interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Statement of Interest

All authors declare that they have no conflict of interest.

References

Almeida OP, Schwab SG, Lautenschlager NT, Morar B, Greenop KR, Flicker L, et al. 2008. KIBRA genetic polymorphism influences episodic memory in later life, but does not increase the risk of mild cognitive impairment. J Cell Mol Med 12(5A):1672–1676.

Bates TC, Price JF, Harris SE, Marioni RE, Fowkes FG, Stewart MC, et al. 2009. Association of KIBRA and memory. Neurosci Lett 458(3):140–143.

Corneveaux JJ, Liang WS, Reiman EM, Webster JA, Myers AJ, Zismann VL, et al. 2010. Evidence for an association between KIBRA and late-onset alzheimer's disease. Neurobiol Aging 31(6): 901–9.

Faul F, Erdfelder E, Buchner A, & Lang A-G. 2009. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. Behav Res Methods 41:1149–1160.

First MB, Spitzer RL, Gibbon M, Williams JBW. 1997. Structured Clinical Interview for DSM-IV Axis I Disorders-Non-patients Edition (SCID-I/NP, Version 2.0–7/97 reversion). Biometrics Research Department, New York State Psychiatric Institute, New York.

Greenwood PM, Parasuraman, R. 2003. Normal genetic variation, cognition, and aging. Behav Cogn Neurosci Rev 2(4):278–306.

Hashimoto R, Numakawa T, Ohnishi T, Kumamaru E, Yagasaki Y, Ishimoto T, et al. 2006. Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology and ERK signaling. Hum Mol Genet 15(20):3024–3033.

Hashimoto R, Hashimoto H, Shintani N, Chiba S, Hattori S, Okada T, et al. 2007. Pituitary adenylate cyclase-activating polypeptide is associated with schizophrenia. Mol Psychiatry 12(11):1026–1032.

Hilton HN, Stanford PM, Harris J, Oakes SR, Kaplan W, Daly RJ, et al. 2008. KIBRA interacts with discoidin domain receptor 1 to modulate collagen-induced signalling. Biochim Biophys Acta 1783(3):383–393.

Kremerskothen J, Plaas C, Buther K, Finger I, Veltel S, Matanis T, et al. 2003. Characterization of KIBRA, a novel WW domain-containing protein. Biochem Biophys Res Commun 300(4):862–867.

Supplementary material available online

Table showing collated results

Nacmias B, Bessi V, Bagnoli S, Tedde A, Cellini E, Piccini C, et al. 2008. KIBRA gene variants are associated with episodic memory performance in subjective memory complaints. Neurosci Lett 436(2):145–147.

Need AC, Attix DK, McEvoy JM, Cirulli ET, Linney KL, Wagoner AP, et al. 2008. Failure to replicate effect of Kibra on human memory in two large cohorts of European origin. Am J Med Genet B Neuropsychiatr Genet 147B(5):667–668.

Need AC, Attix DK, McEvoy JM, Cirulli ET, Linney KL, Hunt P, et al. 2009. A genome-wide study of common SNPs and CNVs in cognitive performance in the CANTAB. Hum Mol Genet 18(23):4650–4661.

Ohi K, Hashimoto R, Yasuda Y, Yoshida T, Takahashi H, Iike N, et al. 2009. Association study of the G72 gene with schizophrenia in a Japanese population: A multicenter study. Schizophr Res 109(1–3):80–85.

Papassotiropoulos A, Stephan DA, Huentelman MJ, Hoerndli FJ, Craig DW, Pearson JV, et al. 2006. Common Kibra alleles are associated with human memory performance. Science 314(5798):475–478.

Preuschhof C, Heekeren HR, Li SC, Sander T, Lindenberger U, Bäckman L. 2009. KIBRA and CLSTN2 polymorphisms exert interactive effects on human episodic memory. Neuropsychologia 48:402–408.

Rayala SK, den Hollander P, Manavathi B, Talukder AH, Song C, Peng S, et al. 2006. Essential role of KIBRA in co-activator function of dynein light chain 1 in mammalian cells. J Biol Chem 281(28):19092–19099.

Rodriguez-Rodriguez E, Infante J, Llorca J, Mateo I, Sanchez-Quintana C, Garcia-Gorostiaga I, et al. 2009. Age-dependent association of KIBRA genetic variation and Alzheimer's disease risk. Neurobiol Aging 30(2):322–324.

Rosenberg SJ, Ryan JJ, Prifitera A. 1984. Rey Auditory-Verbal Learning Test performance of patients with and without memory impairment. J Clin Psychol 40(3):785–787.

Schaper K, Kolsch H, Popp J, Wagner M, Jessen F. 2008. KIBRA gene variants are associated with episodic memory in healthy elderly. Neurobiol Aging 29(7):1123–1125.

Wechsler D. 1987. Wechsler Memory Scale Manual, Revised. San Antonio, TX: Psychological Corporation.

Yoshihama Y, Hirai T, Ohtsuka T, Chida K. 2009. KIBRA Co-localizes with protein kinase Mzeta (PKMzeta) in the mouse hippocampus. Biosci Biotechnol Biochem 73(1): 147–151.

Zhang H, Kranzler HR, Poling J, Gruen JR, Gelernter J. 2009. Cognitive flexibility is associated with KIBRA variant and modulated by recent tobacco use. Neuropsychopharmacology 34(12):2508–2516.

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

The Impact of a Genome-Wide Supported Psychosis Variant in the *ZNF804A* Gene on Memory Function in Schizophrenia

Ryota Hashimoto,[1,2,3]* Kazutaka Ohi,[2,3] Yuka Yasuda,[2,3] Motoyuki Fukumoto,[2,3] Masao Iwase,[2] Naomi Iike,[2,3] Michiyo Azechi,[2] Koji Ikezawa,[2] Masahiko Takaya,[2] Hidetoshi Takahashi,[2] Hidenaga Yamamori,[2,4] Tomo Okochi,[5] Hitoshi Tanimukai,[2] Shinji Tagami,[2] Takashi Morihara,[2] Masayasu Okochi,[2] Toshihisa Tanaka,[2] Takashi Kudo,[2] Hiroaki Kazui,[2] Nakao Iwata,[3,5] and Masatoshi Takeda[2]

[1]Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, Suita, Osaka, Japan

[2]Department of Psychiatry, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

[3]CREST (Core Research for Evolutionary Science and Technology) of JST (Japan Science and Technology Agency), Tokyo, Japan

[4]Department of Molecular Neuropsychiatry, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

[5]Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi, Japan

Received 18 May 2010; Accepted 6 August 2010

A recent genome-wide association study showed that a variant (rs1344706) in the *ZNF804A* gene was associated with schizophrenia and bipolar disorder. Replication studies supported the evidence for association between this variant in the *ZNF804A* gene and schizophrenia and that this variant is the most likely susceptibility variant. Subsequent functional magnetic resonance imaging studies in healthy subjects demonstrated the association of the high-risk *ZNF804A* variant with neural activation during a memory task and a theory of mind task. As these cognitive performances are disturbed in patients with schizophrenia, this gene may play a role in cognitive dysfunction in schizophrenia. The aim of the current study was to investigate the potential relationship between this *ZNF804A* polymorphism and memory function. The effects of the high-risk *ZNF804A* genotype, diagnosis, and genotype–diagnosis interaction on verbal memory, visual memory (VisM), attention/concentration, and delayed recall (measured by the Wechsler Memory Scale-Revised) were analyzed by two-way analysis of covariance in 113 patients with schizophrenia and 184 healthy subjects. Consistent with previous studies, patients with schizophrenia exhibited poorer performance on all indices as compared to healthy control subjects ($P < 0.001$). A significant *ZNF804A* genotype–diagnosis interaction was found for VisM performance ($P = 0.0012$). Patients with the high-risk T/T genotype scored significantly lower on VisM than G carriers did ($P = 0.018$). In contrast, there was no genotype effect for any index in the healthy control subjects ($P > 0.05$). Our data suggest that rs1344706 may be related to memory dysfunction in schizophrenia. © 2010 Wiley-Liss, Inc.

Key words: ZNF804A; memory; schizophrenia; polymorphism; rs1344706

How to Cite this Article:
Hashimoto R, Ohi K, Yasuda Y, Fukumoto M, Iwase M, Iike N, Azechi M, Ikezawa K, Takaya M, Takahashi H, Yamamori H, Okochi T, Tanimukai H, Tagami S, Morihara T, Okochi M, Tanaka T, Kudo T, Kazui H, Iwata N, Takeda M. 2010. The impact of a genome-wide supported psychosis variant in the *ZNF804A* gene on memory function in schizophrenia.
Am J Med Genet Part B 153B:1459–1464.

Grant sponsor: Japanese Ministry of Health, Labor and Welfare; Grant number: H19-kokoro-002; Grant sponsor: Japanese Ministry of Education, Culture, Sports, Science and Technology; Grant number: 18689030; Grant sponsor: CREST of JST; Grant sponsor: MEXT (Scientific Research on Priority Areas—Research on Pathomechanisms of Brain Disorders); Grant number: 18023045; Grant sponsor: Japan Foundation for Neuroscience and Mental Health.

*Correspondence to:
Dr. Ryota Hashimoto, M.D., Ph.D., Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, D3, 2-2, Yamadaoka, Suita, Osaka 5650871, Japan. E-mail: hashimor@psy.med.osaka-u.ac.jp

Published online 18 October 2010 in Wiley Online Library (wileyonlinelibrary.com)
DOI 10.1002/ajmg.b.31123

INTRODUCTION

Schizophrenia (OMIM: 181500) is a common complex psychiatric disease with a lifetime risk of approximately 1%. There are strong genetic components of this disease, with an estimated heritability of approximately 80% [Cardno and Gottesman, 2000; Tsuang, 2000]. In a genome-wide association study and follow-up studies, a single nucleotide polymorphism (SNP) in the *ZNF804A* gene (rs1344706) was found to be associated with schizophrenia and bipolar disorder [O'Donovan et al., 2008]. Subsequent replication studies demonstrated the association between schizophrenia and the *ZNF804A* gene and that rs1344706 remained the most strongly associated marker in the gene after fine mapping of *ZNF804* locus [Riley et al., 2010; Steinberg et al., 2010; Williams et al., 2010; Zhang et al., 2010].

