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^{13}C-phenylalanine breath test and serum biopterin in schizophrenia, bipolar disorder and major depressive disorder

Toshiya Teraishi[a], Masahiro Kajiwara[b,c], Hiroaki Hori[a], Daimei Sasayama[a], Shinsuke Hidese[a], Junko Matsuo[a], Ikki Ishida[a], Yasuhiro Kajiwara[b], Yuji Ozeki[a,d], Miho Ota[a], Kotaro Hattori[a], Teruhiko Higuchi[e], Hiroshi Kunugi[a,*]

[a] Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo, 187-8502, Japan
[b] Yokohama University of Pharmacy, 601 Matano-cho, Totsuka-ku, Yokohama, Kanagawa, 245-0066, Japan
[c] Tri-X Biomedical, Inc., 4-12-5-406, Minamiyawata, Ichikawa, Chiba, 272-0023, Japan
[d] Department of Psychiatry, Dokkyo Medical University School of Medicine, Tochigi, 321-0293, Japan
[e] National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo, 187-8551, Japan

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ABSTRACT

Phenylalanine is required for the synthesis of the neurotransmitters dopamine, noradrenaline, and adrenaline. The rate-limiting step for phenylalanine metabolism is catalyzed by phenylalanine hydroxylase (PAH) and its cofactor tetrahydrobiopterin. We aimed to detect altered phenylalanine metabolism in major psychiatric disorders using the L-[1-^{13}C]phenylalanine breath test (^{13}C-PBT) and serum biopterin levels. We also investigated association of *PAH* mutations with schizophrenia and phenylalanine metabolism. ^{13}C-phenylalanine (100 mg) was orally administered, and the breath $^{13}CO_2/^{12}CO_2$ ratio was monitored for 120 min in four groups: 103 patients with schizophrenia (DSM-IV), 39 with bipolar disorder, 116 with major depressive disorder (MDD), and 241 healthy controls. Serum biopterin levels were measured by high performance liquid chromatography. Mutation screening of *PAH* exons was performed by direct sequencing in 46 schizophrenia patients. Association analysis was performed using six tag single nucleotide polymorphisms and the *PAH* Arg53His mutation by TaqMan assays in 616 schizophrenia patients and 1194 healthy controls. Analyses of covariance controlling for age, sex, and body weight showed that the index for the amount of exhaled $^{13}CO_2$ was significantly lower in the schizophrenia group than in the other three groups (all $p < 0.05$). Biopterin levels in schizophrenia and MDD were significantly lower than those in controls. Biopterin levels correlated with ^{13}C-PBT indices in controls. *PAH* polymorphisms were not associated with schizophrenia or ^{13}C-PBT indices. ^{13}C-PBT revealed reduced phenylalanine metabolism in schizophrenia, though we obtained no evidence of involvement of *PAH* polymorphism. Serum biopterin levels were lower in schizophrenia and MDD, warranting further investigation.

1. Introduction

Since the discovery of psychotropic agents, such as chlorpromazine and iproniazid, and their action mechanisms in 1950s, the monoamine neurotransmitters, dopamine, noradrenaline, and serotonin, have been of interest in the study of psychiatric diseases. An essential aromatic amino acid, phenylalanine, and its immediate metabolite, tyrosine, are required for biosynthesis of dopamine and noradrenaline and compete with tryptophan, the precursor of serotonin, for transport into the brain. The hydroxylation of these amino acid precursors is the rate-limiting step for monoamine biosynthesis and requires the common essential coenzyme, tetrahydrobiopterin (BH4). BH4 is also a cofactor for the conversion of arginine to nitric oxide (NO), which has a role as a neurotransmitter (Snyder and Ferris, 2000) and is involved in the regulation of monoaminergic and glutamatergic systems (Kamisaki et al., 1994; Kiss, 2000; Okusaga, 2014; Richardson et al., 2005, 2007). Phenylalanine influences BH4 synthesis through BH4-dependent feedback inhibition mediated by GTP cyclohydrolase I feedback regulatory protein (GFRP) (Milstien et al., 1996). Therefore, studies have examined potential alterations in the levels or metabolism of phenylalanine and BH4 in psychiatric diseases by obtaining quantitative data from biological fluids, such as peripheral blood and cerebrospinal fluid (CSF), as well as from genetic data.

