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Inter-individual variation in brain phenylalanine concentration in patients with PKU is not caused by genetic variation in the 4F2hc/LAT1 complex

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Abstract

It remains a question why some patients with phenylketonuria (PKU) have high IQ and low brain phenylalanine (Phe) concentrations in spite of high blood Phe levels. One possible explanation for the low brain Phe concentrations in these patients would be a reduced transport of Phe across the blood–brain barrier. The 4F2hc/LAT1 complex has been suggested to be the most important molecular component responsible for this transport. To test the hypothesis that structural variant(s) in the genes encoding 4F2hc and LAT1 might result in a complex with reduced affinity for Phe, we have screened the two genes for sequence variants in a group of 13 PKU patients with a low ratio of brain to blood Phe concentrations. Several common sequence variants were identified, but none of these is predicted to affect the resulting protein product. Our data suggest that individual vulnerability to Phe in patients with PKU is not due to structural variants in the 4F2hc/LAT1 complex.

Keywords: Genotype; Phenotype; Phenylalanine; LAT1; 4F2hc; Blood–brain barrier

Introduction

Phenylketonuria (PKU) is caused by inherited phenylalanine hydroxylase (PAH) deficiency and is characterized by elevated blood levels of phenylalanine (Phe). PKU is the result of mutations in the *PAH* gene and shows a large variation in clinical expression. The metabolic phenotype can be divided into four categories defined on the basis of blood Phe concentration or dietary Phe tolerance: classical, moderate and mild PKU, and mild hyperphenylalaninemia [1]. More than 100 different *PAH* mutations have been assigned to one of these categories [2].

Patients with PKU can be efficiently treated with a Phe-restricted diet, and dietary therapy is preferably guided by blood Phe concentrations. If left untreated, PKU patients with a blood Phe concentration >600 μM will, in general, become mentally retarded [3]. Magnetic resonance imaging studies have revealed a neurotoxic effect of Phe. In patients with blood Phe concentrations >600 μM, an effect can be observed on the posterior/periventricular white matter [4]. In more severely affected patients, an effect on the frontal and subcortical white matter may also be observed [5].

PAH genotype does not always correlate with intellectual phenotype [2]. Several reports have described patients

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with normal IQ in spite of severe PKU and high blood Phe concentrations. Interestingly, a low concentration of brain Phe was found in many of these patients [6,7]. Accordingly, a likely explanation for the different vulnerability to PKU is inter-individual variation in brain Phe concentration. Transport of the large neutral amino acids, including Phe, across the blood–brain barrier is mediated by the amino acid transport system L. One isoform consists of the L-type amino acid transporter 1 (LAT1), which is functionally active only when expressed together with the 4F2hc subunit, forming a heterodimer [8,9]. One possible explanation for the inter-individual variation in blood–brain Phe concentration could be variations in the affinity of the LAT1-4F2hc heterodimer for Phe due to a structural variant(s). To test for this hypothesis, we have analysed the two genes for sequence variants in PKU patients with a low brain to blood Phe ratio.

Patients and methods

Subjects and brain Phe levels

Thirteen PKU patients (age 28–32 years) with a low concentration of brain Phe in spite of high blood Phe concentrations were selected for this study (Table 1). These patients had a relatively high IQ even though they had received no or only poor dietary therapy. Brain Phe concentrations were examined at the Childrens Hospital of Los Angeles, and the patients were *PAH* genotyped at the Kennedy Institute (Table 1) as part of earlier published investigations [7,10]. The mean blood Phe level was 1.334 mmol/L and the mean brain level was 0.23 mmol/L (Table 1). As controls, 8 random blood donors and 8 typical PKU patients with a high ratio of brain to blood Phe levels were examined. In the latter group, the mean blood Phe level was 1.426 mmol/L and the mean brain level was 0.62 mmol/L (Table 2).

Mutation analysis

Genomic DNA was isolated from EDTA blood by the NaCl extraction method [11]. For denaturing gradient gel electrophoresis (DGGE; LAT1 exons 2–10), individual exons were PCR-amplified using HotStarTaq DNA polymerase (Qiagen). The products were analysed in a 6% acrylamide gel with a 0–90% linear gradient of denaturants for 18 h, at 115 V and 58 °C. For sequence analysis (LAT1 exon 1, all exons of 4F2hc, and aberrant DGGE bands), PCR products were subjected to direct sequence analysis using ^{32}P-labelled primers and Thermo Sequenase (USB), or automatic sequence analysis using the BigDye Terminater v3.1 Cycle Sequencing kit (Applied Biosystem). All primers listed are in Table 3.

Results

Thirteen PKU patients with high IQ and low concentrations of brain Phe in spite of high blood Phe concentrations were examined for sequence variants in the entire coding regions and flanking intronic sequences of the LAT1 (gene symbol *SLC7A5*; GenBank Accession No. NM_003486) and 4F2hc (gene symbol *SLC3A2*; GenBank Accession No. BC003000) genes. Three sequence variants were identified in the LAT1 gene,

Table 1
Clinical characteristics, *PAH* mutation genotypes, and LAT1 and 4F2hc variants of the 13 PKU patients included in this study

Subject	IQ	Blood Phe (mmol/L)	Brain Phe (mmol/L)	*PAH* genotype	LAT1 variants			4F2hc variants
					c.31 (p.L11L)	c.345 (p.G115G)	IVS3-31[d]	c.1066 (p.L356L)
DK (E-27)[a]	116	1.650	.23	R408W/F55FS	C/C	C/C	+/+	C/T
SS (E-15)[a]	112	1.122	.18	R408W/I65T	C/C	C/C	+/+	C/T
WS (Pt 2)[b]	120	1.080	.25	Del I94/ND	C/C	C/A	–/–	C/T
LL (Pt 7)[b]	120	1.428	.20	G257V/ND	C/T	C/C	+/+	C/T
SW (Pt 12)[b]	102	1.338	.18	R261Q/IVS12nt1g > a	C/C	C/C	+/–	C/C
CP (Pt 13)[b]	101	1.326	.16	IVS1nt5g > t/IVS1nt5g > t	C/T	C/C	+/+	C/C
TM (L-10)[c]	114	1.591	.16	Y277D/Y204C	C/C	C/C	+/+	C/C
CF (Pt 20)[b]	129	1.608	.31	R261X/IVS12nt1g > a	C/C	C/C	+/+	C/T
CS (I-17)[c]	124	1.965	.19	R408W/L213P	C/C	C/C	+/–	T/T
MB (D18)[a]	123	1.056	.22	A395P/IVS12nt1g > a	C/C	C/A	+/+	C/C
KL (L-18)[c]	123	1.371	.41	IVS12nt1g > a/IVS12nt1g > a	C/C	C/C	+/–	C/T
YW (L-22)[c]	113	.90	.22	R261Q/IVS12nt1g > a	C/C	C/C	+/+	C/T
JS (F10)[a]	136	.90	ND	IVS7nt1g > a/I65T	C/C	C/C	+/–	T/T

ND, not determined.
[a] Unpublished.
[b] Ref. [7].
[c] Ref. [10].
[d] Refers to the lack of 5 bp (c.771-31_35delATCAT).