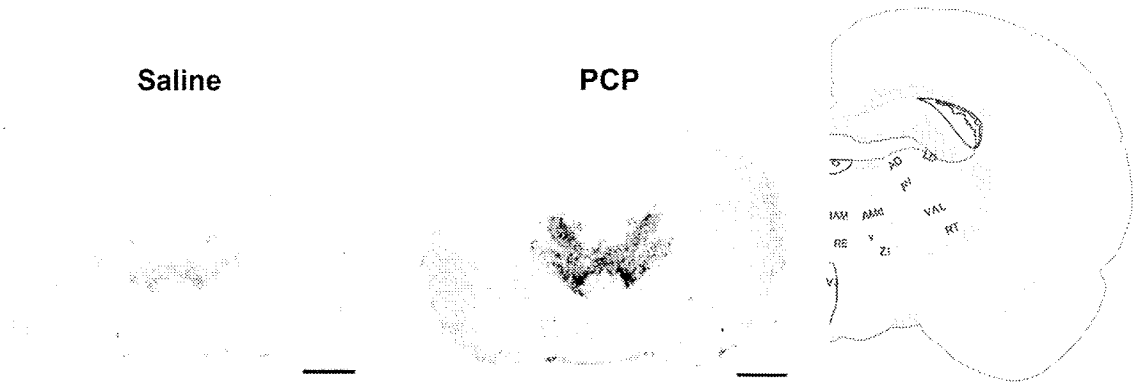


Fig. 4. Effects of acute PCP injection on Lmod2 mRNA expression in the brain regions of adult rats. Thalamic region-restricted Lmod2 mRNA expression in the adult rat (PD 50) brain 60 min after acute PCP administration (7.5 mg/kg s.c.) as revealed by in-situ hybridization histochemistry with 35S-labelled RNA probe for Lmod2.Both of the basal (saline-treated control animals) and PCP-induced Lmod2 mRNA signals were confined to the thalamic regions in the brain (scale bars, 2 mm). Abbreviations: AD, Anterodorsal nucleus ; AMd, Anteromedial nucleus, dorsal part ; AV, anteroventral nucleus; CL, central lateral nucleus; IAM, interanteromedial nucleus; LD, lateral dorsal nucleus; RE, nucleus reunions; RT, reticular nucleus; VAL, ventral anterior-lateral complex; VM, ventral medial nucleus; VPM, ventro posteromedial nucleus; V3, third ventricle ; ZI, zona incerta.

されなかった（データ省略）。すなわち、発達による PCP の影響の差異は、PCP の代謝などの薬物動態の変化によるものではないと推測された。

In situ hybridization においても、視床の *Lmod2* 遺伝子は PCP により発現が増加することを確認した。また、その基礎的発現や PCP に対する応答は、生後 50 日齢の脳において、視床前核群（前内側核，前腹側核，前内側間核），視床腹前外側核群、腹内側視床核，菱形核，髄板内核群 (中心内側核 ，傍中心核、中心外側核)、外側および腹側視床後核等に限局していることが明らかになった（Fig. 4）。

3．PCP，dizocilpine、methamphetamine および haloperidol の成熟ラット視床の *Lmod2* 発現に与える影響

成熟期視床における Lmod2 遺伝子は、PCP と同様に NMDA 受容体を遮断する dizocilpine（MK801）により、PCP 投与後と同程度の発現増加を示した。間接的なドーパミンアゴニストである methamphetamine（MAP）によっても増加したが、その程度は PCP や MK801 より小さかった。一方、強力な D2 型ドーパミン受容体遮断作用を持つ抗精神病薬 haloperidol は、単独で Lmod2 発現に有意な影響を及ぼさなかったが、前処置により、PCP 投与 30 分前に処置することにより、PCP の *Lmod2* 遺伝子発現増強作用を部分的に阻害した。

D. 考察

これまで、本研究者らは RNA arbitrarily primed PDR 法または DNA アレイにより、ラット大脳新皮質において PCP に対して発達依存的応答を示す遺伝子 *prt1*（PCP-responsive transcript 1）や *CCN1*[8] を検出してきた。本研究から、視床においても、PCP が成熟期に発現を誘導するが新生仔期には発現を変化させない遺伝子が存在することが初めて明らかになった。この結果は、研究目的で述べた、精神異常発現薬に応答する特定の神経回路（情報処理システム）の中に、一定の発達期に成熟するものがあるという仮説をさらに支持している。また、視床の *Lmod2* 発現誘導が、統合失調症様症状を引き起こす他の乱用薬物によっても生ずることがわかった。

Lmod2 と同じく Tmod ファミリー[7] に属する

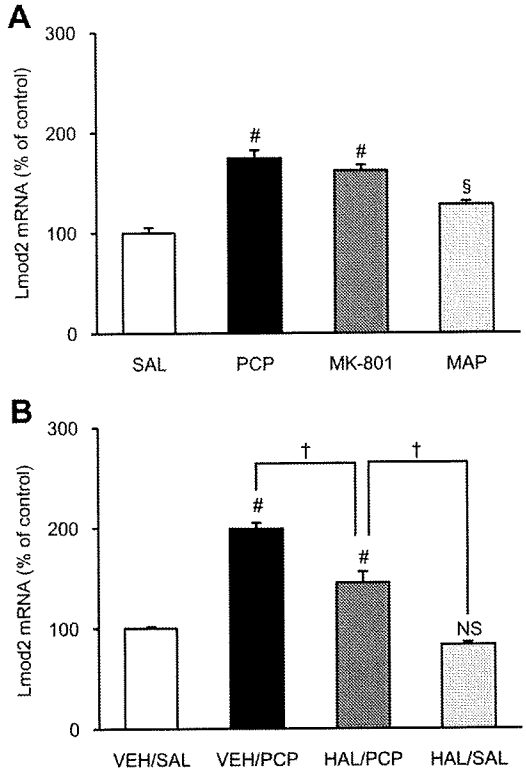


Fig. 5. Effects of acute administration of psychotomimetic and antipsychotic drugs on Lmod2 mRNA expression in the thalamus. (A) Effects of PCP, MK-801 and methamphetamine (MAP) on thalamic Lmod2 mRNA (# $p<0.01$, § $p<0.05$ vs. salinetreated controls). (B) Effects of pretreatment with haloperidol (Hal) on PCP-induced up-regulation of thalamic Lmod2 mRNA. (# $p<0.01$ vs. Veh/Sal controls ; # $p<0.01$ between Veh/PCP and Hal/PCP animals and between Hal/PCP and Hal/Sal animals). n.s., No significant difference. VEH: 0.15% tartaric acid. Relative expression levels of Lmod2 mRNA in the thalamus of the adult (PD 50) rat (Lmod2:GAPDH mRNA ratio) were assayed by the real-time RT–PCR method 60 min after acute PCP (7.5 mg/kg s.c.), MK-801 (0.5 mg/kg s.c.), and MAP (4.8 mg/kg s.c.) administration. HAL or VEH was injected 30 min before PCP treatment. Results are shown as the means with S.E.M of data (Lmod2:GAPDH mRNA ratio) obtained from six or eight rats per group and are expressed as a percentage of the values of the saline-treated controls.

Tmod1 および *Tmod2* の視床の mRNA や、心筋の *Lmod2* mRNA は PCP によって発現変化を受けないことから、*Lmod2* の PCP による発現誘導は非特異的反応ではないと考えられる。この応答は、乱用薬物による精神病状態や依存形成のモデルとされる動物の異常行動が出現するようになる臨界期以降に（研究目的の項および Fig 3 を参照）、視床の特定の核群に限局して見られるため、*Lmod2* は統合失調症類似の精神症状または依存形成に密接に関係する、神経回路（情報処理系）内の分子カスケードを構成する可能性がある。視床 *Lmod2* mRNA が、PCP ・MK801 などの NMDA 受容体遮断や MAP のような統合失調症様異常惹起薬によっても増加し、ketamine、MK801、あるいは MAP 投与後に脳の活動を反映する 2-deoxyglucose 代謝の異常が見出される脳部位と[4,5]、PCP により Lmod2 が発現誘導される部位が類似している点は、上記の仮説と矛盾しない。

視床 *Lmod2* の PCP に対する発達依存的応答のメカニズムは不明であるが、*Lmod2* の発現誘導が NMDA 受容体遮断薬やドーパミン作動薬で引き起こされることは、NMDA 受容体やドーパミン伝達系の生後発達との関連を示唆している。実際に、NMDA 受容体各サブユニットの分布パターン[27]は、PCP による行動異常の臨界期頃に成熟期に近づき、脳内ドーパミン系の機能も生後発達を遂げる[17]。

一方、視床前核群および外側核群にほぼ限局した *Lmod2* 遺伝子の特徴的な分布・応答パターンは、[^{3}H] muscimol を用いて検出した $GABA_A$ 受容体の分布と類似している[15]。興味深いことに、$GABA_A$ 受容体への結合能は、生後 10 日から 21 日にかけて増加していくという報告がある[28]。また、$GABA_A$ 受容体のサブユニットの構成が生後発達に伴って変化し、視床前核群および外側核群では α1 サブユニットが増加することが指摘されている[10]。したがって、PCP による *Lmod2* 遺伝子の発現誘導に、$GABA_A$ 受容体 α1 サブユニットが関与しており、NMDA 受容体遮断薬やドーパミン作動薬による脳の活動性異常に、Lmod2 遺伝子およびコード蛋白や、これらと GABA との相互作用

が関係している可能性がある。

Lmod2 は、アクチンフィラメントの先端に結合するアクチンキャッピング蛋白をコードするtropomodulin ファミリーの一つである[2,7]。本研究でもマウスで報告されて通り、トロポミオシン結合ドメイン、ロイシンリッチリピート、ポリプロリンモチーフを含んでいた。他の Tmod と比較すると、C 末端に、Src-homology 3 (SH3)と相互作用する可能性のあるポリプロリンモチーフを持つことから、シナプスの可塑性に関連する可能性がある[21]。さらに、PSORT プログラムでは核内蛋白である可能性が指摘され、核局在シグナルも有することより、核内で他の遺伝子発現を調節する蛋白として機能し、乱用薬物による依存形成・精神病状態などに伴う遺伝子発現の変化において重要な役割を果たしている可能性がある[6]。

以上のように、今年度の結果は*Lmod2*および本遺伝子または蛋白質を構成員とする分子カスケードや、*Lmod2*を発現する特定の視床核群が、乱用薬物が引き起こす精神障害に深く関与することを示唆している。この視点と一致して、ヒトを対象とした脳機能の画像解析研究においては、覚醒剤依存[22]、コカイン依存[26]、ニコチン依存[1]等に関係する脳部位のひとつとして視床が含まれることが指摘されている。実験動物でも、コカイン[20]、モルヒネ[3]を初め、乱用の対象となる薬物に対する報酬効果増強・嗜好性などの依存形成と密接に関係する脳部位の中に視床が含まれている。

今後は、ヒトゲノム解析により*Lmod2*と薬物依存症との関連をの検討するとともに、本遺伝子のノックアウト・ノックダウン・過剰発現などの操作を行ったマウスや細胞を用いてLmod2と脳神経機能や行動との関連性を調べ、*Lmod2*の薬物依存に対する診断法や予防・治療法の開発における意義を明らかにしたい。

E. 結論

乱用薬物による依存形成や統合失調症様の精神病状態あるいはそれらの動物モデルが、ヒトでは思春期、ラットやマウスでは生後 21ー25 日頃の臨界期以降に生ずる点に着目し、関連候補遺伝子として、ラット視床から、乱用の対象となる PCP 投与時の発現変化が臨界期以後にのみ認められる *Lmod2* を検出した。*Lmod2* は、成熟ラットにおいて他の依存性薬物の dizocipine や MAP によっても異常な発現が誘導され、基礎的発現および PCP による発現変化が視床の前核群を中心とした限局した部位に見られることから、薬物依存に関与する視床内の神経回路に含まれる分子カスケードを構成する可能性が示唆された。したがって、*Lmod2* は薬物依存に関与する視床の神経回路や細胞のキー分子あるいはマーカーとして病態解析に役立つとともに、ヒトゲノムにおける本遺伝子と薬物依存との関連解析や、*Lmod2* 遺伝子改変動物等を使った脳機能・行動との関係の検討により、薬物依存に対する新しい診断・治療・予防法の開発につながることが期待される。

[参考文献]

1. Brody AL (2006). Functional brain imaging of tobacco use and dependence. J Psychiatr Res. 40:404-418.
2. Conley CA, Fritz-Six KL, Almenar-Queralt A, Fowler VM (2001). Leiomodins: larger members of the tropomodulin (Tmod) gene family. Genomics 73, 127-139.
3. David V, Matifas A, Gavello-Baudy S, Decorte L, Kieffer BL, Cazala P (2008). Brain regional Fos expression elicited by the activation of mu- but not delta-opioid receptors of the ventral tegmental area: evidence for an implication of the ventral thalamus in opiate reward. Neuropsychopharmacology.

