Table 1
Biological characteristics of D-serine and glycine in mammalian nervous systems.

	D-Serine	Glycine
Binding selectivity		
GRIN1/GRIN2 NR-associated glycine site	High affinity	High affinity
Inhibitory glycine receptor	Low affinity	High affinity
Effects on GRIN1/GRIN2 NR receptor	Facilitatory	Facilitatory
Effects on GRIN1/GRIN3 NR receptor	Inhibitory, excitatory or no effect	Excitatory
Effects on δ2 glutamate receptor	Inactivation	Inactivation
Distribution: adult period	Brain-enriched	Ubiquitous in CNS and periphery
Adult brain	Forebrain-dominant and GRIN2B-related	Hindbrain-preferential and NR-unrelated
Postnatal development	GRIN2B-related	NR-unrelated
Immunoreactivity	Neuron < glia	Neuron > glia
Synthesis	Serine racemase	Serine hydroxymethyl transferase
Precursor	L-Serine	L-Serine
Effects of L-serine synthesis deficit[a]	Marked loss	Little or moderate reduction
Extracellular release	Depolarization-induced increase (−)	Depolarization-induced increase (+)
Uptake	Na-dependent and -independent	Na-dependent
Transporter	Asc-1, ASCT2	GLYT1, GLYT2
Degradation	D-amino acid oxidase	Glycine cleavage system
Effects of depletion on NR	NR hypofunction	?
Roles in neurotransmission	Neuromodulator	Inhibitory transmitter/neuromodulator

CNS, central nervous system; GLYT, glycine transporter; NR, NMDA receptor; NR-associated glycine site, strychnine-insensitive glycine receptor (cf. inhibitory glycine receptor, strychnine-sensitive glycine receptor).
[a] Mice lacking 3-PGDH (3-phosphoglycerate dehydrogenase).

the view that D-serine is a candidate endogenous regulator of the GRIN1/GRIN3-type NMDA receptor but cannot be a coagonist like for the GRIN1/GRIN2-type.

3.1.2. δ2 glutamate receptor

Orphan receptor δ2 expressed mainly in Purkinje cells of the cerebellum is classified in the ionotropic glutamate receptor family based on its amino acid sequence, and is reported to be involved in the formation of long-term depression (LTD), motor learning, motor coordination, and synaptogenesis [78]. However, it does not form a glutamate-gated channel and its endogenous ligand is not known [78]. By the experiments using the mutated δ2 receptors at the ligand-binding core [102,103], D-serine and glycine have been recognized as potential endogenous regulators because they were demonstrated to bind to the δ2 receptor to cause the conformational changes, thereby inactivating the receptor with ED50 values, 182 and 507 μM, respectively. It is also suggested that the conformational modifications by D-serine and extracellular Ca^{2+} may be conserved between δ1 and δ2 glutamate receptors [104]. Because D-serine is concentrated in the Bergman glia cells in the cerebellum, and because its postnatal cerebellar concentrations are high and low before and after early postnatal days, respectively, the possible functional link between the glial D-serine and δ2 receptors during development has been an important issue to be solved. Very recently, this question has been answered by the observations that D-serine derived from the Bergman glia serves as an endogenous ligand for glutamate δ2 receptors to regulate LTD at synapses between parallel fibers and Purkinje cells in the immature (postnatal days 11–17), but not mature (older than postnatal days 30) cerebellum [105].

3.2. Glia–neuron interaction

Several lines of evidence have been accumulated indicating that D-serine may be involved in the communication between glutamate synapse and glial cells. Firstly, D-serine has a NMDA receptor-like distribution and is noted immunocytochemically in the neurons and glial cells including astrocytes, microglia and oligodendrocytes in the mammalian brains [29,36,37,48] and retinas [40,72]. The contact between the neurons and astrocytes seems to be essential for preserving the cellular D-serine signals, because the D-serine contents are significantly reduced in the cultured astrocytes in the absence of neurons as compared to those mixed with neurons [96]. Moreover, the extracellular D-serine concentrations are altered by modifications of not only neuronal but glial cell activities [66,67]. The D-serine-mediated synapse–glia interaction has indeed been reported in the hypothalamus [106] and hippocampus [107].

In the nucleus supraopticus hypothalami of female rats [106], the degree of astrocytic coverage of neurons governs the level of the NMDA receptor glycine site occupancy by D-serine, thereby affecting the activity dependence of long-term synaptic changes, LTP and LTD. The astrocytes in the hypothalamic area that display the D-serine-like immunoreactivity surround glutamate synapses tightly and loosely during the virgin and lactating period, respectively, in the female rats [106]. In the former period, the increased D-serine release has been found to diminish the threshold for the NMDA receptor-mediated induction of LTP and LTD, and vice versa in the latter period, in the hypothalamic slices with the selective absence of endogenous D-serine, but not glycine, by D-amino acid oxidase application [106].

Furthermore, NMDA receptor-dependent LTP in hippocampal area CA1 is blocked by clamping internal Ca^{2+} in individual CA1 astrocytes in the vicinity of which there is a reduction in the occupancy of the NMDA receptor-glycine sites [107]. This blockade can be reversed by exogenous D-serine or glycine, and mimicked by a selective antagonist for the glycine site and depletion of D-serine by high frequent astrocyte stimulation in combination with a serine racemase inhibitor or disruption of exocytosis by the light-chain tetanus toxin in an individual astrocyte [107]. Ca^{2+}-dependent release of D-serine from an astrocyte, therefore, might regulate NMDA receptor-mediated neuroplasticity in a group of hippocampal excitatory synapses [107].

D-Serine biosynthesis appears to occur predominantly in the neurons in the brain because the immunoreactivity [54] and mRNA [48] of the putative D-serine synthesizing enzyme, serine racemase, has mainly been detected in the neurons although the earlier histochemical studies described the exclusive distribution of serine racemase-like immunoreactivity in astrocytes of the rat brain [108]. In contrast, the synthetic pathway for a major D-serine precursor, L-serine, has been demonstrated in the astrocytes [60]. Together with the foregoing glial origin of extracellular D-serine, these observations indicate that the transfer systems of D-serine and/or its precursor between neurons and glia appear to be present in the brain.

3.3. Neural circuit formation

Because disruption of serine racemase gene that causes a marked loss of brain D-serine leads to alterations in cortical dendritic morphology in the mouse [58], D-serine could directly or indirectly participate in the wiring of certain neuron circuits in mammalian cortical tissues. In the cerebellum, the D-serine concentrations are very low in the adult period but increase transiently to the comparable levels in the adult cerebral cortex around postnatal week 1–2 [46]. This increase is prominent in the Bergmann glia (radial glial cells) that extend their protrusions around the granule cells during the NMDA receptor-mediated migration and synaptic formation of the granule cells [109]. The migration of granule cells is suppressed by the selective degradation of D-serine or inhibition of serine racemase while D-serine rescues this suppression [61]. Further, GRIP (glutamate receptor interacting protein) has been reported to augment serine racemase and D-serine release by binding serine racemase and the AMPA glutamate receptor, and overexpression of GRIP by means of its adenoviral infection in neonatal mouse cerebellum facilitates the above cell migration process [61]. These phenomena suggest an important role of D-serine in neural circuit formation of the developing cerebellum [61].

4. Pathophysiology of neuropsychiatric disorders and D-serine dysfunction

The characteristic metabolism, functions, and developmental changes of mammalian endogenous D-serine suggest the presence of a unique cellular and molecular mechanism, also called the D-serine system, in the central nervous system. The requirement of D-serine in physiological activation of the NMDA receptor indicates that the D-amino acid may play a pivotal role of in the expression and regulation of various higher brain functions related to the major glutamate receptor, and that abnormalities of the D-serine system may be implicated in the pathophysiology of various neuropsychiatric disorders.

4.1. Schizophrenia

As described at the beginning of this review, it has now been widely accepted that the reduced neurotransmission via the NMDA receptor may be involved in the pathophysiology of schizophrenia. This so-called "glutamate hypothesis" is chiefly based upon the following pharmacological observations: (a) antagonists for the NMDA receptor represented by phencyclidine (1(1-phenylcyclohexyl)-piperidine: PCP) and a chemically PCP-related and non-competitive antagonist for the NMDA receptor, ketamine, produce positive and negative symptoms and cognitive disturbances which are indistinguishable from those of schizophrenia [110], (b) a group of schizophrenic patients have been reported to be more sensitive to PCP and ketamine in their psychotomimetic actions than healthy controls because the schizophrenic patients in remission suffer exacerbation of their psychotic symptoms by the challenge doses of these drugs that fail to cause apparent schizophrenia-like symptoms in the controls [110–112], and (c) the psychotomimetic effects of the ketamine stereoisomers are closely correlated with their affinities for the NMDA receptor [113,114]. Because the NMDA receptor hypofunction have been shown to lead to the hyperdopaminergic activity in the brain in experimental animals [13,15,115,116] and humans [117,118], the "glutamate hypothesis" is not conflict with the "dopmanine (DA) hypothesis" of schizophrenia in that the excessive dopaminergic transmission following the reduced NMDA receptor function would produce positive symptoms of schizophrenia in a potent dopamine receptor antagonist antipsychotic-sensitive manner [13,15].