The *ZNF804A* gene (OMIM: 612282) is located on chromosome 2q32.1 and consists of four exons and three introns spanning 341 kb. Although little is known about the encoded protein and its function, the sequence contains predicted zinc ion and DNA-binding domains, suggesting a role in the regulation of gene expression. Two imaging genetics studies using functional magnetic resonance imaging (fMRI) have demonstrated associations between the high-risk *ZNF804A* variant and neural activation during a memory task and a theory of mind task in healthy subjects [Esslinger et al., 2009; Walter et al., 2010]. The high-risk *ZNF804A* variant had impact on brain functional dysconnectivity between dorsolateral prefrontal cortex (DLPFC) and hippocampal formation during an N-back memory task in healthy subjects [Esslinger et al., 2009]. This altered connectivity between DLPFC and hippocampal formation might be a basis of human memory function.

Patients with schizophrenia have pronounced deficits in aspects of neurocognitive function such as speed of processing, attention/vigilance, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving, and social cognition [Nuechterlein et al., 2004]. Cognitive impairments are strongly related to functioning in areas such as work, social relationships, and independent living in schizophrenia. The lack of marked cognitive benefit of present antipsychotics has led to the investigation of alternative drugs and mechanisms for the treatment of these impairments [Buchanan et al., 2007]. Intermediate phenotypes/endophenotypes represent simpler clues to genetic underpinnings than the disease syndrome itself, promoting the view that psychiatric diagnoses can be decomposed or deconstructed, which can result in more straightforward and successful genetic analysis [Gottesman and Gould, 2003; Preston and Weinberger, 2005]. Memory deficits are prominent trait markers of schizophrenia, with impairments also observed in first-degree relatives [Snitz et al., 2006]. Genetic risk for schizophrenia could affect functional activity in the brain; such changes have been shown to mediate disturbed memory function [Meyer-Lindenberg and Weinberger, 2006]. In the present study, we examined the effect of the genome-wide supported variant in the *ZNF804A* gene on memory functions in patients with schizophrenia.

MATERIALS AND METHODS

Sample Description

The subjects of this study consisted of 113 patients with schizophrenia [53.1% males, mean age ± standard deviation: 38.3 ± 12.1 years] and 184 healthy control subjects [47.8% males, 36.2 ± 11.5 years]. The sex ratio and mean age did not differ significantly between patients and control subjects ($P > 0.05$), whereas the years of education were significantly lower among patients with schizophrenia (14.2 ± 2.4) than among control subjects (15.4 ± 2.4) [$z = -4.20$, $P < 0.001$]. All subjects were biologically unrelated Japanese individuals. Subjects were excluded from this study if they had neurological or medical conditions that could affect the central nervous system, such as atypical headache, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, cancer in an active stage, cerebrovascular disease, epilepsy, seizures, substance-related disorders, or mental retardation. Cases were both outpatients and inpatients at Osaka University Hospital. Each patient with schizophrenia had been diagnosed by a trained psychiatrist according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria based on the Structured Clinical Interview for DSM-IV (SCID) for schizophrenia. Healthy control subjects were recruited through local advertisements at Osaka University. Psychiatrically, medically, and neurologically healthy control subjects were evaluated using the DSM-IV-Non-Patient version of the Structured Clinical Interview to exclude individuals who had current or past contact with psychiatric services or had received psychiatric medication [Ohi et al., 2009]. Written informed consent was obtained for all subjects after the procedures had been fully explained. This study was carried out in accordance with the World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University.

Genotyping

We selected rs1344706 in the *ZNF804A* gene because this SNP has been found to be associated with schizophrenia and bipolar disorder in genome-wide association and follow-up studies [O'Donovan et al., 2008] and the four replication studies confirmed the association [Riley et al., 2010; Steinberg et al., 2010; Williams et al., 2010; Zhang et al., 2010]. Furthermore, this SNP was related to functional brain activity in healthy subjects [Esslinger et al., 2009; Walter et al., 2010]. Venous blood was collected from the subjects, and genomic DNA was extracted from whole blood according to standard procedures. The SNP was genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, CA) as described previously [Hashimoto et al., 2006, 2007]. Detailed information on the PCR conditions is available upon request.

Phenotype Measures

A full version of the Wechsler Memory Scale-Revised (WMS-R) [Sugishita, 2001], which is generally used to measure memory functions, was administered to the subjects. The four indices of the WMS-R, that is, verbal memory (VerM), visual memory (VisM), attention/concentration (AC), and delayed recall (DR), were used for the analysis. Psychiatric symptoms in patients with schizophrenia were evaluated using the positive and negative syndrome scale (PANSS) [Kay et al., 1987].

TABLE I. Demographic and Clinical Characteristics of Patients with Schizophrenia and Controls

	Schizophrenia (n = 113)						Control (n = 184)					
	T/T (n = 21)		G carrier (n = 92)				T/T (n = 44)		G carrier (n = 140)			
Variables	**Mean**	**SD**	**Mean**	**SD**	***P*-value**	**z**	**Mean**	**SD**	**Mean**	**SD**	***P*-value**	**z**
Age (years)	38.1	11.2	38.4	12.4	0.99	0.01	36.5	10.8	36.1	11.8	0.68	0.42
Sex (male/female)[a]	10/11		49/43		0.94	0.01	24/20		64/76		0.31	1.05
Education (years)	14.2	2.2	14.2	2.4	0.80	0.25	14.7	1.9	15.6	2.5	**0.05**	1.99
CPZeq (mg/day)	586.2	518.6	535.9	443.1	0.95	0.06	—	—	—	—	—	—
Age at onset (years)	23.7	10.2	24.2	8.6	0.76	0.31	—	—	—	—	—	—
Duration of illness (years)	14.4	9.7	14.2	11.1	0.74	0.33	—	—	—	—	—	—
Positive symptoms[b,c]	16.0	7.9	18.2	5.5	0.10	1.62	—	—	—	—	—	—
Negative symptoms[b,c]	18.6	7.5	18.6	7.0	0.89	0.14	—	—	—	—	—	—

CPZeq, chlorpromazine equivalents of total antipsychotics; [b]PANSS, positive and negative syndrome scale; SD, standard deviation. T/T: individuals with T/T genotype of rs1344706. G carriers: individuals with G/G and G/T genotypes of rs1344706. Differences in clinical characteristics between genotype groups were analyzed using the Mann–Whitney *U*-test, except for [a]χ^2 test. [c]T/T: n = 18; G carrier: n = 84. A significant *P*-value is shown as bold face and underlined.

Statistical Analyses

Statistical analyses were performed using SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan) and PASW Statistics 18.0 software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and control subjects as well as between genotype groups were analyzed using χ^2 tests for categorical variables and the Mann–Whitney *U*-test for continuous variables. The presence of Hardy–Weinberg equilibrium was examined using the χ^2 test for goodness of fit. No deviation from Hardy–Weinberg equilibrium was detected in cases or in controls ($P > 0.05$). To examine the effect of ZNF804A rs1344706 genotype on memory function, the effects of *ZNF804A* genotype, diagnosis, and genotype–diagnosis interactions on four memory domains were analyzed by a two-way analysis of variance (ANOVA). In further analysis to control for confounding factors, the genotype effects, diagnosis effects, and genotype–diagnosis interactions on the memory functions were adjusted by a two-way analysis of covariance (ANCOVA) with sex and years of education as covariates (the scores of indices were previously corrected by age). When genotype–diagnosis interaction was found, cases and controls were separately analyzed by ANOVA and ANCOVA. The Bonferroni correction was applied for multiple testing on four indices of the WMS-R to avoid type I error. Standardized effect sizes were calculated using Cohen's *d* method (http:www.uccs.edu/faculty/lbecker). The significance level for statistical tests was set at two-tailed $P < 0.05$.

RESULTS

The Effect of the High-Risk *ZNF804A* Polymorphism on Memory Functions

We examined potential associations between the *ZNF804A* genotype and memory functions in patients with schizophrenia and healthy controls. There was no difference in age, sex, chlorpromazine equivalents of total antipsychotics, age at onset, duration of illness, or PANSS scores between genotype groups. The only difference in demographic variables was a significantly greater number of years of education in the control groups ($z = 1.99$, $P = 0.05$; Table I). The *ZNF804A* genotype effects, diagnosis effects, and genotype–diagnosis interactions on memory functions are shown in Table II. We found significant effects of diagnosis (VerM: $F_{1,293} = 146.91$, $P < 0.001$; adjusted $F_{1,291} = 133.70$, $P < 0.001$, VisM: $F_{1,293} = 114.30$, $P < 0.001$; adjusted $F_{1,291} = 103.87$, $P < 0.001$, AC: $F_{1,293} = 53.46$, $P < 0.001$; adjusted $F_{1,291} = 48.59$; $P < 0.001$, DR: $F_{1,293} = 200.36$, $P < 0.001$; adjusted $F_{1,291} = 186.09$, $P < 0.001$) and genotype–diagnosis interaction (VisM: $F_{1,293} = 8.21$, $P = 0.0045$, adjusted $F_{1,291} = 10.76$, $P = 0.0012$). Significant genotype effects were only found for VisM ($F_{1,293} = 4.46$, $P = 0.036$, adjusted $F_{1,291} = 3.40$, $P = 0.066$). The effect of diagnosis and the diagnosis–genotype interaction remained positive after correction for multiple tests (corrected *P*-values, VerM: $P < 0.001$, VisM: $P < 0.001$, AC: $P < 0.001$, DR: $P < 0.001$, interaction in VisM: $P = 0.0048$), whereas the genotype effect on VisM did not remain after correction for multiple tests ($P > 0.14$). Patients with schizophrenia displayed lower scores on all memory indices than did controls, and the effect sizes of VerM, VisM, AC, and DR were −1.72, −1.21, −1.17, and −1.89, respectively. As a genotype–diagnosis interaction was found for VisM, we separately analyzed the effects of genotype on VisM in patients and controls (Fig. 1). There was a significant genotype effect in patients with schizophrenia ($F_{1,111} = 5.05$, $P = 0.027$; adjusted $F_{1,109} = 5.82$, $P = 0.018$), whereas there was no genotype effect in controls ($F_{1,182} = 0.88$, $P = 0.35$; adjusted $F_{1,180} = 1.43$, $P = 0.23$). The patients with the high-risk T/T genotype scored significantly lower on VisM than did those who carry a G genotype (effect size: −0.56).

