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EXPERT OPINION

Fibroblast growth factor 21: a novel biomarker for human muscle-manifesting mitochondrial disorders

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Introduction: Diagnosis of mitochondrial disorders is challenging, because of their highly variable clinical manifestations and age-of-onset and the shortage of specific diagnostic tools. Recent molecular studies have found that serum fibroblast growth factor 21 (FGF21) has potential to be a biomarker for muscle-manifesting mitochondrial disease, as well as for follow-up of disease progression and effect of intervention.
Areas covered: Serum FGF21 as a biomarker is compared to conventional serum diagnostic tools for mitochondrial disorders.
Expert opinion: Mitochondrial disorders are a large group of different progressive disorders, with the age-of-onset from neonatal life to late adulthood, and symptoms originating from any organ system but sharing an underlying cause of mitochondrial dysfunction. The prevalence of these disorders is about 1:2000, varying somewhat between different countries. Serum diagnostic tools include lactate, pyruvate, their ratio, creatine kinase and amino acids. However, none of these markers are both sensitive and specific. Increased levels of FGF21 cytokine were recently found in the serum of patients, who have a muscle-manifesting mitochondrial disease, thus providing a promising, novel, sensitive and specific biomarker for these disorders.

Keywords: biomarker, fibroblast growth factor 21, mitochondrial myopathy, respiratory chain

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1. Introduction

Mitochondrial disorders are the most common group of inherited metabolic disorders, consisting of hundreds of different disorders, sharing molecular background of mitochondrial dysfunction. Manifestations are exceptionally variable, ranging from infantile multisystem disorders to cardiomyopathies, epilepsies, progressive neurodegeneration, diabetes or hearing impairment [1]. Mitochondrial dysfunction results in disturbed oxidative energy metabolism, that is, defective utilization of nutrients to generate the chemical energy currency of cells, adenosine triphosphate (ATP). The mitochondrial respiratory chain (RC) passes the nutrient-derived electrons to oxygen and couples the pumping of protons from mitochondrial matrix to intermembrane space. This creates a proton gradient across the inner mitochondrial membrane, utilized to drive phosphorylation of ADP to ATP, by ATP synthase. In this process, called oxidative phosphorylation (OXPHOS), mitochondria use most of the inhaled oxygen. Defects in OXPHOS most often affect tissues with high dependence on oxidative energy production, but this is not always the case, as mitochondrial dysfunction can manifest in any organ system [1].

RC deficiency is the most common cause of mitochondrial disorders and can be caused by mutations in either mitochondrial DNA (mtDNA) or nuclear DNA, in

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genes encoding RC enzyme subunits, components required for their translation, transport, assembly, quality control or maintenance [1]. The most severe defects manifest immediately after birth, or even prenatally, whereas functionally milder defects manifest later in life, at any age. Typically, mitochondrial disorders are progressive in nature, leading to severe disability or premature death.

Diagnosis of mitochondrial disease is challenging, because of the large range of clinical manifestations. Furthermore, the disorders can be tissue-specific, the mechanisms of which are one of the outstanding questions in the field [1]. In clinical practice, commonly used biomarkers include lactate, pyruvate, lactate:pyruvate ratio (L:P), creatine kinase (CK) and amino acids, especially alanine [2]. Increase of lactate and L:P on OXPHOS defects results from energy metabolic balance shifting to favor nonoxidative glycolysis, when pyruvate is converted to lactate instead of being fed to the mitochondrial citric acid cycle and OXPHOS. CK reflects enzyme release from damaged muscle fibers, especially skeletal muscle or the heart. Alanine is a glycogenic amino acid that is released by the skeletal muscle on energy defect and utilized by the liver gluconeogenesis to increase glucose availability – as is also lactate. Blood concentration of lactate as well as L:P, as biomarkers for mitochondrial diseases, shows high specificity for mitochondrial diseases of childhood, and alanine is also often increased [2]. However, in adult patients, lactate and L:P are most often in the normal range. Transient increase of lactate levels in adults can often, however, be provoked by exercise, for example, by ergometry. CK raises most often on muscle dystrophy, but typically not in children with mitochondrial disease. However, myopathic form of mtDNA depletion syndrome, with severe muscle dystrophy, caused by thymidine kinase 2 mutations, does associate with high CK [3]. In adult-onset mitochondrial myopathies, CK is often moderately raised [2,4] but lacks specificity.