Phenylalanine levels in blood and CSF were found to be significantly

* Corresponding author.
E-mail address: hkunugi@ncnp.go.jp (H. Kunugi).

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higher in drug-free (Bjerkenstedt et al., 1985; Poisner, 1960; Rao et al., 1990) and medicated (Okusaga et al., 2014; Reveley et al., 1987) patients with schizophrenia than in healthy controls, although contradictory findings have also been reported (Potkin et al., 1983; Wei et al., 1995). Wei *et al.* reported that the ratio of tyrosine to phenylalanine was significantly lower in patients with early-onset schizophrenia than in healthy controls, suggesting that activity of phenylalanine hydroxylase (PAH), which catalyzes phenylalanine to tyrosine, was inhibited (Wei et al., 1995). Investigations of phenylalanine and tyrosine levels in depression have also yielded inconsistent results (Benkert et al., 1971; Liu et al., 2015; Mayoral-Mariles et al., 2012); however, high ratios of tyrosine to phenylalanine or other large neutral amino acids were found to be protective in depression (Mayoral-Mariles et al., 2012) and related to treatment response in depressed patients (Hoekstra et al., 2001; Lucini et al., 1996; Møller et al., 1985). Plasma phenylalanine and tyrosine levels were reported to be discriminating variables for the Diagnostic and Statistical Manual of Mental Disorders, Third Edition (DSM-III) depressive subgroups (dysthymic disorders, major recurrent depression, and bipolar depression) (Chiaroni et al., 1990). Urinary phenylalanine was identified as one of 20 differential metabolites responsible for the discrimination between major depressive disorder (MDD) and bipolar disorder (BPD) subjects (Chen et al., 2015). Regarding metabolites of phenylalanine, plasma phenylethylamine, an endogenous neuroamine, was significantly higher in patients with schizophrenia (Janssen et al., 1999; O'Reilly et al., 1991), and plasma or urinary phenylacetic acid, a main metabolite of phenylethylamine, was significantly lower in patients with MDD (Sabelli et al., 1986) and BPD (Sabelli et al., 1983) than in controls. Plasma biopterin level, which reflects BH4 level (Fiege et al., 2004; Richardson et al., 2005), was decreased in patients with schizophrenia and schizoaffective disorder compared to that in controls (Richardson et al., 2005, 2007), although a few studies reported no significant differences in urine or CSF samples (Duch et al., 1984; Garbutt et al., 1982). Several studies showed higher biopterin levels in depression than in controls (Duch et al., 1984; Garbutt et al., 1985; Hashimoto et al., 1987, 1994; Knapp and Irwin, 1989), although contradictory findings have also been reported (Abou-Saleh et al., 1995; Coppen et al., 1989; Hoekstra et al., 2001, 2003). Studies on BPD have yielded mixed results (Blair et al., 1984; Duch et al., 1984; Hashimoto et al., 1990a).

Mutations in *PAH* are responsible for over 98% of phenylketonuria (PKU), an autosomal recessive genetic disorder characterized by mental retardation, epilepsy, eczema, and other clinical manifestations, resulting from hyperphenylalaninemia (Erlandsen and Stevens, 1999; Scriver, 2007; Surtees and Blau, 2000). Since the early study by Penrose *et al.* in 1935 (Penrose, 1935), the association between PKU mutation heterozygotes, i.e., parents of patients with PKU, and schizophrenia has been reported (Kuznetsova, 1974; Vogel, 1985). Richardson et al. found a novel mutation of *PAH*, Lys274Glu, which may be associated with psychiatric disorders in African-Americans (Chao and Richardson, 2002; Richardson et al., 1999) and decreased phenylalanine kinetics in schizophrenia (Richardson et al., 2003). *PAH* polymorphisms reportedly confer the risk of schizophrenia (Talkowski et al., 2009), delusions (Bergen et al., 2009), and poor cognitive performance (Teraishi et al., 2013).

