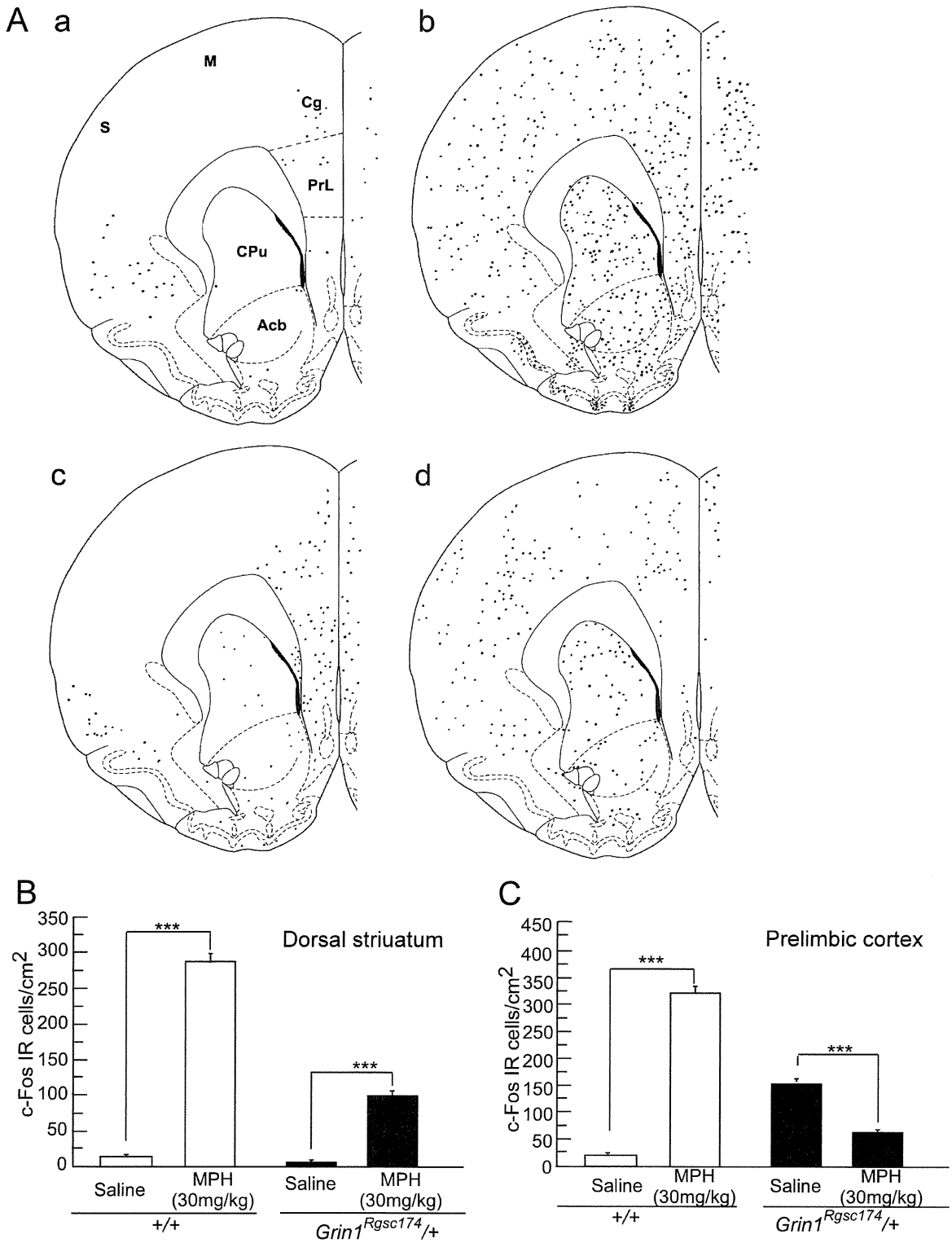

FIG. 10. Effect of MPH on c-Fos expression in *Grin1*^{Rgsc174}/+ and +/+ mice. (A) Diagram of the distribution of c-Fos-IR cells and regions selected for analysis. Dots indicate c-Fos-IR cells 2 h after saline or MPH 30 mg/kg administration. Brain regions are abbreviated as follows: Acb, accumbens nucleus; Cg, cingulate cortex; CPu, caudate-putamen (striatum); M, motor cortex; PrL, prelimbic cortex; S, somatosensory cortex. The diagram was modified from the brain atlas of Paxinos & Franklin (1997). (a) Saline-treated wild type, (b) MPH-treated wild type, (c) saline-treated heterozygote, and (d) MPH-treated heterozygote. (B and C) Quantification of c-Fos-IR cells in the dorsal striatum and prelimbic cortex (E). Error bars represent the SEM. ***$P < 0.0001$, Fisher's PLSD test. Male mice, $n = 7$ in each group at 11 weeks age. (B) ANOVA, effect of genotype $F_{1,24} = 206.7$, $P < 0.0001$; effect of MPH treatment, $F_{1,24} = 735.543$, $P < 0.0001$; interaction between genotype and MPH treatment, $F_{1,24} = 180.89$, $P < 0.0001$. (C) ANOVA, effect of genotype, $F_{1,24} = 76.32$, $P < 0.0001$, effect of MPH treatment, ANOVA, $F_{1,24} = 222.584$, $P < 0.0001$, interaction between genotype and MPH treatment, $F_{1,24} = 761.468$, $P < 0.0001$. Scale bar in A, 500 μm.

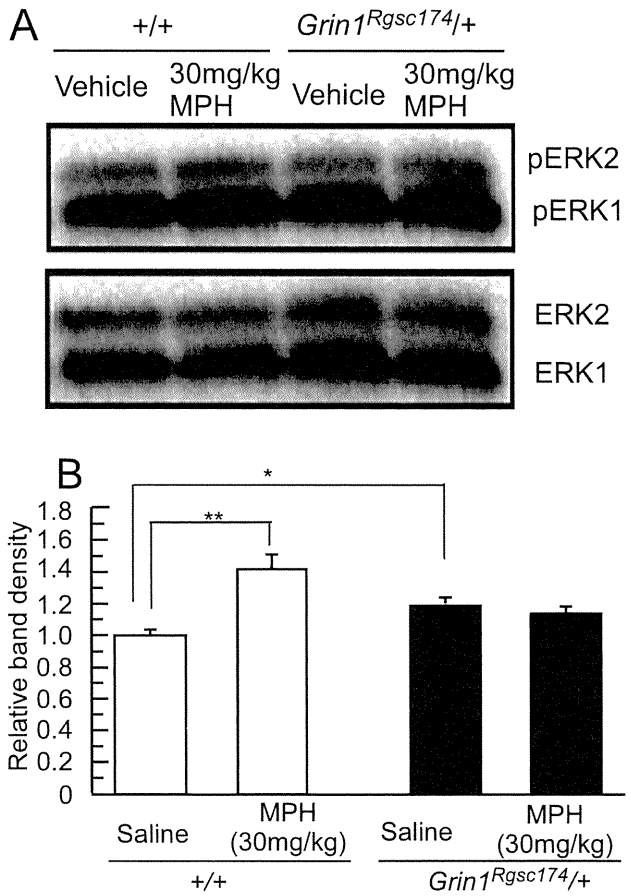

FIG. 11. Phospho-ERK levels after intraperitoneal administration of MPH 30 mg/kg to wild-type and heterozygous mice. (A) Immunoblotting of pERK1 and 2 in nucleus accumbens extracts following MPH administration. (B) Quantitative analysis of the relative band density of pERK2. The baseline level of pERK2 was calculated as the average ratio pERK2/ERK2 prepared from saline-treated wild-type mice, and the data were normalized by using the following formula: pERK level = (band density of pERK2)/(band density of ERK2)/(baseline level). Male mice, $n = 5$ in each group at 12 weeks age. Error bars represent the SEM. ANOVA, effect of genotype, $F_{1,32} = 0.271$, $P > 0.6$; effect of MPH administration, $F_{1,32} = 9.8$, $P < 0.004$; interaction between the MPH effect and genotype, $F_{1,32} = 16.9$, $P < 0.0004$. $^{*}P < 0.05$ and $^{**}P < 0.01$, Fisher's PLSD test.

was not upregulated in comparison with the saline-treated heterozygote (Fig. 11).

Discussion

Phenotype comparison between Grin1^{Rgsc174} and other Grin1 *mutants*

The following *Grin1* mutant mice have been reported previously: *Grin1* null mutants (Forrest *et al.*, 1994; Li *et al.*, 1994), *Grin1^{tm2Blt}* with reduced glycine affinity (Kew *et al.*, 2000; Ballard *et al.*, 2002), *Grin1$^{tm1.1Phs}$* with reduced single-channel conductance (Single *et al.*, 2000), and *Grin1* knockdown mutants (Mohn *et al.*, 1999; Duncan *et al.*, 2006). We observed common phenotypes, including fearfulness, embryonic lethality of homozygote, and social avoidance, in *Grin1^{Rgsc174}*/+ and known *Grin1* mutants (Forrest *et al.*, 1994; Li *et al.*, 1994; Mohn *et al.*, 1999; Kew *et al.*, 2000; Single *et al.*, 2000; Ballard *et al.*, 2002; Duncan *et al.*, 2006). Increased anxiety is a common finding in known *Grin1* mutant mice (Kew *et al.*, 2000; Labrie *et al.*, 2009), but measurements of time spent in the center area of the open field and the results of the light–dark transition test revealed no increase in anxiety in *Grin1^{Rgsc174}*/+ mice. The increased novelty-seeking behavior and the absence of increased anxiety are phenotypes unique to the *Grin1^{Rgsc174}* mutant.

Functional change in GRIN1 *protein*

The missense mutation R844C is located in the intracellular C-terminal domain of NMDAR1, which is referred to the C0 domain. The C0 domain spans amino acid residues 834–863 (Akyol *et al.*, 2004). Previous reports have indicated that the C0 domain is an important regulatory domain of *GRIN1* protein (Holmes *et al.*, 2002; Leonard *et al.*, 2002). The cystein in the wild-type allele that is replaced by arginine in the mutant is very hydrophobic, whereas arginine is a positively charged hydrophilic amino acid and binds to negatively charged amino acid groups. This change in the C0 domain should produce an alteration in the conformation and function of the C0 domain in *GRIN1* protein and, in fact, we observed that after NMDA stimulation calcium influx was increased and prolonged in cortical neurons from the *Grin1^{Rgsc174}* mutant.

Altered interaction between NMDARs and dopamine receptors may be responsible for the phenotypes of the Grin1^{Rgsc174} mutant

NMDAR and dopamine (DA) receptor functions are co-regulated by direct (Lee *et al.*, 2002) and indirect (Cepeda & Levine, 2006) interactions. Morphological evidence suggests that glutamate receptors and DA receptors interact in synaptic complexes or triads in cortical pyramidal neurons (Goldman- Rakic *et al.*, 1989). This type of arrangement is found in striatal neurons (Smith & Bolam, 1990) and provides a morphological basis for close DA receptor–glutamate receptor interaction. As MPH has been reported to be involved in activation of DA signaling, we used MPH to compare the altered DA signaling in the wild-type and *Grin1^{Rgsc174}*/+ mice. The basal level of c-Fos expression in the prelimbic cortex and striatum was very low in the wild type, and MPH administration significantly increased c-Fos expression in both areas. Increased c-Fos expression was observed in the prelimbic cortex of *Grin1^{Rgsc174}*/+ mice at the basal level, and MPH paradoxically reduced c-Fos expression in the prelimbic cortex. Phosphorylation of ERK2, a DA signaling-related protein, was increased in the nucleus accumbens of *Grin1^{Rgsc174}*/+ mice at the basal level, and little change was observed even after the high dose of MPH (30 mg/kg). Thus, NMDAR dysfunction in these regions should underlie the aberrant DA signaling and result in the behavioral phenotypes of *Grin1^{Rgsc174}*/+ mice. In the present study, the difference in MPH-induced behavioral difference between the wild type and *Grin1* mutant was detected only at the high dose of MPH (108 μmol/kg). Taking into consideration that K_I of MPH for the mouse dopamine transporter is < 0.3 μM (Chen *et al.*, 2005), the effect of the high dose of MPH in the present study is considered to be due not to the specific action on the DA transporter but to the effect on any other receptors or transporters that crosstalk with DA signaling system.