Muscle biopsy is still the gold standard diagnostic tool for mitochondrial disorders [2]. It provides material for both histological and biochemical examinations. Biochemical analysis of mitochondrial RC complex activities can specifically assign the defect to a certain complex (e.g., complex I, II, III, IV or ATPase) or indicate combined deficiency (typically complex I + IV) [5]. In histochemical analysis, cytochrome c oxidase (COX; partially mtDNA-encoded and therefore affected upon mtDNA mutations)-deficient muscle fibers may be found, which often also are succinate dehydrogenase (SDH; nuclear-encoded RC complex, not affected by mtDNA mutations)-positive. The activity of the most commonly deficient complex I cannot, however, be histochemically analyzed. In mitochondrial disorders that affect the muscle, analysis of the biopsy sample is highly valuable and points the direction of molecular genetic analyses. However, this procedure is invasive, prone to complications and, on mitochondrial diseases that do not manifest in the muscle, remains uninformative [2].

2. Biomarker identification and preclinical development

2.1 Serum/plasma fibroblast growth factor 21: a potential diagnostic biomarker for muscle-manifesting mitochondrial disease?

Serum biomarkers have long been sought for mitochondrial disorders, but little progress has been made during the past 40 years, after lactate and pyruvate. An optimal biomarker should be sensitive, that is, able to detect mitochondrial disorders from the mixed group of patients entering a clinic, and specific, that is, showing high values only in mitochondrial disease but not in other kind of disorders. Recently, a promising novel serum biomarker, fibroblast growth factor 21 (FGF21) was reported for muscle-manifesting mitochondrial RC deficiencies [4]. FGF21 is a hormone-like cytokine, lacking the heparin-binding domain of most FGF proteins, allowing its secretion [6,7]. FGF21 has been described to be secreted from the liver on fasting and to induce lipolysis and ketogenesis, to replenish the tissues with fuel on low nutritional supply [6,8]. FGF21 induction in mitochondrial disease was initially characterized in the 'Deletor' mouse, with late-onset mitochondrial disease, caused by overexpression of Twinkle helicase, carrying a dominant patient mutation, and replicating well the findings in patients with dominant Twinkle mutations [9-11]. These mice show progressive accumulation of large-scale mtDNA deletions in their muscle, associated with COX-negative, SDH-positive muscle fibers. The muscle of these mice was found to show a pseudo-starvation response, on normal nutritional intake. This response was associated with high expression of FGF21 in the RC-deficient muscle fibers and consequent elevated FGF21 concentration in the mouse blood [11]. If these mice were given ketogenic diet with high fat content, their mitochondrial ultrastructure and serum metabolites improved, which was accompanied by decreased FGF21 serum levels [12]. This study indicated that FGF21 is induced in the skeletal muscle as part of the pseudo-starvation response to RC deficiency, and it reflects the severity of the muscle disease. Furthermore, the FGF21 serum levels in mice reflected a therapy response in the skeletal muscle.

Importantly, a similar FGF21 response was conserved in human patients, not only in adult-onset myopathies but also in a wide range of patients with mitochondrial disease, both in the adults and in children [4]. Increased FGF21 RNA expression was found in patient muscle biopsy samples, suggesting that the origin of serum FGF21 in these patients was the skeletal muscle, similar to mice. The origin of FGF21 from the affected muscle fibers and its presence in the serum raised the question whether FGF21 could be used as a biomarker for muscle-manifesting mitochondrial diseases.

3. Validation and clinical studies

3.1 FGF21 in mitochondrial disorders

A collaborative effort of European and American mitochondrial diagnostic centers for mitochondrial diseases succeeded in collecting a patient material, which included both children and adults with confirmed mitochondrial disorders: all had a DNA diagnosis and/or severe biochemical RC defect in their muscle [4]. In addition, samples from healthy controls and patients with confirmed non-mitochondrial muscle-manifesting neurological and metabolic disorders were collected. The sensitivity and specificity of FGF21 for muscle-manifesting mitochondrial disease in this material was 92% [4]. In the same material, lactate and L:P were also very specific (93% for lactate, 100% for L:P) but lacked sensitivity (63 and 44%, respectively). Pyruvate lacked sensitivity and CK was neither specific nor sensitive [4]. Lactate and L:P performed well in detecting child patients with severe mitochondrial disorders, but in adults with muscle-manifesting mitochondrial disorders these were rarely abnormal [4]. Brain-manifesting mitochondrial disorders, however, such as mitochondrial recessive ataxia syndrome, showed low FGF21 concentrations [4], supporting the source of serum FGF21 to be skeletal muscle.

