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Short Communication

# One-carbon cycle alterations induced by Dyrk1a dosage

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ABSTRACT

Hyperhomocysteinemia due to cystathionine beta synthase deficiency confers diverse clinical manifestations. It is characterized by elevated plasma homocysteine levels, a common amino acid metabolized by remethylation to methionine or transsulfuration to cysteine. We recently found a relationship between hepatic Dyrk1A protein expression, a serine/threonine kinase involved in signal transduction in biological processes, hepatic S-adenosylhomocysteine activity, and plasma homocysteine levels. We aimed to study whether there is also a relationship between Dyrk1a and cystathionine beta synthase activity. We used different murine models carrying altered gene coy numbers for Dyrk1a, and found a decreased cystathionine beta synthase activity in the liver of mice under-expressing Dyrk1a, and an increased in liver of mice over-expressing Dyrk1a. For each model, a positive correlation was found between cystathionine beta synthase activity and Dyrk1a protein expression in the liver of mice, which was confirmed in a non-modified genetic context. The positive correlation found between liver Dyrk1a protein expression and CBS activity in modified and non-modified genetic context strengthens the role of this kinase in one carbon metabolism.



## 1. Introduction

Homocysteine (hcy) is an intermediate in the sulfur amino acid metabolism. Once hcy is formed, it may be metabolized by remethylation to methionine or transsulfuration to cysteine. Cystathionine beta synthase (CBS), which gene is localized on human chromosome 21, is the first enzyme involved in the transsulfuration pathway and catalyzes the condensation of hcy with serine to form cystathionine. Hcy can also turn back to S-adenosylhomocysteine (SAH) via reversal of the SAH hydrolase (SAHH) reaction [1]. Homocystinuria or severe hyperhomocysteinemia, defined by elevated plasma hcy level, is a metabolic disorder with defect in genes encoding for methionine metabolism enzymes. The clinical features consist in ophthalmic, neurologic, orthopedic and vascular manifestations. Homocystinuria is mainly due to CBS deficiency. It is the second metabolic encephalopathy in order of frequency [2]. The disorder is also associated with cognitive dysfunctions such as intellectual disability, cerebral atrophy, and seizures [2,3]. Further, elevated hcy level has also been associated with neurological disorders such as epilepsy [4,5].

The identification of intragenic DYRK1A rearrangements or 21q22 micro-deletions including only DYRK1A in patients with intellectual disability, microcephaly, seizures and epilepsy hypothesized the role of DYRK1A for such a phenotype [6–11]. Dyrk1A, which gene is localized on human chromosome 21, is a protein kinase that belongs to an evolutionarily conserved family of proteins known as DYRK (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase) that might be responsible for intellectual disability in Down's syndrome (DS) patients [12]. We recently analyzed the expression of Dyrk1A in the liver of CBS-deficient mice, a murine model of hyperhomocysteinemia, and found a reduced protein level, concomitant with a decreased hepatic SAHH activity [13–15]. On the contrary, an over-expression of Dyrk1A decreased plasma hcy level and increased the hepatic SAHH activity by a mechanism dependent of NAD(P)H:quinone oxidoreductase (NQO1) activity [15]. We therefore have established a link between hepatic Dyrk1a expression, hepatic SAHH activity and plasma hcy levels. However, the key question asked now is to study whether there is also a relationship between Dyrk1a and CBS. To analyze further the relation between Dyrk1a and CBS, we used two mice models to demonstrate

*Abbreviations:* CBS, cystathionine beta synthase; DS, Down syndrome; DYRK, dual-specificity tyrosine-(Y)-phosphorylation regulated kinase; EGCG, epigallocatechin-gallate; GABA, gamma-amino-butyric-acid; GK, Goto-Kakizaki; hcy, homocysteine; NQO1, NAD(P)H:quinone oxidoreductase; PLP, pyridoxal phosphate; PTZ, pentylenetetrazole; SAH, S-adenosylhomocysteine; SAHH, SAH hydrolase.

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the effect of the over-expression and the under-expression of Dyrk1a on hepatic CBS activity. We also used a spontaneous nonobese model of type 2 diabetes with lower plasma hcy level and increased liver CBS activity [16] to confirm the link established in modified genetic context.

## 2. Materials and methods

### 2.1. Animals

All Animal care was conducted in accordance with internal guidelines of the French Agriculture Ministry for animal handling (authorization number 75–369). Mice and rats were housed in a controlled environment (temperature = 20 ± 1 °C; humidity = 60%) with unlimited access to food and water on 12-h light/dark cycle. A number of mice and rats and suffering were minimized as possible. Mice carrying the mBACtgDYRK1A construct were maintained on a C57BL/6 J background and genotyped as described [17]. Dyrk1a (+/−) mice were maintained on a CD1 background and genotyped as described [18]. The Goto-Kakizaki (GK) line was established by repeated inbreeding from Wistar rats selected at the upper limit of normal distribution for glucose tolerance [19,20]. All experiments were conducted on age-matched control animals.

### 2.2. Sample preparation, tissue collection, and plasma total hcy assay

Following euthanization of mice and rats, blood samples were obtained by retro-orbital sinus sampling with heparinized capillaries, collected into tubes containing a 1/10 volume of 3.8% sodium citrate, and immediately placed on ice. Plasma was isolated by centrifugation at 2500 ×*g* for 15 min at 4 °C. The liver was rapidly removed and snap-frozen in liquid nitrogen before being stored at −80 °C until use. Plasma total hcy, defined as the total concentration of hcy after quantitative reductive cleavage of all disulfide bonds, was assayed using the fluorimetric high-performance liquid chromatography (HPLC) method described by Fortin and Genest [21]. The inter- and intra-assay coefficients of variation for mean total hcy level were 4.2% and 6.3% respectively and the linearity was from 1 to 100 μM [22].

