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Short Communication

The rs113883650 variant of *SLC7A5 (LAT1)* gene may alter brain phenylalanine content in PKU

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ABSTRACT

Functional alteration of the LAT1 amino acid transporter may be responsible for interindividual differences in cerebral phenylalanine content and the lack of intellectual disability in some patients with untreated phenylketonuria. We assessed the effect of the common variant rs113883650 of the *SLC7A5 (LAT1)* gene on brain phenylalanine content, as measured with use of magnetic resonance spectroscopy. Our results suggest that the presence of this variant could influence the amount of phenylalanine in the brain.

1. Introduction

In the individuals diagnosed with phenylketonuria (PKU) brain damage can be caused by the absence of effective dietary treatment. Nevertheless, several reports exist describing interindividual differences of the brain vulnerability to the toxic influence of hyperphenylalaninemia and the lack of intellectual disability in some, but not all, untreated patients with PKU [1–4]. It is speculated that this might result from alteration of the kinetics of phenylalanine across the blood-brain barrier, which is regulated by the LAT1 transporter. Until recently no common candidate variants of the *SLC7A5 (LAT1)* gene with a potential to functionally alter the LAT1-related phenylalanine transport were identified [5–7]. However, our recent report shed a new light on the topic, demonstrating increased risk of obesity in infants with PKU, who are the carriers of the common rs113883650 variant located in the *SLC7A5* gene [8].

Cerebral phenylalanine can be noninvasively quantified using magnetic resonance spectroscopy (MRS) [9,10]. Although this method seems to be not precise enough to detect brain phenylalanine signal in patients with concurrent blood phenylalanine below 1.2 mmol/l, it is much more effective in the case of more severe hyperphenylalaninemia [11].

In this study we hypothesized that the rs113883650 variant alters the content of phenylalanine in the brains of patients with PKU. Therefore, with the use of MRS, we measured the intensity of the brain phenylalanine signal in patients with very high concurrent blood phenylalanine levels, and compared the results obtained in carriers of the rs113883650 variant with the wild-type individuals.

2. Patients and methods

We assessed a group of 28 patients with severe PKU, born in the period 1981–1996, who in the past underwent a diagnostic MRS of the brain at the Department of Medical Genetics, Jagiellonian University, Krakow, Poland. Dietary treatment was established in them at the age of 2–6 months, according to previous PKU treatment scheme. All the patients did not adhere to dietary recommendations prior to the MRS examination and had very high (≥1.2 mmol/l) blood phenylalanine concentrations on the day of magnetic resonance testing.

We performed genotyping for the rs113883650 variant of the *SLC7A5* gene in all of the study participants. We used DNA samples that were collected on the day of MRS examination. We implemented direct sequencing, as described in our previous publication [7].

Next, we assessed the archival brain MRS spectra that were acquired on a 1.5 T magnetic resonance scanner (Magnetom Vision Plus, Siemens) with the use of the Point RESolved Spectroscopy (PRESS) technique. The signal acquisition was performed from a volume of 18 cm^3 of brain white matter (parietal region) with relaxation/echo time of 1500/30 ms and 512 repetitions. The phenylalanine peak was identified on the spectroscopic spectrum at 7.37 ppm. Then, the normalized intensity of the brain phenylalanine signal was calculated as a ratio of the integral of the peak at 7.37 and of the summarized integral of the three major peaks of the spectrum: NAA, creatine and choline (at. 2.0, 3.0 and. 3.2 ppm, respectively), which served as internal control of the spectrum quality (Fig. 1).

Lastly, we compared brain phenylalanine levels between the carriers

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of the rs113883650 variant and wild-type individuals.

The study was accepted by the Jagiellonian University Ethics Committee.

3. Results

The sample consisted of 13 women and 15 men, aged 12–25 years at the time of spectroscopy. On the day of MRS all patients revealed severe hyperphenylalaninemia (1.2–2.16 mmol/l) that is typical for untreated classic PKU.