Grin1^{Rgsc174} mutant as an animal model of psychiatric disorders

The implication of mutations in NMDAR has been suggested in schizophrenia by human association study (Georgi *et al.*, 2007; Galehdari, 2009). The increased locomotor activity observed in the *Grin1^{Rgsc174}*/+ mice may represent fearfulness, and the mutant also

exhibited social isolation in the social interaction test. Thus, the phenotype's fearfulness and social isolation observed in a schizophrenia model (Mohn *et al.*, 1999) were also exhibited by this mutant. MPH is one of the main therapeutic agents used to treat attention deficit hyperactivity disorder (AD/HD) and narcolepsy patients. However, the mechanism of action of MPH is still unclear. According to previous reports, spontaneously hypertensive rat (SHR), a well validated animal model of AD/HD, was found to exhibit the three major characteristics of AD/HD (hyperactivity, impulsivity and poor sustained attention) in a comparison with their progenitor Wistar–Kyoto rat strain, and SHR has been shown to lack responsiveness to MPH in several behavioral tests (Van den Bergh *et al.*, 2006). The *Grin1* mutant mouse described here also exhibited altered pharmacological reactions to MPH. In view of the fact that SHR also exhibits altered glutamatergic functions (Jensen *et al.*, 2009), *Grin1*^{Rgsc174} mice may be a useful model for gaining insight into the mechanism of action of MPH on behavioral disorders in regard to DA receptor-glutamate receptor interactions.

These phenotypes of *Grin1*^{Rgsc174} indicate that this mutant displays some of the signs and symptoms of psychiatric disorders and may be a useful tool for elucidating the molecular mechanisms of abnormal behaviors and the actions of therapeutic agents.

Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1. Strategy of animal production for phenotypic screen, genetic analyses, and detailed phenotypic analyses.

Fig. S2. Results of pilot study for pharmacological analysis using MPH Wild-type male mice.

Fig. S3. Brain histology of *Grin1*^{Rgsc174}/+ and wild-type mice.

Fig. S4. Result of social interaction test.

Fig. S5. Light/dark transition test results.

Fig. S6. Effect of MPH on c-Fos expression in *Grin1*^{Rgsc174}/+ and +/+ mice.

Table S1. SNP markers chosen for genome-wide scanning.

Table S2. Results of the resident intruder test.

Appendix S1. Procedure of pilot study for pharmacological analysis of *Grin1*^{Rgsc174}/+ using MPH.

Appendix S2. Preparation of cells for measurement of intracellular calcium levels.

Appendix S3. Preparation of samples for immunoblotting of NMDAR subunits.

Appendix S4. Preparation of samples for immunoblotting of extracellular signal-regulated kinase2 (ERK2) and phospho-ERK2 (pERK2) proteins.

Appendix S5. Procedure of social interaction test.

Appendix S6. Procedure of resident intruder paradigm.

Appendix S7. Procedure of light/dark transition test.

Please note: As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset by Wiley-Blackwell. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

Acknowledgements

The authors thank Professor Toshihiko Shiroishi of the National Institute of Genetics for kind advice, the animal facility at RIKEN for providing the animals used in this study, the technical staff for assistance with experiments. This study was funded by a National BioResource Project (NBRP), a Research Grant (18A-3) for Nervous and Mental Disorders from the Ministry of Health, Labour and Welfare of Japan to S.Y. and S.W., a research grant to T.F. from the ministry of Education, Culture, Sports, Science, and Technology of Japan (Grant number 09020258), and Grants-in-Aid from the Program for the Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO; Grant number 05-32).

Abbreviations

B6, C57BL/6J; D2, DBA/2J; DA, dopamine; ENU, *N*-ethyl-*N*-nitrosourea; ERK2, extracellular signal-regulated kinase; *Grin1*, glutamate receptor, ionotropic, NMDA1; i.p., intraperitoneal; IR, immunoreactive; MPH, D,L-methylphenidate hydrochloride; NMDA, *N*-methyl-D-aspartate; NMDAR, NMDA receptors; pERK2, phospho-ERK2.

References

Akyol, Z., Bartos, J.A., Merrill, M.A., Faga, L.A., Jaren, O.R., Shea, M.A. & Hell, J.W. (2004) Apo-calmodulin binds with its C-terminal domain to the *N*-methyl-D-aspartate receptor NR1 C0 region. *J. Biol. Chem.*, **279**, 2166–2175.

Bajo, M., Crawford, E.F., Roberto, M., Madamba, S.G. & Siggins, G.R. (2006) Chronic morphine treatment alters expression of *N*-methyl-D-aspartate receptor subunits in the extended amygdala. *J. Neurosci. Res.*, **83**, 532–537.

Ballard, T.M., Pauly-Evers, M., Higgins, G.A., Ouagazzal, A.M., Mutel, V., Borroni, E., Kemp, J.A., Bluethmann, H. & Kew, J.N. (2002) Severe impairment of NMDA receptor function in mice carrying targeted point mutations in the glycine binding site results in drug-resistant nonhabituating hyperactivity. *J. Neurosci.*, **22**, 6713–6723.

Cepeda, C. & Levine, M.S. (2006) Where do you think you are going? The NMDA-D1 receptor trap *Sci. STKE*, **333**, 20.

Chen, R., Han, D.D. & Gu, H.H. (2005) A triple mutation in the second transmembrane domain of mouse dopamine transporter markedly decreases sensitivity to cocaine & methylphenidate. *J. Neurochem.*, **94**, 352–359.

Duncan, G.E., Moy, S.S., Lieberman, J.A. & Koller, B.H. (2006) Effects of haloperidol, clozapine & quetiapine on sensorimotor gating in a genetic model of reduced NMDA receptor function. *Psychopharmacology*, **184**, 190–200.

Forrest, D., Yuzaki, M., Soares, H.D., Ng, L., Luk, D.C., Sheng, M., Stewart, C.L., Morgan, J.I., Connor, J.A. & Curran, T. (1994) Targeted disruption of NMDA receptor 1 gene abolishes NMDA response & results in neonatal death. *Neuron*, **13**, 325–338.

Gainetdinov, R.R., Wetsel, W.C., Jones, S.R., Levin, E.D., Jaber, M. & Caron, M.G. (1999) Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. *Science*, **283**, 397–401.

Galehdari, H. (2009) Association between the G1001C polymorphism in the GRIN1 gene promoter and schizophrenia in the Iranian population. *J. Mol. Neurosci.*, **38**, 178–181.

Georgi, A., Jamra, R.A., Klein, K., Villela, A.W., Schumacher, J., Becker, T., Paul, T., Schmael, C., Höfels, S., Klopp, N., Illig, T., Propping, P., Cichon, S., Nöthen, M., Schulze, T. & Rietschel, M. (2007) Possible association between genetic variants at the GRIN1 gene & schizophrenia with lifetime history of depressive symptoms in a German sample. *Psychiatr. Genet.*, **17**, 308–310.

Glenn, M.J., Kirby, E.D., Gibson, E.M., Wong- Goodrich, S.J., Mellott, T.J., Blusztajn, J.K. & Williams, C.L. (2008) Age-related declines in exploratory behavior & markers of hippocampal plasticity are attenuated by prenatal choline supplementation in rats. *Brain Res.*, **237**, 110–123.

Goldman- Rakic, P.S., Leranth, C., Williams, S.M., Mons, N. & Geffard, M. (1989) Dopamine synaptic complex with pyramidal neurons in primate cerebral cortex. *Proc. Natl. Acad. Sci. USA*, **86**, 9015–9019.

Hattori, K., Yagi, T., Maekawa, M., Sato, T. & Yuasa, S. (2001) *N*-Methyl-daspartate-induced c-Fos expression is enhanced in the forebrain structures related to emotion in Fyn-deficient mice. *Brain Res.*, **905**, 188–198.

Hattori, K., Uchino, S., Isosaka, T., Maekawa, M., Iyo, M., Sato, T., Kohsaka, S., Yagi, T. & Yuasa, S. (2006) Fyn is required for haloperidol-induced catalepsy in mice. *J. Biol. Chem.*, **281**, 7129–7135.

Holmes, K.D., Mattar, P.A., Marsh, D.R., Weaver, L.C. & Dekaban, G.A. (2002) The *N*-methyl-D-aspartate receptor splice variant NR1-4 C-terminal

domain. Deletion analysis & role in subcellular distribution. *J. Biol. Chem.*, **277**, 1457–1468.

Hrabe de Angelis, M.H., Flaswinkel, H., Fuchs, H., Rathkolb, B., Soewarto, D., Marschall, S., Heffner, S., Pargent, W., Wuensch, K., Jung, M., Reis, A., Richter, T., Alessandrini, F., Jakob, T., Fuchs, E., Kolb, H., Kremmer, E., Schaeble, K., Rollinski, B., Roscher, A., Peters, C., Meitinger, T., Strom, T., Steckler, T., Holsboer, F., Klopstock, T., Gekeler, F., Schindewolf, C., Jung, T., Avraham, K., Behrendt, H., Ring, J., Zimmer, A., Schughart, K., Pfeffer, K., Wolf, E. & Balling, R. (2000) Genome-wide, large-scale production of mutant mice by ENU mutagenesis. *Nat. Genet.*, **25**, 444–447.

Inoue, M., Sakuraba, Y., Motegi, H., Kubota, N., Toki, H., Matsui, J., Toyoda, Y., Miwa, I., Terauchi, Y., Kadowaki, T., Shigeyama, Y., Kasuga, M., Adachi, T., Fujimoto, N., Matsumoto, R., Tsuchihashi, K., Kagami, T., Inoue, A., Kaneda, H., Ishijima, J., Masuya, H., Suzuki, T., Wakana, S., Gondo, Y., Minowa, O., Shiroishi, T. & Noda, T. (2004) A series of maturity onset diabetes of the young, type 2 (MODY2) mouse models generated by a large-scale ENU mutagenesis program. *Hum. Mol. Genet.*, **13**, 1147–1157.

Jensen, V., Rinholm, J.E., Johansen, T.J., Medin, T., Storm-Mathisen, J., Sagvolden, T., Hvalby, O. & Bergersen, L.H. (2009) *N*-methyl-D-aspartate receptor subunit dysfunction at hippocampal glutamatergic synapses in an animal model of attention-deficit/hyperactivity disorder. *Neuroscience*, **158**, 353–364.

Justice, M.J., Noveroske, J.K., Weber, J.S., Zheng, B. & Bradley, A. (1999) Mouse ENU mutagenesis. *Hum. Mol. Genet.*, **8**, 1955–1963.

Keays, D.A., Tian, G., Poirier, K., Huang, G.J., Siebold, C., Cleak, J., Oliver, P.L., Fray, M., Harvey, R.J., Molnar, Z., Pinon, M.C., Dear, N., Valdar, W., Brown, S.D., Davies, K.E., Rawlins, J.N., Cowan, N.J., Nolan, P., Chelly, J. & Flint, J. (2007) Mutations in alpha-tubulin cause abnormal neuronal migration in mice and lissencephaly in humans. *Cell*, **128**, 45–57.