33:1746-1759.

4. Dragunow M, Faull RL (1990). MK-801 induces c-fos protein in thalamic and neocortical neurons of rat brain. Neuroscience Letters 111, 39-45.
5. Duncan GE, Miyamoto S, Leipzig JN, Lieberman JA (1999). Comparison of brain metabolic activity patterns induced by ketamine, MK-801 and amphetamine in rats: support for NMDA receptor involvement in responses to subanesthetic dose of ketamine. Brain Research 843, 171-183.
6. Dundr M, Ospina JK, Sung MH, John S, Upender M, Ried T, Hager GL, Matera AG (2007). Actin-dependent intranuclear repositioning of an active gene locus in vivo. Journal Cell Biology 179, 1095-1103.
7. Fischer RS, Fowler VM (2003). Tropomodulins: life at the slow end. Trends in Cell Biology 13: 593-601.
8. Ito T, Hiraoka S, Kuroda Y, Ishii S, Umino A, Kashiwa A, Yamamoto N, Kurumaji A, Nishikawa T (2007). Effects of schizophrenomimetics on the expression of the CCN1 (CYR 61) gene encoding a matricellular protein in the infant and adult neocortex of the mouse and rat. International Journal of Neuropsychopharmacology 10, 717-725.
9. Kajii Y, Muraoka S, Hiraoka S, Fujiyama K, Umino A, Nishikawa T (2003). A developmentally regulated and psychostimulant-inducible novel rat gene mrt1 encoding PDZ-PX proteins isolated in the neocortex. Molecular Psychiatry 8, 434-444.
10. Laurie DJ, Wisden W, Seeburg PH (1992). The distribution of thirteen GABAA receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. J Neurosci. 12:4151-4172.
11. Nagata K, Kiryu-Seo S, Kiyama H (2006). Localization and ontogeny of damage-induced neuronal endopeptidase mRNA-expressing neurons in the rat nervous system. Neuroscience. 141, 299-310.
12. 西川　徹 (2005) 統合失調症の分子薬理学的解析—ドーパミン受容体および NMDA 受容体作用薬を用いたアプローチ—　脳 21, 8: 9-15.
13. Nishikawa T, Umino A, Kashiwa A, Ooshima A, Nomura N, Takahashi K (1993). Stimulant-induced behavioral sensitization and cerebral neurotransmission. In: **Toru M** (Eds.), Neurotransmitters in neuronal plasticity and psychiatric disorders (pp. 53-62). Tokyo: Excerpta Medica.
14. Ohba N, Kiryu-Seo S, Maeda M, Muraoka M, Ishii M, Kiyama H (2004). Expression of damage-induced neuronal endopeptidase (DINE) mRNA in peri-infarct cortical and thalamic neurons following middle cerebral artery occlusion. Journal of Neurochemistry 91, 956-964.
15. Palacios JM, Wamsley JK, Kuhar MJ (1981). High affinity GABA receptors-autoradiographic localization. Brain Research 222, 285-307.
16. Paus T, Keshavan M, Giedd JN (2008). Why do many psychiatric disorders emerge during adolescence? Nat Rev Neurosci. 9:947-957.
17. Perez-Navarro E, Alberch J, Marsal J (1993). Postnatal development of functional dopamine, opioid and tachykinin receptors that regulate acetylcholine release from rat neostriatal slices. Effect of 6-hydroxydopamine lesion. International Journal of Developmental Neuroscience 11, 701-708.
18. Sato D, Umino A, Kaneda K, Takigawa M, Nishikawa T (1997). Developmental changes in distribution patterns of phencyclidine-induced c-Fos in rat forebrain. Neuroscience Letters 239, 21-24.
19. Schmidt HD, Anderson SM, Famous KR, Kumaresan V, Pierce RC (2005). Anatomy and

pharmacology of cocaine priming-induced reinstatement of drug seeking. Eur J Pharmacol. 526:65-76.

20. Segura I, Essmann CL, Weinges S, Acker-Palmer A (2007). Grb4 and GIT1 transduce ephrinB reverse signals modulating spine morphogenesis and synapse formation. Nature Neuroscience 10, 301-310.
21. Sekine Y, Ouchi Y, Sugihara G, Takei N, Yoshikawa E, Nakamura K, Iwata Y, Tsuchiya KJ, Suda S, Suzuki K, Kawai M, Takebayashi K, Yamamoto S, Matsuzaki H, Ueki T, Mori N, Gold MS, Cadet JL (2008). Methamphetamine causes microglial activation in the brains of human abusers. J Neurosci. 28:5756-5761.
22. Takebayashi H, Yamamoto N, Umino A, Nishikawa T. Developmentally-regulated and thalamus-selective induction of leiomodin 2 gene by a schizophrenomimetic, phencyclidine, in the rat. Int J Neuropsychopharmacol. 2009, in press.
23. Tanabe K, Kiryu-Seo S, Nakamura T, Mori N, Tsujino H, Ochi T, Kiyama H (1998). Alternative sBrain Research 53, 291-296.
24. Tanabe K, Nakagomi S, Kiryu-Seo S, Namikawa K, Imai Y, Ochi T, Tohyama M, Kiyama H (1999). Expressed-sequence-tag approach to identify differentially expressed genes following peripheral nerve axotomy. Brain Research Molecular Brain Research 64, 34-40.
25. Tomasi D, Goldstein RZ, Telang F, Maloney T, Alia-Klein N, Caparelli EC, Volkow ND (2007). Thalamo-cortical dysfunction in cocaine abusers: implications in attention and perception. Psychiatry Res. 155:189-201.
26. Umino A, Nishikawa T, Takahashi K (1995). Methamphetamine-induced nuclear c-Fos in rat brain regions. Neurochemistry International 26, 85-90.
27. Watanabe M, Inoue Y, Sakimura K, Mishina M (1992). Developmental changes in distribution of NMDA receptor channel subunit mRNAs. Neuroreport 3, 1138-1140.
28. Xia Y, Haddad GG (1992). Ontogeny and distribution of GABAA receptors in rat brainstem and rostral brain regions. Neuroscience. 49:973-989.

F. 研究発表

1．論文発表

[原著]

1. Kurumaji A, Ito T, Ishii S, Nishikawa T. Effects of FG7142 and immobilization stress on the gene expression in the neocortex of mice. Neurosci Res. 62: 155-159, 2008.
2. Sato J, Shimazu D, Yamamoto N, Nishikawa T. An association analysis of synapse- associated protein 97 (SAP97) gene in schizophrenia. J Neural Transm. 115:1355-1365, 2008.
3. Yamamoto N, Tsutsui, K, Yamamoto M, Arakaki H, Kurumaji A, Nishikawa T: Sliding doors (but not with beans or tofu). Lancet 372(9651): 1782, 2008.
4. Nagase Y, Uchiyam M, Kaneita Y, Li L, Kaji T, Takahashi S, Konno M, Mishima K, Nishikawa T, Ohida T. Coping strategies and their correlates with depression in the Japanese general population. Psychiatry Research, in press, 2008.
5. Takebayashi H, Yamamoto N, Umino A, Nishikawa T. Developmentally-regulated and thalamus-selective induction of *leiomodin 2* gene by a schizophrenomimetic, phencyclidine, in the rat. Int J Neuropsychopharmacol. in press.
6. Hattori E, Toyota T, Ishitsuka Y, Iwayama Y, Yamada K, Ujike H, Morita Y, Kodama M, Nakata K, Minabe Y, Nakamura K, Iwata Y, Takei

N, Mori N, Naitoh H, Yamanouchi Y, Iwata N, Ozaki N, Kato T, Nishikawa T, Kashiwa A, Suzuki M, Shioe K, Shinohara M, Hirano M, Nanko S, Akahane A, Ueno M, Kaneko N, Watanabe Y, Someya T, Hashimoto K, Iyo M, Itokawa M, Arai M, Nankai M, Inada T, Yoshida S, Kunugi H, Nakamura M, Iijima Y, Okazaki Y, Higuchi T, Yoshikawa T. Preliminary genome-wide association study of bipolar disorder in the Japanese population. Am J Med Genet Part B: Neuropsychiatric Genetics, in press.

[総説]

1. 西川 徹. 脳の内在性D-セリンの代謝・機能と精神神経疾患における意義「D-アミノ酸制御システムのニューロバイオロジー」. 生化学. 80: 267-276, 2008.
2. 西川 徹. 精神科薬物療法における将来展望—基礎的観点からー 諏訪・佐野メモリアルシンポジウムー抗精神病薬 50 年を振り返る 臨床精神薬理. 11:547-558, 2008.
3. 西川 徹. 統合失調症の分子薬理学的解析.「連載：統合失調症の脳科学最前線—第４回—」Schizophrenia Frontier. 9: 65-69, 2008.
4. 西川 徹. 動物モデルを用いた統合失調症の病態進行・難治化に関与する分子の検索. 脳と精神の医学. 19: 21-29, 2008.
5. 竹内 崇, 西川 徹. 新規抗精神病薬 quetiapine の薬理作用メカニズムについてーD2 以外の受容体に対する作用を中心にー. 臨床精神薬理 11：921-928, 2008
6. 西川 徹. 統合失調症の病態と治療ー脳科学の進歩により統合失調症はどこまで解明されたかー, 郡山精神医療 23: 27-70, 2008.

[著書]

1. 西川 徹．分子神経科学の視点から. 統合失調症 生物学的背景 『精神医学対話』（松下正明, 加藤 敏, 神庭重信 編集）. pp. 412-435, 弘文堂, 東京, 2008.
2. 竹内 崇, 西川 徹. 69 抑うつ（うつ病）. 病気・病態・重傷度からみた疾患別看護過程（井上智子, 佐藤千史 編集）. pp.1288-1300, 医学書院, 東京, 2008.