In consistent with these data, the facilitation of the NMDA receptor function by its glycine site agonists including glycine, D-serine, D-alanine, D-cycloserine and a glycine transporter inhibitor have been reported not only to improve conventional animal models of schizophrenia that are abnormal behaviors induced by NMDA receptor antagonists and DA agonists, but also to attenuate an NMDA antagonist-induced enhancement of DA transmission in the brain. Furthermore, these NMDA coagonists in combination with various antipsychotics except clozapine have been shown to produce a significant amelioration in currently used antipsychotic-resistant negative symptoms and/or cognitive dysfunctions of schizophrenic patients [119] when compared with conventional antipsychotics alone [119]. It is also worthwhile to note that D-alanine suppresses enhanced dopamine transmission in the frontal cortex of the animals acutely injected with a schizophrennomimetic NMDA receptor blocker [115], and that the repeated co-administration of glycine with PCP eliminates the augmented DA release in response to a challenge dose of amphetamine in the frontal cortex or striatum of the animals repeatedly treated with PCP alone [116]. The hyper-responsiveness of DA neurotransmission to amphetamines has also been observed in a group of schizophrenic patients [120,121], acutely ketamine-administered healthy volunteers [122] and an animal methamphetamine model of schizophrenia [123]. These findings agree with the ideas that the stimulation of the NMDA glycine site is expected to have a therapeutic efficacy on the antipsychotic-resistant symptoms of schizophrenia and that overall abnormalities, including positive symptoms, may be improved with a coagonist alone without the concomitant use of an antipsychotic drug.

The causative mechanisms whereby the NMDA receptor-mediated glutamate transmission is diminished in schizophrenic brains have intensively been investigated from a wide variety of aspects of the regulatory systems of the glutamate receptor. These standpoints contain the possibility that the insufficient D-serine signals could result in the hypofunction of the NMDA receptor because D-serine is an intrinsic coagonist for the glutamate receptor. In fact, the mice lacking serine racemase activity displayed behavioral abnormalities relevant to schizophrenia, including hyperactivity [57] and impairments in prepulse inhibition and sociability, the latter of which were shown to be exacerbated by an NMDA receptor antagonist and ameliorated by D-serine or the atypical antipsychotic clozapine [59].

There are so far no studies to show statistically significant differences in the D-serine contents in the postmortem brain tissues between the patients with schizophrenia and without neuropsychiatric diseases [26,124]. However, an increase in the densities of the glycine-regulatory sites of the NMDA receptor, which are the binding targets for endogenous D-serine, was observed in cerebral neocortical areas of the postmortem schizophrenic brains, such as the angular convolution, supramarginal gyrus, somatosensory area, and premotor area [125]. This increase could be considered as a compensatory change due to the decreased extracellular release of D-serine or glycine in a specific neural circuit. The decreased *in vivo* radioligand binding to the PCP site within the ion channel of the NMDA receptor of the hippocampus of schizophrenic patients could be a consequence of the reduced open frequency of the ion channel due to diminution of D-serine or glycine signaling [126]. Moreover, significant association between schizophrenia and single nucleotide polymorphisms (SNPs) and/or haplotypes of DAO [127] and serine racemase [128,129], and the expressional changes in the protein products or mRNAs of these genes in the postmortem brains of patients with schizophrenia, have been reported [130–133], although other studies fail to replicate these data [134–136].

Interestingly, a primate-specific gene encoding a candidate for a DAO modulator, G72, was isolated from chromosome 13q34 that

has been genetically linked to schizophrenia, and has been found to be associated with schizophrenia [127]. The genetic association was verified by several meta-analyses [137]. Because G72 was originally reported to be an interacting partner and an activator of DAO (DAOA/G72) expressed in the human brain [127] and because the possible physiological interaction has been supported by the observations that DAO- and G72-like immunoreactivity are both detected in astrocytes of the human cortex [138], an anomalous overexpression of DAOA/G72 that might reduce the brain D-serine contents by increasing activity of DAO could have a relation to schizophrenia susceptibility. However, this hypothesis is still debatable in terms of the subsequent studies that suggest alternative roles of G72 in the nervous system such as an inhibitor of DAO [138] and a regulator of mitochondrial functions [139].

Some studies pointed out a decrease in D-serine contents and the ratios of D-serine to the total serine concentrations in the blood and cerebrospinal fluid [124,134,140,141]. Further evaluation, however, is needed to clarify the significance of these results because (a) the results of the quantitative analysis of D-serine in the blood and cerebrospinal fluid of schizophrenic patients are controversial [142–144] and (b) the effects of a variety of factors that may influence the blood D-serine concentrations such as the medication, D-serine-containing foods and diurnal variation of D-serine metabolism cannot be excluded in these studies.

4.2. Bipolar disorder (Manic depressive psychosis)

Relationships with the SNPs and haplotypes of DAOA were noted not only in schizophrenia but also bipolar disorder; the changes in the D-serine system may occur in both psychiatric disorders [136]. This finding suggests the possibility that polymorphism of DAOA relates to common symptoms, such as agitation and delusion, but not to the disease. However, the association of G72 with bipolar disorder is still debatable [137], therefore requiring further investigation.

4.3. Anxiety

Anxiety-related actions emerge in animals receiving a local injection of D-serine in the dorsal periaqueductal gray matter (DPAG) [145]. This effect is mimicked by glycine in a NMDA receptor glycine site antagonist-reversible fashion and microinjections of these amino acids outside the DPAG, or of L-serine inside the DPAG, produced neither of these pro-anxiety effects [145]. These observations suggest that D-serine and/or glycine in specific brain regions may be associated with the manifestation of anxiety [145], and are in line with the results that mutant mice with reduced NMDA-GRIN1 glycine affinity or lack of DAO function exhibit an anxiolytic-like or anxiogenic-like behaviors, respectively [146]. Because DAO deficient mice show an increase in D-serine contents without affecting glycine levels in the brain [89], endogenous D-serine, but not glycine, could participate in the neural mechanisms underlying anxiogenic effects induced by the stimulation of the NMDA glycine site.

4.4. Non-ketotic hyperglycinemia: glycine cleavage enzyme deficiency

In the postmortem cerebral cortical tissues of patients with non-ketotic hyperglycinemia, in which the glycine level in the blood is increased markedly because of lack or marked loss of the activity of the glycine cleavage enzyme system, D-serine and glycine concentrations have been found to be decreased to about one-third and increased up to approximately 7 times as compared with those in the patients without neuropsychiatric disorders as the control group, respectively, but no statistically significant changes in the contents of L-serine are observed [63]. The D-serine concentration of the rat brain is decreased after the administration of cysteamine with an inhibitory action on the glycine cleavage enzyme system [63], while it is increased by the administration of a large amount of glycine [50]; therefore, the above decrease is considered to result from inhibited activity of the glycine cleavage enzyme system, but not due to the secondary effects of the increased glycine levels or of the possible marked loss of L-serine that is supposed to be a major precursor for D-serine, and may be relevant to the various central nervous symptoms observed in the disease, such as mental retardation, convulsive seizure, apneic events, and lethargy. These data indicate that D-serine could have a synthetic pathway unrelated to serine racemase but related to the glycine cleavage enzyme system and/or its associating molecules.

4.5. Serine deficiency syndrome

Cases with severe nervous system disturbances, such microcephaly, convulsive seizure, and psychomotor retardation, are occasionally noted along with a drastic decrease in the L-serine concentrations of the blood and cerebrospinal fluid, which is called serine deficiency syndrome [147]. L-Serine replacement therapy is effective; it has been demonstrated that the activity of 3-phosphoglycerate dehydrogenase (3-PGDH), 3-phosphoserine aminotransferase (3-PSAT) or 3-phosphoserine phosphatase (3-PSP) of the L-serine biosynthesis system is lacking in some serine deficient syndrome cases while no apparent abnormality can be detected in the known L-serine synthetic enzyme in other cases [147]. The D-serine concentration is decreased markedly in the cerebrospinal fluids of patients with this syndrome, suggesting an involvement of lowered D-serine signals in the central nervous system with neuropsychiatric symptoms [147]. These findings suggest that D-serine of the human central nervous system may be mainly derived from L-serine, agreeing with the dramatic reduction in the brain contents of D-serine as well as L-serine in the mice lacking 3-PGDH gene (see Section 2.3) [60].

4.6. Cerebral ischemia

The extracellular D-serine concentration is increased in the rabbit piriform cortex in transient ischemia [148], and ischema-induced neuronal cell death is suppressed in rat hippocampus slices when their D-serine levels are decreased selectively by DAO treatment in the presence of the normal contents of glycine, another coagonist of the NMDA receptor [149,150]. In the rat cerebrocortical slice cultures, sensitivity of NMDA cytotoxicity to an NMDA receptor glycine site antagonist, 5,7-dichlorokynurenic acid, was diminished by L-serine that increased the extracellular levels of D-serine [151]. These observations are consistent with the view that potentiation of the D-serine signal is involved in the neuronal damage caused by overstimulation of the NMDA receptor in cerebrovascular disorders. In support of this hypothesis, a marked reduction of brain D-serine contents led to a decreased neurotoxicity induced by NMDA injections into the right parietal cortex [56] and a diminution in infarct volume following middle cerebral artery occlusion in the serine racemase deficient mice [152].

4.7. Alzheimer's disease

In Alzheimer's disease, the possible involvement of D-serine in the neurodegeneration related to the excessively accumulated amyloid β peptide (Aβ) and NMDA receptor has been examined. Aβ stimulated the release of D-serine as well as glutamate from microglia [153,154] and enhanced the transcription of serine racemase mRNA in microglia *in vitro* [153]. The conditioned medium obtained from the primary microglia treated with Aβ markedly

reduced the viability of the primary hippocampal neuron cultures in a NMDA glycine site antagonist or DAO treatment-reversible manner [153]. Moreover, there was an increased expression of serine racemase mRNA in the postmortem hippocampus of the patients with Alzheimer's disease [153]. These observations seem to be consistent with the idea that the increased synthesis and extracellular release of D-serine by Aβ may promote neuronal cell death [153]. Indeed, serine racemase knock-out mice with an approximately 90% decrease in forebrain D-serine contents have been found to display a reduced neurotoxicity induced by Aβ (1–42)-peptide injections into the right hippocampus [56]. However, no significant change in the tissue concentrations of D-serine has so far been reported in the postmortem brains of patients with Alzheimer's disease [26,28].

