memory which could interfere with task execution. Psychiatry Clin Neurosci, 60:312-318, 2006.

21. 橋本亮太、安田由華、大井一高、武田雅俊、気分安定薬の作用メカニズム. こころの科学, 131:47-52, 2007.
22. 橋本亮太、大井一高、安田由華、武田雅俊、内科医のための脳疾患講座、統合失調症その１, Brain Medical, 18(4):382-387, 2006.
23. 橋本亮太、武田雅俊、統合失調症は神経変性疾患か？ 神経生化学的観点から. 脳21, 9(4):409-412, 2006.
24. 橋本亮太、統合失調症関連遺伝子とその機能. 脳と精神の医学, 17(1):37-46, 2006.

2. 学会発表

1) Hashimoto R, Translational Research for Schizophrenia, 3rdJapanese-German Frontiers of Science Symposium, Heidelberg, Germany, 2 - 5 November, 2006.
2) Iijima Y, Okada T, Hashimoto R, Shizuno T, Hori H, Nakabayashi T, Kato T, Asada T, Tatsumi M, Kosuga A, Kamijima K, Harada S, Arima K, Saitoh O, Kunugi H. An association study of schizophrenia with complex polymorphic region in the BDNF gene. XIV World Congress on Psychiatryc Genetics, Cagliari, Itaria, Oct. 28th-Nov. 1st, 2006
3) Hashimoto R, Noguchi H, Hori H, Nakabayashi T, Hattori S, Chiba S, Harada S, Saitoh O, Takeda M, Kunugi H. Genetic variation in dysbindin influences memory and general cognitive ability. The 36th annual meeting of Society for Neuroscience, Georgia, USA, October 14-18, 2006.
4) Hattori S, Chiba S, Harada S, Saitoh O, Takeda M, Kunugi H, Hashimoto R. Dysbindin knockout mouse reveals abnormal behavior in novel environment. The 36th annual meeting of Society for Neuroscience, Georgia, USA, October 14-18 , 2006.
5) Takebayashi M, Hashimoto R, Hisaoka K, Tsuchioka M, Ryoji M, Knugi H. Increased serum levels of vascular endothelial growth factor in patients with major depressive disorders. The 36th annual meeting of Society for Neuroscience, Georgia, USA, October 14-18 , 2006.
6) S. Numata, S. Ueno, J. Iga, K. Yamauchi, S. Hongwei, S. Kinouchi, S. Shibuya-Tayoshi, S. Tayoshi, S. Sumitani, R. Hashimoto, H. Kunugi, M. Itakura, T. Ohmori. Gene expression and association analysis of LIM (PDLIM5) gene in schizophrenia. The 36th annual meeting of Society for Neuroscience, Georgia, USA, October 14-18 , 2006.
7) Hashimoto R, Dysbindin and pathogenesis of schizophrenia.

The 7th Biennial Meeting of the Asian-Pacific Society for Neurochemistry (APSN), Singapore, July 2-5 (3), 2006.

8) 橋本亮太、尾崎紀夫、オーガナイザー　統合失調症と気分障害の病態生理研究の動向、分子生物学会 2006 フォーラム、名古屋、12.6-8(6), 2006.

9) 橋本亮太、武田雅俊、　統合失調症と気分障害の病態生理研究の動向、統合失調症のトランスレーショナルリサーチ、分子生物学会 2006 フォーラム、名古屋、12.6-8(6), 2006.

10) 飯嶋良味、岡田武也、野口広子、橋本亮太、志津野朋子、堀弘明、中林哲夫、加藤忠史、朝田　隆、巽雅彦、小菅麻子、上島国利、有馬邦正、斉藤治、功刀浩、BDNF 遺伝子における複合繰り返し配列多型と人格傾向および血中 BDNF 濃度との関連、第 14 回日本精神・行動遺伝医学会、筑波、11.18, 2006.

11) 橋本亮太、座長、Panss Training in OSAKA、大阪、10.21, 2006.

12) 安田由華、橋本亮太、武田雅俊、不安障害・側頭葉てんかんの診断で紹介され対照的な転帰を辿った青年期の女子二例についての考察、第 47 回日本児童青年精神医学会総会、千葉、10.18-20(19), 2006.

13) 橋本亮太、岩田仲生、オーガナイザー　合同シンポジウム「エンドフェノタイプを利用したゲノム研究」、第 28 回日本生物学的精神医学会・第 49 回日本神経化学会・第 36 回日本神経精神薬理学会合同年会、名古屋、9.14-16(14), 2006.

14) 橋本亮太、座長、「その他、遺伝子解析」、第 28 回日本生物学的精神医学会・第 49 回日本神経化学会・第 36 回日本神経精神薬理学会合同年会、名古屋、9.14-16(16), 2006.

15) 橋本亮太、功刀浩、馬場明道、合同シンポジウム「エンドフェノタイプを利用したゲノム研究」、PACAP 遺伝子と統合失調症の関連、第 28 回日本生物学的精神医学会・第 49 回日本神経化学会・第 36 回日本神経精神薬理学会合同年会、名古屋、9.14-16(14), 2006.

16) Hashimoto R, Noguchi, H, Hori H, Nakabayashi T, Hattori S, Chiba S, Harada S, Takeda M, Saitoh O, Kunugi H, Genetic variants in dysbindin, a susceptibility gene for schizophrenia, influence cognitive function、第 28 回日本生物学的精神医学会・第 49 回日本神経化学会・第 36 回日本神経精神薬理学会合同年会、名古屋、9.14-16(14), 2006.

17) Yasuda Y, Chiba S, Hattori S, Nakabayashi T, Saito O, Takeda M, Kunugi H, Hashimoto R. Gene expression analysis of SELENBP1 in blood and brain in schizophrenia、第 28 回日本生物学的精神医学会・第 49 回日本神経化学会・第 36 回日本神経精神薬理学会合同年会、名古屋、9.14-16(14), 2006.

18) Chiba S, Hattori S, Nakabayashi T,

Takeda M, Kunugi H, Hashimoto R. Gene expression analysis of dysbindin mRNA in peripheral blood in schizophrenia、第28回日本生物学的精神医学会・第49回日本神経化学会・第36回日本神経精神薬理学会合同年会、名古屋、9.14-16(16), 2006.

19) Hattori S, Chiba S, Takeda M, Kunugi H, Hashimoto R. Dysbindin knockout mouse reveals abnormal locomotor activity in novel environment, 第28回日本生物学的精神医学会・第49回日本神経化学会・第36回日本神経精神薬理学会合同年会、名古屋、9.14-16(16), 2006.

20) 竹林実、橋本亮太、久岡一恵、土岡麻美、龍治 英、功刀 浩、うつ病患者の血中における神経新生関連成長因子に関する検討、第28回日本生物学的精神医学会・第49回日本神経化学会・第36回日本神経精神薬理学会合同年会、名古屋、9.14-16(16), 2006.

21) Hori H, Noguchi H, Hashimoto R, Nakabayashi T, Omori M, Takahashi S, Tsukue R, Anami K, Hirabayashi N, Harada S, Saitoh O, Iwase M, Kajimoto O, Takeda M, Okabe S, Kunugi K. Antipsychotic medication and cognitive function in schizophrenia, 第28回日本生物学的精神医学会・第49回日本神経化学会・第36回日本神経精神薬理学会合同年会、名古屋、9.14-16(14), 2006.

22) 根本清貴、大西隆、森健之、守口善也、橋本亮太、朝田隆、統合失調症における脳形態の共変性変化、第28回日本生物学的精神医学会・第49回日本神経化学会・第36回日本神経精神薬理学会合同年会、名古屋、9.14-16(16), 2006.

23) 森健之、大西隆、根本清貴、守口善也、功刀浩、斎藤治、橋本亮太、松田博史、BDNF遺伝子のVal66Met多型は健常成人における年齢に関連した大脳白質微細構造の変化に影響する、第28回日本生物学的精神医学会・第49回日本神経化学会・第36回日本神経精神薬理学会合同年会、名古屋、9.14-16(14), 2006.

24) 飯嶋良味, 岡田武也, 橋本亮太, 志津野朋子, 堀弘明, 中林哲夫, 加藤忠史, 朝田隆, 巽雅彦, 小菅麻子, 上島国利, 原田誠一, 有馬邦正、斉藤治, 功刀浩、BDNF遺伝子における複合繰り返し配列多型と統合失調症との関連解析、第28回日本生物学的精神医学会・第49回日本神経化学会・第36回日本神経精神薬理学会合同年会、名古屋、9.14-16(16), 2006.

25) 吉田哲彦、渡辺嘉之、長谷川千洋、喜多村祐里、安田由華、関山隆史、栗本龍、大西隆、根本清貴、森健之、木村修代、久保嘉彦、徳永博正、数井裕光、橋本亮太、武田雅俊、統合失調症患者における認知機能障害と脳形態異常について：optimized Voxel-Based Morphometryによる検討、第28回日本生物学的精神医学会・第49回日本神経化学会・第36回日本神経精神薬理学会合同年会、

名古屋、9.14-16(16), 2006.