２．学会発表

特別講演，シンポジウム

1. 西川 徹. 統合失調症の分子病態. 福井大学医学部特別講義，福井, 2008年1月28日.
2. 西川 徹. 脳のD-セリンと統合失調症. 福井大学医学部セミナー，福井, 2008年1月28日.
3. 西川 徹. 抗精神病薬のD2受容体以外の作用と統合失調症状との関連. 第10回新潟統合失調症研究会，新潟，2008年2月5日.
4. 西川 徹. 新規抗精神病薬の薬理作用メカニズムについてーD2以外の受容体に対する作用を中心にー. 第3回日本統合失調症学会 ランチョンセミナー，東京，2008年3月15日.
5. 西川 徹. 統合失調症の新しい治療法開発の可能性と展望 第55回今堀フォーラム『統合失調症治療薬の創薬を考える』，東京，2008年5月7日.
6. 西川 徹. 統合失調症とグルタミン酸伝達系 第 104 回日本精神神経学会 教育講演. 東京, 2008 年 5 月 30 日.
7. Nishikawa T. NMDA receptor dysfunction in schizophrenia: the possible involvement of D-serine system, “NMDA Receptor and Molecular Pathology of Schizophrenia” Joint symposium with the Japanese Society of Neuropsychopharmacology, 2nd WFSBP Asia-Pacific Congress and 30th Annual Meeting of JSBP, Toyama, 9.12, 2008

8. Nishikawa T. NMDA receptor-D-serine system targeted treatment development for schizophrenia, "Drug development for schizophrenia" Translational Research in Neurochemistry, 51st Annual Meeting of JSNC, Toyama, 9.12, 2008
9. 西川　徹，海野麻未，小柄　渚，嶋津　奈，小方茂弘，白久博史，窪田哲朗，仙波禮治，山本直樹　D-セリン含有細胞に関する研究．ミニシンポジウム「脳内D-セリン」　第4回D-アミノ酸研究会，名古屋，2008年9月19日．
10. 西川　徹．統合失調症における情報処理障害の分子科学的理解は可能か　第15回　九州大学　こころと脳のセミナー，福岡，2008年10月4日．
11. 西川　徹．哺乳類で機能しているD-アミノ酸ーD-セリンと脳疾患ー、第15回血液の分子病態研究会、京都，2008年10月10日
12. Nishikawa T. Dysfunction of NMDA receptor-D-serine system and schizophrenia, Symposium "Disturbed glutamate neurotransmission in schizophrenia as a target for development of novel antipsychotics", 13th Pacific Rim College of Psychiatrists Scientific Meeting, Tokyo, 11.1, 2008
13. 西川　徹．セロトニン神経と精神行動，第14回「性と生殖」公開シンポジウム「セロトニンと人間」，東京，2008年11月8日．
14. 西川　徹．統合失調症の分子メカニズムへのアプローチー新しい治療法の開発を目指してー　大会記念講演　日本青年心理学会第16回大会，横浜，2008年11月9日．
15. 西川　徹．薬物依存の発達による変化から見た快・不快情動生成機構の障害　平成20年度生理学研究所研究会「感覚刺激・薬物による快・不快情動生成機構とその破綻」，岡崎，2008年11月27日．
16. 西川　徹．統合失調症の病因論，第18回地域精神保健学講座，東京，2008年11月28日．
17. 西川　徹．NMDA受容体-D-セリン系に作用する既認可薬の難治性精神神経症状治療への応用，第二回創薬シンポジウム-温故知新創薬研究への挑戦—，熊本，2008年12月18日．
18. 西川　徹．Critical period，シンポジウム「統合失調症は神経発達障害か、神経変性疾患か？」第4回統合失調症学会，大阪，2009年1月30日．

国際学会

1. Kurumaji A, Ito T, Ishii S, Nishikawa T., The postnatal development of stress-responsive molecular system in the hippocampus of mice. World Federation of Society of Biological Psychiatry 2nd WFSBP Asia-Pacific Congress and 30th Annual Meeting of JSBP. Toyama, September 11-13, 2008.
2. Shioiri A, Kurumaji A, Takeuchi T, Matsuda H, Arai H, Nishikawa T. White matter abnormalities as a risk factor of postoperative delirium revealed by DTI. World Federation of Society of Biological Psychiatry 2nd WFSBP Asia-Pacific Congress and 30th Annual Meeting of JSBP. Toyama, September 11-13, 2008.
3. Oshima K, Okimura T, Yukizane T, Yasumi K, Iwawaki A, Nishikawa T, Hanamura S. Japanese version of bonn scale for the assessment of basic symptoms, its reliability and its validity. The 14th World Congress of Psychiatry. Prague, (Czech Republic) September 23, 2008.
4. Yamamoto N, Sato J, Shimazu D, Nishikawa T. An association study on synapse-associated protein 97 (SAP97) gene in schizophrenia. XVIth World Congress on Psychiatric Genetics.

Osaka Japan, October 14, 2008.

国内学会

1. 吉池卓也, 竹内　崇, 佐々木健至, 石川洋世, 熱田英範, 正木秀和, 行実知昭, 大島一成, 柏淳, 山本直樹, 車地暁生, 西川　徹. せん妄に対する aripiprazole の使用経験. 第104回日本精神神経学会総会,東京, 2008年5月29日.
2. 熱田英範、車地暁生、大島一成、西川　徹. 併用薬 quetiapine の投与中止を契機として発症したセロトニン症候群の一例. 第 104 回日本精神神経学会総会・東京,2008 年 5 月 30 日.
3. 西川 徹, 海野麻未, 岩間久行, 嶋津 奈, 小方茂弘, 山本直樹, ラット内側前頭葉皮質における組織中・細胞外液中 D-セリン濃度に与える影響, 第31回 日本神経科学大会, 東京, 2008年7月11日.
4. 山本直樹、嶋津　奈、兼松宗太郎、谷口　豪、海野麻未、西川　徹. ラット脳内在性 D-serine 代謝関連遺伝子の発現、局在と発達依存性変化. 第 51 回日本神経化学会大会, 富山, 2008 年 9 月 13 日.
5. 西川　徹, 小方茂弘, 海野麻未, 白久博史, 山本直樹. 内側前頭葉皮質における Asc-1 阻害薬の細胞外 D-セリン濃度増加作用, 第38回日本神経精神薬理学会・第18回日本臨床精神神経薬理学会合同年会, 東京, 2008年11月1日.

G. 知的財産権の出願・登録状況（予定も含む）

1．特許取得

なし

2．実用新案登録

なし

3．その他

本研究者による D-セリンの発見と代謝・統合失調症状との関連等に関する研究について、読売新聞に紹介された：読売新聞　2008年12月7日（日曜日）14版　21ページ　「D-アミノ酸—陰の存在　実は「主役」—」

J Neural Transm (2008) 115:1355–1365
DOI 10.1007/s00702-008-0085-9

BASIC NEUROSCIENCES, GENETICS AND IMMUNOLOGY - ORIGINAL ARTICLE

An association analysis of synapse-associated protein 97 (*SAP97*) gene in schizophrenia

Junko Sato · Dai Shimazu · Naoki Yamamoto · Toru Nishikawa

Received: 29 January 2008 / Accepted: 15 June 2008 / Published online: 30 July 2008

Abstract *SAP97* gene encodes the synaptic scaffolding PDZ proteins that interact with the L-alpha-amino-3-hydroxyl-5-methylisoxazole-4-propionate (AMPA), kainate and *N*-methyl-D-aspartate (NMDA) type glutamate receptors. Because the disturbed glutamate neurotransmission has been implicated in the pathophysiology of schizophrenia, we investigated association between the *SAP97* gene and schizophrenia. We genotyped 23 SNPs capturing the known common haplotype variations of the gene in a sample comprising 229 schizophrenic patients and 214 matched controls. In a single marker analysis, ten SNPs displayed nominally significant ($P < 0.05$) association with schizophrenia, although the P values of these SNPs were non-significant after the Bonferroni correction. We also compared haplotype estimates based on case—control genotypes and observed significant association of eight-two- and three- SNP haplotypes with schizophrenia following permutation-based correction. Further examination of the above series of SNPs or haplotypes in each gender revealed significant associations between some of these SNPs or haplotypes and the disorder only in males. The present findings suggest that the *SAP97* gene may be a susceptibility factor in male schizophrenics and that the modification of the glutamate receptors-SAP97 signaling pathway could be involved in the disease pathophysiology.

J. Sato · D. Shimazu · N. Yamamoto · T. Nishikawa (✉)
Section of Psychiatry and Behavioral Sciences,
Tokyo Medical and Dental University Graduate School,
1-5-45, Bunkyo-ku, Tokyo 113-8519, Japan
e-mail: tnis.psyc@tmd.ac.jp

Keywords Association analysis · Gender · Japanese · Schizophrenia · SNPs · Synapse-associated protein 97 (*SAP97*) gene

Introduction

Schizophrenia is a serious and cryptogenic psychiatric disorder that displays positive and negative symptoms and cognitive disturbances indicating impairments of the specific set of the mental functions (Ross et al. 2006). Pharmacological and biochemical studies have suggested that dysregulation of brain glutamatergic transmission may be involved in the pathophysiology of schizophrenia. Thus, the antagonists for the *N*-methyl-D-aspartate (NMDA) type glutamate receptor such as phencyclidine and ketamine cause a full range of the above symptomtomatologies indistinguishable from those of schizophrenia (Javitt and Zukin 1991; Nishikawa et al. 1991). Moreover, in the cortical and subcortical regions of the postmortem brains from schizophrenic patients, there are accumulating data showing the alterations in the mRNA expressions and/or the amount of proteins of the ionotropic and metabotropic glutamate receptors, glutamate transporters, and the concentrations of glutamate and other amino acids related to the glutamate metabolism and functions (Nishikawa et al. 1983; Harrison et al. 2003; Meador-Woodruff and Healy 2000). In accordance with these results, the schizophrenic symptoms have been reported to be ameliorated by the facilitation of the NMDA receptor-mediated transmission by the direct and indirect agonists for the glycine modulatory site of the NMDA receptor including glycine, D-serine and glycine transporter inhibitor (Javitt 2004), and the selective activation of the mGlu2/3 receptors by LY404039 (Patil et al. 2007).

However, the origin of the disturbed glutamate signaling in schizophrenia is still unclear. One of the probable mechanisms could be a defect in the intracellular integrative molecular cascade for the diverse machineries of the glutamate neurotransmission. From this view point, it appears to be relevant to note the possible pathological role of synapse associated protein 97 (SAP97), in schizophrenia because the synaptic scaffold protein is implicated in the precise targeting and clustering of L-alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) (Nakagawa et al. 2004; Schlüter et al. 2006), kainate (Mehta et al. 2001) and *N*-methyl-D-aspartate (NMDA) (Mauceri et al. 2007; Wang et al. 2005) type ionotropic glutamate receptors. In rat hippocampal slice cultures, overexpression of SAP97 drove GluR1 to synapses, potentisted AMPA receptor excitatory postsynaptic currents (EPSCs) whereas SAP97 knockdown diminished surface expression of both GluR1 and GluR2 and inhibited both AMPA and NMDA EPSCs (Nakagawa et al. 2004). The altered expression of the SAP97 proteins and/or mRNAs (Toyooka et al. 2002) and AMPA, kainate and NMDA receptors (Gao et al. 2000; Ibrahim et al. 2000; Meador-Woodruff et al. 2001; Nishikawa et al. 1983) have indeed been shown in the postmortem brain tissues from schizophrenic patients. We therefore performed genetic analysis of the human *SAP97* gene mapped on the chromosome 3q29 in schizophrenic patients and healthy volunteers.