4.8. Amyotrophic lateral sclerosis

The death of motor neurons in amyotrophic lateral sclerosis (ALS) has been shown to, at least in part, connect to the neuronal toxicity of glia-derived factors including excitatory amino acids. Sasabe et al. [38] recently found in ALS model mice (transgenic mice expressing a high copy number of mutant human superoxide dismutase 1 (SOD1) with a Gly-93-Ala substitution (G93A-SOD1)) that the spinal cord motor neurons, but not astrocytes and microglia, were more susceptible to NMDA toxicity than those of a control group, and that D-serine contents and serine racemase activities in the spinal cord were augmented with the progression of the pathology. In MG5 microglial cells, serine racemase expression was enhanced by transient and enforced expression of G93A-SOD1 [38]. Elevated D-serine-like immunoreactivity in the spinal cords of patients with familial or sporadic ALS were observed, although these data are preliminary due to the limited number of the tissues examined [38]. These results have led to the proposal of a mechanism whereby the overproduction of D-serine in glial cells damages the motor neurons of ALS patients [38].

The possible disturbances in D-serine metabolism has also been supported by the molecular genetic study indicating that familial ALS was associated with a mutation in D-amino acid oxidase and expression of this mutation in neuronal cell lines, primary motor neurons or astrocytes cocultured with motor neurons reduced viability of these neuronal cells [155]. The pathophysiological implication of D-serine in ALS therefore seems to be valuable for further evaluation.

4.9. Cerebellar ataxia

Competitive and non-competitive antagonists for the NMDA receptor, combined gene disruption of the NMDA receptor GRIN2A and GRIN2C subunits [156] and genetic deletion of δ2 glutamate receptor [157] have been demonstrated to produce ataxic movements related to the cerebellum in experimental animals. In humans, PCP-induced motor disturbances are similar to those in the patients with cerebellar ataxia [112]. The ability of a NMDA receptor antagonist, dizocilpine, to induce cerebellar ataxic movements has recently been shown to be attenuated in the mice lacking DAO activity with increased cerebellar D-serine contents [158]. These observations suggest that diminution in NMDA receptor function and/or D-serine signal may also be involved in the pathophysiology of the cerebellar ataxia, whereas there are no studies on the D-serine contents or D-serine- or NMDA receptor-related molecules in the postmortem brain tissues from patients showing cerebellar ataxia.

The plausible disturbances appear to be consonant with the amelioration of the abnormal movements seen in some kinds of the model mice of cerebellar ataxia by D-serine and D-cycloserine, which is a partial agonist for the glycine site of the NMDA receptor [159]. D-Cycloserine has also been reported to attenuate the scores of the International Cooperative Ataxia Rating Scale (ICARS) in a group of the patients with spino-cerebellar degeneration [160].

4.10. Neuropathic pain

Several lines of evidence suggest that endogenous D-serine may play an important role in the mechanisms of central sensitization in NMDA receptor-related neuropathic pain. Thus, (a) D-serine antagonizes the analgesic action of gabapentin and S(+)-3-butyl GABA (γ-aminobutyric acid) against pain produced by the stimuli of heat and formalin [161]; (b) the potentiation of the nociceptive reaction to and the NMDA receptor-mediated excitatory synaptic current by formalin-induced pain is noted in DAO activity-deficient mice [162]; and (c) a selective reduction of D-serine signals in the cingulate cortex by a local injection of a D-serine degrading enzyme DAO or a selective antagonist for the NMDA receptor glycine site suppresses the formation of avoidance behavior in response to the formalin-induced pain [163].

5. Relevance of D-serine system as a target for the development of the NMDA receptor tuning therapy for neuropsychiatric disorders

Based upon growing evidence that NMDA receptor dysfunction may be implicated in the pathophysiology of a variety of neuropsychiatric disorders, the development of a novel class of drugs that tune the glutamate receptor-mediated transmission has been attempted. Also, clinically approved therapeutic agents or the substances used as food additives with some action at the NMDA receptor are applied in the clinical trials for the treatment to the intractable neurologic or psychiatric disturbances or symptoms that are mimicked by the NMDA receptor agonists or antagonists.

Experimental results whereby an NMDA receptor antagonist attenuated neuronal cell death (see Section 4.6) [164,165] and neuropathic pain (see Section 4.10) [166] elicited by cerebral ischemia and an excitatory amino acid were consistently reported. Diminution of the NMDA receptor activity has also been considered to delay or protect the cell dysfunction and loss occurring in neurodegenerative diseases. For acceleration of the NMDA receptor, agonists for its glycine site have been chosen because direct agonists for its excitatory amino acid site, but not the glycine site, have been shown to often produce cell over-excitation or death in the nervous system or convulsion (see Sections 1 and 4.1). These allosteric agonists or coagonists for the NMDA receptor have been demonstrated to improve positive and negative symptoms and cognitive deficits of schizophrenia, and ataxic movements of the patients with spino-cerebellar degeneration (see Sections 4.1 and 4.9).

Recent animal experiments have further been extending the application of the substances acting at the coagonist site of the NMDA receptor for elimination of ill memories in various psychiatric disorders. It has well been established that conditioned fear or drug seeking behavior is eradicated by being replaced by subsequent learning of harmless or alternative cues. This extinction phenomena have been applied as the behavioral therapies to the treatment of phobias, obsessive-compulsive disorder, post-traumatic stress disorder (PTSD) or drug abuse, and have newly been shown to be facilitated by the NMDA receptor glycine site agonists in their animal models [167–172]. In several randomized placebo-controlled clinical trials for phobia [173], social anxiety disorder [174], obsessive-compulsive disorder [175], PTSD [176] or panic disorder [126], low dose of D-cycloserine, a partial agonist for the glycine site of the NMDA receptor, in combination with a conventional cognitive behavioral therapy (CBT) has been reported to be more effective in ameliorating fear or anxiety compared with CBT alone. These experimental and clinical observations suggest

that the enhanced NMDA receptor function may accelerate acquisition and overwriting new memories that lead to extinction of previous harmful memories.

However, modulation of the NMDA receptor functions has not yet been successfully achieved. In clinical trials of prophylactic medication for cerebral ischemia, the competitive and non-competitive NMDA receptor antagonists induced psychotic symptoms [164,165]. Moreover, current clinically applicable coagonists of the NMDA receptor have problems such as: (a) the low BBB permeability that requires a large dose to obtain a sufficient brain content and therapeutic efficacy (e.g., glycine, D-serine, D-alanine, and glycine transporter inhibitors) [30,119], (b) low selectivity for the NMDA receptor due to another potent action at the inhibitory glycine receptor (e.g., glycine and glycine transporter inhibitors) [19], (c) difficulty in dose selection due to a narrow range of therapeutic dosage because of a partial agonist property (i.e., D-cycloserine) [30,119], and (d) nephrotoxicity (i.e., D-serine) [177,178]. Finally, synthesizing a glycine site agonist that specifically binds to the glycine regulatory site with the high BBB permeability has long remained unsuccessful.

To avoid adverse effects by an excessive change in the activity of the NMDA receptor or by toxicity due to large doses from the aforementioned problems, manipulation of the endogenous D-serine signal in the brain seems to have some advantages for the fine-tuning of the NMDA receptor, because an incomplete loss of NMDA receptor currents under the depletion of D-serine by D-amino acid oxidase suggests that the moderate alterations of the NMDA receptor activity could be achieved by an indirect regulation of the NMDA glycine site, and because NMDA receptor-like distribution of endogenous D-serine may lead to the selective effects of the D-serine signal operation on the NMDA receptor. Alternatively, relatively hind brain-selective increase in D-serine contents might be effective on the cerebellar ataxia associated with NMDA receptor hypofunction, and performed by inhibition of D-amino acid oxidase, the distribution of which is inversely related to that of D-serine. Therefore, the molecules specifically participating in the different stages of D-serine metabolism may be suitable targets for the development of a novel class of the excellent therapeutic agents controlling the NMDA receptor functioning. Facilitation of D-serine synthesizing or release machinery members and blockade of transporters and degrading enzymes of D-serine would enhance NMDA receptor-mediated transmission, and vice versa. For this purpose, exact molecular and cellular mechanisms underlying each metabolic step and their localization should further be clarified.

6. Conclusion

In conclusion, a body of evidence has been accumulated indicating that endogenous D-serine differs in many ways, besides simply being a D-amino acid, from other signaling substances in the nervous system examined thus far. First, a peculiar mechanism is required to maintain the extracellular concentration of D-serine within an adequate range, in turn allowing D-serine to play a role as a coagonist for the GRIN1/GRIN1-type NMDA receptors by forming the tonic signaling in the synapse. By contrast, classical neurotransmitters exhibit their nerve impulse-dependent extracellular release and rapid elimination in the synaptic clefts to generate a phasic signaling. The particular type of regulatory system for D-serine remains to be fully elucidated. Furthermore, the exact differences in the physiological roles of glycine and D-serine as ligands at the NMDA receptor glycine site are still unclear. In addition, it is strongly suggested that, unlike most of classical neurotransmitters, D-serine is widely present in both glial cells and neurons and indispensable for their interaction. Certain D-serine-releasing glial cells could function as a fine-tuning station for the excitatory synapse in the nervous system. Such features reveal a host of differences between D-serine and glycine (Table 1), and these differences may not only substantially complicate the elucidation of the metabolism and dynamics of D-serine but also contribute to the elegant control of the NMDA receptor and related molecular cascades that are presumed to be dysfunctional in the various neuropsychiatric symptoms. Therefore, further elucidation of the cellular and molecular mechanisms underlying the metabolism and functions of D-serine in the brain will provide a clue as to unknown information processing systems that regulate higher brain functions, and is expected to markedly advance our understanding of the pathogenesis and pathophysiology of neuropsychiatric disorders: it is hoped that this series of investigations will facilitate the development of novel therapies for these disorders. Furthermore, additional D-serine research will shed light on the biological significance of D-serine in the molecules other than the NMDA receptor and the extra-brain organs, and its differences from glycine.