^{13}C-Breath test is a noninvasive tracer test that can measure changes in the turnover rate of a substrate that is difficult to detect only by use of cross-sectional quantitative data from body fluids (Hasunuma et al., 2009). The L-[1-^{13}C]phenylalanine breath test (^{13}C-PBT) has been shown to be useful in the assessment of liver function (Ishii et al., 2001), PKU (Treacy et al., 1997), and BH4-responsive PAH deficiency (Okano et al., 2004). Previously, we reported the decreased whole-body metabolism of phenylalanine in schizophrenia using the ^{13}C-PBT (Teraishi et al., 2012) in a small number of subjects (20 patients and 20 controls). Further, by using the ^{13}C-tryptophan breath test, we found altered catabolic turnover of tryptophan along the kynurenine pathway in MDD (Teraishi et al., 2015). These studies suggest the possible use of stable carbon isotope ^{13}C-labeled breath tests in psychiatric practice.

In the present study, we demonstrated the use of ^{13}C-PBT in a relatively large sample of major psychiatric disorders. In addition, we examined the possible relevance of serum biopterin levels to phenylalanine metabolism, and we investigated the association of genetic variations of *PAH* with susceptibility to schizophrenia and phenylalanine metabolism.

2. Methods

2.1. Participants

2.1.1. ^{13}C-phenylalanine breath test and biochemical analysis for biopterin

We enrolled 103 patients with schizophrenia or schizoaffective disorder, 39 with BPD, 116 with MDD, and 241 controls. Patients were recruited at the National Center of Neurology and Psychiatry (NCNP) Hospital, Tokyo, Japan and associated psychiatric hospitals nearby (Henmi Hospital and Yamada Psychiatric Hospital). Participants were also recruited through free local information magazine advertisements, word of mouth, and website-based announcements. All participants were biologically unrelated Japanese who resided in the same geographical area, the western part of Tokyo. Patients were diagnosed by the consensus of at least two experienced psychiatrists according to the DSM, Fourth Edition (DSM-IV) criteria (American Psychiatric Association, 1994) and based on the Japanese version of the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Otsubo et al., 2005; Sheehan et al., 1998), a careful examination of medical records, observations, and additional unstructured interviews. Current clinical symptom severity was assessed by the positive and negative symptom scale (PANSS) (Kay et al., 1987) for schizophrenia and schizoaffective disorder and by the 21-item version of the Hamilton Depression Rating Scale (HAMD-21) for MDD and BPD. The mean Young Mania Rating Scale (YMRS) total score in patients with BPD (n = 24) was 4.0. All control participants were screened using the M.I.N.I. by psychiatrists of the research team to confirm no current or past history of major psychiatric disorders. Participants were excluded if they were pregnant or lactating or had a history of central nervous system disease or severe head injury, endocrine disease, respiratory disease, substance abuse/dependence, or a serious physical disorder, especially any diseases of the kidneys or liver, in which phenylalanine is primarily metabolized. All participants were assessed with a blood test to exclude kidney or liver dysfunction, including the following indices: aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholinesterase (ChE), blood urea nitrogen (BUN), and creatinine. Individuals with any abnormal blood test data were not considered eligible in the present study.

2.1.2. Genetic association analysis

For direct DNA sequencing of *PAH*, 46 patients were randomly selected from the schizophrenia patients who participated in the ^{13}C-PBT. For the association study between *PAH* polymorphisms and schizophrenia, we enrolled 616 patients with schizophrenia and 1194 controls. All participants were recruited and psychiatrically screened as described above.

After description of the study, signed informed consent was obtained from all participants or their legal next of kin if patients did not have the capacity or were on medical protection admission. The study protocol was approved by the ethics committee of NCNP.

2.2. ^{13}C-phenylalanine breath test (^{13}C-PBT)

2.2.1. Principle

The kinetic values for L-[1-^{13}C]phenylalanine were assumed to be the same as those for unlabeled phenylalanine (Brown, 1997; Teraishi et al., 2012). Approximately three-fourths of absorbed dietary phenylalanine is metabolized to tyrosine by PAH and its cofactor BH4 in the