When the two genotypes were divided into three genotype groups (patients with T/T genotype, T/G genotype, and G/G genotype), the patients with the high-risk T/T genotype scored significantly lower on VisM than patients with the T/G genotype (adjusted $F_{1,68} = 8.59$, $P = 0.0046$) and marginally lower than patients with the G/G genotype (adjusted $F_{1,58} = 2.89$, $P = 0.09$;

TABLE II. Effects of the *ZNF804A* Genotype on Memory Function Determined Using WMS-R

	Schizophrenia (n = 113)				Control (n = 184)				ANOVA						ANCOVA (adjusted)					
	T/T (n = 21)		G carrier (n = 92)		T/T (n = 44)		G carrier (n = 140)		Diagnosis effect		Genotype effect		Interaction		Diagnosis effect		Genotype effect		Interaction	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*-value	$F_{1,293}$	*P*-value	$F_{1,293}$	*P*-value	$F_{1,293}$	*P*-value	$F_{1,291}$	*P*-value	$F_{1,291}$	*P*-value	$F_{1,291}$
VerM	82.6	14.6	84.6	18.3	110.2	14.1	111.3	13.0	$<10^{-3}$	**146.9**	0.50	0.45	0.83	0.04	$<10^{-3}$	**133.7**	0.78	0.08	0.47	0.52
VisM	81.7	18.2	92.3	19.7	110.5	8.3	108.9	10.3	$<10^{-3}$	**114.3**	**0.036**	**4.46**	**0.0045**	**8.21**	$<10^{-3}$	**103.9**	0.066	3.40	**0.0012**	**10.8**
AC	92.0	16.5	90.9	15.2	105.1	13.6	109.2	13.9	$<10^{-3}$	**53.5**	0.48	0.50	0.23	1.44	$<10^{-3}$	**48.6**	0.54	0.37	0.28	1.17
DR	77.1	18.4	82.6	19.5	111.7	12.7	112.0	11.8	$<10^{-3}$	**200.4**	0.20	1.65	0.25	1.31	$<10^{-3}$	**186.1**	0.35	0.88	0.10	2.71

WMS-R, Wechsler Memory Scale-Revised; VerM, verbal memory; VisM, visual memory; AC, attention/concentration; DR, delayed recall; SD, standard deviation. T/T: individuals with T/T genotype of rs1344706. G carriers: individuals with G/G or G/T genotype of rs1344706. The effects of the ZNF804A genotype and the effects of diagnosis on the memory function were analyzed by a two-way analysis of variance (ANOVA). Adjusted effects of genotype were analyzed by a two-way analysis of covariance (ANCOVA) with sex and years of education as covariates. Significant *P*-values are shown as bold face and underlined.

FIG. 1. The association between the high-risk *ZNF804A* genotype and visual memory in patients with schizophrenia. X-axis: gray bars, individuals with T/T genotype of rs1344706; white bars, individuals with a G allele (G/T and G/G genotypes) of rs1344706. Y-axis: scores of visual memory from the WMS-R. Error bars represent standard errors of the mean. *$P < 0.05$, compared with patients with a G allele.

Table III). However, there was no significant difference in scores between patients with the T/G genotype and G/G genotype ($F_{1,88} = 1.39$, $P = 0.24$).

DISCUSSION

In the present study, we first demonstrated an association between the high-risk *ZNF804A* SNP and memory performance in patients with schizophrenia. We provided evidence that patients with the high-risk T/T genotype had lower performance on VisM than patients who carry a G allele. The effect size of the difference in VisM scores between patients with the T/T genotype and G carriers was −0.56; this effect is typically considered a medium-sized effect. We do not know why we found the genotype effect on only VisM. A possible explanation is that a previous study reported suggestive linkage evidence for the VisM on 2q36 near the locus of the *ZNF804A* gene [Paunio et al., 2004]. Another possibility is that this SNP is associated with connectivity during N-back memory task, which is an fMRI task using visual cue [Esslinger et al., 2009]. This study showed no effect of genotype on a memory task in healthy subjects, which is consistent with our data [Esslinger et al., 2009].

A linear genotype effect on connectivity in DLPFC and hippocampal formation during a memory task was found in healthy control subjects in an fMRI study [Esslinger et al., 2009]. These data

TABLE III. Effects of the *ZNF804A* Genotype on Memory Performance

	Schizophrenia (n = 113)						Control (n = 184)						ANCOVA (adjusted)					
	T/T (n = 21)		T/G (n = 51)		G/G (n = 41)		T/T (n = 44)		T/G (n = 85)		G/G (n = 55)		Diagnosis effect		Genotype effect		Interaction	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*-value	$F_{2,289}$	*P*-value	$F_{2,289}$	*P*-value	$F_{2,289}$
VerM	82.6	14.6	83.9	19.1	85.5	17.3	110.2	14.1	112.5	12.8	109.4	13.2	**<u>$<10^{-3}$</u>**	**<u>168.5</u>**	0.61	0.49	0.52	0.66
VisM	81.7	18.2	93.2	20.2	91.1	19.3	110.5	8.3	108.4	10.0	109.7	10.9	**<u>$<10^{-3}$</u>**	**<u>111.6</u>**	0.15	1.94	**<u>0.0028</u>**	**<u>5.99</u>**
AC	92.0	16.5	89.9	14.6	92.1	15.9	105.1	13.6	109.8	14.8	108.2	12.4	**<u>$<10^{-3}$</u>**	**<u>70.7</u>**	0.84	0.18	0.45	0.81
DR	77.1	18.4	83.1	20.8	82.0	18.1	111.7	12.7	113.2	11.6	110.2	12.0	**<u>$<10^{-3}$</u>**	**<u>227.5</u>**	0.18	1.71	0.23	1.47

WMS-R, Wechsler Memory Scale-Revised; VerM, verbal memory; VisM, visual memory; AC, attention/concentration: DR, delayed recall; SD, standard deviation. T/T, T/G, G/G: individuals with three genotypes of rs1344706. Adjusted effects of three genotypes were analyzed by a two-way analysis of covariance (ANCOVA) with sex and years of education as covariates. Significant *P*-values are shown as bold face and underlined.

might indicate that quantitative traits (i.e., brain physiological activity measured by fMRI) are closer to the genetic substrate than behavioral traits, such as neuropsychological functions and psychiatric disorders, and should be observable in genetically at-risk but behaviorally unaffected individuals [Meyer-Lindenberg and Weinberger, 2006]. Such physiological quantitative traits are likely to influence a neuropsychological trait, memory performance, in patients with schizophrenia, however, they might not affect memory performance in healthy subjects. This phenomena suggests that the high-risk SNP in the *ZNF804A* gene might be related to the neuropsychological disturbance in schizophrenia.

There were several limitations to this study. Although the sample was moderate in size, it might not be representative of the schizophrenic population. A false-positive association cannot be excluded as a possibility in our study, despite the precautions of ethnic matching and correction for multiple testing. The effects of the ZNF804A gene on VisM could be an epiphenomenon of the severity of the disease and/or medication. In conclusion, we found an effect of the high-risk *ZNF804A* SNP on VisM in schizophrenia. Further research will be required to clarify the role of the high-risk *ZNF804A* SNP in the pathophysiology of schizophrenia.

ACKNOWLEDGMENTS

This work was funded in part by Grants-in-Aid from the Japanese Ministry of Health, Labor and Welfare (H19-kokoro-002), the Japanese Ministry of Education, Culture, Sports, Science and Technology (18689030), CREST of JST, Grant-aid for Scientific Research on Priority Areas—Research on Pathomechanisms of Brain Disorders—from the MEXT (18023045), and Japan Foundation for Neuroscience and Mental Health. The authors thank all individuals who participated in this study.

REFERENCES

Buchanan RW, Freedman R, Javitt DC, Abi-Dargham A, Lieberman JA. 2007. Recent advances in the development of novel pharmacological agents for the treatment of cognitive impairments in schizophrenia. Schizophr Bull 33(5):1120–1130.

Cardno AG, Gottesman II. 2000. Twin studies of schizophrenia: From bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet 97(1):12–17.

Esslinger C, Walter H, Kirsch P, Erk S, Schnell K, Arnold C, Haddad L, Mier D, Opitz von Boberfeld C, Raab K, Witt SH, Rietschel M, Cichon S, Meyer -Lindenberg A. 2009. Neural mechanisms of a genome-wide supported psychosis variant. Science 324(5927):605.

Gottesman II, Gould TD. 2003. The endophenotype concept in psychiatry: Etymology and strategic intentions. Am J Psychiatry 160(4):636–645.

Hashimoto R, Numakawa T, Ohnishi T, Kumamaru E, Yagasaki Y, Ishimoto T, Mori T, Nemoto K, Adachi N, Izumi A, Chiba S, Noguchi H, Suzuki T, Iwata N, Ozaki N, Taguchi T, Kamiya A, Kosuga A, Tatsumi M, Kamijima K, Weinberger DR, Sawa A, Kunugi H. 2006. Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology and ERK signaling. Hum Mol Genet 15(20): 3024–3033.