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White Matter Abnormalities as a Risk Factor for Postoperative Delirium Revealed by Diffusion Tensor Imaging

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Objective: *Delirium is a common and critical clinical syndrome in older persons. The authors examined whether any abnormalities in the white matter (WM) assessed by diffusion tensor imaging (DTI) predisposes patients to develop delirium after cardiac surgery and also analyzed other risk factors for delirium.* **Method:** *In 116 consecutive patients who underwent scheduled cardiac operations, fractional anisotropy (FA) values obtained by DTI before the surgery and pre-, peri-, and postoperative factors were evaluated. The postoperative delirium was diagnosed according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, criteria for delirium.* **Results:** *Delirium developed in 19 of 116 patients (16.4%). Eighteen of the patients with delirium (94.7%) were older than 60 years. A multivariate logistic regression analysis showed that advanced age and poor performance on a semantic fluency task (the Word Fluency test animal) were important predictive indicators of the delirium. In addition, a voxel-by-voxel analysis using the Statistical Parametrical Mapping 2 revealed that the FA values of the patients with postoperative delirium were significantly lower than those of the nondelirium patients in the bilaterally widespread deep WMs and bilateral thalamus, whereas the analysis treating age as a nuisance variable indicated a significant change in only four clusters of the brain areas, e.g., the left frontal lobe WM, and left thalamus, when compared with the nondelirium group.* **Conclusion:** *The abnormalities in the deep WMs and thalamus that were mainly accelerated by aging may account for the vulnerability to postoperative delirium, and the semantic word fluency could be a useful predictive indicator of delirium.* (Am J Geriatr Psychiatry 2010; 18:743–753)

Key Words: Delirium, diffusion tensor imaging, word fluency test, white matter

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Delirium is a neurobehavioral syndrome associated with disturbances in consciousness and attention.[1,2] The symptoms of delirium involve an acute generalized impairment of cognitive function (orientation, memory, and abstract thinking), behavioral and psychomotor abnormalities (ranging from apathy and agitation), an altered sleep–wake cycle, and disorganized thinking.[3–5]

Delirium occurs in older hospitalized patients at a higher rate of incidence. The prevalence of delirium at hospital admission ranges from 14% to 24%, and the incidence of delirium during hospitalization ranges from 6% to 56% among the general hospital populations. In particular, delirium occurs in 15%–53% of older postoperative patients.[5] The etiology of delirium may be a complex and multifactorial syndrome.[3,6] It has been reported that the postoperative delirium can be attributed to precipitating factors, e.g., surgery and intercurrent illness, and risk factors predisposed to individual patients including older age, cognitive impairments, sensory impairments, and coexisting medical conditions.[6–11] Circumstantial evidence suggests that advanced age, decline of the cognitive functions, and increased vascular changes are primarily involved in the vulnerable conditions to delirium.[5,12,13]

Magnetic resonance diffusion tensor imaging (DTI) is a noninvasive in vivo method for characterizing the integrity of anatomical connections and white matter (WM) circuitry and provides a quantitative assessment of the microstructure of the WM, e.g., myelin sheath and intra-axonal structures.[14] The diffusion properties that are sensitive to water diffusion can be assessed by means of two indices: fractional anisotropy (FA) and mean diffusivity (MD). FA is a quantitative measure of the anisotropy calculated from the DTI and reflects the degree of directionality of cellular structures within the fiber tracts, whereas MD is a measure of diffusion in the nonlinear direction or free diffusion.[15] FA rather than MD had been used in many studies examining an age-related change in the WM DTI in normal healthy adults,[16–21] with correlation to a decrease in the cognitive function.[22–24] The changes in the WM shown by the FA studies suggest that a deterioration in the cortical circuitry with aging are attributed to the age-related cognitive decline.[25,26]

This study had two primary aims. The first was to examine whether any changes in the WM indicated by FA were predisposed in the patients who developed to delirium after cardiac surgery. We assumed that abnormalities in the WM is involved in the vulnerability to the development of postoperative delirium. The second aim was to investigate whether pre-, peri-, and postoperative risk factors for delirium reported in previous studies[6–13] also behave as a risk factor in this study.

This study demonstrated that there were a widespread reduction of the FA values in the brain of patients with postoperative delirium compared with the nondelirium patients and that advanced age and a neuropsychological test, the Word Fluency test animal (WFTA) were evaluated as important indicators of postoperative delirium. In addition, we analyzed relationships of the age between the FA values, because an age-related linear decline of the FA values was indicated by several studies.[17–19,21–23]

METHODS

Subjects

Subjects were 119 consecutive Japanese patients that underwent scheduled cardiac operations between August 2005 and August 2006 in the Department of Cardiothoracic Surgery, Tokyo Medical and Dental University, University Hospital Faculty of Medicine (Tokyo). Patients who were admitted for an emergency operation in this period were excluded from this study. Approval was obtained from the ethics committee of Tokyo Medical Dental University, and all patients gave written informed consent.

All the patients received an assessment battery for preoperative conditions and a magnetic resonance imaging (MRI) study, whereas 18 of the subjects who did not accept to be examined by a neuropsychological test battery were evaluated by the preoperative assessments excluding the neuropsychological tests. After the operation, all the patients were assessed daily not only by the medical staff of the Department of Thoracic Cardiovascular Surgery but also by a well-trained psychiatrists (either the principle investigator [AS] or a coauthor [TT]) until discharged. The presence of delirium was determined by the psychiatrists according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition-TR criteria for

delirium.[1,27,28] The severity of delirium was evaluated using the severity items of the Delirium Rating Scale—Revised-98.[29]

Preoperative Evaluations

The preoperative evaluation included medical history, impairments in their physical condition, handicaps, alcohol consumption, and smoking habits. Neuropsychological tests, i.e., the Mini-Mental State Examination,[30] the Trail Making tests A and B,[31] the Stroop Color-Word interference test,[32] Word Fluency tests of animal and letter,[33] digit spans of forward and backward, and the Beck Inventory Depression Scale,[34] were examined on the day after the admission for surgery. For the WFTA, participants were asked to name as many animals as possible for 60 seconds. For the Word Fluency test K, participants were asked to name as many words as possible beginning with the letter "Ka (in Japanese)" for 60 seconds.

Peri- and Postoperative Evaluations

The operating procedure, operation time, anesthetic time, use of extracorporeal circulation, amount of blood loss, and requirement of blood transfusion during the surgery were obtained from the flow chart. The postoperative medical factors for the intensive care and any cardiopulmonary complications were also included in the analyses for the risk factors of delirium. No subjects in the study received opioid analgesics or dexmedetomidine (α_2-adrenergic agonist)[35] during the peri- and postoperative period.

Magnetic Resonance Imaging

DTI was performed using a 1.5-tesla General Electric Signa (General Electric, Milwaukee) with a standard head coil. Diffusion weighted echo planar images were acquired in the axial plane (repitition time [TR] = 8,000 milliseconds, echo time [TE] = 78 milliseconds, matrix = 128 × 128, field of view [FOV] = 24 cm × 24 cm, slice thickness = 5 mm, interleave, number of excitations [NEX] = 4) providing whole brain coverage. Six noncollinear directions were sampled using *b* values from 0 to 1,000 s/min and one additional image with a *b* value of zero. The imaging time for the DTI examination was 7.5 minutes. The images were corrected by the GE workstation for eddy current disorientations. Conventional axial T1 and T2-weighted images were also performed to assess WM lesions or lacunar infractions. All subsequent sequences were aligned with the anterior and posterior commissure (AC-PC) plane.

FA was calculated from the diffusion weighted images using the method proposed by Pierpaoli and Basser.[36,37] The FA data were normalized to the Montreal Neurological Institute space using the general linear deformation by Statistical Parametrical Mapping 2 (SPM2, Wellcome Department of Cognitive Neurology, London)[38] as described in the previous reports.[39,40] The FA maps were smoothed with a Gaussian kernel of 12 mm full width at half maximum. The resultant FA values were compared between two groups (patients with or without postoperative delirium) by a voxel-by-voxel analysis using the SPM2. First, to estimate the population effects (diagnostic effects), we used a single subject condition (nondelirium or delirium) and covariate (no covariate of interest) model for the SPM analysis. We next applied the single subject condition (nondelirium or delirium) and covariate (age). For the analyses, we used the one-tailed $p < 0.001$ (uncorrected) as a statistical threshold to search for significant differences between the groups, as we hypothesized that there were reductions in the FA values of patients with postoperative delirium. The voxel-by-voxel analysis in the stereotactic space can provide an unprejudiced view of the result, whereas a region-of-interest technique is limited by the fact that the selection of sample depends on the observer's priori choice and hypothesis. However, image misregistrations from spatial normalization in the voxe-by-voxel analysis can mimic FA alterations, if there are large variations of brain shape in older subjects.[14,41] Thus, we set the masking threshold for the FA values of 0.2 to exclude voxels containing the partial volume of WM and other tissues and to minimize the effect of the misregistrations.

