Table 2 Volumetric measures and statistical results

Variables	Patients with schizophrenia ($n = 29$)		Controls ($n = 29$)		Effect sizes*	Repeated measures analysis of variance			
						Group	Group × Hemisphere	Group × Region	Group × Region × Hemisphere
	Mean	SD	Mean	SD		F (p)	F (p)	F (p)	F (p)
Intracranial volume	1,620	139	1,649	122	0.24				
Left hemisphere									
Area 44	1.92	0.49	2.06	0.48	0.24	4.59 (0.037)	1.96 (0.17)	0.54 (0.47)	1.03 (0.32)
Area 45	2.43	0.67	2.54	0.68	0.10				
Right hemisphere									
Area 44	1.84	0.60	2.10	0.63	0.37				
Area 45	2.27	0.67	2.76	0.86	0.51				

Effect sizes are calculated as: (MEANnc-MEANsc)/SDnc, MEANnc (sc): Group mean of relative volumes of normal controls (patients with schizophrenia)

Table 3 Association between volumetric measures and symptoms

	Left hemisphere		Right hemishere	
	Area 44	Area 45	Area 44	Area 45
Positive symptoms	−0.08	−0.42*	0.02	−0.38*
Negative symptoms	0.02	−0.04	0.08	−0.30
Disorganization symptoms	−0.15	−0.49**	−0.17	−0.49**
Delusional behavior	0.01	−0.49**	−0.13	−0.13

Spearman's rho is presented. * $p < 0.05$, ** $p < 0.01$

healthy male controls. In particular, the present parcellation study found for the first time that the BA 45 volume in right hemisphere showed the largest effect size among the four inferior frontal sub-regions. In addition, the reduced volume of BA 45 specifically showed significant associations with the increased severity of disorganized, positive symptoms and delusional behavior in the patient group, with the specificity being confirmed by Fisher's r to z transformation.

The gray matter volume of both BA 44 and BA 45 was significantly and bilaterally smaller in the patients with schizophrenia than in the controls. The current results are consistent with the previous findings of bilateral inferior frontal gyrus volume reduction without sub-regional segmentation in patients with schizophrenia [7, 31, 38]. In addition, the previous studies employing computational voxel-based analysis also showed regional gray matter volume reduction in the inferior frontal gyrus [3, 4, 6, 13, 19, 27, 30, 36]. Since the present sub-regional segmentation study revealed the highest effect size of gray matter volume reduction in the right BA 45, the present study not only replicated the previous findings of inferior frontal gyrus volume reduction but also extended them to identify the right BA 45 as the site of greatest volume reduction.

The current parcellation study uncovered a correlation between reduced BA 45 volume and positive, disorganized, and delusional behaviors in the patient group. While the severity of disorganized and positive symptoms showed a negative correlation with the volume of BA 45 bilaterally, the severity of delusional behavior showed a negative correlation with the volume of BA 45 in left hemisphere alone. The laterality of the present findings is consistent with previous studies reporting the association between language processing, such as semantic verbal fluency task, and left BA 45 in healthy subjects [28, 40]. In contrast to language processing, inferior frontal gyrus involvement in interpersonal aspect behaviors, such as imitation of other's behavior, is not lateralized to the left hemisphere [5]. F-MRI studies revealed that understanding another person's intention is associated with right inferior frontal gyrus, although the area mainly consists of right pars opercularis [16]. The present study showing correlation between reduced right BA 45 volume and severe psychotic symptoms is also consistent with these previous studies.

The specificity of correlation of BA 45 with psychotic symptom severity including delusional behavior, as confirmed by Fisher's r to z transformation, is consistent with the previous findings of a small number of functional imaging studies that have examined this issue. For example, Wisco et al. [35] recently found that an area within the pars triangularis of the left inferior frontal gyrus showed significantly different cortical folding patterns in the patient group compared with the control group. Dollfus et al. [9] found lower BOLD signal changes during a language task in patients as compared with their control subjects in a network comprising areas of the left middle temporal gyrus, the left angular gyrus, and the pars triangularis of the left inferior frontal gyrus. The present study is consistent with these functional imaging studies,

and in addition, provides support at the brain structural level for the notion that BA 45, mainly consisting of pars triangularis, plays an important role in the pathophysiology of schizophrenia.

