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COMMUNICATION

The TKFC Ala185Thr variant, reported as 'null' for fructose metabolism, is fully active as triokinase

 João M. Ribeiro¹ , María Jesús Costas¹ , Alicia Cabezas¹, Brigitte Meunier², Alexandros Onoufriadis³ and José Carlos Cameselle¹ 

1 Grupo de Enzimología, Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Medicina y Ciencias de la Salud, Universidad de Extremadura, Badajoz, Spain

2 Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France

3 St John's Institute of Dermatology, School of Basic and Medical Biosciences, King's College London, UK

Correspondence

J. M. Ribeiro, Grupo de Enzimología, Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Medicina y Ciencias de la Salud, Universidad de Extremadura, Badajoz, Spain
 Tel: +0034-924289470
 E-mail: jribeiro@unex.es

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TKFC-encoded triokinase catalyses glyceraldehyde phosphorylation in fructose metabolism and favours lipogenesis in mice. In *Tkfc* knockouts or knockdowns, fructose oxidation predominates over lipogenesis. The highly prevalent human variant Ala185Thr-Triokinase/FMN cyclase (TKFC) has been reported to be 'null' for fructose metabolism, since Ala185-TKFC rescues the mouse TKFC-deficient phenotype, whereas Ala185Thr-TKFC does not. Such report implies that most humans would display a noncanonical fructose metabolism, but it ignores the well-characterized triokinase activity of Ala185Thr-TKFC. Here, earlier evidence is summarized, along with new evidence that both human variants are equally active in yeast. Therefore, future research on triokinase in the context of human fructose metabolism should consider that Ala185Thr-TKFC is not biochemically 'null'.

Keywords: fructose metabolism; fructose oxidation; glyceraldehyde crossroads; glyceraldehyde phosphorylation; lipogenesis; rs2260655; single nucleotide polymorphism; TKFC; triokinase and FMN cyclase

The *TKFC* gene encodes for the bifunctional homodimeric human triokinase and FMN cyclase. This enzyme catalyses the ATP-dependent phosphorylation of the trioses D-glyceraldehyde (GA) and dihydroxyacetone (DHA), and the cyclizing lyase splitting of FAD to AMP and riboflavin cyclic-4',5'-phosphate (cyclic FMN or cFMN) [1]. The biological significance of the DHA kinase activity is not clear in mammals, because there is not a known endogenous source of DHA, although it can be acquired exogenously [2]. The same applies to FMN cyclase, as no function has been assigned to cyclic FMN, although it has been reported in rat liver at nanomolar concentration [3]. GA kinase, however, is well known for constituting

the third reaction of the Hers pathway for fructose metabolism [4], as well as one of the three reactions that form the so-called GA crossroads [5], where Triokinase/FMN cyclase (TKFC) competes with alcohol dehydrogenase and aldehyde dehydrogenase (Fig. 1).

The TKFC protein presents with two variants at position 185 in the human population due to single nucleotide polymorphism (SNP) rs2260655 (NM_015533.4:c.553G>A; p.Ala185Thr). The reference allele encodes for Ala185-TKFC, and the alternate one encodes for Thr185-TKFC. According to the gnomAD database [6], rs2260655 has a frequency of approximately 0.92, that is between 0.87 and 0.99 in

Abbreviations

cFMN, cyclic FMN or riboflavin cyclic-4',5'-phosphate; DHA, dihydroxyacetone; GA, D-glyceraldehyde; SNP, single nucleotide polymorphism; TKFC, triokinase/FMN cyclase.

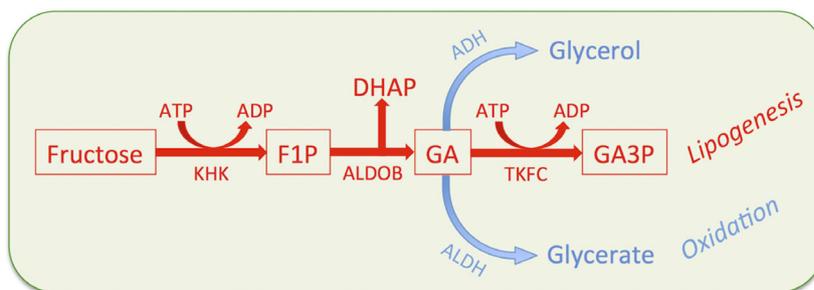


Fig. 1. Schematic for the metabolism of fructose by the Hers pathway and the glyceraldehyde crossroads. The Hers pathway [4] (red) consists of the linear pathway defined by ketohexokinase (KHK), aldolase B (ALDOB) and the GA kinase activity of TKFC. In the glyceraldehyde crossroads [5], two enzymes (blue) compete with TKFC for GA: alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). According to [10], in mice, the GA kinase activity of TKFC favours lipogenesis from fructose, while in the absence of TKFC, fructose is oxidized via ALDH. The DHA kinase and FMN cyclase activities of TKFC are not shown because they are not directly relevant to fructose metabolism, as stated in the Introduction. DHAP, DHA phosphate; F1P, D-fructose-1-phosphate; GA3P, GA-3-phosphate.