Statistical Analysis

We compared the pre-, peri-, and postoperative factors and the neuropsychological assessments between the delirium group and the nondelirium group. The continuous variables are expressed as the mean ± SD, and the categorical data are expressed as

TABLE 1. Comparisons of Pre-, Peri-, and Postoperative Variables Between the Delirium Group and the Nondelirium Group (Univariate Analysis)

Preoperative Data	Delirium Group (n = 19)	Nondelirium Group (n = 97)
Age	73.1 ± 6.4	62.5 ± 11.8[a]
Sex (male)	13 (68.4%)	65 (67.0%)
Body mass index	21.6 ± 3.0	23.6 ± 3.0[b]
Hypertension	18 (94.7%)	65 (67.1%)[c]
Diabetes mellitus	6 (31.6%)	40 (41.2%)
Hemodialysis	5 (26.3%)	5 (5.2%)[c]
Hypercholesteremia	6 (31.6%)	39 (40.2%)
Cerebral vascular disease	2 (10.5%)	15 (15.5%)
Smoking (cigarette/day × year)	647.2 ± 538.9	433.2 ± 713.7
Alcohol daily use	9 (50%)	32 (34%)
Visual disturbance	1 (5.3%)	5 (5.2%)
Auditory disturbance	2 (10.5%)	2 (2.1%)
History of cancer	3 (16.7%)	12 (12.4%)
Cognitive tests[d]		
Mini-Mental State Examination	26.3 ± 3.4	27.2 ± 2.7
Trail making test B-A (seconds)	155.9 ± 131.5	82.3 ± 125.2[c]
Color Stroop test (seconds)	21.4 ± 14.3	18.9 ± 15.9
Word fluency test animal	13.4 ± 4.1	18.6 ± 5.8[a]
Word fluency test "Ka (in Japanese)"	7.6 ± 3.4	10.0 ± 4.2[c]
Digit span forward	7.8 ± 2.4	8.2 ± 2.0
Digit span backward	5.2 ± 1.9	5.9 ± 2.3
Perioperative data		
Operation time (minutes)	404.7 ± 89.2	408.6 ± 113.5
Anesthetic time (minutes)	510.9 ± 92.8	515.4 ± 117.0
Extracorporeal circulation (on)	10 (52.6%)	60 (61.9%)
Blood loss (mL)	797.7 ± 559.4	988 ± 995.1
Blood transfusion	12 (66.7%)	40 (41.2%)[c]
Type of operation		
Coronary artery bypass graft (CABG)	7 (36.8%)	47 (48.5%)
Valve replacement (VR)	5 (26.3%)	24 (24.7%)
CABG + VR	3 (15.8%)	9 (9.3%)
Thoracic aortic aneurysm	3 (15.8%)	9 (9.3%)
The others	1 (5.3%)	8 (8.2%)
Postoperative data		
Incubation period (days)	1.5 ± 1.6	1.3 ± 1.8
Intensive care unit stay (days)	4.2 ± 2.1	4.6 ± 8.3
Cardiopulmonary complication	11 (61.2%)	59 (60.8%)

Notes: Continuous variables are expressed as mean ± SD, and categorical data are expressed by the number of patients. The two-tailed Student's *t*-test or the Mann-Whitney *U* test was used for the continuous variables. The comparison of proportions was analyzed by the χ^2 tests. If the expected cell frequencies were <5, we used Fisher's exact test for the analysis.

[a]$p < 0.001$.

[b]$p < 0.01$.

[c]$p < 0.05$.

[d]The results of the cognitive tests were obtained from 17 patients with delirium and 81 patients with nondelirium (see Methods).

proportions. The two-tailed Student's *t*-test or the Mann-Whitney *U* test was used for the continuous variables. The comparison of proportions was analyzed by the χ^2 tests. If the expected cell frequencies were <5, we used Fisher's exact test for the analysis. The variables with a p value <0.05 were entered into a backward stepwise logistic regression analysis requiring a p value less than 0.05 to remain. In addition, a comparison between the two groups using analysis of covariance (ANCOVA) with the age of the patients treated as nuisance covariate and a correlation analysis with the age were performed. A correlation with the age was analyzed by the Pearson test. The statistical analyses were carried out using the SPSS version 16.0 (SPSS, Inc., Chicago, IL).

RESULTS

General

Of the 119 patients, we eliminated three patients, who had a cerebral vascular embolism after the surgery, from the statistical analyses of this study. The mean age of all the patients (*F*/mean = 38/78) was 64.3 (27–84 years). Nineteen of the 116 patients (16.4%) developed delirium after the cardiac surgery. The mean ± SD of the severity score of Delirium Rating Scale—Revised-98 was 24.3 ± 6.0. Eighteen of the patients with delirium (94.7%) were older than 60 years, whereas 60 of 97 nondelirium patients (61.9%) were older than 60 years. The patients underwent five types of cardiac surgery (number of patients): coronary artery bypass graft surgery (54), valve replacement surgery (29), coronary artery bypass graft surgery and valve replacement surgery (12), thoracic aortic aneurysm surgery (12), and the others (9). The cardiac surgery using extracorporeal circulation was carried out on 70 patients (60.3%).

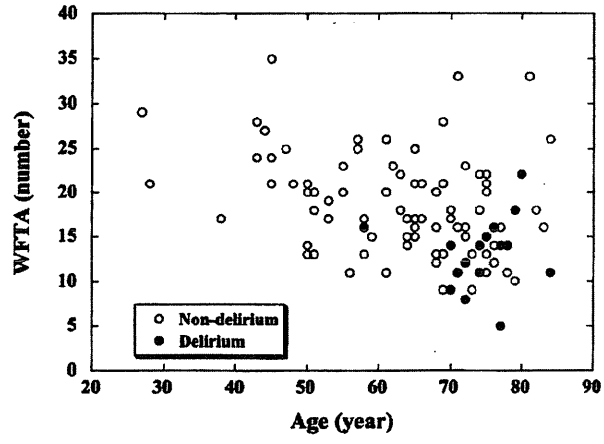
Pre-, Peri-, and Postoperative Variables

The results of the comparison of the pre-, peri-, and postoperative data between the delirium group and the nondelirium group are shown in Table 1. There was a statistically significant difference between the two groups in age, body mass index, high blood pressure, hemodialysis, and blood transfusion

during the operations. Based on the neuropsychological tests, a significant difference between the two groups was observed in the WFTA, the Word Fluency test K, and the Trail Making test B-A, but not in the Mini-Mental State Examination, Color Stroop test, or digit span tests. There was no patient who had a depressive syndrome checked by either the Beck depression scale or a preoperative interview. Neither alcoholic nor dementia was diagnosed by the preoperative assessment in the subjects of the study.

The multivariate logistic regression analysis, in which the significant variables with a $p < 0.05$ based on the univariate comparison test were entered, revealed that advanced age and low WFTA score were important risk factors of postoperative delirium (Table 2). The analysis using ANCOVA with the age of the patient treated as a nuisance covariate showed that the WFTA score of the delirium group was significantly lower than that of the nondelirium group ($F = 5.82$, $df = 1,95$, $p < 0.05$, Fig. 1).

FIGURE 1. Scatter Plots of the Word Fluency Test Animal (WFTA) With Age in Patients With Delirium and Without Delirium



The WFTA score of the delirium group was significantly decreased ($F = 5.82$, $df = 1,95$, $p < 0.05$) compared with that of the nondelirium group. The analysis was performed by ANCOVA with the age of the patient treated as a nuisance covariate.

MRI Study

The comparison of the FA values between the delirium group and nondelirium groups using a single subject condition without the covariate model for the SPM2 is shown in Fig. 2 and Table 3. There was a significant reduction in the FA values of the delirium patients in many sections of the bilateral WM, i.e., frontal lobe, temporal lobe, parietal lobe, limbic lobe, and the bilateral thalamus.

The SPM2 analysis between the two groups with treated age as the covariate demonstrated a significant decrease in the FA values in four clusters of the brain area, i.e., left subgyral of frontal lobe, right cingulate gyrus, left ventral anterior nucleus of the thalamus, and corpus callosum (Fig. 3 and Table 3). The analysis using ANCOVA also indicated that the FA values of the delirium group was significantly decreased in the four brain regions compared with the nondelirium group (left thalamus (ventral anterior nucleus): $F = 11.13$, $df = 1,113$, $p < 0.01$, left frontal lobe (subgyral): $F = 11.87$, $df = 1,113$, $p < 0.001$, right cingulate gyrus: $F = 11.94$, $df = 1,113$, $p < 0.001$, corpus callosum (splenium): $F = 10.00$, $df = 1,113$, $p < 0.01$). In addition, a statistically significant correlation between the FA values and the age was found in all four areas of the nondelirium group (left frontal lobe [$r = 0.394$, $df = 95$, $p < 0.0001$], right cingulate gyrus [$r = 0.376$, $df = 95$, $p < 0.0001$], left thalamus [$r = 0.488$, $df = 96$, $p < 0.0001$] and corpus callosum [$r = 0.398$, $df = 95$, $p < 0.0001$]) but not either of the brain areas of the delirium group (Fig. 4).

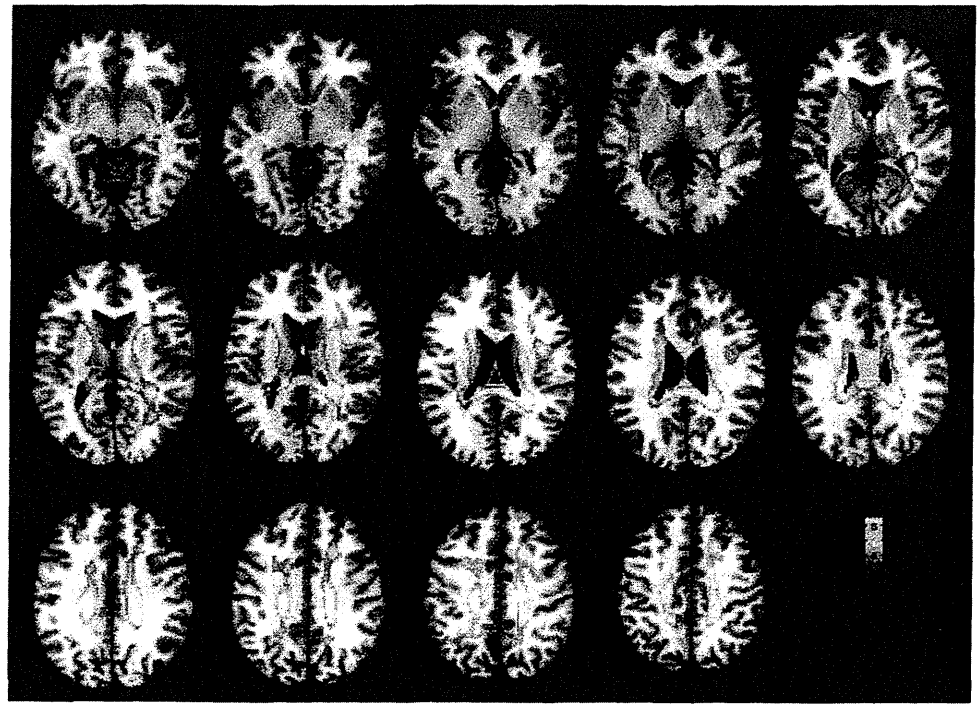
In a subgroup of the subjects older than 60 years, the analysis using ANCOVA also indicated that the FA values of the delirium group was significantly decreased in the four brain regions compared with the nondelirium group (left thalamus (ventral anterior nucleus): $F = 14.6$, $df = 1,74$, $p < 0.01$, left frontal lobe (subgyral): $F = 10.8$, $df = 1,74$, $p < 0.01$, right