FGF21 was high in both isolated and combined RC deficiencies [4]. Importantly, the cytokine was also increased in serum of patients with complex I deficiencies. These disorders are common within the group of mitochondrial diseases, but the diagnosis is challenging, as histochemical tools do not exist and reliable biochemical enzymatic activity analyses are labor-intensive and can be performed only in experienced diagnostic laboratories.

FGF21 concentrations increased along with disease progression in mitochondrial patients, and terminal-stage diseases were associated with higher values than early stage [4]. These preliminary results suggested that FGF21 has potential to be used for follow-up of disease progression, but this conclusions needs to be verified in large patient materials. The correlation with disease progression in human patients, as well as the results from the deleter mouse, indicating that FGF21 decreases on improved mitochondrial function in a therapy trial, raises a possibility that FGF21 analysis could be used for follow-up of therapy effect [4].

FGF21 appeared stabile on processing and storage. No apparent inconsistencies in patient versus control values were found between fresh or frozen samples. This is a valuable characteristic for a biomarker; lactate and pyruvate concentrations are sensitive to sampling effects and cannot be stored [4].

3.2 What is known of FGF21 in controls and in non-mitochondrial disorders?

FGF21 serum levels show some variability in normal population, with no correlation to gender or body mass [4]. In different studies, however, the mean/median level of FGF21 has been found quite consistently to be under 200 pg/mL [4,13-20] (serum-values, review of literature in supplement web appendix of Ref. [4]). Despite high specificity for muscle mitochondrial disease in our cohort, several publications reported increased levels of FGF21 in different common disorders of lipid and glucose metabolism, such as non-alcoholic fatty liver disease, metabolic syndrome and coronary heart disease, as well as HIV-associated lipodystrophy [13-18]. Furthermore, occasional obese individuals with high FGF21 values have been reported, but FGF21 in those cases was shown to correlate with liver fat content, not adiposity *per se* [19]. FGF21 also has been suggested to be raised in type-2 diabetes, but despite the vast literature, little correlation was done with liver fat of those patients, leaving the link between diabetes and raised FGF21 open. Although muscle-manifesting mitochondrial diseases are the only specific disease group, which shows consistently high FGF21 values, it is clear from the literature that serum FGF21 levels can increase especially on fatty liver disease and potentially on reduced kidney clearance [20]. This information and further studies are important when making conclusions from FGF21 concentrations in a patient with potential mitochondrial disease.

3.3 What is known of FGF21 in normal physiology?

FGF21, a hormone-like cytokine, was characterized in 2001, but its function as 'the missing link in the biology of fasting' was suggested in 2005 [6]. Its likely receptor is formed by FGFR-1/Klotho heterodimer [21,22]. FGF21 is induced on fasting in the liver, promotes ketogenesis and is secreted to the bloodstream. The cytokine releases lipids from the adipose tissue, utilizing the body fuel storage to feed the fasting organs [6,23]. Knock-out of FGF21 in mice resulted in viable mice, which have deficient ketogenesis and lowered resistance to fasting [24,25]. In mice, transgenic overexpression of FGF21 from liver was found to reduce liver lipid content, blood lipids and glucose and made the mice resistant to high-fat diet-induced obesity without apparent adverse effects [6,23]. This raised high hopes for FGF21 as a therapeutic agent for metabolic syndrome. Surprisingly in humans, however, FGF21 was found to be very low in patients with anorexia nervosa [26] and sometimes increased in obese individuals with high liver fat [15-17,19] or in metabolic syndrome, indicating major differences in roles of FGF21 between humans and mice. To date, mitochondrial disorders are the only example of a disease group, in which mouse findings can be fully replicated in humans.

4. Conclusion

Serum FGF21 is increased in adult and child patients with mitochondrial diseases, manifesting in the skeletal muscle. The serum FGF21 concentration reflects the disease severity in the skeletal muscle. The sensitivity of FGF21 to detect a mitochondrial disorder is excellent, much better than other

available serum markers, such as lactate, L:P, pyruvate or CK. The specificity is as high as that of lactate and L:P. FGF21 is the first serum marker that reflects the severity of mitochondrial myopathy and follows the disease progression. FGF21 is a novel promising serum marker for the heterogeneous and diagnostically challenging group of mitochondrial disorders.

5. Expert opinion

Biomarkers for mitochondrial disorders have been sought for, for example, through metabolomics analyses [27], but none have been found consistent, sensitive and specific enough to make their way to diagnostic practice. Therefore, muscle biopsy still is the key diagnostic examination. However, many centers prefer not to take biopsies, because of potential complications of the surgical procedure and the risks of general anesthesia especially for children with severe disease. If biopsy material and biochemical and histological results are not available, tools to guide genetic analyses in search for specific diagnosis are scarce.