26) 功刀　浩、野口広子、岡本洋平、柳沢洋子、田中美穂、堀弘明、橋本亮太、廣中直行、音性驚愕反応とそのプレパルスインヒビションは記憶機能・実行機能と関連する、第28回日本生物学的精神医学会・第49回日本神経化学会・第36回日本神経精神薬理学会合同年会、名古屋、9.14-16(14), 2006.

27) Kubota K, Inoue K, Hashimoto R, Kunugi H, Saitoh Osamu, Kosuga A, Tatsumi M, Kamijima K, TOHYAMA M. Identification and functional characterization of TRAP1 as a candidate gene for major depression, 第 28 回日本生物学的精神医学会・第 49 回日本神経化学会・第36回日本神経精神薬理学会合同年会、名古屋、9.14-16(15), 2006.

28) 橋本亮太、Translational Research for Schizophrenia、第３回日独先端科学(JGFoS)シンポジウム事前検討会、東京、8.25, 2006.

29) 橋本亮太、服部聡子、千葉幸恵、安田由華、井池直美、大井一高、武田雅俊、功刀浩、統合失調症脆弱性遺伝子ディスバインジンの遺伝子改変動物による分子病態解析、統合脳５領域「平成１８年度　夏の合同班会議」, 札幌, 8.22-25(22), 2006.

30) 橋本亮太、野口広子、堀弘明、服部聡子、千葉幸恵、原田誠一、斉藤治、功刀浩、統合失調症脆弱性遺伝子であるディスバインジンの遺伝子多型は記憶やIQと関連する、第29回日本神経科学学会年, 京都, 7.19-21(21), 2006.

31) 千葉幸恵、服部聡子、堀弘明、中林哲夫、功刀浩、橋本亮太、統合失調症患者の血中におけるディスバインジン遺伝子の定量解析、第29回日本神経科学学会年, 京都, 7.19-21(21), 2006.

32) 服部聡子、千葉幸恵、功刀浩、橋本亮太、dysbindin 欠損マウスは、新奇環境において自発活動の異常を示す、第29回日本神経科学学会年, 京都, 7.19-21(21), 2006.

33) 橋本亮太、精神疾患の包括的研究、浜松医大精神科セミナー, 浜松, 6.12, 2006.

34) 橋本亮太、千葉幸恵、服部聡子、井池直美、安田由華、野口広子、堀弘明、矢ヶ崎有希、沼川忠広、森健之、根本清貴、大西隆、功刀浩、武田雅俊、統合失調症脆弱性遺伝子ディスバインジンによる統合失調症の分子病態研究、臨床脳神経科学会, 大阪, 6.10, 2006.

35) 橋本亮太、増井拓哉、久住一郎、鈴木克治、田中輝明、中川伸、鈴木竜世、岩田仲生、尾崎紀夫、加藤忠史、功刀浩、小山司、武田雅俊、双極性障害患者におけるリチウム治療反応性と遺伝子多型との関連、第２５回躁うつ病の薬理・生化学的研究懇話会、長崎、6.2-3(3), 2006.

36) 橋本亮太、統合失調症研究総論、第二回統合失調症研究会, 大阪, 5.17, 2006.

37) 橋本亮太、精神疾患研究の最前線-

分子から治療へ-、大阪大学薬学研究科大学院講義，大阪，5.15，2006.

38) 橋本亮太、野口広子、堀弘明、功刀浩、統合失調症脆弱性遺伝子ディスバインジンによる統合失調症の分子病態研究、第102回日本精神神経学会総会，博多，5.11-13(11)，2006.

39) 増井拓哉、橋本亮太、久住一郎、鈴木克治、田中輝明、中川伸、鈴木竜世、岩田仲生、尾崎紀夫、加藤忠史、功刀浩、小山司、双極性障害患者におけるリチウム治療反応性とBCR遺伝子多型との関連、第102回日本精神神経学会総会，博多，5.11-13(13)，2006.

40) 功刀浩、橋本亮太、田中美穂、岡本洋平、堀弘明、中林哲夫、斉藤治、廣中直行、統合失調症における情報処理障害の諸相(ワークショップ)、第102回日本精神神経学会総会，博多，5.11-13(13)，2006.

41) 橋本亮太、統合失調症脆弱性遺伝子ディスバインジンによる統合失調症の分子病態研究，愛知病態脳研究会，名古屋，4.25，2006.

42) 橋本亮太、気分安定薬の薬理作用の新しい知見、第26回リチウム研究会，東京，4.22，2006.

H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

該当なし。

2. 実用新案登録

該当なし。

3. その他

該当なし。

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Iga J, Ueno S, Yamauchi K, Numata S, Motoki I, Tayoshi S, Kinouchi S, Ohta K, Song H, Morita K, Rokutan K, Tanabe H, Sano A, Ohmori T.	Gene expression and association analysis of LIM (PDLIM5) in major depression.	Neurosci Lett	400	203-207	2006
Satsuki Sumitani, Masafumi Harada, Hitoshi Kubo, Tetsuro Ohmori	Proton magnetic resonance spectroscopy reveals an abnormality in the anterior cingulate of a subgroup of obsessive-compulsive disorder patients.	Psychiatry Reserch Neuroimaging	154(1)	85-92	2007
Takahide Taniguchi, Satsuki Sumitani, Michitaka Aono, Junichi Iga, Sawako Kinouchi, Hirosi Aki, Mami Matsushita, Kyoko Taniguchi, Mami Tsuno, Kazunari Yamanishi, Masahito Tomotake, Yasuhiro Kaneda, Tetsuro Ohmori.	Effect of antipsychotic replacement with quetiapine on the symptoms and Quality of Life of schizophrenic patients with extrapyramidal symtoms.	Human Psychopharmacology: Clinical and Experimental.	Oct;21(7)	439-45	2006
Shusuke Numata, Shu-ichi Ueno, Jun-ichi Iga, Ken Yamauchi, Song Hongwei, Koji Ohta, Sawako Kinouchi, Sumiko Shibuya-Tayoshi, Shin-Ya Tayoshi, Michitaka Aono, Naomi Kameoka, Satsuki Sumitani, Masahito Tomotake, Yasuhiro Kaneda, Takahide Taniguchi, Yasuhito Ishimoto, Tetsuro Ohmori.	Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism in schizophrenia is associated with age at onset and symptoms.	Neurosci Lett	401	1-5	2006

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
M. Tomotake, Y. Kaneda, J. Iga, S. Kinouchi, S. Tayoshi, I. Motoki, S. Sumitani, K. Yamauchi, T. Taniguchi, Y. Ishimoto, S. Ueno, T. Ohmori.	Subjective and objective measures of quality of life have different predictors for people with schizophrenia.	Psychological Reports	99	477-487	2006
S. Numata, S. Ueno, J. Iga, K. Yamauchi, S. Hongwei, S. Kinouchi, S. Shibuya-Tayoshi, S. Tayoshi, H. Aki, S. Sumitani, M. Itakura, T. Ohmori.	Interaction between catechol-O-methyltransferase (COMT) Val108/158Met and brain-derived neurotrophic factor (BDNF) Val66Met polymorphisms in age at onset and clinical symptoms in schizophrenia.	J Neural Transm	114	255-259	2006
J. Iga, S. Ueno, K. Yamauchi, S. Numata, S. Kinouchi, S. Tayoshi, H. Song, T. Ohmori.	Altered HDAC5 and CREB mRNA expressions in the peripheral leukocytes of major depression.	Progress Neuro-Psychopharmacol Biological Psychiaty	31	628-632	2007
J. Iga, S. Ueno, K. Yamauchi, S. Numata, S. Tayoshi, S. Kinouchi, M. Nakataki, H. Song, K. Hokoishi, H. Tanabe, T. Ohmori.	Gene expression and association analysis of vascular endothelial growth factor in major depressive disorder.	Progress Neuro-Psychopharmacol Biological Psychiat	31	658-663	2007
S. Numata, S. Ueno, J. Iga, K. Yamauchi, S. Hongwei, S. Kinouchi, Hashimoto R, Takeda M, Kunugi H, Itakura M, T. Ohmori.	Gene expression in the peripheral leukocytes and association analysis of PDLIM5 gene in schizophrenia.	Neurosci Lett	415	28-33	2007

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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.	Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology, and ERK signaling.	Hum Mol Genet	15	3024-3033	2006
Ohnishi T, **Hashimoto R**, Mori T, Nemoto K, Moriguchi Y, Iida H, Yamada M, Noguchi H, Nakabayashi T, Hori H, Ohmori M, Tsukue R, Anami K, Hirabayashi N, Harada S, Arima K, Saitoh O, Kunugi H.	The association between the Val158Met polymorphism of the Catechol-*O*-methyl transferase gene and morphological abnormalities of the brain in chronic schizophrenia.	Brain	129	399-410	2006
Kunugi H, **Hashimoto R**, Okada T, Hori H, Nakabayashi T, Baba A, Kudo K, Ohmori M, Takahashi S, Tsukue R, Anami K, Hirabayashi N, Kosuga A, Tatsumi M, Kamijima K, Asada T, Harada S, Arima K, Saitoh O.	Possible association between nonsynonymous polymorphisms of the anaplastic lymphoma kinase (ALK) gene and schizophrenia in a Japanese population.	J Neural Transm	113	1569-1573	2006