Hashimoto R, Hashimoto H, Shintani N, Chiba S, Hattori S, Okada T, Nakajima M, Tanaka K, Kawagishi N, Nemoto K, Mori T, Ohnishi T, Noguchi H, Hori H, Suzuki T, Iwata N, Ozaki N, Nakabayashi T, Saitoh O, Kosuga A, Tatsumi M, Kamijima K, Weinberger DR, Kunugi H, Baba A. 2007. Pituitary adenylate cyclase-activating polypeptide is associated with schizophrenia. Mol Psychiatry 12(11):1026–1032.

Kay SR, Fiszbein A, Opler LA. 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 13(2):261–276.

Meyer-Lindenberg A, Weinberger DR. 2006. Intermediate phenotypes and genetic mechanisms of psychiatric disorders. Nat Rev Neurosci 7(10):818–827.

Nuechterlein KH, Barch DM, Gold JM, Goldberg TE, Green MF, Heaton RK. 2004. Identification of separable cognitive factors in schizophrenia. Schizophr Res 72(1):29–39.

O'Donovan MC, Craddock N, Norton N, Williams H, Peirce T, Moskvina V, Nikolov I, Hamshere M, Carroll L, Georgieva L, Dwyer S, Holmans P, Marchini JL, Spencer CC, Howie B, Leung HT, Hartmann AM, Möller HJ, Morris DW, Shi Y, Feng G, Hoffmann P, Propping P, Vasilescu C, Maier W, Rietschel M, Zammit S, Schumacher J, Quinn EM, Schulze TG, Williams NM, Giegling I, Iwata N, Ikeda M, Darvasi A, Shifman S, He L, Duan J, Sanders AR, Levinson DF, Gejman PV, Cichon S, Nöthen MM, Gill M, Corvin A, Rujescu D, Kirov G, Owen MJ, Buccola NG, Mowry BJ, Freedman R, Amin F, Black DW, Silverman JM, Byerley WF, Cloninger CR, Molecular Genetics of Schizophrenia Collaboration. 2008. Identification of loci associated with schizophrenia by genome-wide association and follow-up. Nat Genet 40(9):1053–1055.

Ohi K, Hashimoto R, Yasuda Y, Kiribayashi M, Iike N, Yoshida T, Azechi M, Ikezawa K, Takahashi H, Morihara T, Ishii R, Tagami S, Iwase M, Okochi M, Kamino K, Kazui H, Tanaka T, Kudo T, Takeda M. 2009. TATA box-binding protein gene is associated with risk for schizophrenia, age at onset and prefrontal function. Genes Brain Behav 8(4):473–480.

Paunio T, Tuulio-Henriksson A, Hiekkalinna T, Perola M, Varilo T, Partonen T, Cannon TD, Lonnqvist J, Peltonen L. 2004. Search for cognitive trait components of schizophrenia reveals a locus for verbal learning and memory on 4q and for visual working memory on 2q. Hum Mol Genet 13(16):1693–1702.

Preston GA, Weinberger DR. 2005. Intermediate phenotypes in schizophrenia: A selective review. Dialogues Clin Neurosci 7(2):165–179.

Riley B, Thiselton D, Maher BS, Bigdeli T, Wormley B, McMichael GO, Fanous AH, Vladimirov V, O'Neill FA, Walsh D, Kendler KS. 2010. Replication of association between schizophrenia and ZNF804A in the Irish case–control study of schizophrenia sample. Mol Psychiatry 15(1):29–37.

Snitz BE, Macdonald AW III, Carter CS. 2006. Cognitive deficits in unaffected first-degree relatives of schizophrenia patients: A meta-analytic review of putative endophenotypes. Schizophr Bull 32(1): 179–194.

Steinberg S, Mors O, Børglum AD, Gustafsson O, Werge T, Mortensen PB, Andreassen OA, Sigurdsson E, Thorgeirsson TE, Böttcher Y, Olason P, Ophoff RA, Cichon S, Gudjonsdottir IH, Pietiläinen OP, Nyegaard M, Tuulio-Henriksson A, Ingason A, Hansen T, Athanasiu L, Suvisaari J, Lonnqvist J, Paunio T, Hartmann A, Jürgens G, Nordentoft M, Hougaard D, Norgaard-Pedersen B, Breuer R, Möller HJ, Giegling I, Glenthøj B, Rasmussen HB, Mattheisen M, Bitter I, Réthelyi JM, Sigmundsson T, Fossdal R, Thorsteinsdottir U, Ruggeri M, Tosato S, Strengman E, Kiemeney LA, Melle I, Djurovic S, Abramova L, Kaleda V, Walshe M, Bramon E, Vassos E, Li T, Fraser G, Walker N, Toulopoulou T, Yoon J, Freimer NB, Cantor RM, Murray R, Kong A, Golimbet V, Jönsson EG, Terenius L, Agartz I, Petursson H, Nöthen MM, Rietschel M, Peltonen L, Rujescu D, Collier DA, Stefansson H, St Clair D, Stefansson K. 2010. Expanding the range of ZNF804A variants conferring risk of psychosis. Mol Psychiatry (online publication).

Sugishita M. 2001. Japanese Wechsler Memory Scale-Revised. Tokyo: Nihonbunkakagakusha.

Tsuang M. 2000. Schizophrenia: Genes and environment. Biol Psychiatry 47(3):210–220.

Walter H, Schnell K, Erk S, Arnold C, Kirsch P, Esslinger C, Mier D, Schmitgen MM, Rietschel M, Witt SH, Nothen MM, Cichon S, Meyer-Lindenberg A. 2010. Effects of a genome-wide supported psychosis risk variant on neural activation during a theory-of-mind task. Mol Psychiatry (online publication).

Williams HJ, Norton N, Dwyer S, Moskvina V, Nikolov I, Carroll L, Georgieva L, Williams NM, Morris DW, Quinn EM, Giegling I, Ikeda M, Wood J, Lencz T, Hultman C, Lichtenstein P, Thiselton D, Maher BS, Malhotra AK, Riley B, Kendler KS, Gill M, Sullivan P, Sklar P, Purcell S, Nimgaonkar VL, Kirov G, Holmans P, Corvin A, Rujescu D, Craddock N, Owen MJ, O'Donovan MC, Molecular Genetics of Schizophrenia Collaboration (MGS) International Schizophrenia Consortium (ISC), SGENE-plus, GROUP. 2010. Fine mapping of ZNF804A and genome-wide significant evidence for its involvement in schizophrenia and bipolar disorder. Mol Psychiatry (online publication).

Zhang R, Lu SM, Qiu C, Liu XG, Gao CG, Guo TW, Valenzuela RK, Deng HW, Ma J. 2010. Population-based and family-based association studies of ZNF804A locus and schizophrenia. Mol Psychiatry (online publication).

J Inherit Metab Dis
DOI 10.1007/s10545-009-9022-9

RAPID COMMUNICATION

A new case of GABA transaminase deficiency facilitated by proton MR spectroscopy

Megumi Tsuji · Noriko Aida · Takayuki Obata · Moyoko Tomiyasu · Noritaka Furuya · Kenji Kurosawa · Abdellatif Errami · K. Michael Gibson · Gajja S. Salomons · Cornelis Jakobs · Hitoshi Osaka

Received: 2 October 2009 / Revised: 29 October 2009 / Accepted: 23 November 2009

Abstract
Background Deficiency of 4-aminobutyrate aminotransferase (GABA-T) is a rare disorder of GABA catabolism, with only a single sibship reported. We report on a third case, a Japanese female infant with severe psychomotor retardation and recurrent episodic lethargy with intractable seizures, with the diagnosis facilitated by proton magnetic resonance (MR) spectroscopy (^{1}H-MRS).
Methods Neuroimaging was performed at the first episode of lethargy. For ^{1}H-MRS, locations were placed in the semioval center and the basal ganglia. Quantification of metabolite concentrations were derived using the LCModel. We confirmed the diagnosis subsequently by enzyme and molecular studies, which involved direct DNA sequence analysis and the development of a novel multiplex ligation-dependent probe amplification test.
Results ^{1}H-MRS analysis revealed an elevated GABA concentration in the basal ganglia (2.9 mmol/l). Based on the results of quantitative ^{1}H-MRS and clinical findings, GABA-T deficiency was suspected and confirmed in cultured lymphoblasts. Molecular studies of the *GABA-T* gene revealed compound heterozygosity for a deletion of one exon and a missense mutation, 275G>A, which was not detected in 210 control chromosomes.
Conclusions Our results suggest that excessive prenatal GABA exposure in the central nervous system (CNS) was responsible for the clinical manifestations of GABA transaminase deficiency. Our findings suggest the dual

Communicated by: Marinus Duran

M. Tsuji · H. Osaka (✉)
Division of Neurology, Clinical Research Institute,
Kanagawa Children's Medical Center,
2-138-4 Mutsukawa, Minami-ku,
Yokohama 232-8555, Japan
e-mail: hosaka@kcmc.jp

N. Aida
Division of Radiology, Clinical Research Institute,
Kanagawa Children's Medical Center,
2-138-4 Mutsukawa, Minami-ku,
Yokohama 232-8555, Japan

N. Furuya · K. Kurosawa
Division of Genetics, Clinical Research Institute,
Kanagawa Children's Medical Center,
2-138-4 Mutsukawa, Minami-ku,
Yokohama 232-8555, Japan

T. Obata · M. Tomiyasu
Department of Biophysics, Molecular Imaging Center,
National Institute of Radiological Sciences,
Chiba, Japan

A. Errami
MRC-Holland,
Amsterdam, The Netherlands

K. M. Gibson
Department of Biological Sciences,
Michigan Technological University,
Houghton, MI, USA

G. S. Salomons · C. Jakobs
Metabolic Unit, Department of Clinical Chemistry,
VU University Medical Center,
Amsterdam, The Netherlands

H. Osaka
Molecular Pathology & Genetics Division,
Kanagawa Cancer Center Research Institute,
Yokohama, Japan

Published online: 06 January 2010

nature of GABA as an excitatory molecule early in life, followed by a functional switch to an inhibitory species later in development. Furthermore, quantitative ^{1}H-MRS appears to be a useful, noninvasive tool for detecting inborn errors of GABA metabolism in the CNS.