Materials and methods

Subjects

A total of 229 unrelated Japanese schizophrenics [137 males, 44.3 ± 12.2 years (a mean with SD), and 92 females, 44.5 ± 12.7 years] were included in this study. All patients were diagnosed by well-trained psychiatrists, according to the Diagnostic and Statistical Manual of Mental Disorders,fourth edition (DSM-IV Criteria). A Japanese control group consisted of 214 unrelated healthy volunteers (105 males, with age of 42.2 ± 9.2 years, and 109 females, 45.5 ± 14.9 years) who were medical staff members and company employees documented to be free of psychosis. All subjects resided in central Japan.

The present study was approved by the ethics committee of Tokyo Medical and Dental University. All participants and healthy volunteers gave informed and written consent to participate in the study.

Selection and genotyping of single nucleotide polymorphisms (SNPs)

Genomic DNAs were extracted from the peripheral whole blood cells of each subject by the phenol extraction method or by the DNA Extraction Kit (Stratagene, La Jolla, CA, USA). The data of genomic structure and the location of each SNP for human *SAP97* were obtained from the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nml.nih.gov/). We also utilized the International HapMap Project database (http://www.hapmap.org/). The schematic diagram of the *SAP97* and the location of the SNPs examined are shown in Fig. 1. To predict the possible molecular consequences of SNPs, we analyzed the consensus sequences for promoter usage and alternative splicing by using bioinformatic tools, Promoter 2.0 Prediction Server (http://www.cbs.dtu.dk/services/Promoter/) and NetGen2 server (http://www.cbs.dtu.dk/services/NetGene2/), respectively.

At the first stage of the SNP analysis, we chose 10 SNPs (designated as SNP I-1 to SNP I-10) from the *SAP97* genomic interval and the 5′ upstream regions based on the following criteria: (1) minor allele frequencies ≥10%, (2) inclusion of missense polymorphism (SNP I-7), (3) the allele frequencies in Japanese were reported, (4) successful TaqMan probe design and (5) an even as possible spacing between the SNPs. Based on the results obtained from the initial SNPs analysis, in the second, we genotyped nine more polymorphisms (designated as SNP II-1 to SNP II-9). Then, in the third, we further assayed four SNPs (designated as SNP III-1 to SNP III-4). Thus, we have examined total 23 SNPs of the *SAP97* gene (Table 1; Fig. 1).

For the individual SNP analysis, we used the TaqMan SNP Genotyping Assay method (Applied Biosystems, Foster City, CA, USA). Allele-specific probes were labeled with fluorescent dyes VIC and FAM, respectively. PCR reaction was carried out in a total reaction volume of 5 μl with the following amplification protocol: denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 92°C for 15 s, and annealing and extension at 60°C for 1 min. The genotype of each sample was attributed automatically by measuring the allelic specific fluorescence on the ABI 7900HT Sequence Detection System using SDS v2.1 software (Applied Biosystems).

Statistical analysis

Statistical analysis was performed with the SNPAlyse statistical software package (Dynacom, Yokohama, Japan, http://www.dynacom.co.jp/). Deviation from predicted Hardy-Weinberg equilibrium (HWE) was examined by chi-square test. To compare allele and genotype frequencies between schizophrenics and controls, chi-square test, and Fisher's exact test were performed. To measure linkage disequilibrium (LD) between SNPs, D' (normalized D) and r^2 (squared correlation coefficient) values were calculated from the haplotype frequencies using the expectation–

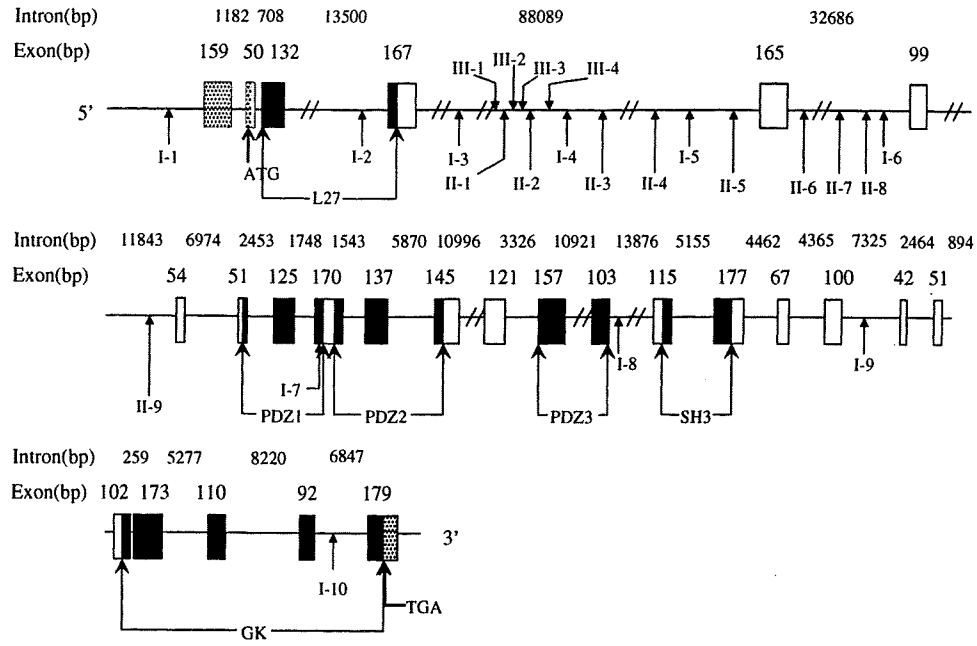


Fig 1 Genomic structures and positions of the SNPs analyzed in the human *SAP97* gene. Exons are denoted by *boxes*, with untranslated regions in *gray*, translated regions in *white* and domain coding regions in *black*. The sizes of exons and introns are shown. *SNP* single nucleotide polymorphism

Table 1 SNPs in *SAP97* genotyped in the present study

SNP name	rs number	Location	Amino acid change
SNP I-1(-2852G>A)	rs338217	5′ upstream region	(−)
SNP I-2(IVS3+6477T>C)	rs382579	intron3	(−)
SNP I-3(IVS4+23954T>C)	rs9843659	intron4	(−)
SNP I-4(IVS4+55103G>T)	rs2122824	intron4	(−)
SNP I-5(IVS4+82369G>T)	rs7650753	intron4	(−)
SNP I-6(IVS5+25682C>G)	rs6583200	intron5	(−)
SNP I-7(833C>T:Q278R)	rs1949471	exon10	Gln → Arg
SNP I-8(IVS15+1842C>T)	rs7616588	intron15	(−)
SNP I-9(IVS19+2398G>A)	rs11710576	intron19	(−)
SNP I-10(IVS25+4092A>G)	rs7638423	intron25	(−)
SNP II-1(IVS4+47221C>A)	rs6805920	intron4	(−)
SNP II-2(IVS4+51473A>G)	rs436564	intron4	(−)
SNP II-3(IVS4+60069T>C)	rs2044862	intron4	(−)
SNP II-4(IVS4+79751G>T)	rs9839886	intron4	(−)
SNP II-5(IVS4+87770G>A)	rs9868546	intron4	(−)
SNP II-6(IVS5+9066A>G)	rs7628045	intron5	(−)
SNP II-7(IVS5+15363A>G)	rs9857189	intron5	(−)
SNP II-8(IVS5+22687A>C)	rs4916461	intron5	(−)
SNP II-9(IVS6+6922A>G)	rs13323530	intron6	(−)
SNP III-1(IVS4+46599C>T)	rs338187	intron4	(−)
SNP III-2(IVS4+48364C>T)	rs10489880	intron4	(−)
SNP III-3(IVS4+48649C>T)	rs4635680	intron4	(−)
SNP III-4(IVS4+52518A>G)	rs447337	intron4	(−)

The rs numbers from dbSNP database and location of each SNP are shown

maximization algorithm. The haplotype block structures were evaluated with Haploview software version 3.32 (Barrett et al. 2005). Haplotype blocks were generated by the default algorithm taken from (Gabriel et al. 2002). Haplotype distributions were evaluated by the permutation test on the basis of 10,000 replications to obtain the empirical significance. Statistical significance was defined at $P < 0.05$.

Results

Association, haplotype and linkage disequilibrium analyses of SNP I

Genotype distribution of the SNPs showed no significant deviations from the HWE in all of the 23 sites in the controls (Table 2). In the schizophrenics, significant deviations from the HWE were detected in the genotypic distributions for SNP I-1 and I-10. We observed genotypic association between schizophrenia and SNP I-10 in co-dominant model. Moreover SNP I-3, I-4, I-5, I-6, I-8 and I-10 displayed nominally significant associations with schizophrenia in recessive model. However, no SNPs showed association with the disease in dominant model (Table 2).

SNP I-2, SNP I-3, SNP I-4, and SNP I-8 showed allelic associations with schizophrenia (Table 2), although differences in the genotype and allele frequencies of these SNPs did not reach the statistically significant levels following the Bonferroni's multiple comparison test. We then considered that these SNPs show nominally significant associations. The minor allele frequencies for the two variants, SNP I-7 and SNP I-9, were found to be less than 13% in both groups. Therefore, we examined LD structures among the eight residual SNPs (minor allele frequencies >13%) (Table 3A). We found that the SNPs I-4, I-5, I-6, and I-8 were in a strong LD with each other ($D' > 0.98$ and $r^2 > 0.86$; D': normalized D; r^2: squared correlation coefficient). The all regions of SNP I-1 to I-10 were shown to be in a relatively strong LD ($D' > 0.79$ and $r^2 > 0.29$).

We also carried out the haplotype analysis using the entire10 SNPs and the 4 SNPs (SNP I-4, I-5, I-6, I-8), which were in a robust LD. However, no significant differences in the frequency of the two SNP sets were observed between schizophrenics and controls (data not shown). In the subsequent haplotype analysis performed by implementing two-locus and three-locus sliding windows spanning SNP I-1 to I-10, and SNP I-4-I-5 showed significant association with schizophrenia (Table 4A).

Association, haplotype and linkage disequilibrium analyses of SNP II and III

Because (1) the above results indicated that the allele frequency of SNP I-4 and the haplotype frequency of SNP I-4-I-5 were significantly different between schizophrenia and controls, (2) SNPs I-4, I-5 and I-6 were considered to be in the same LD block, and (3) these SNPs regions of the human *SAP97* gene coincided with the sites where the expression of rat *SAP97* mRNAs was enhanced in a splicing variant-specific manner when the animal was administered with phencyclidine (our unpublished observation), we next picked up a second set of 9 SNPs (designated as II-1 to II-9) around these 3 SNPs (SNPs I-4 ~ I-6) from the HapMap Project data base (Fig. 1; Table 1). Genotype distribution of all of the 9 SNPs, except for SNP II-1 in schizophrenics, showed no deviations from the HWE (Table 2). Genotype distribution of SNP II-1 and SNP II-8 were significantly different between schizophrenics and controls (Table 2). SNP II-1, II-3, and II-8 indicated the nominal allelic association with schizophrenia (Table 2).