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Hori H, Noguchi H, **Hashimoto R**, Nakabayashi T, Omori M, Takahashi S, Tsukue R, Anami K, Hirabayashi N, Harada S, Saitoh O, Iwase M, Kajimoto O, Takeda M, Okabe S, Kunugi H.	Antipsychotic medication and cognitive function in schizophrenia.	Schizophr Res	86	138-146	2006
Nemoto K, Ohnishi T, Mori T, Moriguchi Y, **Hashimoto R**, Asada T, Kunugi H.	The Val66Met polymorphisı of the BDNF gene affects age-related brain morphology.	Neurosci Lett	397(1-2)	25-29	2006

Available online at www.sciencedirect.com
SCIENCE DIRECT
Neuroscience Letters 400 (2006) 203–207
Neuroscience Letters
www.elsevier.com/locate/neulet

Gene expression and association analysis of LIM (PDLIM5) in major depression

Jun-ichi Iga [a], Shu-ichi Ueno [a,*], Ken Yamauchi [a], Shusuke Numata [a], Ikuyo Motoki [a], Sumiko Tayoshi [a], Sawako Kinouchi [a], Koshi Ohta [a], Hongwei Song [a], Kyoko Morita [b], Kazuhito Rokutan [b], Hirotaka Tanabe [c], Akira Sano [d], Tetsuro Ohmori [a]

[a] *Department of Psychiatry, Course of Integrated Brain Sciences, Medical Informatics, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-18-15 Kuramoto, Tokushima 770-8503, Japan*
[b] *Department of Stress Science, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-18-15 Kuramoto, Tokushima 770-8503, Japan*
[c] *Department of Neuropsychiatry, Ehime University School of Medicine, Shitsukawa, To-on, Ehime 791-0295, Japan*
[d] *Department of Psychiatry, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan*

Received 21 December 2005; received in revised form 16 February 2006; accepted 17 February 2006

Abstract

LIM (PDLIM5) is a small protein that interacts with protein kinase C-epsilon and the N-type calcium channel alpha-1B subunit and modulates neuronal calcium signaling. Recently, the *LIM* mRNA expression in postmortem brains and immortalized lymphoblastoid cells from mood disorder patients was reported to be changed and seems to be involved in its pathophysiology. We hypothesized that the expression of the *LIM* mRNA in the native peripheral leukocytes may be a good candidate for the biological marker for mood disorders. Twenty patients with major depression and age- and sex-matched control subjects were included in this expression study. The *LIM* mRNA levels in the peripheral leukocytes from drug-naive depressive patients were significantly lower than those from control subjects and increased significantly after 4-week paroxetine treatments, to almost the same level as controls'. Hamilton depressive scores (HAM-D) were improved about 50% after 4-week treatment but neither paroxetine concentrations nor the changes of HAM-D scores showed significant correlation with the change of the mRNA levels. Then, we genotyped three single nucleotide polymorphic markers of *LIM* gene, which were reported to be associated with bipolar disorder in patients with major depression and control subjects ($n = 130$, each), but there were no associations between these SNPs and major depression. Our investigation indicates that the lower expression levels of *LIM* mRNA in the peripheral leukocytes are associated with the depressive state and that its recovery after treatment may be an adaptive change induced by the antidepressant.

Keywords: Major depression; *LIM (PDLIM5)*; Gene expression; Leukocytes; Association analysis; Paroxetine

Recent findings from molecular biology suggest that LIM (PDLIM5) may be involved in the pathophysiology of major depression. LIM is expressed at various region of brain such as hippocampus, thalamus, hypothalamus, cortex and amygdala and its cellular localization is identical to Synapsin I, which is known to be involved in neurotransmitter release [17]. LIM is known to interact specifically with N-type calcium channel alpha-1B subunit and protein kinase C-epsilon and is critical for rapid and efficient potentiation of the calcium channel activation by PKC in neurons [4]. Because an extensive clinical literature suggests a role for calcium homeostasis in the pathophysiology of major depression and the actions of antidepressants (reviewed in [10]), the abnormalities of calcium signaling cascade induced by the altered expression of LIM may be involved in the pathophysiology of major depression.

Clinical genetic studies also suggest that LIM may be a potential candidate for one of the etiological factors in major depression. It was reported that the expression level of *LIM* mRNA was significantly increased in the postmortem brain tissues of patients with bipolar disorder, schizophrenia and major depression, and was decreased in the immortalized lymphoblastoid cell lines of patients with bipolar disorder [13,14]. The association of single nucleotide polymorphisms in the upstream region of *LIM* gene with bipolar disorder was confirmed [15]. *LIM* gene

* Corresponding author. Tel.: +81 86 633 7130; fax: +81 86 633 7131.
E-mail address: s-ueno@clin.med.tokushima-u.ac.jp (S.-i. Ueno).

0304-3940/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2006.02.044

is located at 4q22 [25] and there are several linkage studies between this region and mental disorders, bipolar disorder [5], schizophrenia [19] and major depression [3].

Recently, several studies including our own have shown altered mRNA expressions in the native peripheral leukocytes of patients with mental disorders [11,21]. We reported that the expression of serotonin transporter (*5HTT*) mRNA in leukocytes was higher in depressive patients compared with that of control subjects and normalized after antidepressant treatment [11]. Not only neurochemical transmitters, such as serotonin and noradrenaline, but also peptides and proteins including hormones, cytokines and even structural proteins in the whole body may play a part in the depressive states (reviews: [6,20]). Thus, the activity of the circulating blood leukocytes in depressive patients may reflect the brain dysfunction occurring in the depressive state. Since *LIM* mRNA is expressed both in the brain and in the peripheral leukocytes, we hypothesized that the expression level of *LIM* mRNA in the leukocytes would also be a good candidate marker of major depression like that of *5HTT* mRNA. Thus, we compared the *LIM* mRNA levels between major depressive patients and controls. Then, we conducted a genetic association analysis of *LIM* gene polymorphisms.

The subjects consisted of 20 patients with major depression (6 males, 14 females and mean age 41.4 ± 12.7) and 20 age- and sex-matched controls (6 males, 14 females and mean age 42.6 ± 12.8). Before study participation, all subjects signed an informed consent form approved by the Ethical Committee of The University of Tokushima Graduate School. All patients were diagnosed as Major Depressive Disorder according to DSM-IV [1] by at least two trained psychiatrists. All subjects underwent extensive medical, neurological, psychological and laboratory evaluations before participating in the study. The persons who had axis II disorders were removed from the study. The diagnosis and the eligibility of the patients were reconfirmed during follow-up periods. Seventeen patients were in the first and other three were in the recurrent depressive episode. All patients did not receive any antidepressants for the current episode before blood sampling. All patients were treated with paroxetine for 4 weeks. The dose of paroxetine was started with 10 or 20 mg for the first two weeks and gradually increased up to 40 mg based on judgment of the trained clinician. At baseline and 4 weeks, subjects were rated with Structured Interview Guide for the 17-item Hamilton Depression Rating Scale (SIGH-D 17, [27]; Japanese version, Y. Nakane, 2000) before blood collection. Peripheral blood was also collected from 20 sex- and age-matched volunteers who were in good physical health with a history of neither psychiatric nor serious somatic disease and were not taking any medication. Probands who had first-degree relatives with psychiatric disorders were excluded from the control subjects.

Samples were collected in the Tokushima University Hospital and the Ehime University Hospital. These included 130 patients with major depression (61 males, 69 females and mean age 46.1 ± 14.6) and 130 age–sex-matched control subjects (61 males, 69 females and mean age 45.8 ± 14.4). Patients were diagnosed with the consensus of at least two trained psychiatrists using DSM-IV [1]. Controls were selected by the same criteria as the expression study. All these subjects were Japanese, unrelated to each other, and living in the same area (Shikoku Island in Japan). All participants signed an informed consent form approved by the Ethical Committee of The University of Tokushima Graduate School or Ehime University.

The paroxetine quantification was performed using high performance liquid chromatography with 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole (NBDF)-derivatization, according to the method of Irie et al. [12] with slight modification in that the separation was performed on a Phenomenex C18 column (4.6 mm × 250 mm).