TABLE 2. Predictive Factors of Delirium After Cardiac Surgery Resulting From the Multivariate Logistic Regression Analysis

Variables	Odds Ratio	95% Confidence Interval	p
Age (per year)	1.163	1.044-1.296	0.006
WFT A (per number)	0.827	0.707-0.966	0.017

Notes: Variables with significantly different changes between the delirium group and the nondelirium group ($p < 0.05$), i.e., age, body mass index, high blood pressure, hemodialysis, Trail making test B-A, WFTA, WFTK, and blood transfusion, were entered into a backward stepwise logistic regression analysis requiring a p value < 0.05 to remain (Wald χ^2 test, $df = 1$).

FIGURE 2. Comparisons of FA Values Between the Delirium Group and Nondelirium Group by a Single Subject Condition Without the Covariate Model for the SPM2



The SPM{t} values (one-tailed p <0.001, uncorrected) are displayed on the axial FA template images. A significant decrease in the FA values for the delirium group was observed in many brain areas including the bilateral thalamus, bilateral deep white matters of entire cerebral cortices, and corpus callosum.

cingulate gyrus: $F = 7.4$, $df = 1,74$, $p < 0.01$, corpus callosum (splenium): $F = 9.9$, $df = 1,74$, $p < 0.01$).

DISCUSSION

This study demonstrated a significant reduction in the FA values of the WM in many areas of the brain predisposed patients to delirium after the cardiac operation. However, a comparison of the FA values between the delirium group and the nondelirium group analyzing age as the nuisance covariate revealed a statistically significant change in only four clusters of the brain areas. Consequently, it seems that the majority of the brain areas which exhibited a significant decrease in the FA values of the delirium group were involved in the age-related changes in the WM.

Histological studies of postmortem brains have reported age-related alterations in the WM, such as a decline in the volume, number, and length of the myelinated fibers.[42–44] Many cross-sectional, longitudinal, and structural MRI studies showed age-related volume increases in the cerebrospinal fluid (CSF)-filled space that primarily occurred at the expense of cortical gray matter and with most showing little volume change in the WM,[19] whereas a few studies reported that there was a steady decline in the WM volume.[45,46] However, DTI studies including this study indicated age-related declines in the FA values of WM in normal healthy adults in whom volume declines were not necessarily detectable,[16–24] which may be attributable to the changes in the WM observed in the postmortem brains.[42–44]

The core features of delirium are disturbances in consciousness and attention.[1,2] The integrity of the WM in CNS is required to control consciousness and pay attention to objects. The brain states of vigilance are controlled by a system that originates in the

TABLE 3. Decreases in the FA Values of Patients With Delirium

Anatomical Regions	MNI Coordinates			*t*	
	x	*y*	*z*	Model 1[a] (*df* = 114)	Model 2[b] (*df* = 113)
Thalamus					
Left ventral anterior nucleus	−8	−6	6	4.96	3.37
Left ventral lateral nucleus	−10	−12	8	4.42	
Left pulvinar	−20	−28	12	4.31	
Left ventral posterior lateral nucleus	−16	−16	8	3.62	
Left lateral posterior nucleus	−20	−20	12	3.76	
Right anterior nucleus	6	−4	8	4.04	
Right ventral anterior nucleus	12	−6	8	3.39	
Right pulvinar	16	−32	12	4.08	
Right ventral lateral nucleus	16	−16	12	3.28	
Temporal lobe					
Left superior temporal gyrus WM	−36	−40	8	4.44	
Left middle temporal gyrus WM	−56	−36	10	3.21	
Left subgyral WM	−44	−36	−4	3.85	
Right superior temporal gyrus WM	38	−38	8	3.50	
Right subgyral WM	42	−36	−4	3.51	
Frontal lobe					
Left inferior frontal gyrus WM	−36	32	8	3.46	
Left precentral gyrus WM	−36	−4	36	3.41	
Left subgyral WM	−22	−32	34	4.86	3.46
Right subgyral WM	40	0	22	3.53	
Parietal lobe					
Left supramarginal gyrus WM	−48	−42	34	3.42	
Left precuneus WM	−20	−56	36	3.61	
Left subgyral WM	−22	−52	38	3.53	
Right subgyral WM	34	−52	32	3.23	
Limbic lobe					
Left cingulate gyrus WM	−20	−10	42	3.86	
Right cingulate gyrus WM	20	−24	36	4.75	3.47
Left anterior cingulate WM	−12	24	22	3.60	
Corpus callosum (splenium)	−6	−34	20	4.58	3.18

Notes: The FA values were compared between the two groups (paticnts with or without postoperative delirium) by a voxel-by-voxel analysis using the SPM2. MNI: Montreal Neurological Institute.

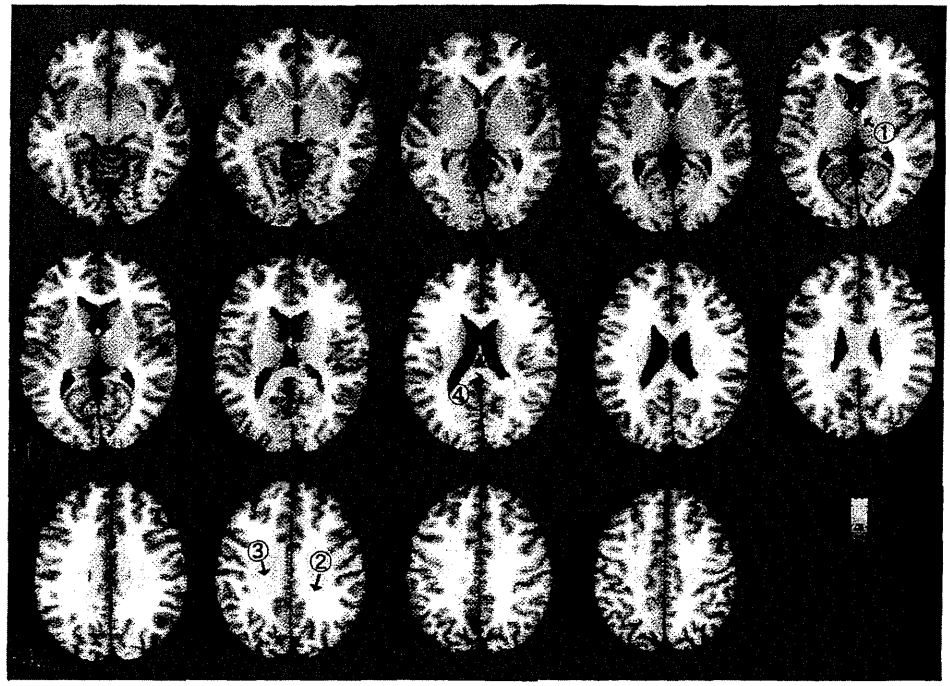
[a]In Model 1, to estimate population effects (diagnostic effects), we used a single subject condition (nondelirium [n = 97] or delirium [n = 19]) and covariate (no covariate of interest) model for the SPM analysis.

[b]In Model 2, we applied the single subject condition (nondelirium [n = 97] or delirium [n = 19]) and covariate (covariate of interest; age). For the analyses, we set the masking threshold for the FA values of 0.2 for excluding voxels containing partial volume of WM and other tissues. We used the one-tailed $p < 0.001$ (uncorrected) as a statistical threshold to search for significant differences between the groups.

brainstem and projects through synaptic relays in the thalamus to the cerebral cortex,[47,48] and neural networks composed of a number of brain areas including the cerebral cortices (e.g., posterior parietal cortex, frontal eye fields, and cingulate cortex) and subcortical areas (e.g., thalamus, striatum, and the reticular activating system) play an important role in visuospatial attention.[49–51] It is also suggested that there is widespread disruption of higher cortical function involving the several brain areas in delirium.[52] Consequently, it is likely that the alterations in the microstructure of the WM underlay a vulnerability of the patients to develop postoperative delirium in this study.