Kew, J.N., Koester, A., Moreau, J.L., Jenck, F., Ouagazzal, A.M., Mutel, V., Richards, J.G., Trube, G., Fischer, G., Montkowski, A., Hundt, W., Reinscheid, R.K., Pauly-Evers, M., Kemp, J.A. & Bluethmann, H. (2000) Functional consequences of reduction in NMDA receptor glycine affinity in mice carrying targeted point mutations in the glycine binding site. *J. Neurosci.*, **20**, 4037–4049.

Kobayashi, N. & Toyoda, T. (2008) Statistical search on the semantic web. *Bioinformatics*, **24**, 1002–1010.

Kohler, S.W., Provost, G.S., Fieck, A., Kretz, P.L., Bullock, W.O., Sorge, J.A., Putman, D.L. & Short, J.M. (1991) Spectra of spontaneous & mutagen-induced mutations in the lacI gene in transgenic mice. *Proc. Natl. Acad. Sci. USA*, **88**, 7958–7962.

Labrie, V., Clapcote, S.J. & Roder, J.C. (2009) Mutant mice with reduced NMDA-NR1 glycine affinity or lack of d-amino acid oxidase function exhibit altered anxiety-like behaviors. *Pharmacol. Biochem. Behav.*, **91**, 610–620.

Lee, F.J.S., Xue, S., Pei, L., Vukusic, B., Che'ry, N., Wang, Y., Wang, Y.T., Niznik, H.B., Yu, X. & Liu, F. (2002) Dual regulation of NMDA receptor functions by direct protein–protein interactions with the dopamine D1 receptor. *Cell*, **111**, 219–230.

Leonard, A.S., Bayer, K.U., Merrill, M.A., Lim, I.A., Shea, M.A., Schulman, H. & Johannes, W. (2002) Regulation of calcium/calmodulin-dependent protein kinase II docking to *N*-methyl-D-aspartate receptors by calcium/calmodulin & alpha-actinin. *J. Chem. Biol.*, **277**, 48441–48448.

Li, Y., Erzurumlu, R.S., Chen, C., Jhaveri, S. & Tonegawa, S. (1994) Whisker-related neuronal patterns fail to develop in the trigeminal brainstem nuclei of NMDAR1 knockout mice. *Cell*, **76**, 427–437.

Masuya, H., Shimizu, K., Sezutsu, H., Sakuraba, Y., Nagano, J., Shimizu, A., Fujimoto, N., Kawai, A., Miura, I., Kaneda, H., Kobayashi, K., Ishijima, J., Maeda, T., Gondo, Y., Noda, T., Wakana, S. & Shiroishi, T. (2005) Enamelin (Enam) is essential for amelogenesis: ENU-induced mouse mutants as models for different clinical subtypes of human amelogenesis imperfecta (AI). *Hum. Mol. Genet.*, **14**, 575–583.

Masuya, H., Nishida, K., Furuichi, T., Toki, H., Nishimura, G., Kawabata, H., Yokoyama, H., Yoshida, A., Tominaga, S., Nagano, J., Shimizu, A., Wakana, S., Gondo, Y., Noda, T., Shiroishi, T. & Ikegawa, S.:. (2007) A novel dominant-negative mutation in Gdf5 generated by ENU mutagenesis impairs joint formation & causes osteoarthritis in mice. *Hum. Mol. Genet.*, **16**, 2366–2375.

Mohn, A.R., Gainetdinov, R.R., Caron, M.G. & Koller, B.H. (1999) Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. *Cell*, **98**, 427–436.

Nolan, P.M., Peters, J., Strivens, M., Rogers, D., Hagan, J., Spurr, N., Gray, I.C., Vizor, L., Brooker, D., Whitehill, E., Washbourne, R., Hough, T., Greenaway, S., Hewitt, M., Liu, X., McCormack, S., Pickford, K., Selley, R., Wells, C., Tymowska-Lalanne, Z., Roby, P., Glenister, P., Thornton, C., Thaung, C., Stevenson, J.A., Arkell, R., Mburu, P., Hardisty, R., Kiernan, A., Erven, A., Steel, K.P., Voegeling, S., Guenet, J.L., Nickols, C., Sadri, R., Nasse, M., Isaacs, A., Davies, K., Browne, M., Fisher, E.M., Martin, J., Rastan, S., Brown, S.D. & Hunter, J. (2000) A systematic, genome-wide, phenotype-driven mutagenesis programme for gene function studies in the mouse. *Nat. Genet.*, **25**, 440–443.

Paxinos, G. & Franklin, K.B.J. (1997) *The Mouse Brain in Stereotaxic Coordinates*, 2nd Edn. Academic Press, San Diego, CA, USA.

Provost, G.S. & Short, J.M. (1994) Characterization of mutations induced by ethylnitrosourea in seminiferous tubule germ cells of transgenic B6C3F1 mice. *Proc. Natl. Acad. Sci. USA*, **91**, 6564–6568.

Single, F.N., Rozov, A., Burnashev, N., Zimmermann, F., Hanley, D.F., Forrest, D., Curran, T., Jensen, V., Hvalby, O., Sprengel, R. & Seeburg, P.H. (2000) Dysfunctions in mice by NMDA receptor point mutations NR1(N598Q) & NR1(N598R). *J. Neurosci.*, **20**, 2558–2566.

Smith, A.D. & Bolam, J.P. (1990) The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. *Trends Neurosci.*, **13**, 259–265.

Tomihara, K., Soga, T., Nomura, M., Korach, K.S., Gustafsson, J.A., Pfaff, D.W. & Ogawa, S. (2009) Effect of ER-beta gene disruption on estrogenic regulation of anxiety in female mice. *Physiol. Behav.*, **96**, 300–306.

Van den Bergh, F.S., Bloemarts, E., Chan, J.S., Groenink, L., Olivier, B. & Oosting, R.S. (2006) Spontaneously hypertensive rats do not predict symptoms of attention-deficit hyperactivity disorder. *Pharmacol. Biochem. Behav.*, **83**, 380–390.

Yano, M. & Steiner, H. (2005) Topography of methylphenidate (regulation, ritalin)-induced gene in the striatum: effects, differential on c-Fos, substance, P and peptides, opioid. *Neuropsychopharmacology*, **30**, 901–915.

Biological Psychology 89 (2012) 117–122

Contents lists available at SciVerse ScienceDirect

Biological Psychology

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

Blood *CADPS2ΔExon3* expression is associated with intelligence and memory in healthy adults

Kotaro Hattori[a,*], Haruko Tanaka[a], Noriko Yamamoto[a], Toshiya Teraishi[a], Hiroaki Hori[a,b], Yukiko Kinoshita[a], Junko Matsuo[a], Yumiko Kawamoto[a], Hiroshi Kunugi[a,b]

[a] *Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Tokyo187-8502, Japan*
[b] *CREST, Japan Science and Technology Agency, Saitama, Japan*

ARTICLE INFO

Article history:
Received 7 June 2011
Accepted 26 September 2011
Available online 12 October 2011

Keywords:
CAPS2
Splicing variant
Intelligence quotient
Memory
Autistic disorder
BDNF
Dopamine

ABSTRACT

Ca^{2+}-dependent activator protein for secretion 2 (CADPS2), a secretory granule associate protein, mediates monoamine transmission and neurotrophin release. Both monoamines and neurotrophins play a crucial role in cognition, learning and memory. An aberrant splice variant of *CADPS2*, *CADPS2ΔExon3*, was reported to be associated with autism. Therefore, we examined the possible association between the expression of *CADPS2/CADPS2ΔExon3* in peripheral blood and brain functions such as intelligence and memory. Quantitative polymerase chain reaction analysis was performed in 271 healthy adults (age range 20–74 years, mean ± SD 43.3 ± 15.3). Data on intelligence quotient (IQ) and memory were obtained by using full versions of the Wechsler Adult Intelligence Scale-Revised (WAIS-R), and the Wechsler Memory Scale-Revised (WMS-R), respectively. *CADPS2* expression levels were not significantly associated with any scores/sub-scores of these scales. However, *CADPS2ΔExon3* expression was significantly associated with lower IQ ($p = 0.022$; effect size: $\eta_p^2 = 0.031$), particularly verbal IQ of WAIS-R ($p = 0.019$; $\eta_p^2 = 0.032$), lower verbal memory ($p = 0.026$; $\eta_p^2 = 0.026$) and delayed recall ($p = 0.042$; $\eta_p^2 = 0.021$) of WMS-R. Our results suggest that CADPS2ΔExon3 affects intelligence and memory in the non-clinical population.

1. Introduction

Ca^{2+}-dependent activator protein for secretion 2 (CADPS2) is a secretory granule-associated protein involved in the release of neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3). Mouse CADPS2 protein is associated with BDNF-containing secretory vesicles and promotes activity-dependent release of BDNF (Sadakata et al., 2004). Accordingly, BDNF release is significantly reduced in the cultured neurons prepared from the cerebellum, neocortex and hippocampus of CADPS2 deficient mice (Sadakata et al., 2007a, 2007b).

BDNF plays a crucial role in the development and maintenance of brain function, including formation of synapses and neural circuits. Reduced long-term potentiation and impaired spatial memory have been reported in conditional BDNF deficient mice or mice after infusion of antisense BDNF (Mizuno et al., 2000; Monteggia et al., 2004). A polymorphism in *BDNF*, Val66Met was reported to affect human memory and hippocampal activity (Egan et al., 2003). That polymorphism may also affect intelligence (Tsai et al., 2004), and susceptibility to psychiatric disorders including depression, schizophrenia (Rybakowski, 2008) and Alzheimer's disease (Fukumoto et al., 2010), although there are also negative reports; i.e. cognition (Houlihan et al., 2009), memory (Strauss et al., 2004), psychiatric disorders (Naoe et al., 2007; Zhang et al., 2006).

CADPS2 also mediates monoamine transmission. CADPS2, together with its family protein, CADPS1, mediates the refilling of catecholamine to the releasable vesicles, and catecholamine secretion is significantly suppressed in the CADPS1/2 double deficient cells (Liu et al., 2008). Another study supports that CADPS2 is involved in monoamine storage as antibodies against CADPS2 inhibit monoamine sequestration by synaptic vesicles (Brunk et al., 2009). Monoamine-containing neurons project to diverse brain regions including the hippocampus, neocortex, amygdala and neocortex, and regulate the mode of their function (Robbins and Arnsten, 2009). Dopamine neurotransmission is critical for basic reinforcement learning, noradrenalin modulate attention/concentration, while serotonin mediates cognitive flexibility (Kehagia et al., 2010). CADPS2's roles in synaptic functions suggest that CADPS2 may also mediate human brain functions, especially in learning, memory and cognition.

The regulation of learning/memory by CADPS2 could also be developmental. A comprehensive voxelwise genome-wide association study (GWAS) study found that a single nucleotide polymorphism (SNP) in *CADPS2* was associated with brain structure (Stein et al., 2010). In that study, the association between whole voxels from brain images of the 740 elderly subjects and SNPs were

* Corresponding author. Tel.: +81 42 341 2712x5831; fax: +81 42 346 1744.
E-mail address: hattori@ncnp.go.jp (K. Hattori).

0301-0511/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.biopsycho.2011.09.017

analyzed, and the *CADPS2* SNP was found to be associated with temporal lobe volume, the region especially crucial for memory.

There is also a link between *CADPS2* expression and human brain disorders. Aberrant splicing of *CADPS2* mRNA was reported in autism; an exon-3 skipped isoform, *CADPS2ΔExon3* was detected in the peripheral blood samples of several autistic patients but not in those of healthy controls (Sadakata et al., 2007b). The authors showed that CADPS2ΔExon3 protein was deficient in proper axonal transport, which results in the loss of local synaptic BDNF release. While the relationship of *CADPS2ΔExon3* expression in the brains and autism is unclear, the aberrant splicing of *CADPS2* could contribute to autism susceptibility by affecting neurotrophin and/or monoamine release.