Total RNA was extracted from peripheral leukocytes of whole blood samples using the PAXgene Blood RNA kit (Qiagen, Tokyo, Japan) according to the manufacturer's recommendations. Residual genomic DNA was digested with RNase-free DNase I (Qiagen). One to five micrograms of total RNA was used for cDNA synthesis by oligo (dT) primers and Powerscript Reverse Transcriptase (BD Biosciences, Japan) after assessing RNA quality and quantity with NanoDrop (NanoDrop Technologies, DE, USA). Primers and hybridization probes were selected and optimized at exon–intron boundary of *LIM* gene (Nihon Gene Research Lab's Inc., Sendai, Japan). Primers were as followed: forward primer: 5′-GATGGTGAACCCTACTGTGA-3′; reverse primer: 5′-GTCTGACCTTCCAAACTTTC-3′. Hybridization probes were as followed: 5′-TCCCATAGAAGCTGGTGACATGTTCCTGG-3′-fluorescein, 5′-LCRed640-AGCTCTGGGCTACACCTGGCATGACACTT-3′-phosphorylation. Quantitative real-time PCR was performed with LightCycler (Roche Diagnostics, Tokyo, Japan). The *G6PD* gene (glucose-6-phosphate dehydrogenase, Qiagen) was used as a housekeeping gene for normalization. Measurements of each gene expression were conducted in duplicate. Proper amplification of the quantitative PCR products of *LIM* and *G6PD* genes was confirmed by agarose gel electrophoresis in all samples.

Genotyping was performed using commercially available TaqMan probes and Applied Biosystems 7500 Fast Real Time PCR System according to the protocol recommended by the manufacturer (Applied Biosystems, CA, USA).

Statistical calculations were carried out using the SPSS Statistical Software Package 11.5 (SPSS, Tokyo, Japan). Expressional differences between patients and control subjects were calculated using the Mann–Whitney *U*-test. Change before and after treatment was calculated with the Wilcoxon rank sum test. Spearman correlation coefficients were used to evaluate the correlations between LIM mRNA levels and either paroxetine concentration or HAM-D score. All significance levels were two-sided. The distribution of genotypes and alleles in the two study groups was compared using the Fisher's exact test. Haplotypic associations were examined with PHASE software [23,24]. The criterion for significance was set at $P < 0.05$ for all tests. Data are presented as mean ± standard deviation.

The relative amount of *LIM* mRNA in the peripheral leukocytes was standardized with G6PD mRNA as an internal standard. The coefficient of variance was less than 20%. There was no significant difference of *LIM* mRNA expression between males and females. *LIM* mRNA levels (*LIM* mRNA/*G6PD* mRNA × 10^3) was in the range of 0.2–19.0 (mean ± S.D.,

Table 1
Association of SNPs in *LIM* gene with major depression

SNP		HWE	*n*	Allele		*P*-value	Genotype			*P*-value
				A	G		A/A	A/G	G/G	
rs10008257	MD	0.91	123	128	118	0.79	33	62	28	0.38
	CT	0.07	125	134	116		41	52	32	
rs2433320	MD	0.73	116	33	199	0.80	2	29	85	0.96
	CT	0.96	123	38	208		3	32	88	
SNP		HWE	*n*	Allele		*P*-value	Genotype			*P*-value
				T	C		T/T	T/C	C/C	
rs2452600	MD	0.88	130	108	152	0.21	22	64	44	0.20
	CT	0.09	130	123	137		24	75	31	

HWE, Hardy–Weinberg equilibrium; MD, major depression; CT, control. *P*-values were calculated by Fisher's exact test.

5.1 ± 4.1) in healthy volunteers, while 0.3–8.2 (mean ± S.D., 2.4 ± 2.0) in 20 medication free depressed patients, showing a statistical difference (Mann–Whitney *U*-test: $P = 0.009$; Fig. 1). *LIM* mRNA levels at baseline was 2.6 ± 2.0 in 17 first episode patients, while 1.0 ± 0.6 in recurrent episode patients, showing no statistical difference. No significant relationship between *LIM* mRNA levels and baseline HAM-D score was observed (Spearman correlation efficient: $P = 0.899$).

Mean paroxetine doses were 29.0 ± 9.7 mg/day (10 mg/day, $n = 1$; 20 mg/day, $n = 7$; 30 mg/day, $n = 5$; 40 mg/day, $n = 7$) at 4-week treatments. Mean paroxetine concentration was 64.8 ± 47.6 ng/ml at that point. Depressive symptoms were improved after 4-week paroxetine treatments (HAM-D scores at baseline and 4 weeks, 22.2 ± 6.5 and 11.3 ± 7.2, respectively; Wilcoxon rank sum test: $P < 0.001$). The *LIM* mRNA level in the leukocytes was significantly increased at 4-week from baseline (2.4 ± 2.0 at baseline; 4.1 ± 4.4 at 4 weeks; Wilcoxon rank sum test: $P = 0.011$; Fig. 1). Neither paroxetine concentrations nor the changes of HAM-D scores showed significant correlation with the change of the mRNA levels.

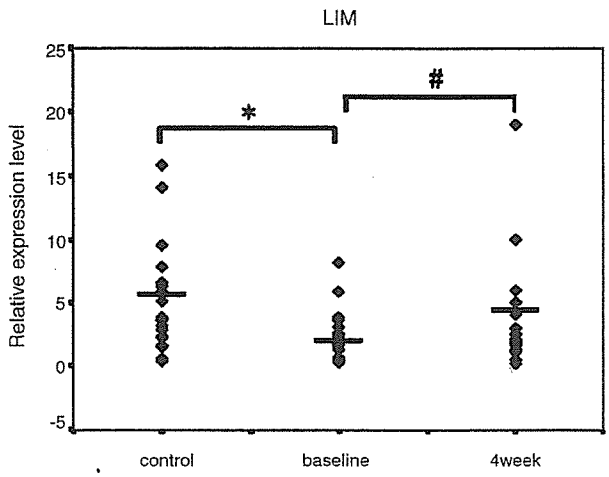

Fig. 1. The measurement of *LIM* mRNA/*G6PD* mRNA × E3 in peripheral leukocytes in depressive patients ($n = 20$) and control subjects ($n = 20$). The mean *LIM* mRNA level was significantly lower in patients (patients: 5.1 ± 4.1, controls: 2.4 ± 2.0, Mann–Whitney *U*-test: $^{*}P = 0.009$). The *LIM* mRNA levels in leukocytes of patients with major depression were significantly increased at 4 weeks compared with those at baseline (baseline: 2.4 ± 2.0, 4-week treatment: 4.1 ± 4.4, Wilcoxon rank sum test: $^{\#}P = 0.011$). The means for controls, baseline and 4-week treatment samples of patients are indicated by horizontal line.

We genotyped three single nucleotide polymorphic (SNP) markers, the two of them (rs10008257 and rs2433320) were reported to be associated with bipolar disorder [15], and the other (rs2452600) was a non-synonymous SNP that results in c to t (Ser136Phe) substitution at codon 136 in LIM protein. The heterozygocity of those three SNPs (rs10008257, rs2433320 and rs2452600) in Japanese population is reported as 0.39, 0.18 and 0.34, respectively and the ratio in the present study was the same as reported. There were no significant deviations in all three SNPs from the Hardy–Weinberg equilibrium in either patients or control subjects. Allele and genotype frequencies of the three SNPs are shown in Table 1. Haplotype analysis of the three SNPs is shown in Table 2. There were no associations between these SNPs and major depression.

The present study is the first report on gene expression in the peripheral leukocytes and association analysis of *LIM* gene in major depression. There are three major findings in our investigation.

First, the mean *LIM* mRNA levels in the peripheral leukocytes of depressive patients before treatment (baseline) were significantly lower than those of age- and sex-matched controls. Our result may be related with previous reports showing that the expression level of *LIM* mRNA was commonly decreased in the immortalized lymphoblastoid cell lines derived from patients with bipolar disorder and schizophrenia [13,14].

Table 2
Haplotypic association of SNPs in LIM with major depression

rs10008257	rs2433320	rs2452600	Frequencies (MD, CT)
A	G	C	0.243, 0.224
A	G	T	0.173, 0.196
A	A	C	0.026, 0.022
A	A	T	0.033, 0.023
G	G	C	0.259, 0.233
G	G	T	0.192, 0.200
G	A	C	0.041, 0.051
G	A	T	0.033, 0.051

P-values were calculated by PHASE software; $P = 0.77$.

However, Iwamoto et al. [13,14] reported that the *LIM* mRNA in the postmortem brains of patients with major depression, bipolar disorder and schizophrenia was commonly high. The cause of these changes in opposite directions is unclear but may be explained by the state-dependent factors such as the regulation of circulating fluid (hormones, cytokines and psychotropic drugs) and clinical symptoms. Further studies with the peripheral leukocytes of bipolar disorder and schizophrenia should be performed. Several findings suggest that N-type calcium channel and PKC epsilon and their adaptor protein LIM may be involved in the pathophysiology of major depression. Decreased anxiety-related behavior was reported in both mice lacking N-type calcium channel [22] and PKC-epsilon [8]. Activity-dependent release of BDNF from hippocampal neuron was triggered by calcium influx specifically through N-type calcium channel [2], which is known to be involved in the pathophysiology of major depression (reviewed in [18]). Because LIM protein is critical for rapid and efficient potentiation of the N-type calcium channel activation by PKC epsilon in neurons [4], down-regulation of *LIM* mRNA in the peripheral leukocytes may reflect the impairments of calcium signaling and neuronal plasticity in neurons.