In the two- and three-SNP-based haplotype analyses, SNP II-5-II-6, SNP II-6-II-7 and SNP II-7-II-8, and SNP II-5-II-6-II-7 and SNP II-7-II-8-II-9 displayed significant global association with the disease (Table 4B). Then, we examined LD structures between the second set of 9 variants, and found that SNP II-2 to II-7 were in a strong LD ($D' > 0.98$ and $r^2 > 0.87$) (Table 3B).

As the SNP II-1 manifested a much greater statistical significance in the association with schizophrenia than the other variants and the SNPs I-4 and II-3 showed allelic association, we genotyped a third set of 4 SNPs (which were designated as SNPs III-1 to III-4) mapped around the SNPs II-1 and I-4 based on the HapMap database (Fig. 1; Table 1). Genotypic distribution of SNP III-2 ($P = 0.026$) was deviated from the HWE in schizophrenics, but not in controls. The other SNPs showed no deviation from the HWE in each group. By the analysis according to co-dominant model, we observed the nominal genotypic association between the SNP III-2 and schizophrenia, and the SNP III-1 and III-2 exhibited the nominal allelic association with the disease (Table 2). The additional genotype analysis revealed that SNP II-1, II-2, II-3, II-4, II-8, III-1, III-2, III-3, and III-4 exhibited nominally significant associations with schizophrenia in recessive model whereas no SNPs in the SNP II and III groups represented association with the disease in dominant model (Table 2).

The calculated LD coefficients, D' and r^2, are shown in Table 3B. Our evaluation of the haplotype block structures for all of the 23 SNPs in the present Japanese samples by using the Haploview version 3.32 software indicated that the SAP97 gene consists of two or one haploblock in schizophrenics and controls (Fig. 2), respectively.

Effects of gender on association and haplotype analyses in schizophrenics and controls

Because distribution of gender was significantly different between the schizophrenic and control groups ($P = 0.023$; χ^2 test), we reexamined the association of SAP97 gene polymorphisms with the risk of schizophrenia in each gender separately. Genotype distributions of SNP I-3, I-10, II-1, II-8 and III-2 in recessive model and allele distributions of SNP I-3, I-10, II-1, II-8 and III-2 significantly differ

Table 2 Genotyping and allele distribution of SNPs on the *SAP97* gene in Japanese controls and schizophrenics

Genotyping(%)							*P* values									Allele(%)		Total	Male	Female
							Dominant model			Recessive model			Co-dominant model							
							Total	Male	Female	Total	Male	Female	Total	Male	Female					
SNPI-1	Group	HWE	*n*	GG	AG	AA										G	A			
rs338217	Control	0.879	214	92(43.0)	98(45.8)	24(11.2)										282(65.9)	146(34.1)			
	Schizophrenia	**0.029**	229	78(34.1)	125(54.6)	26(11.3)	1	0.5564	0.4962	0.0632	0.1439	0.2486	0.1362	0.3302	0.2679	281(61.4)	177(38.6)	0.1633	0.1853	0.6027
SNPI-2	Group		*n*	TT	CT	CC										T	C			
rs382579	Control	1	214	138(64.5)	68(31.8)	8(3.7)										344(80.4)	84(19.6)			
	Schizophrenia	0.705	229	126(55.0)	90(39.3)	13(5.7)	0.3777	0.5928	0.7048	0.0525	0.2421	0.1443	0.1165	0.4928	0.3003	342(74.7)	116(25.3)	**0.0447***	0.2429	0.1697
SNPI-3	Group		*n*	TT	CT	CC										T	C			
rs9843659	Control	0.19	214	84(39.2)	93(43.5)	37(17.3)										261(61.0)	167(39.0)			
	Schizophrenia	0.778	229	69(30.1)	111(48.5)	49(21.4)	0.2823	0.0788	0.7124	**0.0461***	**0.0422***	0.4598	0.1217	0.0643	0.524	249(54.4)	209(45.6)	**0.0488***	**0.016***	0.8397
SNPI-4	Group		*n*	GG	GT	TT										G	T			
rs2122824	Control	0.558	214	135(63.1)	68(31.8)	11(5.1)										338(79.0)	90(21.0)			
	Schizophrenia	0.31	229	119(52.0)	97(42.4)	13(5.7)	0.8367	0.5928	0.7568	**0.021***	0.091	0.1924	0.054	0.2155	0.2838	335(73.1)	123(26.9)	**0.0491***	0.1152	0.3355
SNPI-5	Group		*n*	GG	GT	TT										G	T			
rs7650753	Control	0.557	214	135(63.1)	68(31.8)	11(5.1)										338(79.0)	90(21.0)			
	Schizophrenia	0.338	229	120(52.4)	96(41.9)	13(5.7)	0.8367	0.5928	0.7568	**0.0269***	0.1181	0.1924	0.0706	0.2723	0.2838	336(73.4)	122(26.6)	0.0585	0.1403	0.3355
SNPI-6	Group		*n*	CC	CG	GG										C	G			
rs6583200	Control	1	214	140(65.4)	66(30.8)	8(3.7)										346(80.8)	82(19.2)			
	Schizophrenia	0.298	229	128(55.9)	91(39.7)	10(4.4)	0.8127	0.7607	1	**0.0418***	0.1526	0.2983	0.1234	0.3485	0.4437	347(75.8)	111(24.2)	0.0733	0.1976	0.3741
SNPI-7	Group		*n*	CC	CT	TT										C	T			
rs1949471	Control	0.475	214	169(79.0)	44(20.5)	1(0.5)										382(89.3)	46(10.7)			
	Schizophrenia	0.561	229	175(76.4)	52(22.7)	2(0.9)	1	1	1	0.5688	0.2065	0.8683	0.7331	0.2949	0.87	402(87.8)	56(12.2)	0.5281	0.1862	0.8797
SNPI-8	Group		*n*	CC	CT	TT										C	T			
rs7616588	Control	0.824	214	139(64.9)	68(31.8)	7(3.3)										346(80.8)	82(19.2)			
	Schizophrenia	0.587	229	127(55.5)	90(39.3)	12(5.2)	0.3541	0.4016	1	**0.0423***	0.1916	0.2983	0.1119	0.3259	0.5137	344(75.1)	114(24.9)	**0.043***	0.1386	0.3076
SNPI-9	Group		*n*	GG	AG	AA										G	A			
rs11710576	Control	0.478	214	168(78.5)	45(21.0)	1(0.5)										381(89.0)	47(11.0)			
	Schizophrenia	0.391	229	172(75.1)	55(24.0)	2(0.9)	1	1	1	0.4317	0.1638	0.8683	0.6211	0.186	0.87	399(87.1)	59(12.9)	0.4083	0.1519	0.8797
SNPI-10	Group		*n*	AA	AG	GG										A	G			
rs7638423	Control	0.888	214	101(47.2)	91(42.5)	22(10.3)										293(68.5)	135(31.5)			
	Schizophrenia	**0.034**	229	81(35.4)	123(53.7)	25(10.9)	0.878	0.3071	0.3517	**0.0122***	**0.024***	0.3179	**0.036***	0.0611	0.2194	285(62.2)	173(37.8)	0.0567	**0.0285***	0.7507
SNPII-1	Group		*n*	CC	AC	AA										C	A			
rs6805920	Control	0.665	214	101(47.2)	90(42.1)	23(10.7)										292(68.2)	136(31.8)			
	Schizophrenia	**0.016**	229	76(33.2)	127(55.5)	26(11.3)	0.8803	0.4235	0.4962	**0.00354****	**0.00782****	0.1955	**0.00827****	**0.0245***	0.18	279(60.9)	179(39.1)	**0.0247***	**0.022***	0.5279
SNPII-2	Group		*n*	AA	AG	GG										A	G			
rs436564	Control	0.559	214	135(63.1)	68(31.8)	11(5.1)										338(79.0)	90(21.0)			
	Schizophrenia	0.31	229	120(52.4)	96(41.9)	13(5.7)	0.8367	0.5928	0.7568	**0.0269***	0.1181	0.1924	0.0706	0.2723	0.2838	336(73.4)	122(26.6)	0.0585	0.1403	0.3355

Table 2 continued

Genotyping(%)							*P* values												
							Dominant model			Recessive model			Co-dominant model			Allele(%)		Total	Male	Female
							Total	Male	Female	Total	Male	Female	Total	Male	Female					
SNPII-3	Group		*n*	TT	CT	CC										T	C			
rs2044862	Control	1	214	138(64.5)	68(31.8)	8(3.7)										344(80.4)	84(19.6)			
	Schizophrenia	0.62	229	125(54.6)	91(39.7)	13(5.7)	0.3777	0.5928	0.7048	**0.042***	0.2394	0.1443	0.1014	0.4354	0.3003	341(74.5)	117(25.5)	**0.0371***	0.2045	0.1697
SNPII-4	Group		*n*	GG	GT	TT										G	T			
rs9839886	Control	0.548	214	134(62.6)	69(32.3)	11(5.1)										337(78.7)	91(21.3)			
	Schizophrenia	0.282	229	120(52.4)	96(41.9)	13(5.7)	0.8367	0.5928	0.7568	**0.0345***	0.1181	0.2475	0.0898	0.2723	0.3502	336(73.4)	122(26.6)	0.0703	0.1403	0.4011
SNPII-5	Group		*n*	GG	AG	AA										G	A			
rs9868546	Control	0.815	214	139(64.9)	68(31.8)	7(3.3)										346(80.8)	82(19.2)			
	Schizophrenia	0.619	229	128(55.9)	89(38.9)	12(5.2)	0.3541	0.4016	1	0.0529	0.2394	0.2983	0.1284	0.3763	0.5137	345(75.3)	113(24.7)	0.0516	0.1674	0.3076
SNPII-6	Group		*n*	AA	AG	GG										A	G			
rs7628045	Control	0.824	214	138(64.5)	69(32.2)	7(3.3)										345(80.6)	83(19.4)			
	Schizophrenia	0.574	229	128(55.9)	89(38.9)	12(5.2)	0.3541	0.4016	1	0.0663	0.2394	0.3049	0.1615	0.3763	0.6035	345(75.3)	113(24.7)	0.0627	0.1674	0.3741
SNPII-7	Group		*n*	AA	AG	GG										A	G			
rs9857189	Control	0.672	214	139(65.0)	66(30.8)	9(4.2)										344(80.4)	84(19.6)			
	Schizophrenia	0.628	229	128(55.9)	89(38.9)	12(5.2)	0.6599	0.5928	1	0.0529	0.2394	0.2983	0.1484	0.4354	0.4437	345(75.3)	113(24.7)	0.0756	0.2045	0.3741
SNPII-8	Group		*n*	AA	AC	CC										A	C			
rs4916461	Control	1	214	105(49.1)	90(42.0)	19(8.9)										300(70.1)	128(29.9)			
	Schizophrenia	0.064	229	83(36.2)	122(53.3)	24(10.5)	0.6314	0.4053	0.8016	**0.00711****	**0.0169***	0.2586	**0.0235***	**0.0468***	0.4154	288(62.9)	170(37.1)	**0.0273***	**0.0278***	0.5177
SNPII-9	Group		*n*	AA	AG	GG										A	G			
rs13323530	Control	0.828	214	139(64.9)	68(31.8)	7(3.3)										346(80.8)	82(19.2)			
	Schizophrenia	0.577	229	128(55.9)	89(38.9)	12(5.2)	0.3541	0.4016	1	0.0529	0.2394	0.2983	0.1284	0.3763	0.5137	345(75.3)	113(24.7)	0.0516	0.1674	0.3076
SNPIII-1	Group		*n*	CC	CT	TT										C	T			
rs338187(-)	Control	1	214	138(64.5)	68(31.8)	8(3.7)										344(80.4)	84(19.6)			
	Schizophrenia	0.494	229	124(54.1)	92(40.2)	13(5.7)	0.3777	0.5928	0.7048	**0.0333***	0.2394	0.1087	0.082	0.4354	0.2425	340(74.2)	118(25.8)	**0.0307***	0.2045	0.1357
SNPIII-2	Group		*n*	CC	CT	TT										C	T			
rs10489880(-)	Control	0.634	214	101(47.2)	90(42.1)	23(10.7)										292(68.2)	136(31.8)			
	Schizophrenia	**0.026**	229	77(33.6)	126(55.0)	26(11.4)	0.8803	0.4235	0.4962	**0.00372****	**0.0116**	0.1955	**0.0114***	**0.0324***	0.18	280(61.1)	178(38.9)	**0.0294***	**0.028***	0.5279
SNPIII-3	Group		*n*	CC	CT	TT										C	T			
rs4635680	Control	0.509	214	135(63.1)	68(31.8)	11(5.1)										338(79.0)	90(21.0)			
	Schizophrenia	0.307	229	120(52.4)	96(41.9)	13(5.7)	0.8367	0.5928	0.7568	**0.0269***	0.1181	0.1924	0.0706	0.2723	0.2838	336(73.4)	122(26.6)	0.0585	0.1403	0.3355
SNPIII-4	Group		*n*	AA	AG	GG										A	G			
rs447337	Control	0.533	214	135(63.1)	68(31.8)	11(5.1)										338(79.0)	90(21.0)			
	Schizophrenia	0.402	229	121(52.8)	95(41.5)	13(5.7)	0.8367	0.5928	0.7568	**0.0342***	0.1181	0.2459	0.087	0.2723	0.3584	337(73.6)	121(26.4)	0.0694	0.1403	0.3978