Previously, we found that both *CADPS2* and *CADPS2ΔExon3* expression were increased in the post-mortem brains of schizophrenic patients (Hattori et al., 2011). We also detected *CADPS2ΔExon3* in the blood of both schizophrenic patients and control subjects. There were more *CADPS2ΔExon3* positive subjects in the schizophrenic patients than in the controls, although the difference was not statistically significant.

To get more insight into CADPS2's role in human brain function, the present study examined the possible association between the blood expression levels of *CADPS2/CADPS2ΔExon3* and intelligence/memory in healthy subjects. Considering the continuity between developmental disorders and healthy state (Bishop, 1989; Volkmar et al., 2004), intermediate phenotypes related to the developmental disorders should also be expressed in "healthy" subjects and might be associated with *CADPS2/CADPS2ΔExon3* expression levels. We applied quantitative PCR, a more reliable method, to detect each transcript rather than evaluating electrophoresis bands, applied in the past studies (Eran et al., 2009; Sadakata et al., 2007b). As a result, we found that *CADPS2ΔExon3* expression was associated with lower intelligence and memory. To our knowledge, this is a novel finding, which is likely to have relevance to the susceptibility to autism and learning disorders.

2. Subjects and methods

2.1. Participants

Subjects were 271 healthy volunteers [67 males and 204 females; age range 20–74 years; mean age 43.3 ± 15.3 (standard deviation: SD) years] recruited through advertisements in free local magazines and our website announcement. All subjects were biologically unrelated healthy Japanese from the same geographical area (Western part of Tokyo Metropolitan). They were interviewed by the Japanese version of the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Otsubo et al., 2005; Sheehan et al., 1998) by a research psychiatrist, and those who had a current history of psychiatric disorder were not enrolled in the study. In addition, those individuals who demonstrated one or more of the following conditions in a non-structured interview performed by an experienced psychiatrist were excluded from this study: past or current regular contact to psychiatric services, having a history of regular use of psychotropics or substance abuse/dependence, presenting other obvious self-reported signs of past primary psychotic and mood disorders, and having a prior medical history of central nervous system disease or severe head injury. After the nature of the study procedures had been fully explained, written informed consent was obtained from every subject. The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

2.2. Sample preparation

Blood collection and RNA isolation was performed using the PAXgene blood RNA system (Qiagen, Valencia, CA) as described previously (Hattori et al., 2011). Blood samples were collected around 11 A.M. Extracted RNA was quantified by optical density reading at 260 nm using NanoDrop ND-1000 (Thermo Scientific, Rockford, IL). Samples that contained more than 40 ng/μl of total RNA were used for analysis; 8 μl from each sample was reverse transcribed using SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA).

2.3. Quantitative real-time polymerase chain reaction

Polymerase chain reaction (PCR) amplifications were performed in triplicate (5 μl volume) on 384-well plates using ABI prism 7900HT (Applied Biosystems, Foster City, CA) as described previously (Hattori et al., 2011). Each reaction contained 0.28 μl of cDNA sample, qPCR QuickGoldStar Mastermix Plus (Eurogentec, Seraing, Belgium) and a primer of the target, i.e. *CADPS2* (Hs01095968_m1 at Exons 4–5, on NM_017954.9), *CADPS2ΔExon3* (forward primer: GTAGCTGACGAAGCATTTTGCA, reverse Primer: TGATCTGGGCTGCTTGTTCAT, reporter: CTGCGTTATCCAGCTCAT) and a primer of the housekeeping gene, Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (4326317E), all purchased from Applied Biosystems. Negative control reactions were carried out with "no RNA" samples. The real time PCR reactions ran at 50 °C for 2 min, 95 °C for 10 min and in 40 (for *CADPS2* and *GAPDH*) or 45 (for *CADPS2ΔExon3*) cycles changing between 95 °C for 15 s and 60 °C for 1 min. Data were analyzed using the Sequence Detection System (SDS) 2.0 software (Applied Biosystems) as follows. A standard amplification curve was made by serial dilution of a "standard" pooled cDNA sample in each plate. The mean value of triplicate of each sample was normalized to the standard curve. Then the values of *CADPS2* from each sample were normalized to those of *GAPDH*. With respect to *CADPS2ΔExon3*, we counted the number of tubes in which signals were detected, among triplicates, as reported previously (Hattori et al., 2011). In brief, for each tube, we defined 'detected' if the signal reached a threshold automatically set by the SDS 2.0 software within 45 cycles, and a threshold cycle (Ct) value was obtained. Second, we counted the number of 'detected' tubes of each triplicate (Supplemental Fig. S1). Third, we defined 'positive' when 2 or 3 tubes in triplicate analysis of each sample were detected as we assumed that the 'detection' should be repeated at least once. We defined 'negative' when no tube was detected. The samples with only one-tube detection were excluded from the statistical comparison between individuals with *CADPS2ΔExon3* positive and those with *CADPS2ΔExon3* negative. To avoid an arbitrary interpretation, we also performed statistical analyses including one-tube detection and dividing subjects into 3 groups (negative, one-tube detection, and positive).

Table 1
Demographic information and *CADPS2ΔExon3* expression levels of participants.

	N	Age (SD)	Number of tubes with *CADPS2ΔExon3* detection		
			0	1	2–3
WAIS-R					
Male	54	43.5 (15.6)	36	12	6
Female	185	45.8 (14.6)	114	52	19
Total	239	45.2 (14.8)	150	64	25
WMS-R					
Male	67	40.6 (15.5)	46	13	8
Female	199	43.9 (15.2)	122	56	21
Total	266	43.1 (15.3)	168	69	29

2.4. Neuropsychological test measures

To assess memory and intelligence, the Japanese full versions of the Wechsler Memory Scale-Revised (WMS-R) (Sugishita, 2001) and the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Shinagawa et al., 1990) respectively, were administered.

2.5. Statistical analyses

CADPS2 expression levels were converted to a -10 logarithmic scale before statistical analysis in order to obtain a normal distribution (Castensson et al., 2005) as reported previously (Hattori et al., 2011). One extremely high value of *CADPS2* expression was excluded. The relationship between *CADPS2* expression and each score was analyzed by Spearman correlation test. The effect of *CADPS2* or *CADPS2ΔExon3* expression on intelligence or memory was assessed by multiple analysis of covariance (MANCOVA), controlling for age, sex, and education years. These analyses were performed by SPSS software version 11 (SPSS Japan, Tokyo, Japan).

3. Results

First, we analyzed the association between blood *CADPS2* expression and IQ and memory indices. Spearman correlation analyses did not detect any significant correlation between blood *CADPS2* expression levels and IQ scores (Supplemental Table S1). Among WMS-R scores, verbal memory and general memory tended to correlate with *CADPS2* expression levels (Supplemental Table S1). However, no significant effect of *CADPS2* expression was detected on those scores when age, sex and education years were controlled for ($p = 0.15$ for verbal memory and $p = 0.21$ for general memory).

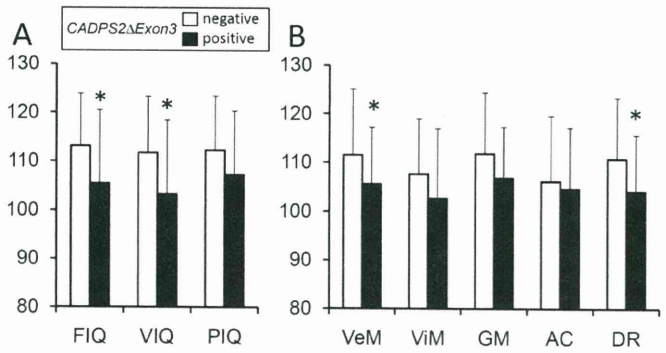

Fig. 1. Association between IQ/memory and *CADPS2 Δ Exon3* expression. WAIS-R scores and WMS-R scores were compared between those who did not (open-bar) and did (filled-bar) express *CADPS2ΔExon3* in the blood. (A) IQ and *CADPS2ΔExon3* expression. FIQ, full-scale IQ; VIQ, verbal IQ; PIQ, performance IQ. (B) Memory and *CADPS2ΔExon3* expression. VeM, verbal memory; ViM, visual memory; GM, general memory; AC, attention and concentration; DR, delayed recall. Data are mean ± SD; $^{*}p < 0.05$, MANCOVA controlled for sex, age, and education years.

Then, we analyzed the possible association of *CADPS2ΔExon3* expression with IQ and memory. As reported previously (Hattori et al., 2011), the expression level was very low and no expression was detected for the majority of samples. Thus, we counted the number of signal-detected tubes among triplicate analyses of each sample (Supplemental Fig. S1, Table 1).

With respect to WAIS-R, full-scale IQ (FIQ) was significantly lower in the *CADPS2ΔExon3* positive group, compared with that of the negative group ($F = 5.3$, df = 1, $p = 0.022$, $\eta_p^2 = 0.031$, Fig. 1A). When verbal IQ (VIQ) and performance IQ (PIQ) were examined separately, VIQ ($F = 5.6$, df = 1, $p = 0.019$, $\eta_p^2 = 0.032$) was significantly lower in the positive group.

With respect to WMS-R, verbal memory ($F = 5.0$, df = 1, $p = 0.026$, $\eta_p^2 = 0.026$) and delayed recall ($F = 4.2$, df = 1, $p = 0.042$, $\eta_p^2 = 0.021$) were significantly lower in the positive group compared with the negative group (Fig. 1B).

Even if one-tube detection was included in the analysis, the results were essentially the same. With respect to WAIS-R, there were marginal effects of *CADPS2ΔExon3* expression levels on FIQ ($F = 2.33$, df = 2, $p = 0.099$, $\eta_p^2 = 0.020$) and VIQ ($F = 2.57$, df = 2, $p = 0.079$, $\eta_p^2 = 0.022$) and the *post hoc* tests detected significant reduction of FIQ ($p = 0.036$) and VIQ ($p = 0.026$) in the positive group compared to negative group (Supplemental Fig. S2A). With respect to WMS-R, significant effects of expression level were detected on verbal memory ($F = 4.5$, df = 2, $p = 0.012$, $\eta_p^2 = 0.034$) and delayed recall ($F = 5.8$, df = 2, $p = 0.003$, $\eta_p^2 = 0.043$). A marginal effect on general memory ($F = 3.0$, df = 2, $p = 0.051$, $\eta_p^2 = 0.023$) was also detected. The *post hoc* tests detected significant reduction of verbal memory ($p = 0.028$) and delayed recall ($p = 0.001$) in the positive group compared to the negative group (Supplemental Fig. S2B).

When males and females were analyzed separately, statistically significant differences were detected only in females with respect to FIQ, VIQ, visual memory, general memory and delayed recall (Supplemental Fig. S3). Nonetheless, average scores of these tests were lower in the *CADPS2ΔExon3* positive group of the male subjects than in the negative group. The failure to reach statistical significance is likely to be ascribed to the lack of statistical power due to the small number of male subjects.

It is possible that we might have removed cognitive ability variance when education years were controlled for. To examine this possibility, we performed an additional analysis in which education was not controlled for. However, the results were essentially unchanged; *CADPS2ΔExon3* expression levels were significantly associated with FIQ ($F = 6.3$, df = 1, $p = 0.013$, $\eta_p^2 = 0.036$), VIQ ($F = 6.7$, df = 1, $p = 0.011$, $\eta_p^2 = 0.038$), verbal memory ($F = 5.1$, df = 1, $p = 0.025$, $\eta_p^2 = 0.026$) and delayed recall ($F = 4.5$, df = 1, $p = 0.035$, $\eta_p^2 = 0.023$).