Here we address the methodological considerations and future direction of our study. First, since the present study sample included patients with chronic schizophrenia and antipsychotic medications, the effect of chronic illness [18] and medication [24] on the present findings cannot be totally ruled out. The volumes of ROIs did not show any significant correlation with duration of illness and daily dose of neuroleptics. In addition, the main effect of antipsychotic medication type was not significant when we employed repeated measures ANOVA in the patients. However, the effect of cumulative dosage still remained unclear. Therefore, future studies should employ patients with first episode schizophrenia and minimum medication. Second, parental SES of the patients was significantly lower than those of controls. Though the parental SES did not show any significant correlation with the volumes of ROIs and the statistical conclusions remained unchanged when we covaried parental SES, the effect of parental SES on the present findings cannot be totally ruled out. Third, the possible sexual-dimorphism in the structural abnormality of inferior frontal gyrus in patients with schizophrenia is still unclear since the present study participants were all male. As significant gender differences in cortical thickness [17] and behavioral correlates [39] of inferior frontal gyrus have been reported, future study should address the issue. Finally, since cytoarchitectonic borders with significant inter-individual variability do not consistently coincide with sulcal contours as shown by Amunts et al. [2], it should be noted that the current definition utilizing sulcal pattern cannot exactly reflect cytoarchitectonic borders.

In conclusion, the present findings employing a reliable manual-tracing method demonstrated that the male patients with schizophrenia had significantly reduced volumes of both the BA 44 and BA 45 compared with the healthy male controls bilaterally. In particular, the BA 45 volume in right hemisphere revealed the largest effect size among the inferior frontal sub-regions. In addition, the reduced volume of BA 45 showed significant associations with the increased severity of disorganized, positive symptoms and delusional behavior in the patient group. Of note, the Fisher's Z-transformation confirmed a differential relationship of delusional behavior with reduced volume of BA 45, rather than BA 44. These results indicate a significant role of inferior frontal gyrus, especially BA 45, in the pathophysiology of schizophrenia.

Acknowledgments This study was supported by grants-in-aid for scientific research (No. 18390319 and 20023009 to KK) from the JSPS and the MEXT, and by grants-in-aid (H18-Shi 7, H19-Kokoro-Ippan 012, H20-3 and H20-Kokoro-Ippan 001 to KK) from the Ministry of Health, Labor and Welfare, Japan. We thank Dr. Mark A. Rogers for his helpful comments and English editing on the manuscript.

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Glycine Transporter-1: A New Potential Therapeutic Target for Schizophrenia

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Abstract: The hypofunction hypothesis of glutamatergic neurotransmission via *N*-methyl-D-aspartate (NMDA) receptors in the pathophysiology of schizophrenia suggests that increasing NMDA receptor function via pharmacological manipulation could provide a new therapeutic strategy for schizophrenia. The glycine modulatory site on NMDA receptor complex is the one of the most attractive therapeutic targets for schizophrenia. One means of enhancing NMDA receptor neurotransmission is to increase the availability of the obligatory co-agonist glycine at modulatory site on the NMDA receptors through the inhibition of glycine transporter-1 (GlyT-1) on glial cells. Some clinical studies have demonstrated that the GlyT-1 inhibitor sarcosine (*N*-methylglycine) shows antipsychotic activity in patients with schizophrenia. Currently, a number of pharmaceutical companies have been developing novel and selective GlyT-1 inhibitors for the treatment of schizophrenia. A recent double blind phase II study demonstrated that the novel GlyT-1 inhibitor RG1678 has a robust and clinically meaningful effect in patients with schizophrenia. In this article, the author reviews the recent findings on the GlyT-1 as a potential therapeutic target of schizophrenia.

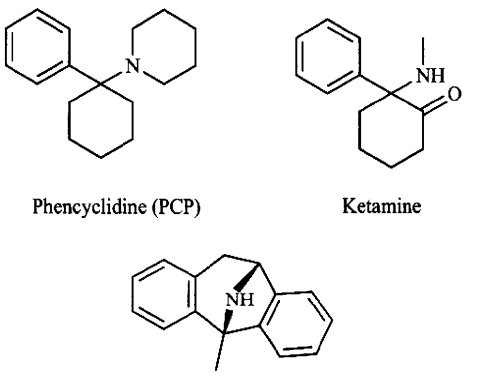
Keywords: Schizophrenia, NMDA receptor, Glutamate, Glycine, Transporter, Glia.