Abbreviations

GABA-T	Gamma aminobutyric acid transaminase
^{1}H-MRS	Proton magnetic resonance spectroscopy
CNS	Central nervous system
SSADH	Succinic semialdehyde dehydrogenase
GHB	4-hydroxybutyrate
EEG	Electroencephalogram
CSF	Cerebrospinal fluid
DWI	Diffusion-weighted image
Glx	Glutamine/glutamate complex

Introduction

Disorders of gamma aminobutyric acid (GABA) metabolism are rare and manifest prominent neurological sequelae; 4-aminobutyrate aminotransferase (γ-aminobutyrate: GABA transaminase, or GABA-T; OMIM 137150) deficiency is characterized by severe psychomotor retardation, hypotonia, hyperreflexia, seizures, high-pitched cry, and growth acceleration, associated with early infantile death in two siblings (one family) (Jaeken et al 1984; Jakobs et al 1993). Succinic semialdehyde dehydrogenase (SSADH) deficiency [or 4-hydroxybutyric (GHB) aciduria] is the most prevalent of the GABA degradation disorders and one in which pharmacologically active GHB, as well as GABA, accumulate in patient body fluids (Jakobs et al 1993; Pearl et al 2007). Homocarnosinosis (homocarnosine is the GABA:L-histidine

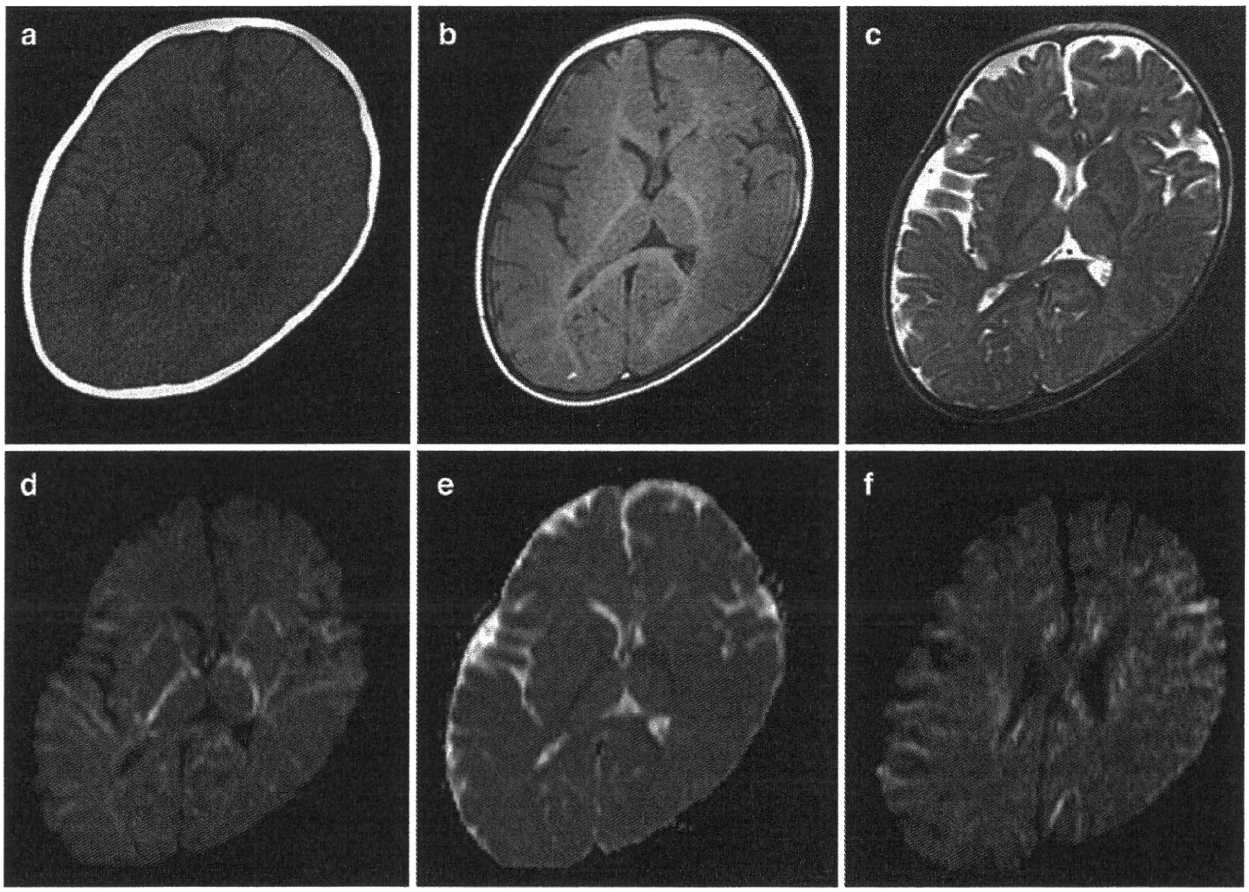

Fig. 1 Initial computed tomography (CT) and magnetic resonance imaging (MRI) findings at 8 months. Baseline CT (**a**), T1-weighted (**b**), T2-weighted (**c**), diffusion-weighted (**d**) axial MRI images, and apparent diffusion coefficients (ADC) map (**e**) at the level of the basal ganglia, and diffusion-weighted images (DWI) of the semioval center (**f**). CT (**a**) shows no particular abnormality, whereas T1-weighted (**b**) and T2-weighted (**c**) images suggest delayed myelination. Subcortical high white-matter signal on the T1-weighted image was not observed, and low signal on the T2-weighted image was limited to the posterior portion of the internal capsules and splenium of the corpus callosum. DWI (**d**, **f**) shows widespread high signals in the internal and external capsules and many parts of the subcortical white matter, with restricted diffusion (**e**)

conjugate) is very rare (two cases) and may represent an allelic form of carnosinase deficiency(Pearl et al 2007). Considering the inhibitory nature of GABA activity in the central nervous system (CNS), the paradoxical neurological phenomenon associated with seizures in cases of GABA excess is of interest. In this study, we detected elevated GABA in a patient by proton magnetic resonance spectroscopy (^{1}H-MRS) using the LCModel to quantify the spectra automatically. This method has potential application to neurological disorders such as GABA-T deficiency.

Case report

The patient was a Japanese female infant, born full term with normal delivery. She was the second child of healthy parents. There was no consanguinity or family history of neurological disorders. A 6-year-old sister was normal. Early infancy was unremarkable. At 7 months, she was evaluated for psychomotor retardation, hypotonia, bilateral intermittent esotropia, hyperreflexia, and positive Babinski reflex. There was no dysmorphy. At age 8 months, she was admitted with decreased consciousness 48 h after an acute febrile illness. Respiratory distress developed that required mechanical ventilation. Steroid pulse therapy was initiated for a suspected acute encephalopathy of unknown etiology. Segmental myoclonic jerks occurred and were difficult to control, but consciousness returned. Electroencephalography (EEG) revealed diffuse slow spike and wave discharges with 1- to 2-s periods of suppression. Phenobarbital, clonazepam, valproate, and midazolam could not completely control seizures. Limb motor conduction velocities were