Second, the *LIM* mRNA levels after 4 weeks of paroxetine treatment were significantly increased from the baseline levels and almost the same levels as those of healthy controls. Although our results fail to reveal the relationship among LIM expression, clinical symptoms and medication, the increase of LIM expression after treatment may be a consequence of state-dependent factors such as clinical improvement and pharmacological effects of antidepressant. Because administration of serotonin selective reuptake inhibitor was reported to decrease the plasma serotonin in patients with major depression [26], the action of serotonin may be involved in this change. Serotonin inhibits N-type calcium channels via 5-HT1A receptors in lamprey spinal neurons [7] and activates PKC epsilon via 5-HT7 receptors in human U373 MG astrocytoma cells [16]. These findings suggest that the alteration of serotonergic signaling induced by the pharmacological effects of antidepressant may influence the *LIM* mRNA expression via N-type calcium channel and PKC epsilon.

Third, there were no associations between major depression and *LIM* gene polymorphisms which were associated with bipolar disorder [15]. Recently, Horiuchi et al. [9] reported that the same SNP (rs2433320) was associated with schizophrenia as well as bipolar disorder. They also reported the different allele of this SNP showed different DNA-protein complexes on electrophoreic mobility shift assay and GA genotype might have higher transcriptional activity. In our study, however, neither patients (GG: 2.3 ± 2.0, $n = 16$; GA: 2.9 ± 2.0, $n = 4$ Mann–Whitney U-test: $P = 0.68$) nor controls (GG: 5.0 ± 4.4, $n = 18$; GA: 6.2 ± 0.5, $n = 2$: $P = 0.38$) showed significant difference of *LIM* mRNA expression between GG and GA genotype of this SNP. These results suggest that the altered expression level of *LIM* mRNA in major depression may be caused by the state-dependent factors. The major limitation of our association study may be the relatively small sample size and we cannot exclude the type II error.

In conclusion, our investigation revealed that the mean *LIM* mRNA levels in depressive patients were significantly decreased at baseline and were significantly increased up to healthy control levels after paroxetine treatment. There were no associations between major depression and *LIM* gene polymorphisms which were reported to be associated with bipolar disorder and schizophrenia. These results suggest that the levels of *LIM* mRNA in native leukocytes may be a useful biological marker of major depression. Further studies are necessary to confirm and extend the present results.

Acknowledgements

The authors are extremely grateful to the volunteers for participating in this research. The authors thank Mrs. Akemi Okada for her technical assistance. This work was supported by a Health and Labor Science Research Grant from the Japanese Ministry of Health, Labor and welfare (kokoro H16-002), a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology (No. 16390325) and a Grant-in-Aid for Scientific Research from the 21st Century COE program, Human Nutritional Science on Stress Control, Tokushima, Japan.

References

[1] American Psychiatric Association Quick reference to the diagnostic criteria from DSM-IV (1994).
[2] A. Balkowiec, D.M. Katz, Cellular mechanisms regulating activity-dependent release of native brain-derived neurotrophic factor from hippocampal neurons, J. Neurosci. 22 (2002) 10399–10407.
[3] N.J. Camp, M.R. Lowry, R.L. Richards, A.M. Plenk, C. Carter, C.H. Hensel, V. Abkevich, M.H. Skolnick, D. Shattuck, K.G. Rowe, D.C. Hughes, L.A. Cannon-Albright, Genome-wide linkage analyses of extended Utah pedigrees identifies loci that influence recurrent, early-onset major depression and anxiety disorders, Am. J. Med. Genet. B Neuropsychiatr. Genet. 135 (2005) 85–93.
[4] Y. Chen, M. Lai, Y. Maeno-Hikichi, J. Zhang, Essential role of the LIM domain in the formation of the PKC epsilon-ENH-N-type Ca^{2+} channel complex, Cell. Signal. 18 (2006) 215–224.
[5] S.D. Detera-Wadleigh, J.A. Badner, T. Yoshikawa, A.R. Sanders, L.R. Goldin, G. Turner, D.Y. Rollins, T. Moses, J.J. Guroff, D. Kazuba, M.E. Maxwell, H.J. Edenberg, T. Foroud, D. Lahiri, J.I. Nurnberger Jr., O.C. Stine, F. McMahon, D.A. Meyers, D. MacKinnon, S. Simpson, M. McInnis, J.R. DePaulo, J. Rice, A. Goate, T. Reich, M.C. Blehar, E.S. Gershon, Initial genome scan of the NIMH genetics initiative bipolar pedigrees: chromosomes 4, 7, 9, 18, 19, 20, and 21q, Am. J. Med. Genet. 74 (1997) 254–262.
[6] F.A. Henn, B. Vollmayr, Stress models of depression: forming genetically vulnerable strains, Neurosci. Biobehav. Rev. 29 (2005) 799–804.
[7] R.H. Hill, E. Svensson, Y. Dewael, S. Grillner, 5-HT inhibits N-type but not L-type Ca(2+) channels via 5-HT1A receptors in lamprey spinal neurons, Eur. J. Neurosci. 18 (2003) 2919–2924.
[8] C.W. Hodge, J. Raber, T. McMahon, H. Walter, A.M. Sanchez-Perez, M.F. Olive, K. Mehmert, A.L. Morrow, R.O. Messing, Decreased anxiety-like behavior, reduced stress hormones, and neurosteroid supersensitivity in mice lacking protein kinase Cepsilon, J. Clin. Invest. 110 (2002) 1003–1010.
[9] Y. Horiuchi, M. Arai, K. Niizato, S. Iritani, E. Noguchi, T. Ohtsuki, M. Koga, T. Kato, M. Itokawa, T. Arinami, A polymorphism in the PDLIM5 gene associated with gene expression and schizophrenia. Biol. Psychiatry 59 (2006) 434–439.

[10] A.P. Ian, Antidepressant activity and calcium signaling cascades, Hum. Psychopharmacol. Clin. Exp. 16 (2001) 71–81.
[11] J. Iga, S. Ueno, K. Yamauchi, I. Motoki, S. Tayoshi, K. Ohta, H. Song, K. Morita, K. Rokutan, T. Ohmori, Serotonin transporter mRNA expression in peripheral leukocytes of patients with major depression before and after treatment with paroxetine, Neurosci. Lett. 389 (2005) 12–16.
[12] H. Irie, M. Fujita, Y. Inokawa, Y. Narita, Phase I clinical study of paroxetine HCl (Study 3): pharmacokinetics after single oral administration of paroxetine HCl 10, 20 and 40 mg to healthy adult male volunteers, Jpn. Pharmacol. Ther. 28 (2000) S47–S68.
[13] K. Iwamoto, M. Bundo, S. Washizuka, C. Kakiuchi, T. Kato, Expression of HSPF1 and LIM in the lymphoblastoid cells derived from patients with bipolar disorder and schizophrenia, J. Hum. Genet. 49 (2004) 227–231.
[14] K. Iwamoto, C. Kakiuchi, M. Bundo, K. Ikeda, T. Kato, Molecular characterization of bipolar disorder by comparing gene expression profiles of postmortem brains of major mental disorders, Mol. Psychiatry 9 (2004) 406–416.
[15] T. Kato, Y. Iwayama, C. Kakiuchi, K. Iwamoto, K. Yamada, Y. Minabe, K. Nakamura, N. Mori, K. Fujii, S. Nanko, T. Yoshikawa, Gene expression and association analyses of LIM (PDLIM5) in bipolar disorder and schizophrenia, Mol. Psychiatry 10 (2005) 1045–1055.
[16] K. Lieb, L. Biersack, A. Waschbisch, S. Orlikowski, R.S. Akundi, E. Candelario-Jalil, M. Hull, B.L. Fiebich, Serotonin via 5-HT7 receptors activates p38 mitogen-activated protein kinase and protein kinase C epsilon resulting in interleukin-6 synthesis in human U373 MG astrocytoma cells, J. Neurochem. 93 (2005) 549–559.
[17] Y. Maeno-Hikichi, S. Chang, K. Matsumura, M. Lai, H. Lin, N. Nakagawa, S. Kuroda, J.F. Zhang, A PKC epsilon-ENH-channel complex specifically modulates N-type Ca^{2+} channels, Nat. Neurosci. 6 (2003) 468–475.
[18] H.K. Manji, J.A. Quiroz, J. Sporn, J.L. Payne, K.A. Denicoff, N. Gray, C.A. Zarate Jr., D.S. Charney, Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression, Biol. Psychiatry 53 (2003) 707–742.
[19] B.J. Mowry, K.R. Ewen, D.J. Nancarrow, D.P. Lennon, D.A. Nertney, H.L. Jones, M.S. O'Brien, C.E. Thornley, M.K. Walters, R.R. Crowe, J.M. Silverman, J. Endicott, L. Sharpe, N.K. Hayward, M.M. Gladis, S.J. Foote, D.F. Levinson, Second stage of a genome scan of schizophrenia: study of five positive regions in an expanded sample, Am. J. Med. Genet. 96 (2000) 864–869.
[20] C.L. Raison, A.H. Miller, When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders, Am. J. Psychiatry 160 (2003) 1554–1565.
[21] P. Rocc, C. De Leo, C. Eva, L. Marchiaro, A.M. Milani, R. Musso, L. Ravizza, E. Zanalda, F. Bogetto, Decrease of the D4 dopamine receptor messenger RNA expression in lymphocytes from patients with major depression, Prog. Neuropsychopharmacol. Biol. Psychiatry 26 (2002) 1155–1160.
[22] H. Saegusa, T. Kurihara, S. Zong, A. Kazuno, Y. Matsuda, T. Nonaka, W. Han, H. Toriyama, T. Tanabe, Suppression of inflammatory and neuropathic pain symptoms in mice lacking the N-type Ca^{2+} channel, EMBO J. 20 (2001) 2349–2356.
[23] M. Stephens, P. Donnelly, A comparison of Bayesian methods for haplotype reconstruction from population genotype data, Am. J. Hum. Genet. 73 (2003) 1162–1169.
[24] M. Stephens, N. Smith, P. Donnelly, A new statistical method for haplotype reconstruction from population data, Am. J. Hum. Genet. 68 (2001) 978–989.
[25] N. Ueki, N. Seki, K. Yano, Y. Masuho, T. Saito, M. Muramatsu, Isolation, tissue expression, and chromosomal assignment of a human LIM protein gene, showing homology to rat enigma homologue (ENH), J. Hum. Genet. 44 (1999) 256–260.
[26] M. Urbina, S. Pineda, L. Pinango, I. Carreira, L. Lima, [3H]Paroxetine binding to human peripheral lymphocyte membranes of patients with major depression before and after treatment with fluoxetine, Int. J. Immunopharmacol. 21 (1999) 631–646.
[27] J.B. Williams, A structured interview guide for the Hamilton Depression Rating Scale, Arch. Gen. Psychiatry 45 (1988) 742–747.