INTRODUCTION

The hypofunction of glutamatergic neurotransmission via the *N*-methyl-D-aspartate (NMDA) receptors might play a role in the pathophysiology of schizophrenia [1-10]. The non-competitive NMDA receptor antagonist phencyclidine (PCP; Fig. **1**) and ketamine (Fig. **1**) have been shown to produce a wide range of transient schizophrenia-like symptoms, including estrangement, loss of body boundaries, formal thought disorder, hallucinations, and psychosis [1,11-15]. In addition, the NMDA receptor antagonists, including PCP or ketamine, are known to dramatically exacerbate the symptoms of schizophrenia [1,16]. Thus, it seems that blockade of NMDA receptors by PCP or ketamine could cause schizophrenia-like symptoms in humans.

Glycine (Fig. **2**) is among the well-characterized amino acid neurotransmitters in the mammalian central nervous system (CNS), where it is well known to act as an inhibitory transmitter via its interaction with strychnine-sensitive glycine receptors [17]. It also plays an important role in excitatory neurotransmission via strychnine-insensitive glycine site located on the NMDA receptors [17,18]. In the CNS, synaptic levels of glycine are regulated by specific sodium/chloride-dependent transporters. The effects of glycine in the synapse are terminated by its rapid reuptake into the nerve terminal and adjacent glial cells via high-affinity glycine transporters referred to as GlyT-1 and GlyT-2. GlyT-1 and GlyT-2 possess 12 putative transmembrane spanning domains, and share approximately 50% amino acid sequence identity [17,18]. GlyT-1 is widely expressed in the CNS. Although GlyT-1 is predominantly present on glial cells, some immunohistochemical studies suggested the existence of GlyT-1 in neurons, particularly on pre-synaptic nerve endings of glutamatergic neurons [19]. It is likely that GlyT-1 is responsible for glycine reuptake in forebrain areas, and in some regions, it may be co-localized with strychnine-insensitive glycine modulatory site on the NMDA receptors [21-23]. It is shown that GlyT-1 might maintain local synaptic glycine at very low levels, suggesting that GlyT-1 could play a role in regulating glutamatergic neurotransmission via NMDA receptors [24].

In an attempt to clarify the *in vivo* functional roles of GlyT-1 in the CNS, knockout mice deficient in the GlyT-1 gene have been generated [25,26]. Newborn mice deficient in the GlyT-1 gene are anatomically normal, but show severe motor and respiratory deficits and die during the first postnatal day [25]. Furthermore, heterozygous knockout mice (reduced expression of GlyT-1) show enhanced hippocampal NMDA receptor function and memory retention, and are protected against disruptions of sensory gating induced by amphetamine, which suggests that GlyT-1 inhibitors might bring about both cognitive enhancement and antipsychotic effects [26]. These findings highlight the importance of GlyT-1 in the regulation of glutamatergic neurotransmission. Taken together, the data suggest that increasing synaptic levels of glycine by inhibition of GlyT-1 will lead to enhanced NMDA receptor activation, in turn suggesting a potential role for GlyT-1 inhibitors as a novel treatment for schizophrenia [27-39]. This review article provides an overview of GlyT-1 inhibitors as a potential therapeutic approach to the treatment of schizophrenia.



Phencyclidine (PCP) Ketamine

Dizocilpine ((+)MK-801)

Fig. (1). Chemical structures of phencyclidine (PCP), ketamine, and dizocilpine ((+)-MK-801).

SARCOSINE

Sarcosine (*N*-methylglycine) (Fig. **2**) is generated by the enzymatic transfer of a methyl group from *S*-adenosylmethionine (SAM) to glycine, and this reaction is catalyzed by the enzyme glycine *N*-methyltransferase (Fig. **3**) [40]. It is known that sarcosine is a natural GlyT-1 inhibitor with functional antagonist activity in glycine uptake assays. Sarcosine is a safe drug since it is synthesized in the human body.