All the subjects of this study had a minimal impairment of cognitive function. A sensitive decline in the delirium group was observed in the executive functions such as the Trail Making tests and the word fluency tests, in accordance with a recent study reporting that the mildly impaired cognitive performance can be an independent risk factor for postoperative delirium.[13] The decrease in the functions with normal aging is supposed to be paralleled with the anatomical changes of the frontal lobe and its connection with other brain areas.[23,53] Moreover, the multivariate stepwise logistic analysis indicated that the lower WFTA score was an important predisposed risk factor for the postoperative delirium. It has been reported that the semantic fluency

FIGURE 3. Comparisons of FA Values Between the Delirium and Nondelirium Group by a Single Subject Condition With the Covariate (Age) Model for the SPM2



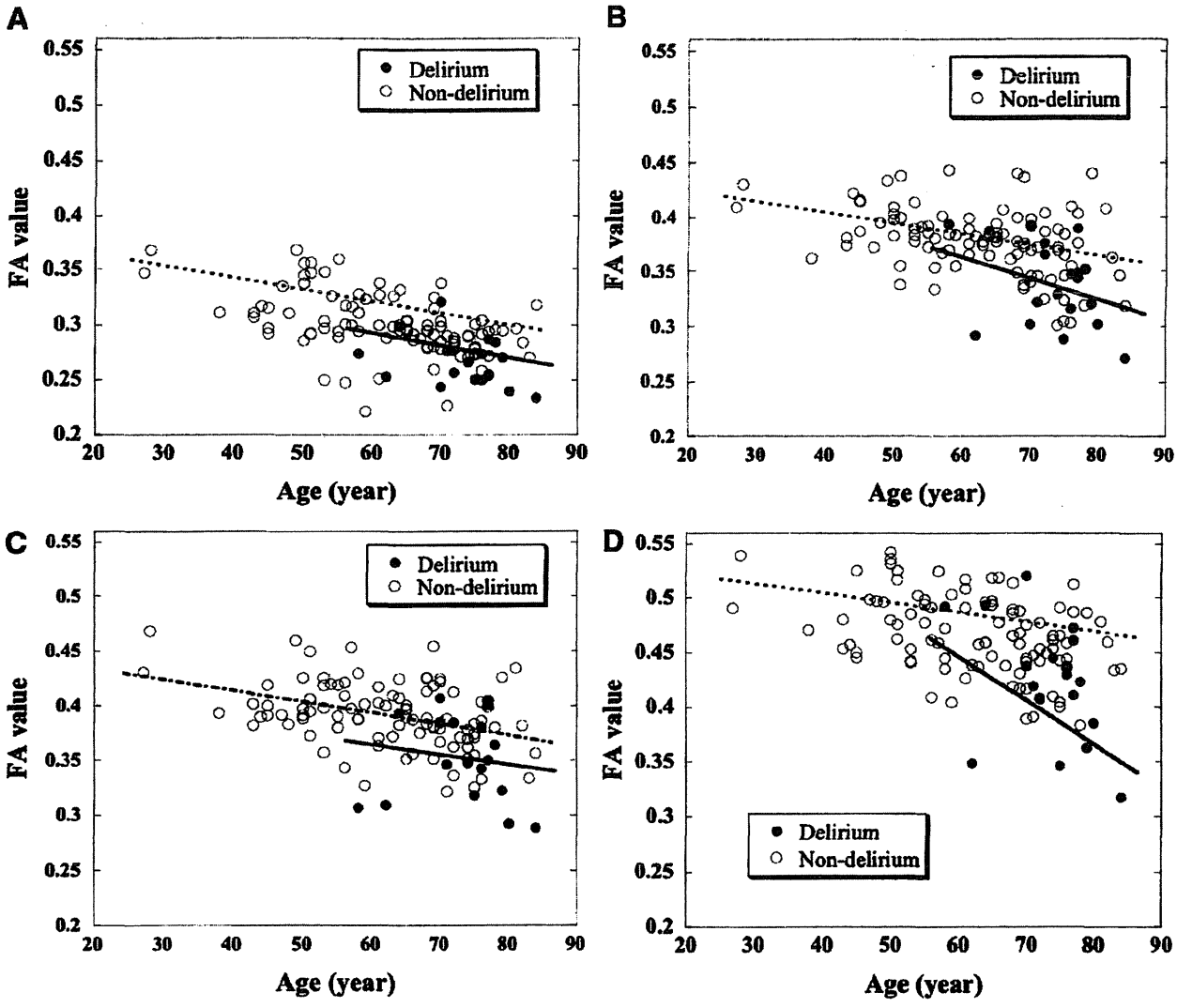
The SPM{t} values (one-tailed p <0.001, uncorrected) are displayed on the axial FA template images. A significant decrease in the FA values was observed in four clusters of the brain area. The area of maximum change in each cluster was ① the left thalamus (nucleus ventralis anterior)(the Montreal Neurological Institute (MNI) coordinates; $x = -8, y = -6, z = 6$), ② the left frontal lobe (subgyral white matter) (MNI coordinates; $x = 22. y = -32, z = 34$), ③ the right limbic lobe (cingulate gyrus white matter) (MNI coordinates; $x = 20, y = -24, z = 36$) and ④ the corpus callosum (splenium) (MNI coordinates; $x = -6, y = -34, z = 20$) (Table 3). The brain area is marked by an arrow with the number.

task such as WFTA was more useful than the letter fluency task for discriminating between healthy aging and mild dementia of the Alzheimer type.[33,54] Thus, this study suggests a similar usefulness of the word fluency tests for predicting a risk for postoperative delirium.

A number of limitations of our study ought to be mentioned. Because this study was carried out in a limited number of patients who underwent the planned cardiac operation, further examinations carried out in a larger group of subjects or with any modification to the design of the study, such as a noncardiac operation, are required to confirm and generalize the observation of this study. Secondary, although the assessment by the T1 and T2-weighed images could not find any quantitative difference between the two groups in this study (data not shown), it is likely that small and silent insults of cerebrovascular arteriosclerosis observed in the older persons interfere the FA values of the WM. Thus, the relationship between the fiber integrity and the pathological changes needs to be explored further. Another limitation is the spatial normalization of WM regions in the voxel-based analysis of FA data,[14,41] whereas many studies using the analysis have already provided an important insight into microstructural WM abnormalities in neuropsychiatric disorders.[39–41,55,56] Large variations of brain shape in older subjects might limit an ability to achieve the normalization across the subjects. The FA values in some regions surrounding the ventricles (Figs. 2 and 3) could be distorted by the misregistrations of image and underlying variation of brain shapes.

In conclusion, this study revealed that the advanced age and the cognitive decline were important predictive indicators of the postoperative delirium and suggests that the abnormalities of the microstructure in the

FIGURE 4. Scatter Plots of the FA Values With Age in Four Brain Areas



[A] The left thalamus (nucleus ventralis Anterior), [B] the left frontal lobe (subgyral white matter), [C] the right limbic lobe (cingulated gyrus white matter), and [D] the corpus callosum (splenium). The FA values of the delirium group were significantly decreased in the four brain areas (left thalamus [nucleus ventralis anterior]: $F = 11.13$, $df = 1,113$, $p < 0.01$, left frontal lobe [subgyral]: $F = 11.87$, $df = 1,113$, $p < 0.001$, right cingulate gyrus: $F = 11.94$, $df = 1,113$, $p < 0.001$, corpus callosum: $F = 10.00$, $df = 1,113$, $p < 0.01$) compared with those of the nondelirium group. The analysis was performed by ANCOVA with the age of the patients treated with as a nuisance covariate. The linear regression lines between the age (x) and the FA values (y) of each brain area for the nondelirium group (the thalamus [$y = -0.001 \times x + 0.383$, $r = 0.488$, $df = 95$, $p < 0.0001$], the frontal lobe [$y = -0.001 \times x + 0.352$, $r = 0.394$, $df = 95$, $p < 0.0001$], the cingulate gyrus [$y = -0.001 \times x + 0.465$, $r = 0.376$, $df = 95$, $p < 0.0001$] and the corpus callosum [$y = -0.002 \times x + 0.465$, $r = 0.398$, $df = 95$, $p < 0.0001$]) and the delirium group (the thalamus [$y = -0.001 \times x + 0.352$, $r = 0.346$, $df = 17$, $p = 0.147$], the frontal lobe [$y = -0.002 \times x + 0.51$, $r = 0.396$, $df = 17$, $p = 0.0936$], the cingulate gyrus [$y = -0.001 \times x + 0.394$, $r = 0.089$, $df = 17$, $p = 0.716$], and the corpus callosum [$y = -0.004 \times x + 0.685$, $r = 0.431$, $df = 17$, $p = 0.0656$]) are indicated by the dotted lines and solid lines, respectively.

deep WMs and thalamus are predisposed in the patients with delirium and putatively account for the underlying mechanism of age-related vulnerability to delirium. On the other hand, the FA values in the four brain areas such as left ventral anterior nucleus of the thalamus may be affected by factors other than aging,

supported by the additional analysis in the subgroup of the subjects older than 60 years indicating that the decreased FA values of the brain areas in the delirious patients were also statistically significant. Thus, further DTI studies to clarify factors other than aging affecting the microstructure of the WM, e.g., arteriosclerosis, can provide a new insight into the brain condition vulnerable to delirium.

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ORIGINAL ARTICLE

Genome-wide association study identifies a potent locus associated with human opioid sensitivity

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Opioids, such as morphine and fentanyl, are widely used as effective analgesics for the treatment of acute and chronic pain. In addition, the opioid system has a key role in the rewarding effects of morphine, ethanol, cocaine and various other drugs. Although opioid sensitivity is well known to vary widely among individual subjects, several candidate genetic polymorphisms reported so far are not sufficient for fully understanding the wide range of interindividual differences in human opioid sensitivity. By conducting a multistage genome-wide association study (GWAS) in healthy subjects, we found that genetic polymorphisms within a linkage disequilibrium block that spans 2q33.3–2q34 were strongly associated with the requirements for postoperative opioid analgesics after painful cosmetic surgery. The C allele of the best candidate single-nucleotide polymorphism (SNP), rs2952768, was associated with more analgesic requirements, and consistent results were obtained in patients who underwent abdominal surgery. In addition, carriers of the C allele in this SNP exhibited less vulnerability to severe drug dependence in patients with methamphetamine dependence, alcohol dependence, and eating disorders and a lower 'Reward Dependence' score on a personality questionnaire in healthy subjects. Furthermore, the C/C genotype of this SNP was significantly associated with the elevated expression of a neighboring gene, *CREB1*. These results show that SNPs in this locus are the most potent genetic factors associated with human opioid sensitivity known to date, affecting both the efficacy of opioid analgesics and liability to severe substance dependence. Our findings provide valuable information for the personalized treatment of pain and drug dependence.