4. Discussion

In the present study, we examined the possible association between the expression of *CADPS2* transcripts (*CADPS2* and *CADPS2ΔExon3*) in the peripheral blood and higher brain functions such as intelligence and memory in healthy subjects. While *CADPS2* expression levels were not associated with the scores of these measurements, *CADPS2ΔExon3* expression was significantly associated with lower IQ, lower verbal memory and delayed recall of WMS-R.

4.1. Evaluation of CADPSΔExon3 expression levels

Because there were relatively large number of 1-tube detection samples, we suppose that there are continuity between negative and positive samples, and 1-tube detection might stochastically reflects the expression levels between negative (0) and positive (>1). Since inclusion or exclusion of 1-tube detection samples in the criteria did not affect the results essentially, our conclusion; the expression of *CADPS2Δ3* was associated with cognition and memory, was supported. However, because the expression levels might continuous rather than qualitative values, future studies should improve the sensitivity of analyses, i.e. by using larger sample volume.

4.2. Did the participants include autism?

It has been reported that *CADPS2ΔExon3* was present in individuals with autism but not in controls (Sadakata et al., 2007b). In the present study, all participants were screened for current and past psychiatric histories by experienced psychiatrists using structured (M.I.N.I.) and unstructured interviews. As the M.I.N.I. is not designed to diagnose autism, there remains the possibility that some patients with mild, high functioning autism could have been included. However, interviews by experienced psychiatrists did not detect any subject who could be diagnosed as autism or other pervasive developmental disorders. Thus, our results suggest that *CADPS2ΔExon3* may be positive even in non-autistic individuals. Rather, *CADPS2ΔExon3* is likely to be present in individuals with lower VIQ and lower memory function which may be intermediate phenotypes of autism (see below).

4.3. Autism and intelligence

Approximately three-quarters of individuals with autism have low (<70) full-IQ scores (Yeargin-Allsopp et al., 2003). With respect to profiles of IQ, Lincoln et al. reported depressed verbal IQ relative to performance IQ (VIQ < PIQ) in autism (Lincoln et al., 1988), although inconsistent findings (no difference or VIQ < PIQ) have also been reported (Ehlers et al., 1997; Siegel et al., 1996; Williams et al., 2008). Thus, *CADPS2ΔExon3* positive subjects partly share similar cognitive deficits with autism.

4.4. Autism and memory

Similar to *CADPS2ΔExon3* positive subjects, adults with high functioning autism were reported to have impaired memory functions (Bennetto et al., 1996; Minshew and Goldstein, 2001; Steele et al., 2007; Williams et al., 2005b). Not all but several studies have shown that the impairments were prominent in visual memories especially for human faces rather than verbal memories (Hillier et al., 2007; Williams et al., 2005a, 2006). In the case of *CADPS2ΔExon3* positive subjects in the present study,

although visual memory also tended to be lower, the significant reduction was rather detected in verbal memory. The different memory profiles between previous findings in autistic adults and our *CADPS2ΔExon3*-positive subjects might be partly due to the reduced IQ in our *CADPS2ΔExon3* positive subjects, while the previous studies compared memory between patients with high functioning autism and IQ-matched controls.

4.5. Implications on schizophrenia susceptibility

Previously, we analyzed *CADPS2/CADPS2ΔExon3* expression both in the post-mortem brains of psychiatric patients from the Stanley neuropathology consortium, consisting of 15 patients with schizophrenia, 15 with depression, 15 with bipolar disorder and 15 control subjects and bloods of 121 schizophrenic patients and 318 controls (Hattori et al., 2011). In the brain samples, we found that not only *CADPS2* but also *CADPS2ΔExon3* was significantly increased in the schizophrenic group. These changes were not observed in other psychiatric disorders. We also found that the ratio of blood *CADPS2ΔExon3* positive subjects was higher in the schizophrenic group (21 out of 121 patients, ratio = 0.17) compared to the control group (36 out of 318, ratio= 0.11), although the difference in the ratio was not statistically significant.

Of all cognitive domains, verbal memory is one of the most frequently and severely affected in schizophrenia (Aleman et al., 1999; Heinrichs and Zakzanis, 1998; Leeson et al., 2009). Thus, *CADPS2ΔExon3* positive subjects and schizophrenic patients share a similar phenotype. On the other hand, with respect to IQ, many studies showed PIQ is lower than VIQ in schizophrenic patients (Amminger et al., 2000; Aylward et al., 1984), suggesting that *CADPS2ΔExon3* positive subjects also have a different feature to schizophrenia.

Because autism and schizophrenia are supposed to be heterogeneous disorders, *CADPS2ΔExon3* could be a susceptibility marker for a sub-type, especially the patients with affected verbal functions. Alternatively, *CADPS2ΔExon3* expression might be merely related to verbal functions or associated with verbal learning disorder.

4.6. How does blood CADPS2ΔExon3 affect brain function?

The mechanism of how peripheral *CADPS2ΔExon3* expression affects brain functions is unclear. Although *CADPS2ΔExon3* was shown to express in the human brain (Eran et al., 2009; Hattori et al., 2011), there is no evidence that blood *CADPS2ΔExon3* expression reflects its high expression in the brain. In case of autism, high *CADPS2ΔExon3* expression might have genetic basis as *CADPS2* has been suggested to be a susceptibility gene for autism (Cisternas et al., 2003). Sadakata et al. (2007b) found several non-synonymous SNPs in *CADPS2* from autistic patients but such SNPs were not detected in healthy subjects. Although CADPS2ΔExon3 retains BDNF releasing activity, it lacks ability to be transported to axons, which would result in the loss of local synaptic BDNF release (Sadakata et al., 2007b). Therefore, higher *CADPS2ΔExon3* expression in the brain, may affect BDNF release through the dominant-negative effect.

CADPS2 mediates the release of monoamine neurotransmission as well. CADPS2 promotes monoamine uptakes and storage (Brunk et al., 2009; Liu et al., 2008) and mediates priming process of monoamine-containing dense core vesicles, so that it facilitates Ca^{2+}-triggered release of neurotransmitters (Jockusch et al., 2007). Therefore, it is also plausible that *CADPS2ΔExon3* expression affects brain function through altered monoamine transmission.

Among monoamines, dopamine's roles on cognition and learning have been established by both animal and human studies (Kehagia et al., 2010). The dopamine neurotransmission in the hippocampus and the prefrontal cortex plays an essential role in working memory (Goldman-Rakic, 1998) and that in striatum also mediates reinforcement and reversal learning (Kehagia et al., 2010). Several studies suggest a link between human verbal function and dopamine D2 receptor. Striatal dopamine D2/D3 receptor availability was reported to correlate with VIQ assessed by WAIS-R (Guo et al., 2006). Hippocampal D2/D3 receptor availability was also related to verbal memory (Takahashi et al., 2007). In addition, dopamine release was enhanced in the frontal cortex, the amygdala and the hippocampus during verbal working memory task (Aalto et al., 2005). Thus, the features of *CADPS2ΔExon3* positive subjects have some similarity with deficits in the D2 receptor function. Because CADPS2 is highly expressed in the dopamine-rich brain areas such as ventral tegmental area and substantia nigra of mice brain (Sadakata et al., 2006), and it is reported to interact with dopamine D2 receptor (Binda et al., 2005), the features of *CADPS2ΔExon3* positive subjects could be ascribed, at least in part, to impaired dopamine transmission.

Although no significant association was detected between wild-type *CADPS2* expression levels and WMS-R scores, *CADPS2* expression level tended to correlate with verbal/general memory. Thus, CADPS2 and CADPS2ΔExon3 might have opposite effects on memory, which is consistent with the above discussion about CADPS2/CADPS2ΔExon3 functions. On the other hand, with respect to intelligence, the mechanism of CADPS2/CADPS2ΔExon3 effects might be more complicated or CADPS2ΔExon3 might have functions independent from wild-type CADPS2.

4.7. Limitation

A limitation is that the mean IQ of our sample was relatively high (mean full-scale IQ: 111.7 ± 12.1). This may have arisen by the geographic area of the sample (i.e. Western part of Tokyo Metropolitan) because average income in Tokyo is approximately 20% higher than national average (Ministry of Health Labour and Welfare, 2009) and income correlates with education level. Therefore, our sample was overrepresented by individuals with higher IQ relative to the Japanese population as a whole. This may have missed the possible effects of *CADPS2* and *CADPS2ΔExon3* on brain functions in people with relatively low IQ.

5. Conclusion

We found that expression of the splice variant of *CADPS2*, *CADPS2ΔExon3*, in the peripheral blood is associated with human brain functions such as intelligence and memory. Those individuals who expressed *CADPS2ΔExon3* had lower IQ and memory. Because CADPS2 mediates the release of BDNF and monoamines, impaired function of CADPS2 due to CADPS2ΔExon3 might exert detrimental effects on BDNF and monoamine functions which are crucial in the brain development and higher brain functions. Further studies to elucidate the mechanisms of production of CADPS2ΔExon3 and its effects at the molecular level are warranted.

Acknowledgements

This work was supported by a Health and Labor Science Research Grant (H21-KOKORO-WAKATE-20 and H21-KOKORO-001), CREST of JST, Grant-in-Aid for Scientific Research (KAKENHI, 22591269), Intramural Research Grant for Neurological and Psychiatric Disorders of NCNP, Takeda Science Foundation, and Mitsubishi Pharma Research Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biopsycho.2011.09.017.

References

Aalto, S., Bruck, A., Laine, M., Nagren, K., Rinne, J.O., 2005. Frontal and temporal dopamine release during working memory and attention tasks in healthy humans: a positron emission tomography study using the high-affinity dopamine D2 receptor ligand [11C]FLB 457. Journal of Neuroscience 25, 2471–2477.

Aleman, A., Hijman, R., de Haan, E.H., Kahn, R.S., 1999. Memory impairment in schizophrenia: a meta-analysis. American Journal of Psychiatry 156, 1358–1366.

Amminger, G.P., Schlogelhofer, M., Lehner, T., Looser Ott, S., Friedrich, M.H., Aschauer, H.N., 2000. Premorbid performance IQ deficit in schizophrenia. Acta Psychiatrica Scandinavica 102, 414–422.

Aylward, E., Walker, E., Bettes, B., 1984. Intelligence in schizophrenia: meta-analysis of the research. Schizophrenia Bulletin 10, 430–459.

Bennetto, L., Pennington, B.F., Rogers, S.J., 1996. Intact and impaired memory functions in autism. Child Development 67, 1816–1835.

Binda, A.V., Kabbani, N., Levenson, R., 2005. Regulation of dense core vesicle release from PC12 cells by interaction between the D2 dopamine receptor and calcium-dependent activator protein for secretion (CAPS). Biochemical Pharmacology 69, 1451–1461.

Bishop, D.V., 1989. Autism Asperger's syndrome and semantic-pragmatic disorder: where are the boundaries? British Journal of Disorders of Communication 24, 107–121.

Brunk, I., Blex, C., Speidel, D., Brose, N., Ahnert-Hilger, G., 2009. Ca2+-dependent activator proteins of secretion promote vesicular monoamine uptake. Journal of Biological Chemistry 284, 1050–1056.

Castensson, A., Aberg, K., McCarthy, S., Saetre, P., Andersson, B., Jazin, E., 2005. Serotonin receptor 2C (HTR2C) and schizophrenia: examination of possible medication and genetic influences on expression levels. American Journal of Medical Genetics B: Neuropsychiatry and Genetics 134B, 84–89.

Cisternas, F.A., Vincent, J.B., Scherer, S.W., Ray, P.N., 2003. Cloning and characterization of human CADPS and CADPS2, new members of the Ca^{2+}-dependent activator for secretion protein family. Genomics 81, 279–291.

Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., Weinberger, D.R., 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112, 257–269.

Ehlers, S., Nyden, A., Gillberg, C., Sandberg, A.D., Dahlgren, S.O., Hjelmquist, E., Oden, A., 1997. Asperger syndrome, autism and attention disorders: a comparative study of the cognitive profiles of 120 children. Journal of Child Psychology and Psychiatry and Allied Disciplines 38, 207–217.

Eran, A., Graham, K.R., Vatalaro, K., McCarthy, J., Collins, C., Peters, H., Brewster, S.J., Hanson, E., Hundley, R., Rappaport, L., Holm, I.A., Kohane, I.S., Kunkel, L.M., 2009. Comment on "Autistic-like phenotypes in Cadps2-knockout mice and aberrant CADPS2 splicing in autistic patients". Journal of Clinical Investigation 119, 671–680, 679–680; author reply.

Fukumoto, N., Fujii, T., Combarros, O., Kamboh, M.I., Tsai, S.J., Matsushita, S., Nacmias, B., Comings, D.E., Arboleda, H., Ingelsson, M., Hyman, B.T., Akatsu, H., Grupe, A., Nishimura, A.L., Zatz, M., Mattila, K.M., Rinne, J., Goto, Y., Asada, T., Nakamura, S., Kunugi, H., 2010. Sexually dimorphic effect of the Val66Met polymorphism of BDNF on susceptibility to Alzheimer's disease: new data and meta-analysis. American Journal of Medical Genetics B: Neuropsychiatry and Genetics 153B, 235–242.

Goldman-Rakic, P.S., 1998. The cortical dopamine system: role in memory and cognition. Advances in Pharmacology 42, 707–711.

Guo, J.F., Kuang Yang, Y., Tsing Chiu, N., Lieh Yeh, T., See Chen, P., Lee, I.H., Lin Chu, C., 2006. The correlation between striatal dopamine D2/D3 receptor availability and verbal intelligence quotient in healthy volunteers. Psychological Medicine 36, 547–554.

Hattori, K., Tanaka, H., Wakabayashi, C., Yamamoto, N., Uchiyama, H., Teraishi, T., Hori, H., Arima, K., Kunugi, H., 2011. Expression of Ca(2+)-dependent activator protein for secretion 2 is increased in the brains of schizophrenic patients. Progress in Neuro-Psychopharmacology and Biological Psychiatry 35, 1738–1743.

Heinrichs, R.W., Zakzanis, K.K., 1998. Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. Neuropsychology 12, 426–445.

Hillier, A., Campbell, H., Keillor, J., Phillips, N., Beversdorf, D.Q., 2007. Decreased false memory for visually presented shapes and symbols among adults on the autism spectrum. Journal of Clinical and Experimental Neuropsychology 29, 610–616.

Houlihan, L.M., Harris, S.E., Luciano, M., Gow, A.J., Starr, J.M., Visscher, P.M., Deary, I.J., 2009. Replication study of candidate genes for cognitive abilities: the Lothian Birth Cohort 1936. Genes, Brain and Behaviour 8, 238–247.

Jockusch, W.J., Speidel, D., Sigler, A., Sorensen, J.B., Varoqueaux, F., Rhee, J.S., Brose, N., 2007. CAPS-1 and CAPS-2 are essential synaptic vesicle priming proteins. Cell 131, 796–808.

Kehagia, A.A., Murray, G.K., Robbins, T.W., 2010. Learning and cognitive flexibility: frontostriatal function and monoaminergic modulation. Current Opinion in Neurobiology 20, 199–204.

Leeson, V.C., Barnes, T.R., Hutton, S.B., Ron, M.A., Joyce, E.M., 2009. IQ as a predictor of functional outcome in schizophrenia: a longitudinal, four-year study of first-episode psychosis. Schizophrenia Research 107, 55–60.

Lincoln, A.J., Courchesne, E., Kilman, B.A., Elmasian, R., Allen, M., 1988. A study of intellectual abilities in high-functioning people with autism. Journal of Autism and Developmental Disorders 18, 505–524.

Liu, Y., Schirra, C., Stevens, D.R., Matti, U., Speidel, D., Hof, D., Bruns, D., Brose, N., Rettig, J., 2008. CAPS facilitates filling of the rapidly releasable pool of large dense-core vesicles. Journal of Neuroscience 28, 5594–5601.

Ministry of Health, Labour and Welfare, 2009. Basic Survey on Wage Structure, http://www.e-stat.go.jp/SG1/estat/List.do?bid=000001025026&cycode=0.

Minshew, N.J., Goldstein, G., 2001. The pattern of intact and impaired memory functions in autism. Journal of Child Psychology and Psychiatry and Allied Disciplines 42, 1095–1101.

Mizuno, M., Yamada, K., Olariu, A., Nawa, H., Nabeshima, T., 2000. Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. Journal of Neuroscience 20, 7116–7121.

Monteggia, L.M., Barrot, M., Powell, C.M., Berton, O., Galanis, V., Gemelli, T., Meuth, S., Nagy, A., Greene, R.W., Nestler, E.J., 2004. Essential role of brain-derived neurotrophic factor in adult hippocampal function. Proceedings of the National Academy of Sciences of the United States of America 101, 10827–10832.

Naoe, Y., Shinkai, T., Hori, H., Fukunaka, Y., Utsunomiya, K., Sakata, S., Matsumoto, C., Shimizu, K., Hwang, R., Ohmori, O., Nakamura, J., 2007. No association between the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and schizophrenia in Asian populations: evidence from a case-control study and meta-analysis. Neuroscience Letters 415, 108–112.

Otsubo, T., Miyaoka, H., Kamijima, K. (Eds.), 2005. M.I.N.I. Mini International Neuropsychiatric Interview. Seiwa Shoten Publishers, Tokyo.

Robbins, T.W., Arnsten, A.F., 2009. The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. Annual Review of Neuroscience 32, 267–287.

Rybakowski, J.K., 2008. BDNF gene: functional Val66Met polymorphism in mood disorders and schizophrenia. Pharmacogenomics 9, 1589–1593.

Sadakata, T., Itakura, M., Kozaki, S., Sekine, Y., Takahashi, M., Furuichi, T., 2006. Differential distributions of the Ca^{2+}-dependent activator protein for secretion family proteins (CAPS2 and CAPS1) in the mouse brain. Journal of Comparative Neurology 495, 735–753.

Sadakata, T., Kakegawa, W., Mizoguchi, A., Washida, M., Katoh-Semba, R., Shutoh, F., Okamoto, T., Nakashima, H., Kimura, K., Tanaka, M., Sekine, Y., Itohara, S., Yuzaki, M., Nagao, S., Furuichi, T., 2007a. Impaired cerebellar development and function in mice lacking CAPS2, a protein involved in neurotrophin release. Journal of Neuroscience 27, 2472–2482.

Sadakata, T., Mizoguchi, A., Sato, Y., Katoh-Semba, R., Fukuda, M., Mikoshiba, K., Furuichi, T., 2004. The secretory granule-associated protein CAPS2 regulates neurotrophin release and cell survival. Journal of Neuroscience 24, 43–52.

Sadakata, T., Washida, M., Iwayama, Y., Shoji, S., Sato, Y., Ohkura, T., Katoh-Semba, R., Nakajima, M., Sekine, Y., Tanaka, M., Nakamura, K., Iwata, Y., Tsuchiya, K.J., Mori, N., Detera-Wadleigh, S.D., Ichikawa, H., Itohara, S., Yoshikawa, T., Furuichi, T., 2007b. Autistic-like phenotypes in Cadps2-knockout mice and aberrant CADPS2 splicing in autistic patients. Journal of Clinical Investigation 117, 931–943.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. Journal of Clinical Psychiatry 59 (Suppl. 20), 34–57, 22–33; quiz.

Shinagawa, F., Kobayashi, S., Fujita, K., Maekawa, H., 1990. Japanese Wechsler Adult Intelligence Scale-Revised. Nihonbunkakagakusha, Tokyo.

Siegel, D.J., Minshew, N.J., Goldstein, G., 1996. Wechsler IQ profiles in diagnosis of high-functioning autism. Journal of Autism and Developmental Disorders 26, 389–406.

Steele, S.D., Minshew, N.J., Luna, B., Sweeney, J.A., 2007. Spatial working memory deficits in autism. Journal of Autism and Developmental Disorders 37, 605–612.

Stein, J.L., Hua, X., Lee, S., Ho, A.J., Leow, A.D., Toga, A.W., Saykin, A.J., Shen, L., Foroud, T., Pankratz, N., Huentelman, M.J., Craig, D.W., Gerber, J.D., Allen, A.N., Corneveaux, J.J., Dechairo, B.M., Potkin, S.G., Weiner, M.W., Thompson, P., 2010. Voxelwise genome-wide association study (vGWAS). Neuroimage 53, 1160–1174.

Strauss, J., Barr, C.L., George, C.J., Ryan, C.M., King, N., Shaikh, S., Kovacs, M., Kennedy, J.L., 2004. BDNF and COMT polymorphisms: relation to memory phenotypes in young adults with childhood-onset mood disorder. Neuromolecular Medicine 5, 181–192.

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

Takahashi, H., Kato, M., Hayashi, M., Okubo, Y., Takano, A., Ito, H., Suhara, T., 2007. Memory and frontal lobe functions; possible relations with dopamine D2 receptors in the hippocampus. Neuroimage 34, 1643–1649.

Tsai, S.J., Hong, C.J., Yu, Y.W., Chen, T.J., 2004. Association study of a brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and personality trait and intelligence in healthy young females. Neuropsychobiology 49, 13–16.

Volkmar, F.R., Lord, C., Bailey, A., Schultz, R.T., Klin, A., 2004. Autism and pervasive developmental disorders. Journal of Child Psychology and Psychiatry and Allied Disciplines 45, 135–170.

Williams, D.L., Goldstein, G., Carpenter, P.A., Minshew, N.J., 2005a. Verbal and spatial working memory in autism. Journal of Autism and Developmental Disorders 35, 747–756.

Williams, D.L., Goldstein, G., Kojkowski, N., Minshew, N.J., 2008. Do individuals with high functioning autism have the IQ profile associated with nonverbal learning disability? Research in Autism Spectrrm Disorders 2, 353–361.

Williams, D.L., Goldstein, G., Minshew, N.J., 2005b. Impaired memory for faces and social scenes in autism: clinical implications of memory dysfunction. Archives of Clinical Neuropsychology 20, 1–15.

Williams, D.L., Goldstein, G., Minshew, N.J., 2006. The profile of memory function in children with autism. Neuropsychology 20, 21–29.

Yeargin-Allsopp, M., Rice, C., Karapurkar, T., Doernberg, N., Boyle, C., Murphy, C., 2003. Prevalence of autism in a US metropolitan area. Journal of the American Medical Association 289, 49–55.

Zhang, H., Ozbay, F., Lappalainen, J., Kranzler, H.R., van Dyck, C.H., Charney, D.S., Price, L.H., Southwick, S., Yang, B.Z., Rasmussen, A., Gelernter, J., 2006. Brain derived neurotrophic factor (BDNF) gene variants and Alzheimer's disease, affective disorders, posttraumatic stress disorder, schizophrenia, and substance dependence. American Journal of Medical Genetics B: Neuropsychiatry and Genetics 141B, 387–393.