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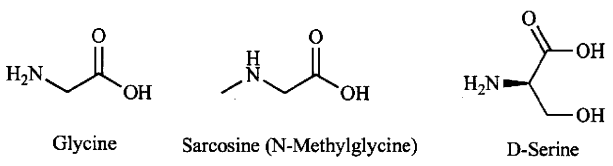


Fig. (2). Chemical structures of glycine, sarcosine (*N*-methylglycine), and D-serine.

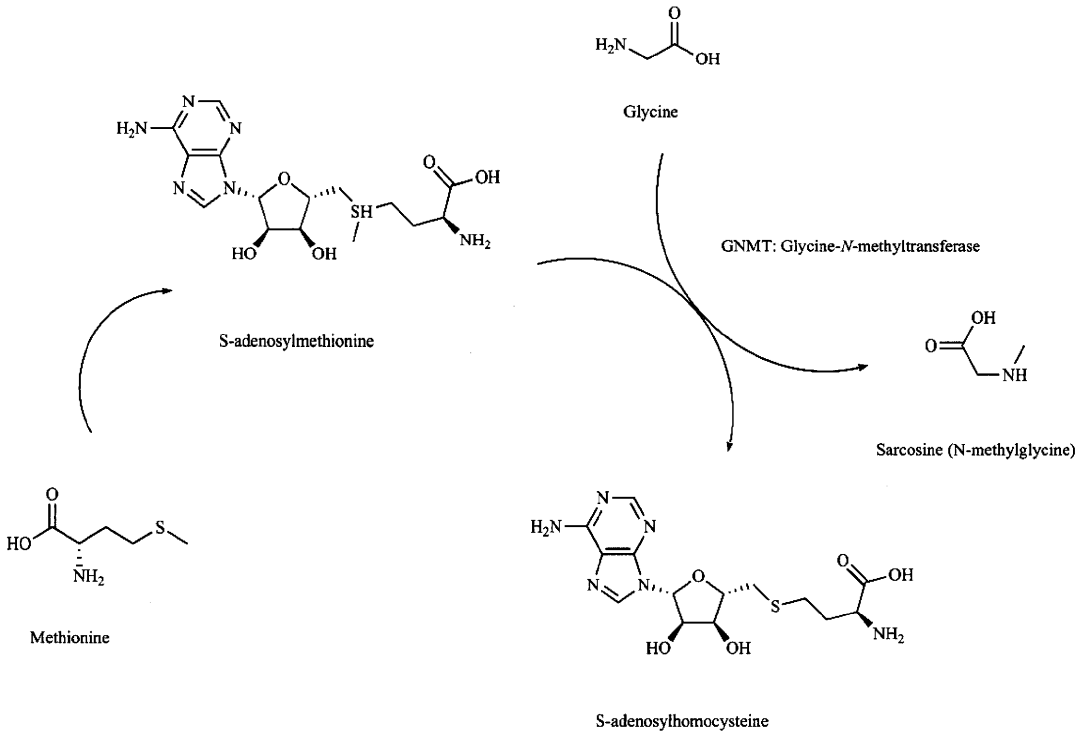


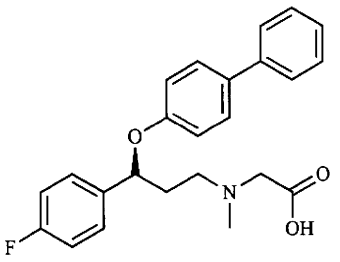
Fig. (3). Pathway of synthesis of sarcosine. Sarcosine (*N*-methylglycine) is generated by the enzymatic transfer of a methyl group from *S*-adenosylmethionine to glycine. This reaction is catalyzed by the enzyme glycine *N*-methyltransferase (GNMT).