Table 1. Human population frequencies for the rs2260655 SNP according to the gnomAD database version 2.1.1 [6].

Population	Allele number	Number of homozygotes	Frequency
African/African American	18008	2050	0.4734
European (non-Finnish)	79674	39 142	0.9912
European (Finnish)	18984	9228	0.9861
Ashkenazi Jewish	8906	4356	0.9891
Latino/Admixed American	25998	12 254	0.9705
East Asian	13410	6259	0.9662
South Asian	23132	8860	0.8737
Other	5636	2595	0.9576

non-African populations and 0.47 in Africans/African American (Table 1). The variant p.Ala185Thr is predicted to be ‘tolerated/benign’ by the SIFT [7] PolyPhen [8] and EVE [9] pathogenicity software tools.

Recently, in an interesting study of the role of TKFC in fructose metabolism in mice, Liu et al. suggested that the human Thr185-TKFC variant is ‘null’ as triokinase [10]. This would be unexpected given that this variant is highly prevalent in the human population (Table 1) and that it would lead to a hepatic fructose metabolism different from the widely recognized canon [11–13]. This has to be carefully considered in the light of all available evidence, especially that the alterne Thr185-TKFC variant (and not the reference one Ala185-TKFC), is the only human TKFC that has been expressed from a cloned cDNA and characterized in detail, showing that it is fully active as triokinase [1]. This is an essential point that Liu et al. did not take into consideration nor discuss in their work [10].

Thus, the aim of this communication is to make aware those interested in human fructose metabolism, a topic of widespread attention [11–13].

The role of TKFC in controlling lipogenesis from fructose metabolism in mice

Liu et al. [10] investigated the role of TKFC in fructose metabolism in mice. The authors concluded that TKFC, through its GA kinase activity, favours lipogenesis from fructose and hepatic accumulation of triglycerides. In the absence of TKFC, as in *Tkfc* knockout mice or in liver-specific knockdown hepatocytes, due to the alternative enzymes of the GA crossroads, fructose oxidation via aldehyde dehydrogenase predominates over lipogenesis, leading to oxidative stress and to decreased hepatic triglyceride accumulation (Fig. 1). These important results are not questioned in the present communication.

Reported differential behaviour of the Ala185-TKFC and Thr185-TKFC human variants in the rescue of the TKFC-deficient phenotype of mouse *Tkfc* knockouts or knockdowns

Part of the study by Liu et al. was however devoted to test the ability of the above-mentioned human TKFC variants to rescue the mouse TKFC-deficient phenotype [10]. Ala185-TKFC worked as expected and displaced the balance of fructose metabolism from toxic oxidation (typical of *Tkfc* knockouts or knockdowns) towards lipogenesis (typical of wild-type mice). In contrast, Thr185-TKFC failed to do so. Upon these results, it was concluded that the Thr185-TKFC

variant behaves as triokinase ‘null’ for fructose metabolism and that amino acid Ala185 has ‘an important enzymatic role’ in human TKFC. Although these conclusions were reached in the absence of direct measurements of triokinase activity, this seems to imply that the Thr185-TKFC variant is catalytically inactive, since the effects of TKFC in fructose metabolism depend on its GA kinase activity. Therefore, it is unclear how the majority of humans would have an inactive TKFC, leading to a hepatic fructose metabolism different from the widely recognized canon [11–13]. In addition, the possible ‘null’ behaviour of Thr185-TKFC would have to be reconciled with the characterization of this variant as a fully active triokinase.

Human Thr185-TKFC is fully active as triokinase

Thr185-TKFC is so far the only human triokinase expressed from a cloned cDNA and characterized in detail. It was cloned by Cabezas et al. [14] while looking for the molecular identity of rat liver FAD-AMP lyase (cyclic FMN forming) or FMN cyclase, previously studied in the same laboratory [15,16]. Peptide-mass fingerprinting of the purified rat liver enzyme pinpointed rodent and human translations of cDNAs putatively classified as DHA kinases by homology to biochemically proven DHA kinases of plants, yeasts and bacteria. Next, Cabezas et al. implemented a PCR amplification from a human cDNA library using primers designed from human mRNA accession number NM_015533, which codes for Ala185-TKFC (accession number NP_056348). However, the PCR amplicon obtained (accession number DQ138290) contained three SNPs compared to NM_015533, only one (rs2260655-A) resulting in non-synonymous codons, that is Ala185Thr. The finding of this variant, that displayed FMN cyclase and DHA kinase activities, was explicitly mentioned [14]. The same protein was later characterized in full detail, including its GA kinase activity showing hyperbolic kinetics with an 18 μM K_M value for GA, thus completing its identification as triokinase, thereon named triokinase and FMN cyclase (TKFC) [1]. The kinetic parameters of the triokinase activity of Thr185-TKFC agree with those reported for the enzyme purified from human blood [17].