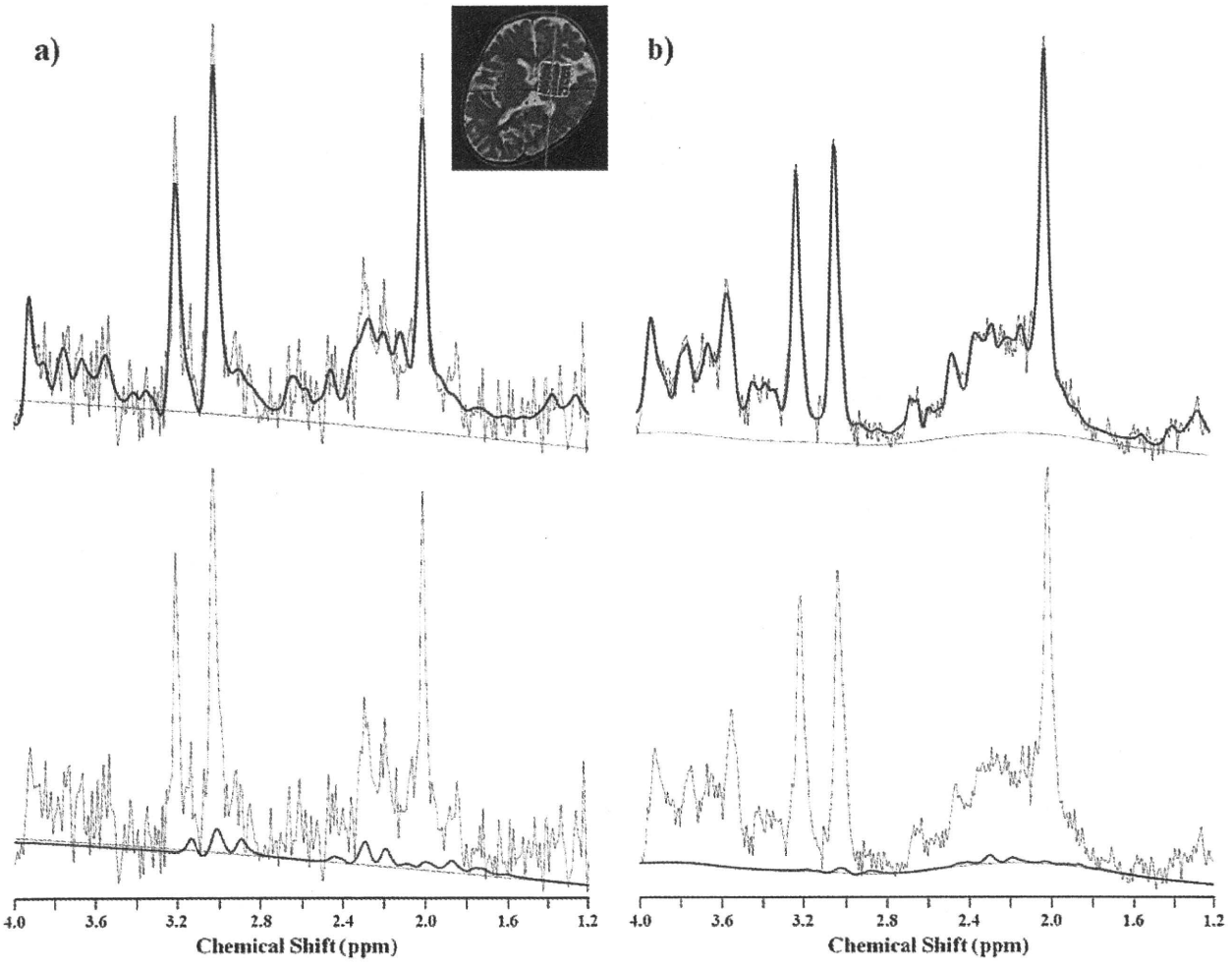

Fig. 2 LCModel outputs of in vivo proton magnetic resonance (MR) spectra from the basal ganglia (volume, 10–17.5 ml; TE/TR, 20–30/5000 ms; number of excitations 6). *Bold lines* indicate LCModel fitting, and *thin lines* indicate the original spectra. Patient with gamma aminobutyric acid transaminase (GABA-T) deficiency (8 months) (**a**), a control individual (7 months) (**b**). *Bold lines* in the *upper row* are the fitting curves of total spectra including all metabolites, and those in the *lower row* are fitting curves for GABA. The estimated absolute concentrations of GABA in patient and control are 2.9 and 0.8 mmol/l, respectively. Normal GABA spectrum exhibits a quintet ($^3CH_2$) at 1.89 ppm, a triplet ($^4CH_2$) at 2.28 ppm, and a multiplet resembling a triplet ($^2CH_2$) at 3.01 ppm(Govindaraju et al 2000)

within normal limits. Anthropomorphic parameters revealed accelerated height in late infancy [+2.5–3.0 standard deviation (SD)], with normal head circumference and decreased weight gain. At 8 months, her height was 76 cm (+3.0 SD), weight 6745 g (−1.7 SD), and head circumference 44 cm (−0.4 SD). Nasogastric tube feeding was started due to recurrent aspiration pneumonia. At 11 months, domiciliary oxygen was introduced because of chronic respiratory failure. At the age of 28 months, her height was 96 cm (+2.9 SD), weight 10.3 kg (−1.1 SD), and head circumference 46.5 cm (−0.7 SD). Febrile illness was consistently associated with neurological deterioration, and the patient progressed to opisthotonic posturing with generalized dystonia and segmental myoclonic jerks, which never resolved while awake.

Methods and results

Laboratory data

Routine laboratory tests were normal. Amino acid analysis showed elevated free GABA in the serum and cerebral spinal fluid (CSF) at 9 months of age (2.1 μmol/l and 1.26 μmol/l, respectively; normal range serum 0.12–0.50, CSF 0.04–0.12) (Jaeken et al 1984). Serum growth hormone was elevated (8.84 ng/ml; normal range 0.28–1.64). Insulin-like growth factor levels were relatively low (54 ng/ml; normal range 37–229). An absence of GHB in urine organic acid analysis precluded SSADH deficiency as a cause of increased GABA. β-alanine and homocarnosine were not detectable on the chromatogram, and their quantitative analyses were not performed.

Radiological findings

Bone age was 1 year 8 months at the age of 1 year 10 months (TW2 method). Initial brain computed tomography (CT) was unremarkable (Fig. 1a), and brain magnetic resonance imaging (MRI) (1.5 T) suggested mild delay in myelination (Fig. 1b, c) without structural anomalies. Diffusion weighted images (DWI) revealed high signal intensity in the internal and external capsules and much of the subcortical white matter, with restricted apparent diffusion coefficiency (Fig. 1d–f). For quantitative ^{1}H-MRS (age 8 months), locations were placed in the white matter (semioval center) and the basal ganglia (10 ml). ^{1}H-MR spectra were obtained using the stimulated-echo acquisition mode (STEAM) sequence (Frahm et al 1987) (TE/TR= 20/5,000 ms). To quantify the spectra, the LCModel (Provencher 1993) was used. The LCModel facilitates metabolite separation based upon differing linear combinations of spectra of individual metabolites and estimates the concentration of each metabolite concentration by comparing the proton concentration of water in identical voxels. The GABA concentration in the basal ganglia (Fig. 2) was

Table 1 Clinical, enzymatic, and molecular characteristics of gamma aminobutyric acid transaminase (GABA-T)-deficient patients

Sign/symptom	Patient 1	Patient 2 (sib of patient 1)	This report
Intractable seizures	+	+	+
Psychomotor retardation	+	+	+
Hypotonia	+	+	+
High-pitched cry	+	+	–
Hyperreflexia	+	+	+
Lethargy	+	+	+
Acceleration of height growth	+	+	+
Age of death	25 months	12 months	Alive at 28 months
EEG/MRI/CT abnormalities	+	+	+
GABA-T (liver)[a]	70 (310–690)	–	–
GABA-T (white cells)[b]	1.2 (20–58)	–	2 (23–64)
Genotype [c]	c.[659G>A (+) 1433T>C][d]	–	c.[275G>A]+[199–?_316+?][e]
Deduced effect	p.[Arg220Lys (+) Leu478Pro]	–	p.[Arg92Gln]+[?]

EEG electroencephalograph, *MRI* magnetic resonance imaging, *CT* computed tomography, + present; – absent or not determined.

[a] Protein pmol/h/mg (control range in parentheses). [b] Protein pmol/min/mg (control range in parentheses). [c] Reference sequence NM_000663.3; missense mutations are considered to be pathogenic, as they were not encountered in 210 control chromosomes and involve highly conserved amino acids among GABA-T species. [d] Following the original publication (Jaeken et al 1984), we identified the second mutation (c.1433T>C) in the first described patient, confirming GABA-T deficiency at the DNA level. [e] In our patient, a presumed homozygous mutation was detected by direct sequence analysis; however, this was in contrast to the findings in DNA of the mother. The heterozygous mutation could not be detected in DNA of the father, therefore, a specific multiplex probe amplification test was developed. This showed the presence of a heterozygous exon deletion in DNA of the patient confirming compound heterozygosity

significantly elevated (2.9 mmol/l; normal 1.1 mmol/l±0.3, n=9), but in the semioval center, GABA elevation was slight (0.8 mmol/l; normal 0.5 mmol/l±0.2, n=9). Glutamine/glutamate complex (Glx) concentration was also slightly elevated in the semioval center (11.3 mmol/l in the basal ganglia, 8.3 mmol/l in the semioval center; normal 10.1 mmol/l ±1.5, 6.6 mmol/l±1.0, n=9, respectively). Follow-up ^{1}H-MRS analysis (at 9 months of age) revealed a more pronounced GABA elevation both in the basal ganglia and in the semioval center (5.9 mmol/l and 2.9 mmol/l, respectively). Based on these data and the results of quantitative ^{1}H-MRS, we suspected GABA-T deficiency, which was confirmed by enzyme and molecular studies in cultured lymphoblasts (Schor et al 2001) (Table 1).

Discussion

This report is on the third patient (second family) with GABA-T deficiency and the first patient in whom ^{1}H-MRS was performed. All three patients showed severe, nonspecific neurological manifestations, including psychomotor retardation, epilepsy, hypotonia, and hyperreflexia (Table 1), but our patient appeared less severely affected than the reported patients. All three also showed growth acceleration associated with increased serum growth hormone levels.

The underlying pathophysiology in GABA-T deficiency remains to be elucidated, and there is no animal model available. Evidence from animal studies indicates a neurotoxic role for supraphysiological GABA levels. For example, inhibition of GABA-T by the irreversible inhibitor, vigabatrin, induces intramyelinic edema in dogs via GABA elevation (Peyster et al 1995). Both GABA-T and SSADH deficiencies manifest seizures, which is paradoxical, as activation of the GABAergic system is predicted to be anticonvulsive. Nonetheless, it is important to remember that GABA is excitatory in the developing rodent brain and remains so for the first 1–2 weeks of life. Along these lines, the switch of GABA from a depolarizing to a hyperpolarizing response is critically important in the rodent substantia nigra pars reticulata (SNR), which has one of the highest concentrations of GABAergic neurons in the CNS (Iadarola and Gale 1982). In the murine model of SSADH deficiency, Jansen and coworkers (2008) demonstrated a significant increase in GABA in E10 embryos, which may predispose these animals to a hyperexcitatory state during development. This result, along with GABA(A) and GABA (B) receptor anomalies detected in developing SSADH-deficient mice, may reduce the seizure threshold in SSADH deficiency (Buzzi et al 2006; Wu et al 2006). Both disorders occupy juxtaposed positions in GABA degradation, and accordingly we speculate that the pathophysiological mechanisms observed in SSADH deficiency may be likely to be caused by high GABA levels, as observed in GABA-T deficiency. Neuropathology of the two index cases revealed spongy leukodystrophy, which may correspond to the white matter lesions seen on DWI in our patient. This observation may reflect changes in water motion in the axonal direction and/or axonal swelling associated with cortical neuronal damage.

Whereas ^{1}H-MRS estimates in vivo neurotransmitter concentrations (Provencher 1993), quantifying GABA in nonpathological states is difficult due to interference by much larger peaks of the glutamine–glutamate complex, creatine, and large peaks of N-acetylaspartic acid (Novotny et al. 2003). However, utilizing the LCModel facilitates separation of even low-concentration species (such as GABA) from other major compounds. Screening of the metabolite concentration by ^{1}H-MRS may readily reveal the pathological state, however, as in our patient. Moreover, the addition of a short exposure to ^{1}H-MRS may be acceptable, even in infants and children. Increased intracranial GABA detected by ^{1}H-MRS has been reported in SSADH deficiency (Ethofer et al 2004), but additional GABA-T-deficient patients require identification in order to determine how the concentrations of intracranial GABA compare to those in the same regions of SSADH-deficient patients.