P values were evaluated by Fisher's exact test. * $P < 0.05$, ** $P < 0.01$. *SAP97* synapse-associated protein-97, *HWE* Hardy-Weinberg equilibrium

Table 3 Pairwise linkage disequilibrium between polymorphisms of *SAP97*

SNP	I-1	I-2	I-3	I-4	I-5	I-6	I-8	I-10
A. r^2 and D' in controls (schizophrenia) for the first set of 8 SNPs; SNP I-1 ~ I-10 except I-7, I-9								
I-1		0.452 (0.486)	0.506 (0.571)	0.514 (0.513)	0.514 (0.506)	0.438 (0.472)	0.458 (0.439)	0.870 (0.837)
I-2	0.979 (0.950)		0.346 (0.404)	0.889 (0.924)	0.889 (0.934)	0.912 (0.897)	0.941 (0.910)	0.469 (0.454)
I-3	0.791 (0.872)	0.952 (1.000)		0.298 (0.373)	0.298 (0.369)	0.335 (0.365)	0.353 (0.363)	0.575 (0.608)
I-4	1.000 (0.938)	0.985 (1.000)	0.847 (0.924)		1.000 (0.989)	0.862 (0.871)	0.890 (0.880)	0.537 (0.499)
I-5	1.000 (0.937)	0.985 (1.000)	0.847 (0.923)	1.000 (1.000)		0.862 (0.881)	0.890 (0.891)	0.537 (0.493)
I-6	0.978 (0.965)	0.969 (0.976)	0.951 (0.978)	0.984 (1.000)	0.984 (1.000)		0.970 (0.965)	0.494 (0.509)
I-8	1.000 (0.914)	0.985 (0.964)	0.976 (0.958)	1.000 (0.988)	1.000 (0.988)	0.985 (1.000)		0.514 (0.528)
I-10	0.988 (0.932)	0.941 (0.901)	0.893 (0.917)	0.964 (0.909)	0.964 (0.908)	0.980 (0.983)	1.000 (0.984)	

SNP	I-1	I-2	I-3	III-1	II-1	III-2	III-3	II-2	III-4	I-4	II-3	II-4	I-5	II-5	II-6	II-7	II-8	I-6	II-9	I-7	I-8	I-9	I-10
B. r^2 and D' for all of the 23 SNPs calculated using the genotype data from all subjects																							
I-1		0.471	0.540	0.469	0.885	0.890	0.511	0.511	0.508	0.515	0.474	0.515	0.511	0.455	0.449	0.443	0.807	0.458	0.455	0.217	0.449	0.227	0.852
I-2	0.963		0.379	0.974	0.519	0.522	0.915	0.915	0.908	0.909	0.981	0.909	0.915	0.930	0.923	0.917	0.499	0.904	0.930	0.038	0.923	0.020	0.463
I-3	0.833	0.978		0.384	0.627	0.632	0.337	0.337	0.334	0.339	0.390	0.339	0.337	0.366	0.360	0.354	0.661	0.353	0.366	0.177	0.360	0.184	.0.594
III-1	0.954	0.994	0.979		0.526	0.529	0.927	0.927	0.920	0.921	0.994	0.921	0.927	0.943	0.936	0.930	0.506	0.918	0.943	0.038	0.936	0.021	0.470
II-1	0.959	0.991	0.915	0.991		0.995	0.570	0.570	0.567	0.574	0.532	0.574	0.570	0.512	0.505	0.500	0.909	0.495	0.512	0.236	0.505	0.246	0.946
III-2	0.964	0.991	0.921	0.991	1		0.573	0.573	0.569	0.577	0.535	0.577	0.573	0.514	0.508	0.502	0.913	0.498	0.514	0.237	0.508	0.248	0.951
III-3	0.966	0.993	0.888	0.993	1	1		1	0.994	0.994	0.933	0.994	1	0.897	0.891	0.884	0.470	0.873	0.897	0.041	0.891	0.023	0.515
II-2	0.966	0.993	0.888	0.993	1	1	1		0.994	0.994	0.933	0.994	1	0.897	0.891	0.884	0.470	0.873	0.897	0.041	0.891	0.023	0.515
III-4	0.965	0.987	0.887	0.987	1	1	1	1		0.988	0.926	0.988	0.994	0.890	0.884	0.878	0.466	0.866	0.890	0.041	0.884	0.023	0.511
I-4	0.966	0.993	0.889	0.993	1	1	1	1	1		0.927	0.988	0.994	0.892	0.885	0.879	0.474	0.868	0.892	0.041	0.885	0.023	0.519
II-3	0.963	0.994	0.989	1	1	1	1	1	0.993	1		0.927	0.933	0.949	0.943	0.936	0.512	0.924	0.949	0.038	0.943	0.021	0.476
II-4	0.966	0.993	0.889	0.993	1	1	1	1	1	0.994	1		0.994	0.892	0.885	0.879	0.474	0.868	0.892	0.041	0.885	0.023	0.519
I-5	0.966	0.993	0.888	0.993	1	1	1	1	1	1	1	1		0.897	0.891	0.884	0.470	0.873	0.897	0.041	0.891	0.023	0.515
II-5	0.962	0.980	0.978	0.993	1	1	1	1	0.993	1	0.993	1	1		0.994	0.987	0.557	0.974	1	0.037	0.994	0.019	0.520
II-6	0.952	0.973	0.967	0.987	0.991	0.991	0.993	0.993	0.986	0.993	0.987	0.993	0.993	1		0.981	0.551	0.967	0.994	0.037	0.987	0.019	0.514
II-7	0.943	0.967	0.956	0.980	0.982	0.982	0.986	0.986	0.980	0.986	0.980	0.986	0.986	1	0.993		0.545	0.961	0.987	0.037	0.981	0.019	0.508
II-8	0.956	0.931	0.981	0.932	0.995	0.995	0.870	0.870	0.870	0.871	0.940	0.871	0.870	1	0.991	0.983		0.540	0.557	0.257	0.551	0.268	0.931
I-6	0.971	0.973	0.966	0.986	0.991	0.991	0.993	0.993	0.986	0.993	0.986	0.993	0.993	0.993	0.993	0.993	0.991		0.974	0.036	0.967	0.029	0.504
II-9	0.962	0.980	0.978	0.993	1	1	1	1	0.993	1	0.993	1	1	1	1	1	1	0.993		0.037	0.994	0.019	0.520
I-7	0.978	−1	−1	−1	1	1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	1	−1	−1		0.037	0.957	0.244
I-8	0.952	0.973	0.967	0.987	0.991	0.991	0.993	0.993	0.986	0.993	0.987	0.993	0.993	1	0.993	0.993	0.991	0.993	1	−1		0.019	0.524
I-9	0.979	−0.717	1	−0.723	1	1	−0.740	−0.740	−0.737	−0.729	−0.720	−0.729	−0.740	−0.699	−0.703	−0.707	1	−0.880	−0.699	1	−0.703		0.255
I-10	0.958	0.920	0.906	0.921	0.990	0.990	0.934	0.934	0.934	0.934	0.929	0.934	0.934	0.991	0.982	0.973	0.990	0.982	0.991	1	0.991	1	

Values above the diagonal shows r^2 (squared correlation coefficient), and values below the diagonal shows standardized D'

SNP single nucleotide polymorphism

Table 4 Two- and three-SNP-based haplotype analyses of SNP I and II in Japanese controls and schizophrenics

A. SNP I: First set of 10 SNPs; (SNPI-1~I-10)

	SNPI-1	SNPI-2	SNPI-3	SNPI-4	SNPI-5	SNPI-6	SNPI-7	SNPI-8	SNPI-9	SNPI-10
2SNPs	0.169				0.14				0.129	
		0.088				0.087				
			0.103				0.054			
				0.045*				0.127		
3SNPs	0.307				0.15					
		0.176				0.09				
			0.106				0.102			
				0.123				0.166		

B. SNP II: Second set of 9 SNPs; SNPII-1~II-9

	SNPII-1	SNPII-2	SNPII-3	SNPII-4	SNPII-5	SNPII-6	SNPII-7	SNPII-8	SNPII-9
2SNPs	0.064				0.048*				
		0.105				0.037*			
			0.088				0.047*		
				0.154				0.059	
3SNPs	0.098				0.036*				
		0.108				0.054			
			0.072				0.049*		
				0.158			-		

Results are the global P values evaluated by the permutation test on the basis of 10,000 replications using SNPAlyse. Significant global P values are shown as * ($P < 0.05$)

SNP single nucleotide polymorphisms

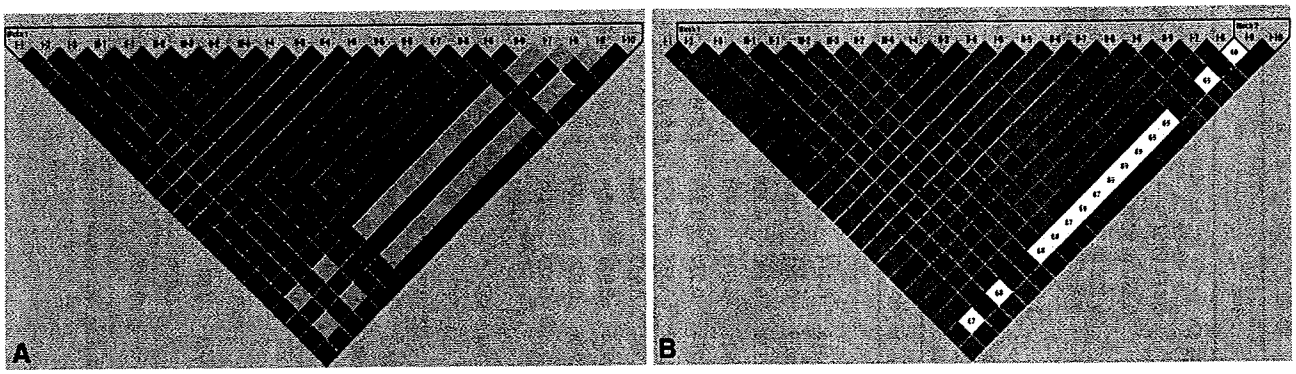


Fig 2 Linkage disequilibrium of the SAP97 in Japanese. The number in each box represents the D' (×100), blank means $D' = 1$. **a** Haplotype Block Pattern of Control Samples. Note that SAP97 gene consists of only one haplotype block. **b** Haplotype Block Pattern of Schizophrenia. SAP97 gene consists of two haplotype blocks in schizophrenia

in males, but not in females, between the schizophrenic and control groups (Table 2). Genotype distribution of all 23 SNPs showed no significant deviations from the HWE in each sex (data not shown).