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Keywords: analgesia; dependence; opioids; pharmacogenetics

INTRODUCTION

The opioid system has important roles in both antinociception and reward.[1,2] Therefore, opioids, such as morphine and fentanyl, are widely used not only as effective analgesics for the treatment of acute and chronic pain but also as abused drugs. The opioid system is also involved in the rewarding effects of morphine,[3] ethanol,[4] cocaine,[5] and various other drugs[6–8] or behaviors. However, opioid sensitivity is well known to vary widely among individual subjects,[9] resulting in differences in the effectiveness of opioid analgesics and vulnerability to dependence on opioids and other drugs or behaviors. Individual differences may be attributable to both genetic and environmental factors,[10] although the relative influence of each of these factors can be diverse. To date, several candidate genetic polymorphisms have been reported to be associated with opioid sensitivity in human studies.[10–14] However, such polymorphisms have not sufficiently explained the wide range of interindividual variance observed in the sensitivity to opioid analgesics. A genome-wide approach has not yet been adopted to explore the best candidates, although this approach has been applied to other pharmacogenomics-related traits. Several genetic polymorphisms have been found to be associated with the sensitivity to pharmacotherapies.[15–17] In this study, we sought to comprehensively identify genetic polymorphisms in the human genome that could greatly contribute to individual differences in opioid sensitivity by conducting a genome-wide association study (GWAS) of healthy subjects and further analyses.

MATERIALS AND METHODS

Subjects

Enrolled in this multistage GWAS were 355 healthy patients who were scheduled to undergo cosmetic orthognathic surgery for mandibular prognathism at Tokyo Dental College Suidoubashi Hospital. The surgical

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protocol and subsequent postoperative pain management were fundamentally the same as a previous study[12] and are detailed in the Supplementary Information.

The subjects recruited in the additional analysis to confirm the association between the rs2952768 single-nucleotide polymorphism (SNP) and postoperative opioid analgesic requirements were 112 patients who underwent major open abdominal surgery at several related hospitals. The surgical protocol and subsequent postoperative pain management were fundamentally the same as a previous study[11,18] and are detailed in the Supplementary Information. Enrolled in the study to investigate the contribution of the rs2952768 SNP to the symptoms of drug dependence or related personality traits were 203 patients with methamphetamine (METH) dependence with clinical data that included their multisubstance abuse status, 438 patients with alcohol dependence with clinical data that included the number of drugs used, 228 patients with eating disorders with clinical data including the presence or absence of other psychiatric disorders such as substance dependence and 500 healthy volunteer subjects with personality profile data from the temperament and character inventory (TCI).[19–21] To examine the mRNA expression levels of the *METTL21A* (*FAM119A*) and *CREB1* genes, 100 post-mortem human brain specimens, from which DNA and RNA were extracted for experimental use, were obtained from the Stanley Medical Research Institute (SMRI; Bethesda, MD, USA) as samples independent of those in the association study with opioid sensitivity (SMRI samples).

All of the individuals included in the study originated from Japan, with the exception of those from whom the SMRI samples were obtained, whose racial background was mostly European American (see Supplementary Information).

The study protocol was approved by the Institutional Review Boards at the related hospitals, Tokyo Institute of Psychiatry (currently Tokyo Metropolitan Institute of Medical Science) and the ethics committee of each participating institute of the Japanese Genetics Initiative for Drug Abuse.[22,23] All of the subjects provided informed, written consent for the genetic studies. The detailed demographic and clinical data of the subjects are provided in Supplementary Tables 1, 5–8 and 10.

Genotyping

After total genomic DNA was extracted from whole-blood samples using standard procedures, whole-genome genotyping was performed using the Infinium assay II with an iScan system (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. The data for the whole-genome genotyped samples were analyzed using BeadStudio or GenomeStudio with the Genotyping module v3.3.7 (Illumina) to evaluate the quality of the results. In the data-cleaning process, the samples with a genotype call rate of <0.95 were excluded from further analyses. As a result, one sample was excluded from further analyses. Markers with a genotype call frequency of <0.95 or 'Cluster sep' (that is, an index of genotype cluster separation) of <0.1 were excluded from the subsequent association study. A total of 295 036 SNP markers survived the filtration process and were used for the GWAS (Supplementary Figure S1).

For additional genotyping of the rs2952768 and rs2254137 SNPs, the TaqMan allelic discrimination assay (Life Technologies, Carlsbad, CA, USA) was mostly conducted after total genomic DNA was extracted from whole-blood or oral mucosa samples using standard procedures. For samples that were not appropriately genotyped by this assay, direct sequencing was alternatively adopted to genotype the rs2952768 SNP. A total of 112, 203, 438, 228, 500 and 105 DNA samples from patients who underwent major abdominal surgery, patients with METH dependence/psychosis, patients with alcohol dependence, patients with eating disorders, healthy volunteer subjects and SMRI, respectively, were used to genotype the rs2952768 SNP. In addition, a total of 105 DNA samples from the post-mortem specimens for the expression analysis were used to genotype the rs2254137 SNP, although genotyping this SNP for other samples was not conducted because of the strength of the linkage disequilibrium (LD) with the rs2952768 SNP. The genotype distribution of the rs2952768 SNP in patients with METH dependence/psychosis, patients with alcohol dependence and patients with eating disorders is provided in Supplementary Table 9.

Quantitative PCR procedure

The SMRI RNA samples were treated with DNase I using the RNase-Free DNase Set (Qiagen, Hilden, Germany), and clean-up was then performed using the RNeasy MinElute Cleanup Kit (Qiagen). First-strand complementary DNA for use in the real-time quantitative PCR was synthesized with the SuperScriptIII First-Strand synthesis system for quantitative reverse transcriptase-PCR (Life Technologies) with 100 ng purified total RNA according to the manufacturer's protocol and diluted properly with diethylpyrocarbonate-treated H_2O before the experiments.

To perform real-time quantitative PCR with a LightCycler 480 (Roche Diagnostics, Basel, Switzerland), TaqMan Gene Expression Assays (Life Technologies) were used as a probe/primer set specified for the *FAM119A* (*METTL21A*) gene and *CREB1* gene and a probe/primer set for the *ACTB* gene, a house-keeping gene that encodes β-actin. The expression level of the *FAM119A* (*METTL21A*) gene or *CREB1* gene was normalized to the expression level of the *ACTB* gene for each sample, and relative mRNA expression levels were compared between the genotype subgroups for each gene. The experiments were performed in triplicate (separate experiments) for each sample, and average values were calculated for normalized expression levels.

Statistical analysis

A three-stage GWAS was conducted for the patients who underwent painful cosmetic surgery to investigate the association between opioid sensitivity and the 295 036 SNPs that passed the quality control criteria in a total of 353 subjects (118, 117 and 118 subjects for the first-, second- and final-stage analyses, respectively). As an index of opioid sensitivity, postoperative patient-controlled analgesia fentanyl use during the first 24-h postoperative period was used because analgesic requirements likely reflect the efficacy of fentanyl in each individual. To explore the association between the SNPs and phenotype, linear regression analyses were conducted in each stage of the analysis, in which postoperative fentanyl use ($\mu g\,kg^{-1}$; log transformed) and the genotype data of each SNP were incorporated as dependent and independent variables, respectively. Additive, dominant and recessive genetic models were used for the analyses because of the previously insufficient knowledge about the genetic factors associated with opioid sensitivity. The GWAS procedure is summarized in Supplementary Figure S1. In the first-stage analysis of 118 subjects, the SNPs that showed statistical *P*-values of <0.05 were selected as the candidate SNPs for the second-stage analysis among the 295 036 SNPs. For these SNPs, the second-stage analysis was conducted; again, the SNPs that showed $P<0.05$ were considered potent candidates and selected for further final-stage analysis. In the final stage of the three stages, the *Q*-values of the false discovery rate were calculated to correct for multiple testing in addition to the *P*-values based on previous reports.[24,25] The SNPs that showed $Q<0.05$ in the analysis were considered genome-wide significant.

Additional analyses were conducted using the samples of the patients who underwent major abdominal surgery, patients with METH dependence/psychosis, patients with alcohol dependence, patients with eating disorders, healthy volunteers with personality profile data and post-mortem specimens for the expression analysis. For all of the genotype data used in these analyses, the distributions were checked using the χ^2 test, and the absence of significant deviation from the theoretical distribution expected from Hardy–Weinberg equilibrium was confirmed (Supplementary Table 13).

In the analysis of the patients who underwent major abdominal surgery, the calculated total dose of rescue analgesics administered during the first 24-h postoperative period was used as an index of opioid sensitivity. To explore the association between the SNPs and phenotype, Student's *t*-test or analysis of variance was performed, in which Bonferroni correction for multiple comparisons was used for the *post hoc* tests. For these analyses, postoperative analgesic use ($\mu g\,kg^{-1}$; log transformed) and the genotype data of each SNP were incorporated as dependent and independent variables, respectively. χ^2 Tests were performed to investigate the contribution of the SNPs to the vulnerability to the presence of serious symptoms of substance dependence. For the analyses in which the number of subjects in a cell in 2×2 contingency tables was <5, Fisher's exact tests were conducted instead of χ^2 tests. In the analysis of healthy volunteers with personality profile data, raw TCI scores were properly processed (see Supplementary Information). To explore the association between the SNPs and phenotype, linear regression analyses were conducted, in which the endpoint TCI score (log transformed) and genotype data of each SNP were incorporated as dependent and independent variables, respectively. The correction of multiple tests for the analyses of the seven phenotypes was not performed in the present exploratory study. In the analysis of the post-mortem specimens for the expression analysis, the calculated expression level of the *FAM119A* (*METTL21A*) gene or *CREB1* gene normalized to the *ACTB* gene for each