In summary, GABA transaminase deficiency represents a human model of endogenous GABA elevation, which likely occurs during critical periods of human CNS development. This disorder may offer valuable insights into the role of the GABAergic system in human brain development. Our studies further suggest that quantitative ^{1}H-MRS may be clinically applicable to the inborn errors of GABA metabolism.

Acknowledgements We thank Lyuba Bayukanskaya, Ana Pop, and Rizkat Yilmaz for technical assistance. This work was supported in part by Grants-in-Aid from Scientific Research from the Ministry of Health, Labor and Welfare of Japan, Health and Labor Science Research Grant of Japan (Kokoro-Ippan-015), Takeda Science Foundation and Kanagawa Municipal Hospital Pediatric Research.

References

Buzzi A, Wu Y, Frantseva MV et al (2006) Succinic semialdehyde dehydrogenase deficiency: GABAB receptor-mediated function. Brain Res 1090:15–22

Ethofer T, Seeger U, Klose U et al (2004) Proton MR spectroscopy in succinic semialdehyde dehydrogenase deficiency. Neurology 62:1016–1018

Frahm J, Merboldt KD, Hänicke W (1987) Localized proton spectroscopy using stimulated echoes. J Magn Reson 72:502–508

Govindaraju V, Young K, Maudsley AA (2000) Proton NMR chemical shifts and coupling constants for brain metabolites. NMR Biomed 13:129–153

Iadarola MJ, Gale K (1982) Substantia nigra: site of anticonvulsant activity mediated by gamma-aminobutyric acid. Science 218:1237–1240

Jaeken J, Casaer P, de Cock P et al (1984) Gamma-aminobutyric acid-transaminase deficiency: a newly recognized inborn error of neurotransmitter metabolism. Neuropediatrics 15:165–169

Jakobs C, Jaeken J, Gibson KM (1993) Inherited disorders of GABA metabolism. J Inherit Metab Dis 16:704–715

Jansen EE, Struys E, Jakobs C, Hager E, Snead OC 3rd, Gibson KM (2008) Neurotransmitter alterations in embryonic succinate semialdehyde dehydrogenase (SSADH) deficiency suggest a heightened excitatory state during development. BMC Dev Biol 8:112

Novotny EJ Jr, Fulbright RK, Pearl PL, Gibson KM, Rothman DL (2003) Magnetic resonance spectroscopy of neurotransmitters in human brain. Ann Neurol 54(Suppl 6):S25–S31

Pearl P, Jakobs C, Gibson K (2007) Disorders of β- and γ-amino acids in free and peptide-linked forms. In The metabolic and molecular bases of inherited disease Part 8, amino acids, Ch 91. McGraw-Hill, New York

Peyster RG, Sussman NM, Hershey BL et al (1995) Use of ex vivo magnetic resonance imaging to detect onset of vigabatrin-induced intramyelinic edema in canine brain. Epilepsia 36:93–100

Provencher SW (1993) Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med 30:672–679

Schor DS, Struys EA, Hogema BM, Gibson KM, Jakobs C (2001) Development of a stable-isotope dilution assay for gamma-aminobutyric acid (GABA) transaminase in isolated leukocytes and evidence that GABA and beta-alanine transaminases are identical. Clin Chem 47:525–531

Wu Y, Buzzi A, Frantseva M et al (2006) Status epilepticus in mice deficient for succinate semialdehyde dehydrogenase: GABAA receptor-mediated mechanisms. Ann Neurol 59:42–52

ELSEVIER

Brain & Development xxx (2010) xxx–xxx

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Case report

5,10-Methylenetetrahydrofolate reductase deficiency with progressive polyneuropathy in an infant

Megumi Tsuji[a], Atsushi Takagi[b], Kiyoko Sameshima[a], Mizue Iai[a], Sumimasa Yamashita[a], Hiroko Shinbo[a], Noritaka Furuya[b], Kenji Kurosawa[b], Hitoshi Osaka[a,*]

[a] *Division of Neurology, Kanagawa Children's Medical Center, Yokohama, Japan*
[b] *Genetics, Kanagawa Children's Medical Center, Yokohama, Japan*

Received 10 June 2010; received in revised form 24 August 2010; accepted 26 August 2010

Abstract

5,10-Methylenetetrahydrofolate reductase (MTHFR) deficiency is the most prevalent inborn error of folate metabolism, and has variable clinical manifestations from asymptomatic to severe psychomotor retardation, microcephalus and seizure. In untreated infantile cases, it predominantly affects the central nervous system, which is sometimes fatal. On the other hand, peripheral nerve involvement is uncommon. We present a severe infantile case of MTHFR deficiency that manifested unilateral phrenic nerve palsy with communicating hydrocephalus, developmental delay and died at 11 months of age. An enzymatic study confirmed MTHFR deficiency with residual activity of 0.75% of mean control values in cultured fibroblasts. Mutation analysis of the *MTHFR* gene revealed homozygous, tandem missense mutations c.[446G>T; 447C>T] in exon 3 of the *MTHFR* gene converting glycine to valine (Gly149Val). In MTHFR deficiency, betaine may improve the symptoms if started immediately after birth by reducing the level of serum homocysteine and increasing that of methionine. Our results show that we should be aware of possible inborn errors of folate metabolism such as MTHFR deficiency, in infants with unexplained developmental delay manifesting rapidly progressive polyneuropathy.

Keywords: Methylenetetrahydrofolate reductase deficiency; MTHFR; Hyperhomocysteinemia; Autosomal recessive inheritance; Polyneuropathy; Developmental delay

1. Introduction

5,10-Methylenetetrahydrofolate reductase (MTHFR) catalyses the reduction of methylenetetrahydrofolate to methyltetrahydrofolate. MTHFR deficiency (MIM#-236250), an autosomal recessive disorder, is known as the most prevalent inborn error of folate metabolism. MTHFR deficiency results in homocysteinemia showing a wide range of clinical manifestations from asymptomatic to severe psychomotor retardation, epilepsy and microcephalus depending on the residual enzyme

Abbreviations: MTHFR, 5,10-methylenetetrahydrofolate reductase; CNS, central nervous systems; CT, computed tomography; MRI, magnetic resonance imaging; EEG, electroencephalogram; SWI, susceptibility weighted imaging; CSF, cerebrospinal fluid; SAM, *S*-adenosylmethionine; FAD, flavin adenine dinucleotide.

* Corresponding author. Address: Division of Neurology, Kanagawa Children's Medical Center, 2-138-4 Mutsukawa, Minami-ku, Yokohama 232-8555, Japan. Tel.: +81 45 711 2351; fax: +81 45 721 3324.
E-mail address: hosaka@kcmc.jp (H. Osaka).

0387-7604/$ - see front matter © 2010 Published by Elsevier B.V.
doi:10.1016/j.braindev.2010.08.013

Please cite this article in press as: Tsuji M et al. 5,10-Methylenetetrahydrofolate reductase deficiency with progressive polyneuropathy in an infant. Brain Dev (2010), doi:10.1016/j.braindev.2010.08.013

activities [1]. More than 100 cases of MTHFR deficiency have been reported. One case of subclinical peripheral neuropathy in an MTHFR deficient infant has been reported [2], although most cases are seen in older children or young adults [3]. We report here a patient with severe MTHFR deficiency with progressive polyneuropathy and underscore the importance of early diagnosis of this rare disorder.

2. Case report

The patient was a Japanese female infant, born full term with vacuum extraction due to uterine inertia. There was no consanguinity or family history of neurological disorders. She manifested failure to thrive due to feeding difficulty in the neonatal period, and was referred to our hospital for sunset phenomenon at the age of 2 months. She was unable to gaze or smile with muscle hypertonia and increased deep tendon reflexes. The brain MRI showed communicating hydrocephalus (Fig. 1). Because of arrested development, ventriculoperitoneal shunting was performed at the age of 4 months. Generalized brief tonic seizures started within a month after surgery with EEG findings of multifocal spikes with low background activities, which responded well to carbamazepine. At the age of 8 months, acute respiratory distress developed due to unilateral phrenic nerve palsy (Fig. 1), which resulted in total dependence on mechanical ventilation for the rest of her life. Deep tendon reflexes diminished over a period of 2 months and subsequently became absent. Urinary retention, hypothermia and cessation of spontaneous breathing occurred within a month. In the measurement of motor conduction velocity of the limbs, no M-waves were detected. Auditory brainstem response showed abnormality with an elimination of waves II–V. Rapidly progressive clinical exacerbation appeared in two months with unconsciousness and generalized edema, despite adequate nutrition by way of nasogastric tube feeding. She died of bacterial infection of unknown focus at 11 months of age.