FGF21 is a promising tool for mitochondrial disease diagnosis. However, some obstacles remain to be solved.

1) Tools for routine diagnostic analysis. Currently available commercial enzyme-linked immunosorbent assay kits for analysis of FGF21 are not validated for clinical diagnostic use. Our own experience has shown that lot-to-lot and manufacturer-to-manufacturer variation exists in all currently available kits and results of different studies are best comparable with each other, if analyzed with the same kit. Even the range of normal FGF21 concentration in healthy controls varies in publications that used different kits, despite the fact that all should measure concentration. Because of the clear potential for diagnostic use of FGF21, high-quality, monoclonal antibody-based tools should be developed and validated to fill high diagnostic standards.
2) Differential diagnosis on increased FGF21. Literature indicates that some common disorders, especially involving fatty liver, show moderately increased FGF21 values. These diseases are not typically associated with muscle symptoms, and therefore should not complicate the use of FGF21 in diagnostics of muscle weakness and mitochondrial diseases. Moreover, these patient groups are heterogeneous, typically without definitive genetic diagnosis, and therefore could include patients with mitochondrial dysfunction. More studies in well-characterized patient materials need to be performed to gain knowledge, whether other physiological states or disorders consistently associated with elevated FGF21 levels exist. These disorders should be considered as differential diagnoses, on utilizing FGF21 test in mitochondrial disease diagnosis.
3) FGF21 in routine diagnosis. Prospective studies in mitochondrial patients should be conducted, carefully correlating serum FGF21 level analysis with histological findings of muscle sample, to gain experience of the ability of FGF21 to predict severity of muscle mitochondrial disease.
4) The tissue source of serum FGF21 in human patients. In mice with mitochondrial myopathy, only skeletal muscle was found to induce expression of the cytokine. It remains, however, possible that in other disorders, different tissues could contribute to FGF21 raise. In the original study of FGF21 in mitochondrial disorders, no correlation between raised FGF21 values and serum markers of liver dysfunction was found. However, as the liver is the source of FGF21 on fasting, the possibility of hepatic FGF21 origin cannot be excluded. More studies of the potential tissue sources of FGF21 are needed in different disease models.
5) The contribution of FGF21 in mitochondrial disease progression is still unknown. FGF21 has the potential to tune organs to shift their metabolism to face periods of starvation. When chronically induced on mitochondrial disease, FGF21 could promote disease progression by stimulating defective mitochondrial oxidative metabolism or be protective through downregulation of energy metabolism. In mice, and suggestively in humans, FGF21 concentrations increase on disease progression. On terminal stage of mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) and mitochondrial recessive ataxia syndrome (MIRAS), FGF21 levels were highly increased. It is possible that FGF21 is a marker of metabolic crisis and that RC deficiency represents a severe cellular crisis.

Based on current evidence, the author proposes FGF21 to be used as a tool to prioritize patients for the invasive surgical biopsy but not to replace the procedure: patients with high FGF21 should be forwarded to biopsy, as their muscle histology and/or biochemistry is likely to be informative. However, those patients, whose FGF21 is low, should be prioritized for other examinations. Especially children with mitochondrial disorders in their muscles show very high serum FGF21 values, up to 10- to 20-fold high, compared to the reference values. Therefore, a child with low FGF21 value is not likely to have a muscle-manifesting mitochondrial disease and other diagnostics paths should be considered before muscle sampling.

Declaration of interest

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Ylikallio E, Suomalainen A. Mechanisms of mitochondrial diseases. Ann Med 2011;44:41-59
2. Suomalainen A. Biomarkers for mitochondrial respiratory chain disorders. J Inherit Metab Dis 2011;34:277-82
3. Götz A, Isohanni P, Pihko H, et al. Thymidine kinase 2 defects can cause multi-tissue mtDNA depletion syndrome. Brain 2008;131:2841-50
4. Suomalainen A, Elo JM, Pietiläinen KH, et al. FGF-21 as a biomarker for muscle-manifesting mitochondrial respiratory chain deficiencies: a diagnostic study. Lancet Neurol 2011;10:806-18

•• **The original description of increased FGF21 concentrations in patients with muscle-manifesting mitochondrial RC disorders.**

5. Rustin P, Chretien D, Bourgeron T, et al. Biochemical and molecular investigations in respiratory chain deficiencies. Clin Chim Acta 1994;228:35-51
6. Kharitonenkov A, Shiyanova TL, Koester A, et al. FGF-21 as a novel metabolic regulator. J Clin Invest 2005;115:1627-35