Available online at www.sciencedirect.com
ScienceDirect
Psychiatry Research: Neuroimaging 154 (2007) 85–92
PSYCHIATRY RESEARCH NEUROIMAGING
www.elsevier.com/locate/psychresns

Proton magnetic resonance spectroscopy reveals an abnormality in the anterior cingulate of a subgroup of obsessive–compulsive disorder patients

Satsuki Sumitani[a,*], Masafumi Harada[b], Hitoshi Kubo[b], Tetsuro Ohmori[a]

[a]*Department of Psychiatry, Institute of Health Biosciences, University of Tokushima Graduate School, 18-15 Kuramoto-cho 3, Tokushima 770-8503, Japan*
[b]*Department of Radiologic Technology, School of Health Sciences, University of Tokushima, Tokushima, Japan*

Received 29 August 2005; received in revised form 30 January 2006; accepted 13 February 2006

Abstract

Numerous neuroimaging studies have suggested that obsessive–compulsive disorder (OCD) patients had a neurobiological abnormality in the frontal-subcortical circuits. On the other hand, there are distinct differences in the responses to pharmacological treatment among OCD patients. In the present study, we measured the concentration of *N*-acetyl aspartate (NAA), a putative marker of neuronal viability, with proton magnetic resonance spectroscopy (MRS) in OCD patients with different pharmacological responses. Participants comprised 20 patients and 26 healthy control subjects. OCD patients were divided into three groups according to the pharmacological response; responders to a selective serotonin reuptake inhibitor (SSRI) (group A: $n=7$), responders to SSRI with an atypical antipsychotic (group B: $n=8$) and non-responders to either SSRI or SSRI with an atypical antipsychotic (group C: $n=5$). Short echo proton MRS was used to measure NAA concentrations in the anterior cingulate, the left basal ganglia and the left prefrontal lobe of subjects. A significantly lower NAA concentration was observed only in group B compared with control subjects in the anterior cingulate. Our results suggest that a subgroup of OCD patients who respond to an SSRI with an atypical antipsychotic have distinct biological abnormalities in the anterior cingulate.

Keywords: Selective serotonin reuptake inhibitor (SSRI); Atypical antipsychotic; Pharmacological response; *N*-acetyl aspartate (NAA); Basal ganglia; Prefrontal lobe

1. Introduction

Although selective serotonin reuptake inhibitors (SSRIs) are the mainstay of pharmacological treatment for obsessive–compulsive disorder (OCD), 40–60% of OCD patients fail to show improvement after an adequate trial with SSRIs (Goodman et al., 1993). When OCD patients experience little improvement with SSRIs, the addition of a low dose of an atypical antipsychotic, such as risperidone or olanzapine, to ongoing SSRI treatment has been shown to be effective (McDougle et al., 2000; Bystritsky et al., 2004). However, there are patients who continue to show little or no improvement after the trial with this augmentation therapy. These differences of pharmacological response suggest the existence of biological differences among

* Corresponding author. Tel.: +81 88 633 7130; fax: +81 88 633 7131.
E-mail address: satsuki@clin.med.tokushima-u.ac.jp (S. Sumitani).

0925-4927/$ - see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.pscychresns.2006.02.003

OCD patients. Recently, numerous neuroimaging studies in OCD implicate dysfunction within frontal-subcortical circuits (Saxena et al., 2001). Functional magnetic resonance imaging (fMRI), single photon emission computed tomography (SPECT) or positron emission tomography (PET) indicated abnormalities in structures such as the orbitofrontal cortex, anterior cingulate, caudate nucleus, and thalamus (Baxter et al., 1987, 1988; Swedo et al., 1989; Rauch et al., 1994; Hollander et al., 1995; Breiter et al., 1996). Magnetic resonance spectroscopy (MRS) can be used to obtain information about several metabolites that are highly relevant to our understanding of OCD. The aim of this study is to examine *N*-acetyl aspartate (NAA) concentrations in OCD patients who show different responses to pharmacotherapy with proton MRS.

2. Methods

2.1. Subjects

Twenty patients were diagnosed with OCD according to DSM-IV criteria, in addition, 26 healthy comparison subjects participated in this study. The OCD patients were recruited at the Department of Psychiatry, Tokushima University Hospital. The diagnosis of UCD was confirmed by at least two trained psychiatrists. Patients comorbid with other axis I disorders were excluded. OCD patients were subclassified into the following three groups according to pharmacological response: group A, SSRI responders (7 cases in 20 participants); group B, SSRI plus atypical antipsychotic responders (8/20); and group C, non-responders to either SSRI or SSRI plus an atypical antipsychotic (5/20). Severity of OCD symptoms was assessed using the Yale-Brown Obsessive–Compulsive Scale (Y-BOCS). SSRI responders were those who showed a >50% decrease on the Y-BOCS at the end of an 8- to 12-week treatment with a high dose of either fluvoxamine or paroxetine. Then, patients who had no response to SSRI were treated by adding a low dose of either risperidone or olanzapine. When SSRI-refractory OCD patients showed a >50% decrease on the Y-BOCS after institution of augmentation therapy, they were regarded as responders to SSRI plus an atypical antipsychotic. The remaining patients were regarded as non-responders to either SSRI or SSRI plus an atypical antipsychotic. When the MRS scans were performed, 8 patients were initially either drug-naive or drug-free and 12 patients were receiving pharmacological treatment (8 patients took SSRI and 4 patients took both SSRI and antipsychotic medication) and 4 of them already showed

Table 1
Subject characteristics

	OCD			Control	*P*-value
	A	B	C		
No. of subjects	7	8	5	26	
Age	38.4±13.9	25.0±5.6	26.2±7.5	25.2±7.4	0.036
Gender					0.033
Male	6	6	1	10	
Female	1	2	4	16	
Treatment status at MRS					0.978
Before treatment	3	3	2	–	
During treatment[a]	4 (1)	5 (3)	3 (0)	–	
Medication status at MRS					0.095
SSRIs	4	1	3	–	
SSRI+neuroleptics	0	4	0	–	
Drug-naive	3	3	2	–	
Y-BOCS					
Before treatment	21.1±5.6	25.0±4.4	25.6±6.5	–	0.293
At MRS	19.7±5.3	18.1±6.2	24.4±5.4	–	0.183
Percent volume of tissue types in the anterior cingulate					
CSF	19.9±5.4*	18.2±3.5	13.6±2.2*	15.5±3.3	0.011[b]
Gray matter	69.0±8.7	73.6±8.3	77.7±10.4	74.5±7.6	0.150
White matter	11.1±8.5	8.2±6.2	8.7±9.9	8.6±3.9	0.771

A, SSRI responders; B, SSRI with atypical antipsychotics responders; C, non-responders.
Comparisons were made by ANOVA with Bonferroni's post hoc test or Fisher's exact test. The level of statistical significance is $P<0.05$.
[a]Within the parentheses is the number of patients who already showed >50% decrease on the Y-BOCS at the time MRS was performed.
[b]Group C showed a significantly lower CSF percentage than group A ($P=0.03$).

a >50% decrease on the Y-BOCS (Table 1). The mean length of augmentation therapy with atypical antipsychotic was 52.0 ± 36.2 weeks (12–96 weeks). None of the OCD subjects were participating in formal cognitive behavioral therapy at the time of the study. Neither the subjects with OCD nor the healthy control subjects had a history of significant head injury or seizures. No subjects showed anatomical abnormalities on MRI.