More recently, TKFC mutations that inactivate the triokinase activities have been found in patients who are also homozygous for the rs2260655 polymorphism, namely Gly445Ser, Arg543Ile (associated with cataracts and multisystem disease; [18]), Gly192Arg and Arg228Trp (associated with autosomal recessive

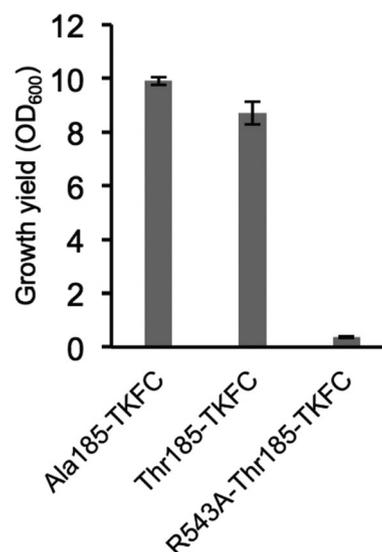


Fig. 2. Yeast growth assays showing that Thr185-TKFC promotes growth of Δdak1 using DHA as the sole carbon source. Yeast Δdak1 cells, overexpressing the indicated human genes, freshly grown on pre-culture plates were inoculated in DHA medium at an initial optical density at 600 nm (OD₆₀₀) of 0.25. The cultures were incubated for 3 days at 28 °C with vigorous agitation, and OD₆₀₀ values were then recorded. Growth assays were repeated twice and the data averaged. Error bars represent standard deviation. Yeast strains and culture media were described in [18].

hypotrichosis with loose anagen hairs; [19]). Importantly, Thr185-TKFC was used as a control in assays of DHA kinase and GA kinase activities of Gly192Arg and Arg228Trp TKFC mutants, showing full activity when added to reaction mixtures at the end of incubations with mutant enzyme [19]. Moreover, it was previously shown that the overexpression of Ala185-TKFC, but not of its inactive mutants, enabled yeast devoid of the endogenous *DAK1* and/or *DAK2* genes (homologous to human *TKFC*) to grow on DHA as the sole carbon source [18]. Using the same assay, we observed that expression of Ala185-TKFC and Thr185-TKFC resulted in similar growth competences, whereas R543A-Thr185-TKFC, a mutant devoid of triokinase activity [19], did not promote yeast growth (Fig. 2). This assay does not detect GA kinase activity, but it should be noticed that all the point mutations applied to Thr185-TKFC affect similarly DHA kinase and GA kinase [1,18,19].

Discussion

We conclude that human Thr185-TKFC cannot be considered as a ‘null’ variant in terms of triokinase activity. When Liu et al. [10] referred to the characterization of human TKFC by Rodrigues et al. [1], they

may have not realized that it was Thr185-TKFC what they were referring to. Otherwise, they would have been conscious of the disparity between their results with Thr185-TKFC and its known catalytic behaviour. Our manuscript adds this essential aspect to the picture that emerged from the work of Liu et al. [10].

To reconcile the 'null' character of Thr185-TKFC in the systems employed by Liu et al. [10] with its full activity *in vitro* [1] and in yeast cells (Fig. 2), one has to assume in mouse hepatocytes a regulatory mechanism dependent on the difference between alanine and threonine, such that Ala185-TKFC would be active *in vivo*, but Thr185-TKFC would not. Perhaps a threonine kinase could do the job, by phosphorylating Thr185. Even if this hypothetical regulation would occur in the systems of Liu et al. [10], important questions would remain to be answered, such as its function in different human tissues. This is a matter of global interest, since the vast majority of human genomes encode for Thr185-TKFC. Anyone intending to approach these questions from the point of view of human metabolism of fructose would need to be aware that Thr185-TKFC, if it actually fails to favour lipogenesis and hepatic accumulation of triglycerides in humans as it does in mice [10], is not biochemically 'null' as triokinase ([1,19] and Fig. 2).

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Data accessibility

Data that support the findings of this study are available on request from the authors.

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