Genotyping of the *SLC7A5* gene identified 15 wild type individuals, 12 heterozygotes and one homozygote with regard to the rs113883650 variant. The normalized intensity of the brain phenylalanine signal ranged from 0.0041 to 0.0072 in the whole sample. The mean intensity was significantly higher in the carriers of the rs113883650 variant (0.0055) compared to the wild-type individuals (0.0048); $t(26) = 3.2$, $p = 0.0035$. On contrary, the mean blood phenylalanine concentration in patients with the variant was slightly lower than in wild-type individuals (1.57 mmol/l vs. 1.66 mmol/l), although the difference did not reach statistical significance ($p = 0.18$). We did not observe significant correlations between blood and brain phenylalanine concentrations in the entire group or in the two subgroups of patients.

Table 1 provides anthropometric data on the patients, details on PKU-causing mutations of the phenylalanine hydroxylase gene (*PAH*), PKU treatment initiation time, brain MRS spectra and blood phenylalanine concentrations on the day of MRS examination.

4. Discussion

The phenomenon of PKU patients who somehow seem to have escaped from severe intellectual disability despite high blood phenylalanine concentrations still remains unexplained. Two hypothetical mechanisms have been proposed, which could explain the lower (but probably not absent) brain vulnerability to hyperphenylalaninemia: the presence of interindividual variability of the cerebral transport of phenylalanine and/or the presence of an escape mechanism in one of the metabolic pathways mediating the cerebral response to hyperphenylalaninemia [3,4]. Our findings seem to support the first hypothesis. We showed that the presence of the rs113883650 variant of the *SLC7A5* gene has the potential to increase the concentration of brain phenylalanine in PKU patients with severe hyperphenylalaninemia. Additional rise of the cerebral content of phenylalanine in some of the patients would exacerbate the deficits in the production of dopamine and serotonin, further inhibit mitochondrial energy production and alter the protein metabolism in brain, which is observed in case of prolonged hyperphenylalaninemia [12–14].

The mechanism of the functional alteration of the LAT1 transporter remains to be elucidated. The genomic position of the rs113883650 variant overlaps with the binding site of the transcriptional repressor CTCF [15]. Thus, the presence of this variant could alter the *SLC7A5* gene expression and, consequently, the abundance of the LAT1 transporter. Interestingly, the membrane expression of LAT1 was shown to be inducible when stimulated by interferon γ [16]. Although hypothetical, similar effect of hyperphenylalaninemia should be tested in further cellular studies.

We did not observe significant correlations between blood and brain phenylalanine concentrations in our patients. However, since their blood phenylalanine concentrations at the time of MRS were very high, we believe that this finding could be explained by the effect of saturation of the transport of phenylalanine across the blood-brain barrier. Previously published data on the kinetics of transport of this amino acid across blood-brain barrier microvascular endothelial cells seem to support our hypothesis [17].

The limitations of the present study relate mainly to the diagnostic potential of the MRS technique, and to the relatively small size of the assessed group of patients.

The weak spectroscopic signal at 7.37 ppm overlaps with the signal of other substances, such as tyrosine, which together form a background noise. This makes detection of small amounts of phenylalanine problematic [11]. In addition, interindividual reproducibility of the