2.2. Proton MR spectroscopy

All proton MRS studies were performed at Tokushima University Hospital using a Signa Horizon (1.5T GE, Milwaukee, WI, USA) scanning system. Proton MRS was performed using the STEAM sequence with water suppression by CHESS pulses (TE=18 ms, TR=5000 ms, acquisition=64 times) to minimize the longitudinal and transverse relaxation effect. Neurochemical compounds that can be identified in short-echo proton MRS include *N*-acetyl aspartate (NAA), complex of glutamate and glutamine (Glx), creatine and phosphocreatine (Cr), choline-containing compounds (Cho) and myoinositol (mI). The area under each of the magnetic resonances is proportioned to the concentration of the particular compound. On the basis of previous reports of functional anomalies, the volumes of interest for proton MRS were set at the anterior cingulate, the left basal ganglia and the left frontal lobe as shown in Fig. 1A–C. The anatomical positions of the voxels were chosen from axial localizer images. The voxel of the anterior cingulate was placed to encompass bilateral anterior cingulate (Brodmann area 24/32), left basal ganglia was placed to include the caudate and putamen, and left frontal lobe was placed rostrolaterally from the anterior horn of the lateral ventricle (contained white matter exclusively). (ROI size= 1.7 cm × 1.7 cm × 1.5 cm).

2.3. Processing of spectroscopic data

Metabolite concentrations were estimated using the linear combination model (LCModel) (Provencher, 1993) (Fig. 2). Our basis set for prior knowledge derived from in vitro original data for each metabolite. The accepted shimming criterion was 3–5 Hz of FWHM on the water peak. We excluded data with extremely high spectra (% S.D.>30%). The spectroscopic voxels were volumetrically segmented and quantified by tissue types. The anterior cingulate voxel contained gray matter, white matter and cerebrospinal fluid (CSF) (Fig. 1A). The left basal ganglia voxel was mainly gray matter, including the caudate (Fig. 1B) and putamen,

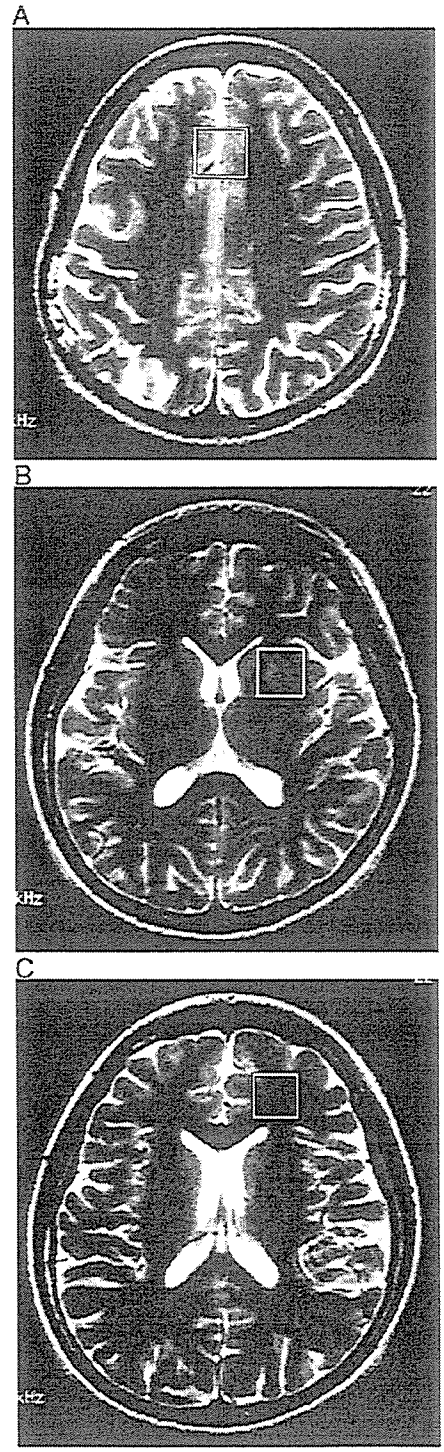

Fig. 1. Location and size of the MRS voxels. (A) anterior cingulate (B) left basal ganglia (deep gray matter) (C) left frontal lobe (white matter).

while the left frontal lobe voxel contained white matter exclusively (Fig. 1C). We adjusted NAA, Cho, and Cr for the anterior cingulate voxel assuming that the

metabolite concentrations in CSF were equal to zero. These corrected NAA, Cho and Cr values were compared among subject groups in the anterior cingulate. Considering that metabolite concentrations varied between white and gray matter tissues (Wiedermann et al., 2001), we compared the percentage of gray matter and white matter in the anterior cingulate among subject groups. However, there were no significant differences in the percentages among subject groups (Table 1).

2.4. Statistics

All statistical tests were performed with SPSS version 11.5 (Tokyo, Japan). All data are expressed as mean± S.D. Means of the metabolite concentrations between all of the OCD patients and healthy subjects were compared with Student's *t*-test. Analyses of variance (ANOVAs) were performed with Bonferroni's post-hoc PLSD to look for significant differences in four groups (three OCD groups and a healthy control group). Pearson's correlation coefficient was used to study the relationship between NAA concentrations and age in the healthy controls, and Student's *t*-test was performed to compare the mean NAA concentrations between male and female healthy subjects.

Table 2
Concentrations of metabolites in three brain regions for OCD patients and healthy controls

	OCD (n=20)	Control (n=26)	P-value
Anterior cingulate cortex (concentrations corrected for CSF)			
NAA	9.14±1.24	9.88±0.99	0.031*
Cho	2.58±0.75	2.36±0.59	0.705
Cr	7.73±1.49	8.07±1.25	0.263
Left basal ganglia			
NAA	8.47±1.29	8.35±0.98	0.739
Cho	2.14±0.33	2.04±0.46	0.470
Cr	7.44±1.19	7.31±0.98	0.692
Left frontal lobe			
NAA	7.86±0.91	8.40±1.16	0.092
Cho	1.97±0.64	1.86±0.53	0.561
Cr	6.13±1.40	5.46±1.27	0.126

Comparisons were made by unpaired *t*-test.
*The level of statistical significance is $P<0.05$.

To confirm pharmacological treatment would not significantly change NAA concentration, ANOVAs were performed among three OCD subgroups (no medication, taking SSRI or taking both SSRI and antipsychotic medication) when proton MRS was

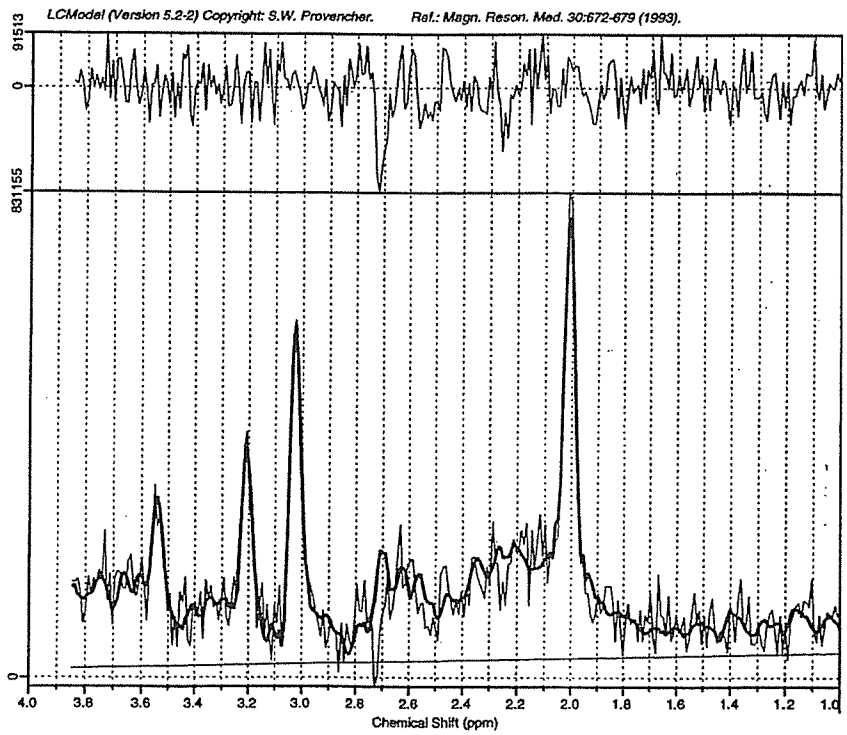

Fig. 2. Representative proton MRS spectrum for the anterior cingulate showing the three largest peaks: NAA, Cre and Cho. The spectrum was collected using a PRESS-CHESS sequence. Chemical shifts are indicated in part per million (ppm).

performed. Pearson's correlation coefficient was used to study the relationship between NAA concentrations and Y-BOCS scores (both before treatment and at MRS scan) to examine the relationship between severity of illness and NAA level. The level of statistical significance was set at $P<0.05$.

