНІ МАРКЕРИ М'ЯЗОВОЇ ДИСТРОФІЇ

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Анотація. М'язові дистрофії характеризуються прогресуючою втратою м'язової тканини та/або м'язової функції. Хоча метаболічні зміни були описані в біоптатах м'язів пацієнтів, для об'єктивного моніторингу стану м'язів необхідні неінвазивні показники, здатні описати ці зміни. Використано метаболічний підхід для дослідження концентрації метаболітів у сироватці крові пацієнтів з множинними формами м'язової дистрофії. Показано, що понад 10 метаболітів беруть участь у виробництві енергії, обміні амінокислот, метаболізмі тестостерону та відповіді на лікування глюкокортикоїдами. Близько 5 метаболітів змогли розрізнити інші форми м'язової дистрофії. Зокрема, креатинін і співвідношення креатин/креатинін були достовірно пов'язані з пацієнтами. Отримані результати свідчать про те, що метаболічний аналіз зразків сироватки крові може надати корисну інформацію щодо стану м'язів та відповіді на лікування, наприклад, глюкокортикоїдами.

Ключові слова: біорідина, біомаркер, м'язова дистрофія, дистрофінопатія, метаболізм.

Abstract. Muscular dystrophies are characterized by a progressive loss of muscle tissue and/or muscle function. While metabolic alterations have been described in patients' derived muscle biopsies, non-invasive readouts able to describe these alterations are needed in order to objectively monitor muscle condition. Used a metabolomic approach to study metabolites concentration in serum of patients affected by multiple forms of muscular dystrophy. Show that over 10 metabolites involved in energy production, amino acid metabolism, testosterone metabolism and response to treatment with glucocorticoids. About 5 metabolites were able to discriminate other forms of muscular dystrophy. In particular, creatinine and the creatine/creatinine ratio were significantly associated with patients. The obtained results provide evidence that metabolomics analysis of serum samples can provide useful information regarding muscle condition and response to treatment, such as to glucocorticoids.

Key words: biofluid, biomarker, muscular dystrophy, dystrophinopathy, metabolomics.

Introduction. Inherited Muscular Dystrophies (MDs) are caused by mutations in multiple genes [1]. The disease is characterized by progressive loss of muscle tissue and muscle function. Duchenne Muscular Dystrophy (DMD) is the most common and most severe MD. It is a lethal disease caused by lack of dystrophin due to protein-truncating mutations in the DMD gene [2, 3]. The identification of non-invasive biomarkers able to monitor disease progression and response to therapy would enable better patients' management and faster evaluation and marketing authorization of medicinal products for DMD and MDs in general. Towards this aim, multiple groups are working to identify biomarkers in body fluids such as blood-derived samples and urine [4, 5, 6]. Most of the available information was obtained by studying protein concentrations in different samples matrices and miRNAs, while less information is available for metabolites

concentration in body fluids, even though DMD was considered in the past and recently re-evaluated to be a metabolic myopathy. Recently markers of metabolic syndrome such as serum levels of leptin [7], creatine, arginine, branched amino acids and phosphatidylcholine [8] were reported to be elevated in DMD, leading us to further study metabolites profiles in DMD patients.

The aim of the study. The aims of this study were to identify metabolites able to discriminate between patients and controls, and to test associations between biomarkers levels and clinical performance.

Materials and methods. A literature search was conducted in PubMed, Scopus and Web of Science databases using keywords.