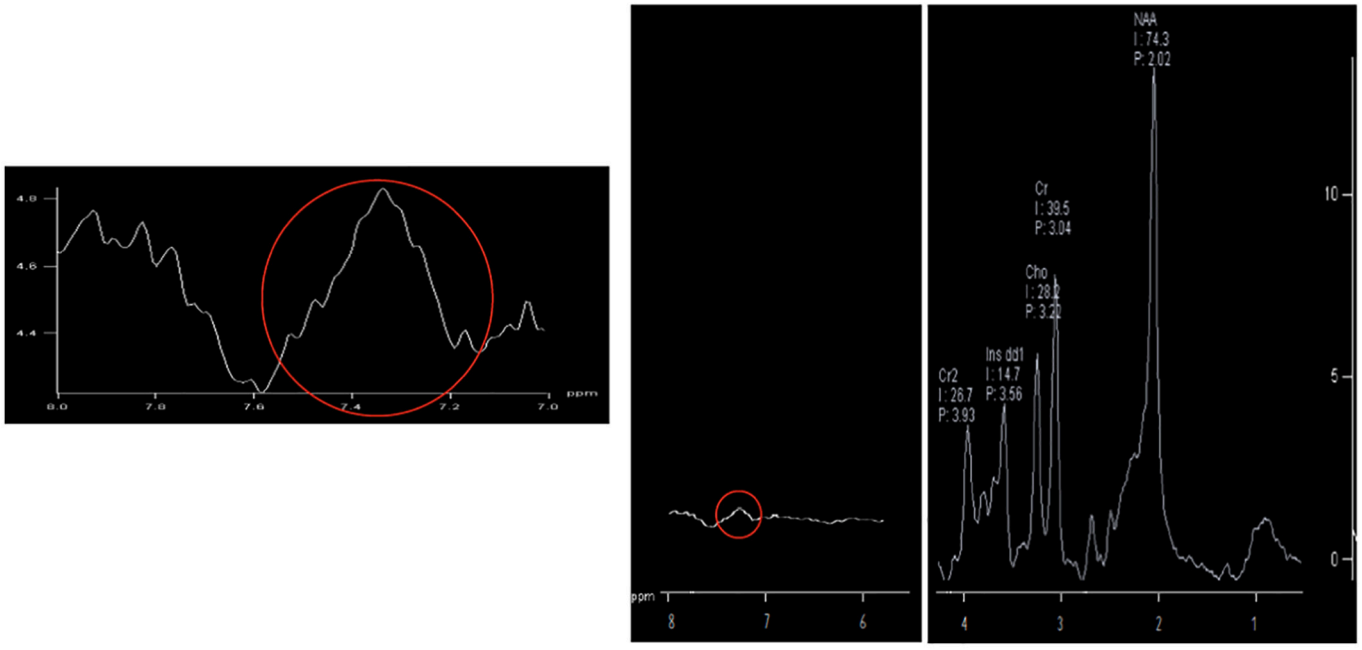

Fig. 1. MRS chromatogram of the brain in a patient with PKU.
The peak at 7.37 ppm (marked with a circle) represents the signal of phenylalanine (Phe). Comparison of intensities of this relatively weak signal between patients requires prior normalization of the intensity of their spectroscopic spectra. This can be done by using the signal of the three major peaks: NAA, creatine (Cr) and choline (Cho) as a frame of reference. The normalized brain phenylalanine signal (Phe_{norm}) is then given by:
Phe_{norm} = Phe integral/(NAA + Cr + Cho integrals).

Table 1
Participants of the study, their genotypes and blood phenylalanine (Phe) concentration and brain signal of this amino acid.