3. Results

Metabolite concentrations of three brain regions are given for OCD patients and healthy control in Table 2. OCD patients showed a significantly lower NAA concentration than healthy controls in the anterior cingulate ($t_{44}=-2.2$, $P=0.031$). There were no significant differences in NAA concentrations between OCD patients and healthy controls in either the left basal ganglia or the left frontal lobe.

Metabolite concentrations of the three brain regions are given for three subtypes of OCD patients and healthy control in Table 3. ANOVAs revealed a significant intergroup difference in NAA concentration [$F(3, 42)=4.88$, $P=0.005$] in the anterior cingulate and significant intergroup differences in NAA [$F(3, 42)=3.91$, $P=0.015$] and Cho concentrations [$F(3, 42)=3.84$, $P=0.016$] in the left basal ganglia. In the frontal lobe, no significant intergroup differences were observed in any metabolites.

In the anterior cingulate, group B showed significantly lower NAA concentrations compared with group A (8.51±1.08 vs. 10.06±1.16 mmol/l, $P=0.035$) and to the healthy control group (8.51±1.08 vs. 9.88±0.99 mmol/l, $P=0.013$). In the basal ganglia, group B showed significantly lower NAA and Cho concentrations compared with group A (NAA, 7.74±0.69 vs. 9.50±1.29 mmol/l, $P=0.011$ and Cho, 1.83±0.14 vs. 2.49±0.13 mmol/l, $P=0.013$).

No significant correlation was found between the NAA concentrations and age in healthy controls in the anterior cingulate ($r=-0.025$, $P=0.91$), the left basal ganglia ($r=0.031$, $P=0.88$) or the left frontal lobe ($r=-0.076$, $P=0.71$). There were no significant differences in NAA concentrations between male and female healthy controls in the anterior cingulate ($t_{24}=-1.3$, $P=0.20$), the left basal ganglia ($t_{24}=-0.27$, $P=0.79$) or the left frontal lobe ($t_{24}=0.12$, $P=0.90$). There were no significant differences in NAA concentrations according to medication status (no medication or taking SSRI or taking SSRI with antipsychotic) in the anterior cingulate, in the left basal ganglia or in the left frontal lobe

Table 4
NAA concentrations in different medication status of OCD patients when MRS was performed

	OCD			P-value
	No medication ($n=8$)	SSRI ($n=8$)	SSRI and antipsychotics ($n=4$)	
Anterior cingulate	8.94±1.41	9.51±1.13	8.79±1.25	0.570
Left basal ganglia	8.49±1.43	8.66±1.42	8.05±0.87	0.761
Left frontal lobe	7.79±0.96	7.93±1.03	7.88±0.73	0.959

Table 3
Concentrations of metabolites in three subtypes of OCD patients and healthy controls

	OCD			Controls ($n=26$)	P-value
	A ($n=7$)	B ($n=8$)	C ($n=5$)		
Anterior cingulate (concentrations corrected for CSF)					
NAA	10.06±1.16*	8.51±1.08*	8.87±0.99	9.88±0.99*	0.005[a]
Cho	2.90±0.97	2.29±0.64	2.60±0.46	2.36±0.59	0.229
Cr	8.41±1.73	7.38±1.44	7.33±1.10	8.07±1.25	0.333
Left basal ganglia					
NAA	9.50±1.29*	7.74±0.69*	8.20±1.26	8.36±0.98	0.015[b]
Cho	2.49±0.13*	1.83±0.14*	2.08±0.18	2.05±0.47	0.016[c]
Cr	7.39±0.87	7.01±1.14	8.10±1.55	7.31±0.98	0.368
Left frontal lobe					
NAA	7.59±1.04	7.96±1.71	8.08±0.27	8.40±2.87	0.319
Cho	1.83±0.83	1.82±0.36	2.50±0.40	1.85±0.53	0.315
Cr	5.58±2.02	6.55±0.95	6.30±0.64	5.46±1.27	0.266

A, SSRI responders; B, SSRI plus atypical antipsychotic responders; C, non-responders.
Comparisons were made by ANOVA and Bonferroni's post hoc test.
*The level of statistical significance is $P<0.05$.
[a] Group B showed significantly lower NAA concentrations than group A ($P=0.035$) and the healthy control group ($P=0.013$), respectively.
[b] Group B showed significantly lower NAA concentrations than group A ($P=0.011$).
[c] Group B showed significantly lower Cho concentrations than group A ($P=0.013$).

(Table 4). There were no significant differences in NAA concentrations between unmedicated and medicated OCD patients in the anterior cingulate ($t_{18}=0.45$, $P=0.661$), in the left basal ganglia ($t_{18}=0.29$, $P=0.778$) or in the left frontal lobe ($t_{18}=-0.83$, $P=0.418$). No significant correlation was found between the NAA concentrations and Y-BOCS scores of the OCD patients before treatment in the anterior cingulate ($r=-0.17$, $P=0.48$), the left basal ganglia ($r=-0.04$ $P=0.84$) or the left frontal lobe ($r=-0.02$, $P=0.94$). There were also no significant correlation between the NAA concentrations and Y-BOCS scores of the OCD patients at the time of MRS scans in the anterior cingulate ($r=-0.004$, $P=0.99$), in the left basal ganglia ($r=-0.02$, $P=0.95$) or in the left frontal lobe ($r=-0.25$, $P=0.28$).

4. Discussion

Proton MRS is an important tool to study in vivo biochemical aspects of brain disorders. By using signals from excitation of the nucleus of hydrogen, proton MRS allows the acquisition of signals from several biochemical compounds such as NAA, Glx, Cr, Cho and mI. Although the roles of these metabolites are not certain, there were several hypotheses about the nature of NAA. NAA was thought to be abundant in neurons and scarce in mature glial cells, and a reduction of NAA was initially considered to reflect a loss of neurons and neural dysfunction. Later, it was reported that NAA might act via glutamatergic NMDA receptors to elevate intracellular calcium (Rubin et al., 1995). More recent evidence suggests a possible role of NAA as a molecular water pump in the brain operating between neurons and oligodendrocytes (Baslow, 2002, 2003). It is thought that NAA levels are sensitive to pathological processes affecting the functioning of neurons, and reduction is often reversible (Baker, 2001).

In agreement with Ebert et al. (1997), who reported a significantly lower NAA/Cr ratio in OCD patients than in normal control subjects in the anterior cingulate, we found significantly lower NAA levels in the same region of OCD patients compared with those in controls. Recently, Rosenberg et al. (2004) reported reduced glutamate concentrations in the anterior cingulate in pediatric OCD patients. Although no other MRS study has focused on the anterior cingulate, other functional imaging studies such as fMRI, SPECT and PET have revealed abnormalities in that region in OCD patients. Our results, together with these, suggest a functional change in the region in OCD patients.

Interestingly, when we divided OCD patients into three groups according to pharmacological response (group A, SSRI responders; group B, SSRI plus atypical antipsychotic responders and group C, non-responders to either SSRI or SSRI plus atypical antipsychotic), we found that only patients who responded to atypical antipsychotics combined with ongoing SSRI (group B) but not other patients showed significantly lower NAA concentration compared with healthy controls in the anterior cingulate. The lower NAA concentration was not accounted for by the effect of antipsychotics or SSRI, since NAA concentration did not differ according to medication status. Besides, antipsychotics have been shown to have no effects on the levels of NAA (Deicken et al., 1997; Bustillo et al., 2004; Bertolino et al., 1998), and SSRI have been reported to have no effects on NAA concentration in OCD patients after paroxetine intervention (Rosenberg et al., 2000).

In the basal ganglia, previous MRS studies have produced different results in the levels of NAA. A significantly lower NAA/Cr ratio in the right striatum (Ebert et al., 1997) or a lower NAA level in the left striatum (Bartha et al., 1998) has been reported in OCD patients compared with controls, while no difference in the NAA/Cr ratio has been observed in either the right or the left lenticular nucleus (Ohara et al., 1999). We found no difference in NAA concentration between the total group of OCD patients and the healthy controls. When the patients were divided into three groups, OCD patients who responded to atypical antipsychotics combined with ongoing SSRI treatment (group B) showed significantly lower NAA concentrations compared with the SSRI responders (group A) in the left basal ganglia. Interpretation of this finding is not easy, since neither group A nor group B showed a significant difference compared with the control group. However, the results suggest that NAA concentrations are different in the left basal ganglia as well as the anterior cingulate among OCD patients with different pharmacological responses. Differences between subtypes of OCD may explain in part the inconsistent results of NAA levels in the basal ganglia in the previous studies.

We divided OCD patients in terms of the response to SSRI and the augmentation with atypical antipsychotics. Recently, a large number of studies have been conducted to find clinical features predictive of treatment response in OCD. It is suggested that positive predictors of response to serotonin reuptake inhibitors (SRIs) and SSRIs are female gender, late onset and lower severity, and that negative predictors are male gender, early onset, poor insight, higher severity, and comorbidity such as tics or schizotypal personality disorder (McDougle et al., 1993; Ravizza et al., 1995; Ackerman et al., 1998, 1999; Steketee et al., 1999; Mundo et al., 1999; Erzegovesi et