Results. DMD is a lethal disease caused by the absence of dystrophin resulting in substitution of muscle mass by adipose tissue [9] Downstream effects of lack of dystrophin have largely been studied in muscles samples from patients and animal models enabling the identification of morphological alterations and pathological pathways behind the clinical presentation [10]. Clear metabolic alterations have been described in DMD muscle tissue affecting the energy metabolism (eg glycolysis) and mitochondrial alterations (eg the tricarboxylic acid cycle and electron transport chain). Interestingly, several enzymes involved in these processes have been found to be differentially present in serum and plasma of DMD patients compared to healthy controls, providing evidence that certain metabolic alterations can be detected peripherally by studying protein concentration in serum. Given the very limited data on circulating metabolites concentration in DMD patients, we studied a large proportion of metabolites in fasted patients (to avoid possible food-related confounders) and compared the signature observed in DMD patients with the profiles observed in the milder allelic form Becker Muscular Dystrophy (BMD) and other forms of muscular dystrophy.

The results of our analysis confirm a decrease in creatinine and an increase in creatine serum levels likely due to the insufficient creatine utilization by muscles [8, 11]. Creatine (from the Greek κρέας, krèas, "meat") is an intermediate compound of energy metabolism synthesized by the liver (1.0 g/day) starting from arginine, S-adenosyl-methionine and glycine. In mammalian muscles, it serves to regenerate ATP during the first few seconds of muscle contraction. Creatinine, that represents its degradation product and is present in urine and blood, is usually considered a marker of renal function. As in Duchenne patients, creatine is normally synthesized by the liver but not metabolized in muscles, high creatine and low creatinine values are usually observed in both blood and serum. Similar profiles were observed for other forms of muscular dystrophy such as BMD, LGMD2A and LGMD2B. Interestingly, creatinine levels showed intermediate levels in BMD patients compared to DMD and healthy individuals indicating a possible relationship between dystrophin levels in muscle and creatinine in serum. An involvement of the creatine downstream metabolism is supported by the finding that the creatine precursor, the guanidinoacetic acid is reduced in DMD as well as in other MDs. It was recently reported that the creatine/creatinine ratio is particularly elevated in older, more severely affected DMD patients postulating its use as a marker of disease progression. Our data suggest that there is a negative correlation between the ratio and the performance of patients, as indicated by the significant association with 6-Minute Walk Test (6-MWT) and North Star Ambulatory Assessment (NSAA) figures. However, further research in bigger cohorts of patients is needed to confirm this association and its potential use as a surrogate end-point.

Data regarding the testosterone metabolism confirmed the reduction of 2 testosterone-related steroids (5α-DHT and dehydroisoandrosterone 3-sulphate), most probably caused by treatment with glucocorticoids. We further expand this observation by reporting a reduction in other metabolites involved in testosterone metabolism such as isohomovanillic acid, which is a product of catecholamine metabolism, mainly found in urine as a product of adrenal glands. Reduced levels of isohomovanillic acid could be due to the treatment with glucocorticoids suppressing the Hypothalamus-Pituitary-Adrenal (HPA) axis resulting in adrenal glands suppression. More

studies are needed to evaluate whether isohomovanillic acid levels could be a prognostic marker of HPA suppression.

Several metabolites involved in amino acids metabolism were affected in DMD patients such as isohomovanillic acid, p-coumaric acid, L-Aspartic acid, serine, ornithine, 2-hydroxycaproic acid and indoleacetic acid. This observation could be the direct effect of increased muscle protein degradation and resynthesis in accordance of what has been observed in dystrophic animal models [12].

Finally, citrulline levels were found to be reduced in DMD patients; interestingly, citrulline is being tested in a single-centre, randomized, placebo-controlled trial in combination with metformin [13]. This therapeutic approach aims to stimulate mitochondrial function and to compensate oxidative stress by increasing the production of nitric oxide (NO). In fact, NO is synthesized from the precursor arginine, which is in turn synthesized from citrulline. It was recently shown that both arginine and citrulline can boost the production of NO in humans [14]. Restoration of citrulline serum levels and NO levels in DMD patients could be used as pharmacodynamics biomarkers to study the effect of this ongoing combination therapy.

Conclusions. The obtained results provide evidence that metabolomics analysis of serum samples can provide useful information regarding muscle condition and response to treatment, such as to glucocorticoids treatment.

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