Patient ID	Sex	Age at treatment initiation (months)	Age at MRS assessment (years)	Intellectual status (IQ)	*PAH* gene mutation	Blood Phe (mmol/l)	Phe signal in brain MRS (A)	NAA + Creatine + Choline signals in brain MRS (B)	Normalized brain Phe signal (A/B)
Patients with the rs113883650 variant of the *SLC7A5* gene									
SA	F	2	13	Low average	p.R408W/p.R408W	2.16	1.17	162	0.0072
SMA	M	2	12	Average	p.R408W/p.R408W	1.78	0.95	212	0.0045
SK	F	6	15	Low average	p.R408W/p.R408W	1.78	1.01	216	0.0047
WM	M	2	19	Average	p.R408W/p.R408W	1.75	0.89	155	0.0058
KS	F	2	12	Average	p.R408W/IVS10-11 g > a	1.63	1.46	231	0.0063
MKL	F	2	19	Average	p.R408W/p.R408W	1.61	0.97	207	0.0047
BB	M	2	20	Average	p.R408W/p.R408W	1.55	1.11	202	0.0055
KAN	F	2	15	Low average	p.R408W/p.R408W	1.48	1.21	222	0.0054
KAD	M	2	12	Average	p.R408W/p.R408W	1.43	1.15	197	0.0058
RJ	M	2	12	Average	p.R408W/p.R408W	1.39	0.95	196	0.0048
KH	M	2	21	Low average	p.R408W/IVS10-11 g > a	1.37	1.36	213	0.0069
ST	M	3	18	Average	p.R408W/p.R408W	1.31	1.2	210	0.0057
PE	F	2	15	Average	p.R408W/p.R408W	1.2	1.03	182	0.0057
Wild-type individuals (no rs113883650 variant of the *SLC7A5* gene)									
SMO	F	6	25	Low average	p.R408W/p.G272*	2.02	1.07	200	0.0054
LG	M	2	12	Average	p.R408W/p.R408W	1.96	1.15	224	0.0051
LA	F	2	18	Low average	p.R408W/IVS10-11 g > a	1.91	1.09	220	0.0049
UP	F	2	12	Average	p.R408W/p.R408W	1.88	1.22	220	0.0055
SP	F	3	21	Low average	p.R408W/p.G272*	1.87	0.96	227	0.0042
CD	M	3	19	Average	p.R408W/p.F55Lfs*6	1.8	0.67	163	0.0041
MP	M	2	17	Average	p.L194P/p.Y277C	1.77	1.06	216	0.0049
CM	M	3	22	Low average	p.R408W/not identified	1.75	1.08	238	0.0045
TS	F	3	23	Low average	p.R408W/p.Q283*	1.7	1.05	198	0.0053
MKI	F	2	16	Average	p.R408W/p.R158Q	1.53	1.1	221	0.005
SMT	M	2	13	Average	p.R408W/p.R408W	1.52	1.11	233	0.0048
GP	M	2	14	Low average	p.R408W/not identified	1.41	0.86	190	0.0045
RM	F	2	22	Average	p.R408W/p.R408W	1.31	0.83	195	0.0043
SB	M	3	21	Average	IVS12 + 1 g > a/IVS12 + 1 g > a	1.26	0.89	217	0.0041
KM	M	3	12	Very high (136)	p.R408W/p.R408W	1.26	1.06	196	0.0054

spectroscopic spectra is not perfect (which is visible in Table 1). Therefore, it is necessary to normalize the phenylalanine signal. Thus, we only measured the relative amount of brain phenylalanine using a ratio of the integrals of the peaks of phenylalanine and of NAA + Creatine+Choline, which served as a frame of reference. More powerful magnetic resonance scanners and novel techniques of spectroscopic signal acquisition, such as correlated spectroscopy [18] could be useful in resolving the above technical problems in future studies.

It should be stressed, that the above-described cohort of patients was relatively small. In addition, they were treated according to historical therapeutic schemes with significantly delayed treatment start, which could result in decline of their intellectual status (Table 1). Moreover, their treatment adherence before the MRS study was very low. This makes the comparison of the above-described cohort with early and continuously treated PKU patients difficult. Therefore, additional studies in bigger groups of early treated patients should be performed to further build upon the findings of the present study.

In conclusion, our results show that the rs113883650 variant of the *SLC7A5 (LAT1)* gene may influence the amount of phenylalanine in brain in patients with PKU and could be one of the factors responsible for interindividual differences of the brain vulnerability to the toxicity of hyperphenylalaninemia.

Informed consent

Informed consent was obtained from the patients participating in the study.

CRediT authorship contribution statement

Miroslaw Bik-Multanowski: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Writing - original draft, Supervision, Funding acquisition, Writing - review & editing. **Kinga Bik-Multanowska:** Conceptualization, Formal analysis, Writing - original draft. **Iwona Betka:** Methodology, Investigation. **Anna Madetko-Talowska:** Methodology, Investigation.

Declaration of Competing Interest

The authors declare that they have no competing financial interests and no other conflicts of interest in connection with this paper.

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