脳脊髄液（CSF）からみた統合失調症のモノアミン代謝産物の解析

服部功太郎*,**　寺石　俊也*　篠山　大明*　功刀　浩*

抄録：1970年代以降，統合失調症のドーパミン仮説を検討するために，脳脊髄液（CSF）を用いたモノアミン代謝産物の解析が行われてきた。薬剤フリーの統合失調症CSFを用いた研究において，ドーパミン代謝産物であるホモバニリン酸（HVA）は，健常対照群と同等という報告が多く，減少しているとする報告も散見された。このことから統合失調症の脳において，少なくともドーパミン放出量は増加していないことが示唆された。また，薬剤フリーの統合失調症患者に抗精神病薬の投与を数週間行うと，CSF中のHVAは投与前に比べて上昇し，HVA上昇と陽性症状の改善とが相関するという報告も多かった。このようにCSFモノアミン代謝の解析は統合失調症分子病態の解明には貢献するが，臨床マーカーとしての有用性は，現状では限られる。しかし，蛋白質解析技術の進歩に従い，近年CSFの脳神経疾患のバイオリソースとしての価値が再評価されており，統合失調症バイオマーカーの開発に役立つことが期待される。

精神科治療学　26(12)：1557-1563，2011

Key words：*cerebrospinal fluid, schizophrenia, dopamine, homovanillic acid*

I．はじめに

脳脊髄液（cerebrospinal fluid：CSF）は脳室内や脳・脊髄のくも膜下腔に約150m*l*存在し，1日約500m*l*脈絡叢などで産生され，矢状洞などから吸収される（図1）。CSF検査は現在でも，髄膜炎，脳炎，多発性硬化症，正常圧水頭症などの診療で日常的に用いられているが，扱う疾患の変化や無侵襲の脳画像検査が進歩したこともあり，特に精神科診療では，以前に比べ実施されることが少なくなった。一方，CSFは主に脳の周りを循環し，一部は脳実質からも産生されるため脳由来の物質を多く含んでおり，血液に比べ他の臓器の影響が少ない。さらに質量分析法をはじめとする解析技術が進歩したこと等から，近年，脳神経疾患のバイオリソースとしての有用性が再認識されている。たとえばアルツハイマー型認知症の診断においては，各国で大規模な研究が行われ，感度・特異度とも90%以上のマーカー（タウ，リン

Monoamine metabolites in the cerebrospinal fluid of patients with schizophrenia.
*国立精神・神経医療研究センター神経研究所疾病研究第3部
〔〒187-8502　東京都小平市小川東町4-1-1〕
Kotaro Hattori, M.D., Ph.D., Toshiya Teraishi, M.D., Daimei Sasayama, M.D., Hiroshi Kunugi, M.D.: Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry. 4-1-1, Ogawahigashi-cho, Kodaira-shi, Tokyo, 187-8502 Japan.
**国立精神・神経医療研究センタートランスレーショナル・メディカル・センター
Kotaro Hattori, M.D., Ph.D.: Translational Medical Center, National Center of Neurology and Psychiatry.

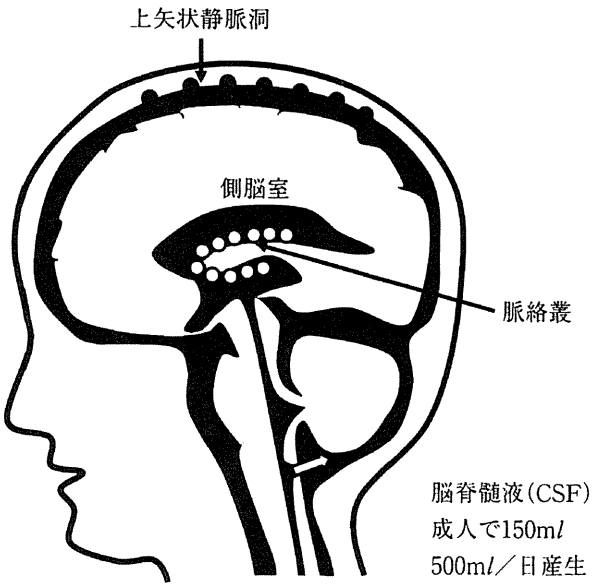

図1　頭蓋内における脳脊髄液の分布

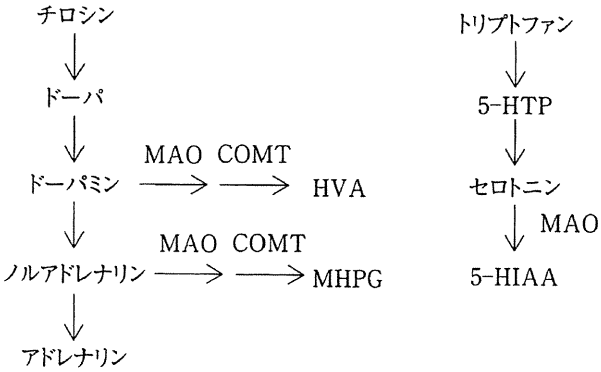

図2　主なアミン系伝達物質の代謝経路
MAO：モノアミン酸化酵素，COMT：カテコール-O-メチル基転移酵素

酸化タウ，アミロイドβ等）が開発されている[2]。もし，統合失調症などの精神科疾患の病態にも，ある程度広範な分子の変化が伴うならば，CSFにも反映される可能性がある。

本稿では，これまでの統合失調症のCSFにて最も多く調べられてきた，モノアミン代謝産物の研究を概観し，統合失調症分子病態における意義や臨床的な意義を考察したうえで，今後の統合失調症CSF研究の方向性についても触れたい。

Ⅱ．モノアミン系伝達物質の代謝経路

中枢神経系における主要なモノアミン系伝達物質であるドーパミン，ノルアドレナリン，セロトニンの代謝経路を図2に示す。ホモバニリン酸（HVA）はドーパミンの最終代謝産物であり，CSF中のHVA値は脳内のドーパミン放出量を反映していると考えられている。同様にCSF中の3-メトキシ-4-ハイドロキシフェニルエチレングリコール（MHPG）は脳内ノルアドレナリン，5-ハイドロキシインドール酢酸（5-HIAA）はセロトニンの放出量を反映すると考えられている。

ドーパミン，ノルアドレナリン，セロトニンは血液脳関門（および血液CSF関門）を通過しない。HVA，5-HIAAは中枢から血液への移行はあるが，血液からCSFへは移行しない。MHPGは血液脳関門を通過するが，ノルアドレナリンは末梢では主にVMAに代謝され，やはり中枢から末梢血に移行することはあっても（末梢血中のMHPGの大半は中枢神経由来），血液のCSFへの影響は小さい。また，HVA，5-HIAAおよびMHPGの濃度は脳室内から腰椎CSFに至るまでに暫減し，脊柱管内のCSFの流れを中途で阻害すると，そこから下流のCSF中では濃度が低下することなどから，腰椎CSF中のHVA，5-HIAA，MHPGは主に脳に由来すると考えられている。さらに動物実験にて，脳内のドーパミン放出を促進するとCSF中のHVA値が上昇すること，黒質等ドーパミン産生部位の破壊により低下すること等，脳内のモノアミン系伝達物質の放出と，CSF中の代謝産物を結び付ける証拠が蓄積されている。

Ⅲ．研究方法

被検者は病院内に掲示したポスターやフリーペーパー等を通じて募集し，図や写真を用いて，研究目的や検体の流れ，副作用の頻度等について説明し，書面にて同意を得ている。同意は検査中・検査後であっても撤回できることを伝え，またいったん検査を予約した場合でも，検査直前に意思の確認を再度行っている。

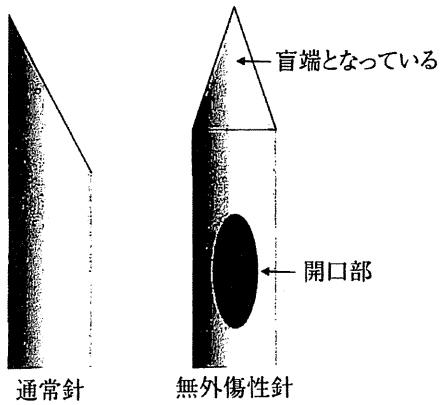

図3　穿刺針先端部の模式図

研究目的で腰椎穿刺を行う場合，臨床上必要な検査よりも厳しい安全性の確保と苦痛・副作用の軽減が求められる。我々は必要な問診を行ったのち，Jolt accentuation（頭を左右に素早く振って頭痛の出現をみる。感度がきわめて高い）にて髄膜兆候を除外し，眼底検査（乳頭浮腫だけでなく，可能な限り眼底静脈の拍動を確認する）にて頭蓋内圧亢進を除外している。WelchAllyn社のパンオプティック眼底鏡は，熟練者でなくとも広い視野を得ることができ便利である。

丁寧に麻酔を行えば，腰椎穿刺は実はそれほど「痛い」検査ではない。これまでの被検者の多くから，穿刺の痛みは採血と同等以下であったという感想を得ている。我々は，まず皮内・皮下の麻酔を29G針で行い，さらに靭帯などの穿刺経路の麻酔を23G針にて十分に時間をかけて行うようにしている。通常，最大の痛みは最初の皮膚の穿刺時に生じ，靭帯部分で痛みが生じることは少ない。本穿刺の際に，万一，左右の神経根に触れると電撃痛が生じるため，矢状面から針が逸れないよう極力注意を払っている。また，穿刺後頭痛のリスクが高い若年者に対しては，できるだけ先端が円錐形の無外傷性針（図3，ユニシス社，UNIEVER穿刺針など）を使用している。これは頭痛の発生を半減すると報告されているが[5]，先端が鋭利でないため，力をかけると針が湾曲して矢状面より逸れやすく少し熟練を要する。

検査後の安静は穿刺後頭痛の有無に関係しないと報告されているため[5]，我々も長時間の安静は不要であると考えている。一方，脱水はCSFの産生低下，圧力低下をもたらすため，穿刺前から十分に水分を摂取するよう勧めている。外来で穿刺を行った場合は，24時間連絡可能な電話番号を被検者に伝え，電話応対から往診・入院まで行う体制を整えている。モノアミン代謝産物の解析は高速液体クロマトグラフィー（HPLC）法による測定を業者に委託している。

Ⅳ．今までの研究の歴史
—どこまでわかったか—

1．統合失調症でドーパミンは増えているのか，減っているのか？

統合失調症CSFを用いた研究は意外に古く，すでに1940年代には行われていた。研究が盛んになったのは1970年代以降で，ドーパミンが精神症状や抗精神病薬の作用に関わることが示されてからである。実験動物にドーパミンの放出を促進する覚醒剤を投与すると，盛んに動きまわったり，首を左右に繰り返し振ったり（常同行動）といった行動変化が現れるが，抗精神病薬投与（ドーパミン受容体拮抗薬）で，それらの行動変化は改善する。このような現象から統合失調症の脳内ではドーパミンが過度に放出されているのではないかというドーパミン過剰説が生まれた。そのドーパミン過剰説を検証する手段の一つとして1970～90年代に薬剤フリーの統合失調症患者を対象としたモノアミン代謝産物の解析が行われた（表1）。当初の仮説に反し，いずれの研究においてもCSF中のHVAの亢進は認められず，むしろ減少しているという報告もいくつかみられた。すなわち，薬剤フリーの統合失調症ではドーパミン放出は少なくとも増えていないことが示唆された。

一方，脳画像の研究ではドーパミン過剰説を裏付ける報告も相次いだ。たとえば，ドーパミンの前駆物質を標識しPETで解析したところ統合失調症では線条体でドーパミンの蓄積が増えており，ドーパミン合成が亢進していると考えられた[4]。また，あらかじめ標識物質をドーパミンD2受容体に結合させておき，アンフェタミン（覚醒剤，前シナプスのドーパミン放出を促進し再取り込み