The haplotype using the entire10 SNPs and the 4 SNPs (SNP I-4, I-5, I-6, I-8) in the SNP I set showed no significant association with schizophrenia in males and female (data not shown). In the two- and three-SNP-based haplotype analysis for all 23 SNPs, SNP III-2-II-1, SNP III-3-II-2, SNP II-5-II-6, SNP II-6-II-7 and SNP II-7-II-8, and SNP III-1-II-1-III-2, SNP II-2-III-4-I-4 and SNP II-5-II-6-II-7 displayed significant global association with schizophrenia (Table 5A). SNP III-1-II-1 and SNP III-1-II-1-III-2 were shown to be associated with the disease only in males. However, in males and females, none of the SNP III-2-II-1, SNP III-3-II-2, SNP II-5-II-6, SNP II-6-II-7, SNP II-7-II-8, SNP II-2-III-4-I-4 and SNP II-5-II-6-II-7 indicated significant association with the disease (Table 5B).

Analysis of the alternative promoter or splicing consensus sequences related to the SNPs

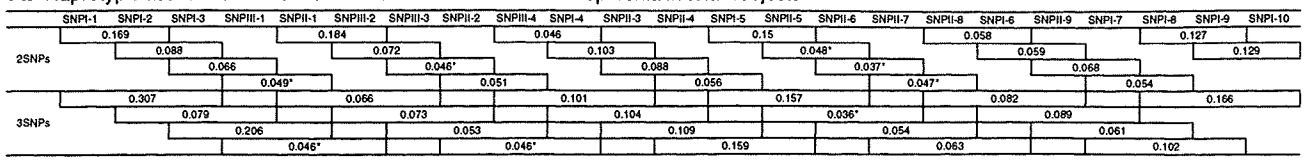
Using prediction algorithms, we examined whether any of the schizophrenia-associated SNPs may influence the alternative promoter or alternative splicing consensus sequences. However, no possible consensus sites were detected within these SNP positions.

Discussion

This is the first case-control study of the polymorphisms of the SAP97 gene encoding a synaptic and ionotropic

Table 5 Two- and three-SNP-based haplotype analyses of the 23 SNPs in SNP I, II and III in Japanese controls and schizophrenics

A. Haprotypic associations between SAP97 SNPs sets and schizophrenia in total subjects



B. Haprotypic associations between SAP97 SNPs sets and schizophrenia in each gender group

associated haplotypes	male	female
SNPIII-1-II-1	0.0424*	0.2954
SNPIII-3-II-2	0.1421	0.3356
SNPII-5-II-6	0.1738	0.3
SNPII-6-II-7	0.164	0.3042
SNPII-7-II-8	0.0733	0.5358
SNPIII-1-II-1-III-2	0.0477*	0.2914
SNPII-2-III-4-I-4	0.1165	0.391
SNPII-5-II-6-II-7	0.1608	0.3062

Results are the global *P* values evaluated by the permutation test on the basis of 10,000 replications using SNPAlyse. Significant global *P* values are shown as * ($P < 0.05$)

SNP single nucleotide polymorphisms

glutamatereceptor-interacting scaffold protein in schizophrenia. Here, we show a nominally significant association of ten (genotype or allele frequency: co-dominant model) of the selected 23å SNPs of the gene with schizophrenia in the Japanese population. We further observe that schizophrenic and control group have different frequencies in eight from the 32 two- or three- SNP haplotypes. It should be noted that significant genetic associations between *SAP97* and schizophrenia have been detected in males, but not in females.

These associations could be solely due to the possible population stratification. In fact, the values of the genotype distributions of the SNPs I-1, I-10, II-1, and III-2 deviated from the HWE estimation in the schizophrenic patients while those of the all 23 SNPs agree with the expected values from the HWE. However, the above possibility is unlikely because the previous studies failed to detect the significant associations of the nucleotide sequence polymorphisms in the genes for the peripheral benzodiazepine receptor (Kurumaji et al. 2000) and tyrosine hydroxylase (Kurumaji et al. 2001) with schizophrenia in the same sample set as used in the present analysis.

The male-selective genetic associations between *SAP97* gene and schizophrenia might be related to the observed gender differences in the development and/or the risk of schizophrenia (Leung and Chue 2000). For schizophrenia, there are significant sex differences in the age of onset, premorbid functioning, symptomatic characteristics, and course of illness, which may have arisen from interplay between sex hormones, neurodevelopmental and psychosocial sex differences (Leung and Chue 2000). Yet the genetic basis of sex differences in schizophrenia has not been identified, several genes have been suggested to have sex-specific associations with the disease (Shifman et al. 2002, Hennah et al. 2003). There is a large-scale genome-wide association study that has demonstrated the replication of the sex-specific genetic association between *reelin* and schizophrenia (Shifman et al. 2008). Because it cannot be totally excluded that the sexually dimorphic effect of *SAP97* might be due to the differences between the number of males and females which could influence the statistical analyses of case-control study, further investigation on larger number of subjects is needed to clarify the possible gender-selective association (Leung and Chue 2000).

The fact that the SNPs having nominal association with schizophrenia or included in the disorder-associated haplotypes are found in the introns, but not in the functional domains or motifs, seems to render their biological consequences obscure. In these intron regions, bioinformatics tools we used failed to reveal any consensus sequences that may play a role in the alternative promoter usage or alternative splicing.

The differences in the distribution patterns of the SNPs between schizophrenic and control group might lead to the aberrant expression and/or functions of the *SAP97* gene in schizophrenia through certain higher structural changes in the gene besides the nucleotide sequence variations. The assumed structural modification appears to be supported by the distinct haploblock compositions between the two groups (Fig. 2). Moreover, the reduction of the SAP97 protein have been observed selectively in the prefrontal cortex of the postmortem schizophrenic brains (Toyooka et al. 2002; Dracheva et al. 2005) whereas another postmortem study found no significant changes in the *SAP97*

mRNA expression in the dorsolatreral prefrontal cortex (Toyooka et al. 2002; Dracheva et al. 2005). The expressional changes in the prefrontal SAP97 proteins could be linked to the results that some of the schizophrenia-associated SNPs are positioned in the vicinity of the functional domain regions of the SAP97 gene including the PDZ and L27 domains (Fig. 1).

The SAP97 protein has three PDZ domains by which the various intracellular proteins associate with the membrane molecules such as neurotransmitter receptors. The SAP97 PDZ domains have been reported to bind to the C-terminal tail of the AMPA receptor subunit GluR1 (Cai et al. 2002; Leonard et al. 1998) and the C-termini of NR2A and NR2B NMDA receptor subunits (Bassand et al. 1999; Niethammer et al. 1996). In the present study, we indicate that the allelic, genotypic, and haplotypic associations between schizophrenia and several SNPs located in the up-stream region of the PDZ domain encoding regions (Fig. 1).

The N-terminal of the SAP97 protein contains a L27 domain that can function as an organization center of large protein assemblies (Feng et al. 2004). The scaffold protein shows a propensity for multimerization via its N-terminal L27 domain, which has been demonstrated to be important for the maintenance and control of the delivery, cell surface expression and activities of the AMPA and NMDA glutamate receptors (Nakagawa et al. 2004). Furthermore, the activity-dependent regulation of the AMPA receptor is suggested to be governed by the N-terminal L27 domain in the hippocampal slice culture (Schlüter et al. 2006). These data buttress the crucial role of the SAP97 L27 domain in the integration of the excitability of the glutamate synapse. Interestingly, we found a significant association between schizophrenia and SNP I-2 of the *SAP97* gene, located between the exons 3 and 4 that encode L27 domain (Fig. 1).

Yet the schizophrenia-associated SNPs situated near the PDZ or L27 domain region are on the intervening sequences and expected to be "silent" to date, it is possible that the "silent" SNPs might affect the levels or final conformation of the *SAP97* mRNA and protein (Kimchi-Sarfaty et al. 2007) including the structure of PDZ domains. These plausible changes lead to abnormal AMPA and NMDA receptor-mediated excitatory postsynaptic currents.

The in vivo functional interactions of the *SAP97* gene with the NMDA receptor are also suggested by the upregulating effects of the toxic or psychotomimetic doses of the NMDA antagonists on the *SAP97* mRNA expression in the entorhinal cortex (Lindén et al. 2001) or neocortex (our unpublished data), respectively, in the rats. These upregulation have been proposed to link to the NMDA antagonist-induced neurotoxicity and the abnormal behavior as a model of schizophrenia.

In conclusion, the present findings provide the evidence indicating that the SNP variation at the *SAP97* gene may have a sexually dimorphic effect of giving susceptibility to schizophrenia. The potential deficits of the gene resulted from the *SAP97* variations could cause the distorted glutamate neurotransmission including the NMDA receptor dysfunction that is presumed to be connected to the pathophysiology of schizophrenia (Javitt and Zukin 1991). To elucidate the exact causative and pathophysiological roles of the *SAP97* gene in schizophrenia, further replication studies are needed in independent and larger samples. The effects of the SNP variations on the brain *SAP97* expressions and functions and their psychological consequences are also required to be clarified.