•• **This manuscript highlighted for the first time the role of FGF21 as a regulator of mouse lipid and glucose levels.**

7. Kharitonenkov A. FGFs and metabolism. Curr Opin Pharmacol 2009;9:805-10
8. Badman MK, Pissios P, Kennedy AR, et al. Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. Cell Metab 2007;5:426-37
9. Tyynismaa H, Mjosund KP, Wanrooij S, et al. Mutant mitochondrial helicase Twinkle causes multiple mtDNA deletions and a late-onset mitochondrial disease in mice. Proc Natl Acad Sci USA 2005;102:17687-92
10. Suomalainen A, Majander A, Haltia M, et al. Multiple deletions of mitochondrial DNA in several tissues of a patient with severe retarded depression and familial progressive external ophthalmoplegia. J Clin Invest 1992;90:61-6
11. Tyynismaa H, Carroll CJ, Raimundo N, et al. Mitochondrial myopathy induces a starvation-like response. Hum Mol Genet 2010;19:3948-58

•• **The first report of FGF21 in mitochondrial dysfunction, in a late-onset mitochondrial myopathy mouse.**

12. Ahola-Erkkila S, Carroll CJ, Peltola-Mjosund K, et al. Ketogenic diet slows down mitochondrial myopathy progression in mice. Hum Mol Genet 2010;19:1974-84
13. Li H, Dong K, Fang Q, et al. High serum level of fibroblast growth factor 21 is an independent predictor of non-alcoholic fatty liver disease: a 3-year prospective study in China. J Hepatol 2013;58:557-63
14. Domingo P, Gallego-Escuredo JM, Domingo JC, et al. Serum FGF21 levels are elevated in association with lipodystrophy, insulin resistance and biomarkers of liver injury in HIV-1-infected patients. AIDS 2010;24:2629-37
15. Dushay J, Chui PC, Gopalakrishnan GS, et al. Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. Gastroenterology 2010;139:456-63
16. Yilmaz Y, Eren F, Yonal O, et al. Increased serum FGF21 levels in patients with nonalcoholic fatty liver disease. Eur J Clin Invest 2010;40:887-92
17. Zhang X, Yeung DC, Karpisek M, et al. Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. Diabetes 2008;57:1246-53
18. Lin Z, Wu Z, Yin X, et al. Serum levels of FGF-21 are increased in coronary heart disease patients and are independently associated with adverse lipid profile. PLoS One 2010;5:e15534
19. Tyynismaa H, Raivio T, Hakkarainen A, et al. Liver fat but not other adiposity measures influence circulating FGF21 levels in healthy young adult twins. J Clin Endocrinol Metab 2011;96:E351-5

•• **An important study showing that the increased values of FGF21 in occasional obese individuals were not associated with obesity as such but to liver fat.**

20. Lin Z, Zhou Z, Liu Y, et al. Circulating FGF21 levels are progressively increased from the early to end stages of chronic kidney diseases and are associated with renal function in Chinese. PLoS One 2011;6:e18398
21. Suzuki M, Uehara Y, Motomura-Matsuzaka K, et al. betaKlotho is required for fibroblast growth factor (FGF) 21 signaling through FGF receptor (FGFR) 1c and FGFR3c. Mol Endocrinol 2008;22:1006-14
22. Kurosu H, Choi M, Ogawa Y, et al. Tissue-specific expression of betaKlotho and fibroblast growth factor (FGF) receptor isoforms determines metabolic activity of FGF19 and FGF21. J Biol Chem 2007;282:26687-95
23. Badman MK, Pissios P, Kennedy AR, et al. Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. Cell Metab 2007;5:426-37
24. Hotta Y, Nakamura H, Konishi M, et al. Fibroblast growth factor 21 regulates lipolysis in white adipose tissue but is not required for ketogenesis and triglyceride clearance in liver. Endocrinology 2009;150:4625-33

•• **FGF21 knock-out mouse report, one of the two first ones.**

25. Potthoff MJ, Inagaki T, Satapati S, et al. FGF21 induces PGC-1alpha and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. Proc Natl Acad Sci USA 2009;106:10853-8

•• **FGF21 knock-out mouse report, one of the two first ones.**

26. Dostálová I, Kaválková P, Haluzíková D, et al. Plasma concentrations of fibroblast growth factors 19 and 21 in patients with anorexia nervosa. J Clin Endocrinol Metab 2008;93:3627-32
27. Shaham O, Slate NG, Goldberger O, et al. A plasma signature of human mitochondrial disease revealed through metabolic profiling of spent media from cultured muscle cells. Proc Natl Acad Sci USA 2010;107:1571-5

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