### 2.3. Dyrk1a protein analysis by slot blot

Protein samples were prepared by homogenizing the liver in 500 μL phosphate-buffered saline with a cocktail of proteases inhibitors. Protein concentrations were detected with the Bio-Rad Protein Assay reagent (Bio-Rad). To assess the relative amount of Dyrk1a, we used a slot blot method previously developed [23]. Protein preparations were blotted on Hybond-C Extra membrane (GE Healthcare Europe GmbH) using Bio-Dot SF Microfiltration Apparatus (Bio-Rad). After transfer, membranes were saturated by incubation in 5% w/v nonfat milk powder in Tris-saline buffer (1.5 mM Tris base, pH 8; 5 mM NaCl; 0.1% Tween-20), and incubated overnight at 4 °C with an antibody directed against DYRK1A (1/500) (Abnova corporation, Tebu, France). Binding of the primary antibody was detected by incubation with horseradish peroxidase (HRP)-conjugated secondary antibody using the Western Blotting Luminol Reagent (Santa Cruz Biotechnology, Tebu, France). Ponceau-S coloration (Sigma-Aldrich, France) was used as an internal control. Digitized images of the immunoblots obtained using an LAS-3000 imaging system (Fuji Photo Film Co., Ltd.) were used for densitometric measurements with an image analyzer (UnScan It software, Silk Scientific Inc.).

### 2.4. CBS enzyme activity assays

Determination of CBS activity was assayed on 400 μg of total proteins obtained from liver samples, as described [24]. Proteins were incubated for 1 h at 37 °C with 1 mM of propargylglycine, 0.2 mM of pyridoxal phosphate (PLP), 10 mM of L-serine, 10 mM of DL-Hcy, 0.8 mM of SAM, using DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)) based-assay. All the chemical products were obtained from Sigma (Sigma-Aldrich, France).

### 2.5. Data analysis

Statistical analysis was performed with one-way analysis of variance (ANOVA) followed by Fisher's post-hoc test using Statview software. The results are expressed as medians with interquartile ranges. Data were considered significant when $p \leq 0.05$. Correlations between Dyrk1a protein level and CBS activity were determined by using Spearman's rank correlation, as data were not normally distributed according to Shapiro–Wilk test.

## 3. Results

### 3.1. CBS activity is decreased in liver of mice under-expressing Dyrk1a

We first conducted the analysis of CBS activity in the liver of mice carrying only one copy of Dyrk1A, the Dyrk1a (+/−) mice [18]. The decreased protein expression of Dyrk1a (Fig. 1A), analyzed by slot blot method previously validated [23], was associated with a decreased CBS activity in liver of Dyrk1a (+/−) mice compared to control (wild type) mice (Fig. 1B). We observed a significant positive correlation between the liver CBS activity and liver Dyrk1A protein expression ($\rho = 0.867$, $p < 0.009$). Commensurate with the decreased hepatic CBS activity, plasma hcy levels were increased in Dyrk1a (+/−) mice compared to control (wild type) mice (Fig. 1C).

### 3.2. CBS activity is increased in liver of mice over-expressing Dyrk1a

We also conducted the analysis of CBS activity in the liver of mice carrying the murine BAC containing one copy of the entire murine Dyrk1a gene, the mBACtgDyrk1a mice [17]. The increased protein expression of Dyrk1a (Fig. 2A) was associated with an increased CBS activity in the liver of mBACtgDyrk1a mice compared to control (wild type) mice (Fig. 2B). We also observed a significant positive correlation between the liver Dyrk1A protein expression and CBS activity ($\rho = 0.603$, $p < 0.006$). As previously described [15], plasma hcy levels were decreased in mBACtgDyrk1a mice compared to control (wild type) mice (Fig. 2C).

### 3.3. Dyrk1a protein expression is increased in liver of GK rats with increased CBS activity

We previously found a lower plasma level of hcy concomitantly with an increased liver activity of CBS in GK rats, a spontaneous nonobese model of type 2 diabetes [16]. We therefore used this rat model in order to analyze the hepatic Dyrk1a protein expression in a non-modified genetic context. The increased protein expression of Dyrk1a (Fig. 3A) was associated with an increased CBS activity in the liver of GK rats compared to nondiabetic Wistar (wild type) rats (Fig. 3B). We also observed a significant positive correlation between liver Dyrk1A protein expression and CBS activity ($\rho = 0.625$, $p < 0.02$). As previously described [15], plasma hcy levels were decreased in GK rats compared to Wistar (wild type) rats (Fig. 3C).

## 4. Discussion

Dyrk1a encodes a dual-specificity tyrosine-phosphorylation-regulated kinase, which has been shown to play an important role in signaling transduction in biological processes. Along with quantifying the link between CBS activity and Dyrk1a, we used two different models carrying altered gene copy numbers for Dyrk1a: a mBACtgDyrk1a model with three copies of the murine Dyrk1a gene and a Dyrk1a (+/−) model with only one copy. For each model, a positive correlation was found between the liver CBS activity and liver Dyrk1a protein