Acknowledgments This study was partly supported by research grants-in-aid from the Research Development Corporation of Japan, the Ministry of Health, Labour and Welfare, and the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

Barrett J, Fry B, Maller J, Daly M (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265

Bassand P, Bernard A, Rafiki A, Gayet D, Khrestchatisky M (1999) Differential interaction of the tSXV motifs of the NR1 and NR2A NMDA receptor subunits with PSD–95 and SAP97. Eur J NeuroSci 11:2031–2043

Cai C, Coleman S, Niemi K, Keinänen K (2002) Selective binding of synapse-associated protein 97 to GluR-A alpha-amino-5-hydroxy-3-methyl-4-isoxazole propionate receptor subunit is determined by a novel sequence motif. J Biol Chem 277:31484–31490

Dracheva S, McGurk S, Haroutunian V (2005) mRNA expression of AMPA receptors and AMPA receptor binding proteins in the cerebral cortex of elderly schizophrenics. J Neurosci Res 79:868–878

Feng W, Long J, Fan J, Suetake T, Zhang M (2004) The tetrameric L27 domain complex as an organization platform for supramolecular assemblies. Nat Struct Mol Biol 11:475–480

Gabriel S, Schaffner S, Nguyen H, Moore J, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero S, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander E, Daly M, Altshuler D (2002) The structure of haplotype blocks in the human genome. Science 296:2225–2229

Gao X, Sakai K, Roberts R, Conley R, Dean B, Tamminga C (2000) Ionotropic glutamate receptors and expression of N-methyl-D-aspartate receptor subunits in subregions of human hippocampus: effects of schizophrenia. Am J Psychiatry 157:1141–1149

Harrison P, Law A, Eastwood S (2003) Glutamate receptors and transporters in the hippocampus in schizophrenia. Ann N Y Acad Sci 1003:94–101

Hennah W, Varilo T, Kestilä M, Paunio T, Arajärvi R, Haukka J, Parker A, Martin R, Levitzky S, Partonen T, Meyer J, Lönqvist J, Peltonen L, Ekelund J (2003) Haplotype transmission analysis provides evidence of association for DISC1 to schizophrenia and suggests sex-dependent effects. Hum Mol Genet 12:3151–3159

Ibrahim H, Hogg AJ, Healy D, Haroutunian V, Davis K, Meador-Woodruff J (2000) Ionotropic glutamate receptor binding and

subunit mRNA expression in thalamic nuclei in schizophrenia. Am J Psychiatry 157:1811–1823
Javitt D (2004) Glutamate as a therapeutic target in psychiatric disorders. Mol Psychiatry 9:984–997, 979
Javitt D, Zukin S (1991) Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 148:1301–1308
Kimchi-Sarfaty C, Oh J, Kim I, Sauna Z, Calcagno A, Ambudkar S, Gottesman M (2007) A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 315:525–528
Kurumaji A, Nomoto H, Yoshikawa T, Okubo Y, Toru M (2000) An association study between two missense variations of the benzodiazepine receptor (peripheral) gene and schizophrenia in a Japanese sample. J Neural Transm 107:491–500
Kurumaji A, Kuroda T, Yamada K, Yoshikawa T, Toru M (2001) An association of the polymorphic repeat of tetranucleotide (TCAT) in the first intron of the human tyrosine hydroxylase gene with schizophrenia in a Japanese sample. J Neural Transm 108:489–495
Leonard A, Davare M, Horne M, Garner C, Hell J (1998) SAP97 is associated with the alpha-amino–3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit. J Biol Chem 273:19518–19524
Leung A, Chue P (2000) Sex differences in schizophrenia, a review of the literature. Acta Psychiatr Scand Suppl 401:3–38
Lindén A, Väsänen J, Storvik M, Lakso M, Korpi E, Wong G, Castrén E (2001) Uncompetitive antagonists of the N-methyl-D-aspartate (NMDA) receptors alter the mRNA expression of proteins associated with the NMDA receptor complex. Pharmacol Toxicol 88:98–105
Mauceri D, Gardoni F, Marcello E, Di Luca M (2007) Dual role of CaMKII-dependent SAP97 phosphorylation in mediating trafficking and insertion of NMDA receptor subunit NR2A. J Neurochem 100:1032–1046
Meador-Woodruff J, Healy D (2000) Glutamate receptor expression in schizophrenic brain. Brain Res Brain Res Rev 31:288–294
Meador-Woodruff J, Davis K, Haroutunian V (2001) Abnormal kainate receptor expression in prefrontal cortex in schizophrenia. Neuropsychopharmacology 24:545–552
Mehta S, Wu H, Garner C, Marshall J (2001) Molecular mechanisms regulating the differential association of kainate receptor subunits with SAP90/PSD-95 and SAP97. J Biol Chem 276:16092–16099
Nakagawa T, Futai K, Lashuel H, Lo I, Okamoto K, Walz T, Hayashi Y, Sheng M (2004) Quaternary structure, protein dynamics, and synaptic function of SAP97 controlled by L27 domain interactions. Neuron 44:453–467
Niethammer M, Kim E, Sheng M (1996) Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. J Neurosci 16:2157–2163
Nishikawa T, Takashima M, Toru M (1983) Increased [3H]kainic acid binding in the prefrontal cortex in schizophrenia. Neurosci Lett 40:245–250
Nishikawa T, Umino A, Tanii Y, Hashimoto A, Hata N, Takashima M, Takahashi K, Toru M (1991) Dysfunction of excitatory amino acidergic systems and schizophrenic disorders. In: Nakazawa T (ed) Biological Basis of Schizophrenic Disorders. Japan Scientific Societies Press, Tokyo
Patil S, Zhang L, Martenyi F, Lowe S, Jackson K, Andreev B, Avedisova A, Bardenstein L, Gurovich I, Morozova M, Mosolov S, Neznanov N, Reznik A, Smulevich A, Tochilov V, Johnson B, Monn J, Schoepp D (2007) Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. Nat Med 13:1102–1107
Ross C, Margolis R, Reading S, Pletnikov M, Coyle J (2006) Neurobiology of schizophrenia. Neuron 52:139–153
Schlüter O, Xu W, Malenka R (2006) Alternative N-terminal domains of PSD-95 and SAP97 govern activity-dependent regulation of synaptic AMPA receptor function. Neuron 51:99–111
Shifman S, Bronstein M, Sternfeld M, Pisante-Shalom A, Lev-Lehman E, Weinzman A, Renznik I, Spivak B, Grisaru N, Karp L, Schiffer R, Kotler M, Strous R, Swartz-Vanetik M, Knobler H, Shinar E, Beckmann J, Yakir B, Risch N, Zak N, Darvasi A (2002) A highly significant association between a COMT haplotype and schizophrenia. Am J Hum Genet 71:1296–1302
Shifman S, Johannesson M, Bronstein M, Chen S, Collier D, Craddock N, Kendler K, Li T, O'donovan M, O'neill F, Owen M, Walsh D, Weinberger D, Sun C, Flint J, Darvasi A (2008) Genome-wide association identifies a common variant in the reelin gene that increases the risk of schizophrenia only in women. PLoS Genet 4:e28
Toyooka K, Iritani S, Makifuchi T, Shirakawa O, Kitamura N, Maeda K, Nakamura R, Niizato K, Watanabe M, Kakita A, Takahashi H, Someya T, Nawa H (2002) Selective reduction of a PDZ protein, SAP–97, in the prefrontal cortex of patients with chronic schizophrenia. J Neurochem 83:797–806
Wang L, Piserchio A, Mierke D (2005) Structural characterization of the intermolecular interactions of synapse-associated protein-97 with the NR2B subunit of N-methyl-D-aspartate receptors. J Biol Chem 280:26992–26996

Neuroscience Research 62 (2008) 155–159

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Effects of FG7142 and immobilization stress on the gene expression in the neocortex of mice

Akeo Kurumaji *, Takashi Ito, Sumikazu Ishii, Toru Nishikawa

Section of Psychiatry and Behavioral Sciences, Tokyo Medical and Dental University Graduate School, 1-5-45 Yushima, Bunkyou-ku, Tokyo 113-8519, Japan

ARTICLE INFO

Article history:
Received 19 May 2008
Received in revised form 1 August 2008
Accepted 4 August 2008
Available online 14 August 2008

Keywords:
FG7142
Stress
Neocortex
Gene expression
Microarray
RT-PCR

ABSTRACT

Several psychiatric disorders are often precipitated or exacerbated by exposure to stressors. FG7142 (*N*-methyl-β-carboline-3-carboxamide), a partial inverse agonist of benzodiazepine receptors, mimics the physiological (an increased release in the adrenal steroid hormone) and neurochemical (an enhanced neurotransmission of monoamines) changes induced by stressful stimuli.

We examined the effects of FG7142 and immobilization stress on the gene expression of the mouse neocortex in order to obtain a new insight into the molecular stress-responsive system.

The effect of FG7142 (20 mg/kg, i.p.) on the gene expression of the brain area was examined using a DNA microarray method. The genes showing a significant change in expression were investigated in further experiments using the quantitative RT-PCR method.

There was an increase in the mRNA of seven genes in the neocortex of mice 1 h after treatment with FG7142. In addition, there was an increase in the mRNAs of five of the seven genes (Fos, Cyr61, Btg2, Adamts1, and Gem) in the neocortex of mice exposed to the stress for 1 h.

The up-regulation of these five genes by both FG7142 and immobilization stress indicates that these genes may be involved in the stress-responsive system. Dysfunctions of the system may be associated with the pathophysiology of psychiatric disorders.

1. Introduction

Several psychiatric disorders, such as schizophrenia (Norman and Malla, 1993) and mood disorders (Ellicott et al., 1990; Kendler et al., 1999), are often precipitated or exacerbated by exposure to the stress of life events. Stress has been applied as a model to study changes in the brain function and structure to clarify the pathophysiology of the psychiatric disorders (Moghaddam, 2002; Duman and Monteggia, 2006).

FG7142 (*N*-methyl-β-carboline-3-carboxamide) acts at the benzodiazepine sites of the $GABA_A$ receptors as a partial inverse agonist and allosterically inhibits the ability of GABA to bind and activate the receptors, which can be blocked by a benzodiazepine antagonist, e.g., flumazenil (Ro 15-1788) (Atack et al., 2005). This compound produces anxiety in humans (Dorow et al., 1983) and rodents (Pellow and File, 1986; Rodgers et al., 1995) and mimics some physiological and neurochemical responses to stress in animals (Evans and Lowry, 2007), including an increase in adrenal steroid hormones (Pellow and File, 1985; Mikkelsen et al., 2005). In the cerebral cortex of rodents, both FG7142 and stress, such as foot-shock and restraint stress, similarly produce an increased release of dopamine (Bradberry et al., 1991; Dazzi et al., 2003, 2004), noradrenaline (Nakane et al., 1994), and glutamate (Moghaddam, 1991; Karreman and Moghaddam, 1996). In addition, FG7142 produces the selective activation of mesocortical dopaminergic transmission as well as an impairment of the working memory in monkeys and rodents (Tam and Roth, 1990; Murphy et al., 1996a,b; Birnbaum et al., 2004), and an increased Fos-like immunoreactivity in the cortex of rats along with some brain areas associated with neuronal circuits mediating stress (Kurumaji et al., 2003; Singewald et al., 2003), and wide-spread reductions in the cortical metabolisms in monkeys (Takamatsu et al., 2003). Consequently, FG7142 is considered a useful pharmacological tool to investigate anxiety-related and stress-related responses in experimental animals (Evans and Lowry, 2007).

The main purpose of the present study was the identification of new candidate genes related to the stress response in the neocortex. A microarray analysis was performed on the mouse neocortex after FG7142 administration to screen for the candidate genes. Seven candidate genes were identified as being up-regulated by the drug. A quantitative RT-PCR method verified all

* Corresponding author. Tel.: +81 3 5803 5242; fax: +81 3 5803 0135.
E-mail address: 0724.psyc@tmd.ac.jp (A. Kurumaji).

0168-0102/$ – see front matter © 2008 Elsevier Ireland Ltd and the Japan Neuroscience Society. All rights reserved.
doi:10.1016/j.neures.2008.08.001