2.1. Laboratory and radiological findings

The brain MRI obtained at 2 months revealed severe ventriculomegaly with T2 elongation in the bilateral anterior periventricular white matter. Mild pontine and cerebellar hypoplasia were also noted (Fig. 1). MRI of the cervical spine was unremarkable. The cerebrospinal fluid (CSF) findings showed mild elevation of protein (30 mg/dl) without pleocytosis, and hypoglycorrhachia (42.2 mg/dl) in the absence of hypoglycemia. We did not detect microbial infections of the CNS. Routine laboratory tests were unremarkable except for chronic hypoproteinemia and transient hyperlactacidemia (60 mg/dl). Serum amino acid analysis at 8 months revealed a marked decrease of methionine (2.9 nmol/ml) and increased homocystine (29.1 nmol/ml; total

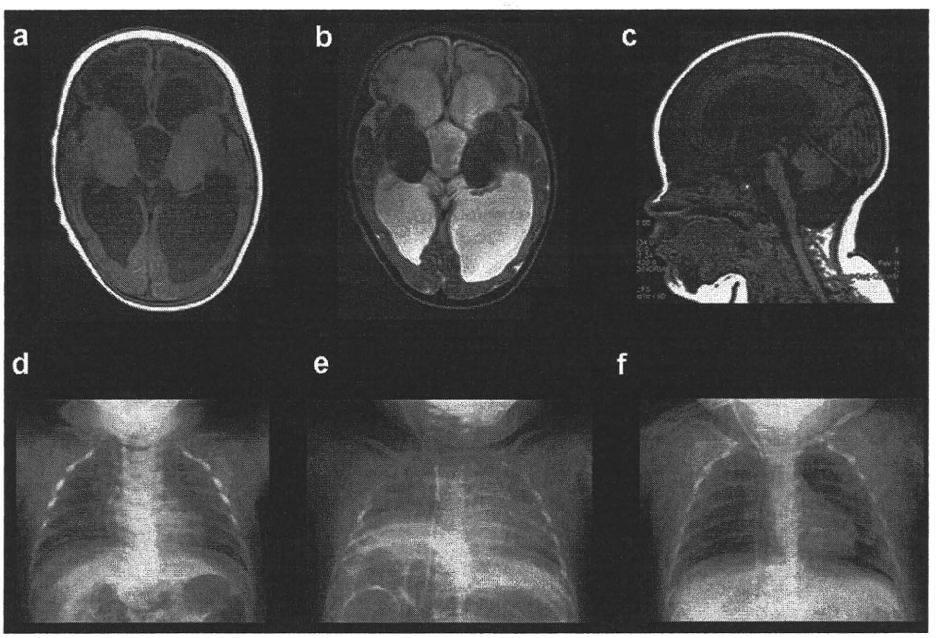

Fig. 1. Brain MRI obtained at 2 months and chest radiographs. (a) Axial T1-weighted image shows marked dilatation of all ventricles indicating communicating hydrocephalus. (b) T2-weighted image shows hyperintensity of the bilateral frontal periventricular white matter. (c) Sagittal T1-weighted image shows mild hypoplasia of pons and cerebellum. (d) Chest radiograph reveals no abnormality at 3 months. (e) Chest radiograph at 8 months shows the right diaphragm elevation suggesting unilateral phrenic nerve palsy. (f) Intubation and mechanical ventilation effectively inflated the patient's lungs.

Please cite this article in press as: Tsuji M et al. 5,10-Methylenetetrahydrofolate reductase deficiency with progressive polyneuropathy in an infant. Brain Dev (2010), doi:10.1016/j.braindev.2010.08.013

homocysteine 153.8 nmol/ml). Because of the normal findings of the urine organic acid profiles and the absence of megaloblastic anemia, cobalamin deficiencies seemed to be unlikely.

2.2. Enzymatic and molecular analysis of MTHFR

The residual activity of MTHFR in cultured fibroblast from the patient was 0.19 nmol/h/mg protein (0.75% of mean control values) and showed no responsiveness to flavin adenine dinucleotide (FAD) *in vitro*. We identified a homozygous, tandem missense substitution c. [446G>T; 447C>T] in exon 3 of the *MTHFR* gene, which converted glycine to valine (Gly149Val). This mutation was considered to be pathogenic, because it was not observed in 100 control alleles, and the glycine residue is located in a highly conserved region of MTHFR proteins from eukaryotic and prokaryotic origin (Fig. 2).

3. Discussion

In MTHFR deficiency, early onset of neurological symptoms have been described as severe phenotypes, however, progressive polyneuropathy in infants is uncommon with only one reported infantile case of subclinical peripheral neuropathy [2]. One case report with MTHFR deficiency presenting polyneuropathy in a young adult revealed demyelination and spheroid formation in peripheral nerves [3]. Demyelination of the brain in homocysteine remethylation defects has been considered to be associated with *S*-adenosylmethionine (SAM) deficiency in the CSF [4]. We speculate that the demyelination also occurs in peripheral nerves in cases of SAM deficiency, as SAM is the most prevalent methyl donor in the human body and its deficiency reportedly affects broad systemic organs.

The present case manifested with failure to thrive beginning at neonatal stages and communicating hydrocephalus without any evidence of intracranial hemorrhage or CNS infections. Most of the previously reported cases of MTHFR deficiency manifesting hydrocephalus did not require surgical intervention [5]. Although the mechanism of communicating hydrocephalus remains to be clarified, one possibility is that microthrombi in cerebral capillaries, caused by thrombotic tendency with hyperhomocysteinemia, may impair CSF absorption. In addition, we presume that the surgical intervention was related to the worsening of the neurological impairments. Nitrous oxide anesthesia has been reported as a risk factor for acute deterioration of neurological symptoms inducing even death to those with severe MTHFR deficiency by inactivating methionine synthase [6]. Our patient was given general anesthesia with the use of NO at the anesthesia induction, and we suggest that this procedure together with physical stress by the surgery may have worsened her condition.

Our patient had a homozygous, tandem missense mutation in exon 3 of the *MTHFR* gene that was reported previously in two Japanese patients, one of which was a homozygote and the other was a heterozygote with a Thr139Met substitution and an Ala222Val polymorphism. The homozygous patient with the same mutation reportedly manifested developmental delay and died at 9 months without a detailed clinical course [7], and the heterozygote was a 15-year-old male patient who was mentally retarded with pyramidal signs and sensory disturbance in lower limbs [8]. This mutation seems to be more common in the Japanese population than in other ethnic groups.

Betaine is a substrate of betaine methyltransferase that converts homocystine to methionine, constituting an alternative folate dependent remethylation pathway. Betaine is the only agent shown to prevent further neurological deterioration in patients with MTHFR deficiency [9]. However, one patient with severe MTHFR deficiency with neonatal onset is reportedly responded only to methionine supplement [10].

In conclusion, in cases of unexplained, neurological deterioration in infants with progressive polyneuropathy and CNS involvement, it is important not to miss such disorders as homocysteine remethylation defects including MTHFR deficiency early in life.

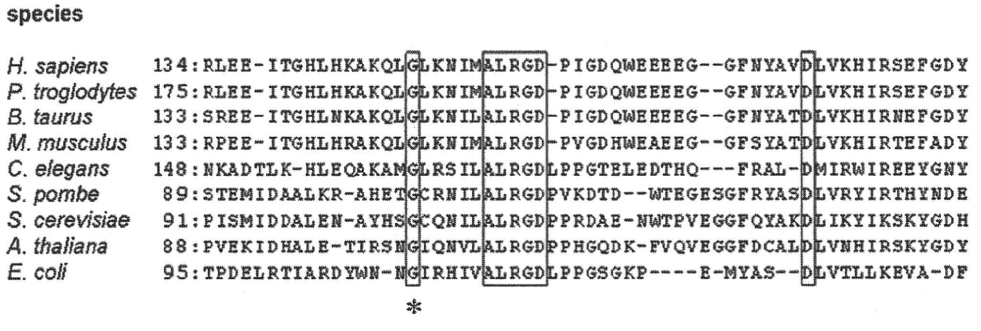

Fig. 2. Amino acid conservation of 5,10-methylenetetrahydrofolate reductase (MTHFR) protein in eukaryotic and prokaryotic species.

Please cite this article in press as: Tsuji M et al. 5,10-Methylenetetrahydrofolate reductase deficiency with progressive polyneuropathy in an infant. Brain Dev (2010), doi:10.1016/j.braindev.2010.08.013

Acknowledgement

We thank Prof. Dr. Brian Fowler (Basel University Children's Hospital) for enzymatic studies.

References

[1] Rosenblatt DS, Fenton WA. Part17, vitamins, Ch 155: inherited disorders of folate and cobalamin transport and metabolism. In: Valle D, Beaudet AL, Vogelstein B, Kinzler KW, Antonarakis SE, Ballabio A, editors. The metabolic and molecular bases of inherited disease. New York: McGraw-Hill; 2007.

[2] Al Tawari AA, Ramadan DG, Neubauer D, Heberle LC, Al Awadi F. An early onset form of methylenetetrahydrofolate reductase deficiency: a report of a family from Kuwait. Brain Dev 2002;24:304–9.

[3] Nishimura M, Yoshino K, Tomita Y, Takashima S, Tanaka J, Narisawa K, et al. Central and peripheral nervous system pathology of homocystinuria due to 5,10-methylenetetrahydrofolate reductase deficiency. Pediatr Neurol 1985;1:375–8.

[4] Surtees R, Leonard J, Austin S. Association of demyelination with deficiency of cerebrospinal-fluid *S*-adenosylmethionine in inborn errors of methyl-transfer pathway. Lancet 1991;338:1550–4.

[5] Baethmann M, Wendel U, Hoffmann GF, Göhlich-Ratmann G, Kleinlein B, Seiffert P, et al. Hydrocephalus internus in two patients with 5,10-methylenetetrahydrofolate reductase deficiency. Neuropediatrics 2000;31:314–7.

[6] Selzer RR, Rosenblatt DS, Laxova R, Hogan K. Adverse effect of nitrous oxide in a child with 5,10-methylenetetrahydrofolate reductase deficiency. N Engl J Med 2003;349:45–50.

[7] Goyette P, Christensen B, Rosenblatt DS, Rozen R. Severe and mild mutations in *cis* for the methylenetetrahydrofolate reductase (MTHFR) gene, and description of five novel mutations in MTHFR. Am J Hum Genet 1996;59:1268–75.

[8] Yano H, Nakaso K, Yasui K, Wakutani Y, Nakayasu H, Kowa H, et al. Mutations of the *MTHFR* gene (428C>T and [458G>T + 459C>T]) markedly decrease MTHFR enzyme activity. Neurogenetics 2004;5:135–40.

[9] Wendel U, Bremer HJ. Betaine in the treatment of homocystinuria due to 5,10-methylenetetrahydrofolate reductase deficiency. Eur J Pediatr 1984;142:147–50.

[10] Abeling NGGM, van Gennip AH, Blom H, Wevers RA, Vreken P, van Tinteren HLG, et al. Rapid diagnosis and methionine administration: basis for a favourable outcome in a patient with methylene tetrahydrofolate reductase deficiency. J Inherit Metab Dis 1999;22:240–2.

Please cite this article in press as: Tsuji M et al. 5,10-Methylenetetrahydrofolate reductase deficiency with progressive polyneuropathy in an infant. Brain Dev (2010), doi:10.1016/j.braindev.2010.08.013