表1　未治療もしくは薬剤フリーの統合失調症患者を対象としたCSFモノアミンの主要な研究

著者，発表年	診断基準	対象人数		薬剤フリー期間	健常群との比較結果		
		健常者	統合失調症		HVA	5-HIAA	MHPG
Wieselgren and Lindström, 1998	DSM-Ⅲ-R	47	90	2週間以上	↓	→	
Hsiao et al., 1993	DSM-Ⅲ-R RDC	33	50	2週間以上	→	→	↑
Csernansky et al., 1990	RDC	9	21*	2週間以上	→	→	
Lewine et al., 1991	DSM-Ⅲ	91	45	平均7日	→	→	
Lindström, 1985	RDC	21	40	2週間以上	↓	→	
Bjerkenstedt et al., 1985	RDC	65	37	3日以上	↓	→	↑
Nybäck et al., 1983	RDC	43	26*	3週間以上	→	→	→
Gattaz et al., 1982	RDC	16	28	4週間以上	→	↓	→
Post et al., 1975	DSM-Ⅱ	10	20*	2週間以上	→	→	→
Gottfries et al., 1971	—	7	7	4日以上	→		
		12	12			→	
Persson and Roos, 1969	—	29	40	4日	→	→	

RDC：研究用診断基準，*統合失調感情障害を若干名含む，P＝0.05以下を有意とした。Probenecidを使用している研究は除外した。

を阻害する）でドーパミンを放出させた際に，どれくらい標識物質が剥がれ落ちるかSPECTで測定したところ，統合失調症は健常者の約2倍剥がれ落ちた。すなわち，アンフェタミンで放出を促した際のドーパミン放出量は統合失調症の方が多いと考えられた[3]。

CSFの所見と脳画像の所見からどのような説明が可能であろうか。一つには，脳の部位によってドーパミン放出が異なるという仮説がある。すなわち，線条体では放出が亢進し，陽性症状の基盤となる一方，大脳皮質では放出が低下し，前頭葉機能低下の基盤となっている。そして，それらの合計となるため，CSF中のHVAは変わらない，ないし大脳皮質の方が大きいので若干減少しているという仮説である。動物実験でも前頭葉のドーパミン阻害によって，線条体のドーパミン放出が増すこと等が示されており，この仮説は広く支持されている。

しかし，ヒトの死後脳やCSFの研究からは，この仮説と矛盾する証拠も提示されている。すなわち，線条体は大脳皮質より100倍程度ドーパミンおよびHVAの濃度が高く[1]，脳室CSF中のHVA値は線条体組織中のドーパミン量と相関があることが判明している[6]。このことから線条体がCSF中HVAの主要な供給源である可能性が高く，線条体においてもドーパミン放出量は上昇していないかもしれない。合成が亢進しており，放出が変わらないか低下しているとすれば，プレシナプスに蓄積しているとも考えられる。もし，プレシナプスに蓄積しているのであれば，覚醒剤で放出させた際の放出が増加していることも説明できるため，これも有力な仮説の一つである。

統合失調症では前頭葉のドーパミン代謝が変化しているという仮説もある[7]。線条体においては，

シナプス間隙におけるドーパミン量は，再取り込みやモノアミン酸化酵素（MAO）で調節されているのに対し，前頭葉ではカテコール-O-メチル基転移酵素（COMT）により調節される（図2）。COMTは統合失調症と関係の深い遺伝子領域22q11に存在し，COMTの活性に影響を及ぼす遺伝子多型と疾患の関連が解析されてきた。しかし，現在までのところ，関連解析における疾患と対照の差はほとんど認められていない。

その他，病期によって変化するという説，前シナプスにあるドーパミン受容体が関与するという説，ドーパミン放出の時間的・空間的なパターンで考えるべきであるという説等もある。

2. 抗精神病薬治療のCSF中HVAに与える影響

次に，治療薬（抗精神病薬）のCSF中モノアミン代謝産物に対する効果を検討する。表2に示すように，大半の研究で4週間以上の抗精神病薬投与はCSF中のHVA値を増加させる。さらに，複数の研究で治療後のHVA値の上昇と，PANSSの陽性症状とは負の相関を示すことが示されており，HVAが上昇した人ほど，薬が「効いている」ことが示唆される。抗精神病薬がHVAを上昇させるメカニズムは不明ではあるが，ポストシナプスのD2受容体を遮断したことで，フィードバック機構などで，プレシナプスの放出が亢進した可能性がある（図4）。

他の抗精神病薬と異なりclozapineはHVAを上昇させないと報告されている（表2）。Clozapineではドーパミン遮断作用とは別のメカニズムが関わっているのかもしれない。MHPG，5-HIAAについては，知見がやや乏しいが陰性症状や副作用と相関するという報告もある。

V．研究の問題点・困難な点

CSFを用いる研究の最も大きな障害は侵襲性を伴うこと，特に高頻度（10～30%）に生じる腰椎穿刺後頭痛である。立位で増悪し臥位で軽減（ないし消失）することで他の頭痛と区別できる。鎮痛剤の効果はなく，安静臥床と水分補給で対応し，通常数日以内には治癒する。予防のため，

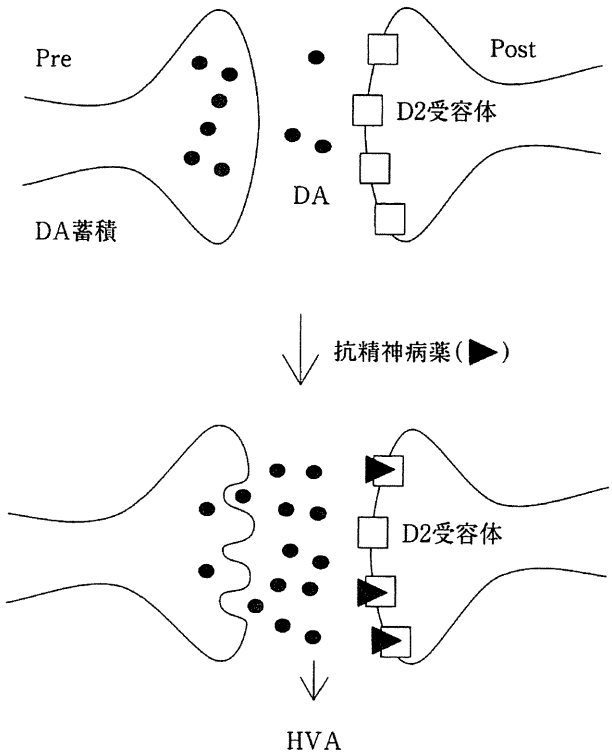

図4　抗精神病薬によるHVA亢進のメカニズム

できるだけ細め（22G程度）の穿刺針を用いるとともに，内針を元に戻してから抜去すること，無外傷性針の使用などを心がけている[5]。また，他の研究検査でも問題になることではあるが，研究上重要性の高い未治療例や重症例については同意能力の問題に加え，採取に協力が必要ということも大きな障壁となっている。

VI．今後の展望

上述のようにCSFアミン代謝産物の測定は，統合失調症分子病態や抗精神病薬作用機序の解析には重要な意義を持っているものの，現状では診療における有用性はあまり期待できない。我々の研究においてもCSF収集の主な目的は新たなマーカー分子の探索である。CSFには脳由来の物質が多く含まれており，蛋白質やアミノ酸などは，検出感度，解析技術が近年，飛躍的に進歩している。現在，我々は施設内の共同研究で網羅的な蛋白質解析やアミノ酸解析などを行っており，CSF中で数百種類の蛋白質の中から探索を行っ

表2　統合失調症患者を対象とした薬物投与前後のCSFモノアミンの主要な研究

著者，発表年	患者数	診断基準	薬物投与前薬剤フリー期間	投与薬物の種類と投与量（mg／日）	投与期間	薬物投与後の変化		
						HVA	5-HIAA	MHPG
Nikisch et al., 2010	22	DSM-Ⅳ	3週間以上	quetiapine 600	28日	↑	↑	↑
Wieselgren and Lindström, 1998	37	DSM-Ⅲ-R	2週間以上	chlorpromazine 換算 300～600	平均53日	↑	→	
Hsiao et al., 1993	22	DSM-Ⅲ-R RDC	2週間以上	fluphenazine もしくは他の定型抗精神病薬	平均38日	→	↓	→
Kahn et al., 1993	19*	DSM-Ⅲ-R RDC	2週間以上	haloperidol 20	35日	↑	→	
Sharma et al., 1993	19*	RDC	平均13.7日	chlorpromazine 換算平均1,118.86	平均33.1日	→		
Doran et al., 1989	14	DSM-Ⅲ	3週間以上	fluphenazine 平均18.4	平均32.9日	↑		
Härnryd et al., 1984	11	RDC	—	sulpiride 800	8週間	↑	→	↓
	12			chlopromazine 400	8週間	↑	→	↓
Sedvall, 1980	8	—	2週間以上	chlopromazine 200～600	4週間	↑		↓
	15			tiotixene 30		↑		→
	13			melperone 300		↑		↓
	6			sulpiride 800		↑		→
	7			clozapine 600		→		→
Wode-Helgodt et al., 1977	8	—	4週間以上	chlorpromazine 200	4週間	↑	→	↓
	8			chlorpromazine 400	4週間	↑	→	↓
	6			chlorpromazine 600	4週間	↑	→	↓
Bjerkenstedt et al., 1977	29	—	2日以上	melperone 300	4週間	→	→	↓
	34			tiotixene 30	4週間	↑	→	→
Rüther et al., 1976	17	—	4週間以上	haloperidol 平均11.6	15日間	→	→	

RDC：研究用診断基準，*統合失調感情障害を若干名含む，P＝0.05以下を有意とした。Probenecidを使用している研究は除外した。

ている。

また，そのように検体に含まれる情報を深く掘り下げる一方，検体に付随する臨床情報を充実させることも同程度に重要である。症候群と考えられる統合失調症を単に健常者と対照者で比較するだけでは有用なマーカー開発につながる可能性は低い。当研究部では，症状評価は無論，副作用の評価，fMRIやプレパルス抑制（PPI），遺伝子検査，神経心理学的検査など多面的な情報を極力得るようにしている。そうした中から，これまで埋もれていた単一病態が浮かび上がる可能性も否定できない。

CSFを用いた研究は侵襲性を伴う点でハードルが高いものの，特殊な設備を必要としないた

め，病院であれば研究が可能である。また，マーカーが開発された際には，発展途上国でも対応できる可能性がある。

文　献

1) Adolfsson, R., Gottfries, C.G., Roos, B.E. et al.: Post-mortem distribution of dopamine and homovanillic acid in human brain, variations related to age, and a review of the literature. J. Neural. Transm., 45; 81-105, 1979.
2) Blennow, K., Hampel, H., Weiner, M. et al.: Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nat. Rev. Neurol., 6; 131-144, 2010.
3) Laruelle, M., Abi-Dargham, A., van Dyck, C.H. et al.: Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. Proc. Natl. Acad. Sci. USA, 93; 9235-9240, 1996.
4) McGowan, S., Lawrence, A.D., Sales, T. et al.: Presynaptic dopaminergic dysfunction in schizophrenia: a positron emission tomographic [18F] fluorodopa study. Arch. Gen. Psychiatry, 61; 134-142, 2004.
5) Straus, S.E., Thorpe, K.E. and Holroyd-Leduc, J.: How do I perform a lumbar puncture and analyze the results to diagnose bacterial meningitis? JAMA, 296; 2012-2022, 2006.
6) Wester, P., Bergström, U., Eriksson, A. et al.: Ventricular cerebrospinal fluid monoamine transmitter and metabolite concentrations reflect human brain neurochemistry in autopsy cases. J. Neurochem., 54; 1148-1156, 1990.
7) Williams, H.J., Owen, M.J. and O'Donovan, M.C.: Is COMT a Susceptibility Gene for Schizophrenia? Schizophr. Bull., 33; 635-641, 2007.