Some clinical studies on sarcosine in patients with schizophrenia have been reported. Treatment with sarcosine (2 g/day) can benefit schizophrenic patients also being treated with antipsychotics, including risperidone [41], but not clozapine [42]. A randomized, double-blind, placebo-controlled study demonstrated that members of a sarcosine (2 g/day) group showed greater reductions in their Positive and Negative Syndrome Scale (PANSS) total scores than members of a placebo group or D-serine (2 g/day) group, suggesting that sarcosine is superior to D-serine in that benefits both patients with long-term stable disease and also acutely ill persons with schizophrenia [43]. Furthermore, a randomized, double-blind study reported that sarcosine (2 g/day) alone was effective in the treatment of acutely symptomatic drug-free patients with schizophrenia [44]. Moreover, a randomized, double-blind, placebo-controlled comparison study of sarcosine and D-serine demonstrated that sarcosine was superior to placebo at all four outcome measures of PANSS total (p=0.005), Scale for the Assessment of Negative Symptoms (SANS)(p=0.021), Quality of Life (QOL) (p=0.025), and Global Assessment of Functioning (GAF)(p=0.042). However, D-serine did not differ significantly from placebo in any measures [45]. This study suggests that GlyT-1 inhibitors might be more efficacious than the NMDA/glycine site agonist D-serine in treatment for schizophrenia, including QOL and global function [45]. Further large-sized, placebo-controlled, dose-finding studies are needed to fully assess the effects of sarcosine, since these reports regarding the beneficial effects of sarcosine were based on small studies from the same group in Taiwan. In contrast, Zhang *et al.* [46] reported that sarcosine is an NMDA receptor co-agonist that differs from glycine, suggesting that co-agonistic activity at the NMDA receptors of sarcosine may, in part, be involved in the clinical benefits of this drug in schizophrenia. Nonetheless, these all findings suggest that GlyT-1 inhibition could be a novel therapeutic target for enhancing NMDA receptor function.

A PROTOTYPE OF SARCOSINE-DERIVED GlyT-1 INHIBITOR NFPS

The first sarcosine derivative, (*R*)-(*N*-[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl])sarcosine (NFPS, ALX5407; Fig. 4), has been a key pharmacological tool to study GlyT-1 pharmacology [47,48]. Therefore, NFPS has been widely used as a tool of GlyT-1 pharmacology in animal models of schizophrenia.

The NMDA receptor antagonists including PCP, ketamine, and dizocilpine ((+)-MK-801; Fig. 1), has been widely used in studies of animal models of schizophrenia [49]. Previously, we reported that repeated administration of PCP (10 mg/kg/day for 10 days) caused long-term cognitive deficits in mice (more than 6 weeks after the final administration of PCP), and that PCP-induced cognitive deficits could be improved by subsequent subchronic (2 weeks) administration of the atypical antipsychotic drug clozapine, but not



NFPS (or ALX 5407)

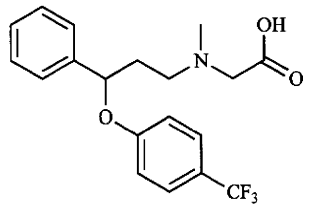
Fig. (4). Chemical structure of (*R*)-(*N*-[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl])sarcosine (NFPS, ALX 5407).

the typical antipsychotic drug haloperidol [50]. Using this model, we reported that subsequent subchronic administration of NFPS significantly improved PCP-induced cognitive deficits in mice [51]. Furthermore, we also found that repeated administration of PCP caused increased levels of GlyT-1 protein in the mouse hippocampus, and that extracellular glycine levels in the hippocampus of PCP-treated mice were lower than those of control mice [51]. Moreover, NFPS inhibited PCP-induced hyperactivity in mice, and NFPS reversed PCP-induced changes in electroencephalogram (EEG) power spectra in conscious rats [52]. It has been reported that NFPS induced a pattern of c-Fos immunoreactivity comparable with the atypical antipsychotic drug clozapine, and that NFPS enhanced prepulse inhibition (PPI) of the acoustic startle response in DBA/2J mice, a strain with low basal levels of PPI [53]. To the same degree as by clozapine or D-serine, NFPS was able to reverse persistent latent inhibition (LI) [53] and cognitive deficits [54] induced by dizocilpine. Manahan-Vaughan *et al.* [55] reported that NFPS could rescue hippocampal long-term potentiation and learning deficits in freely behaving rats after systemic administration of dizocilpine. Recently, Shimazaki *et al.* [56] reported that NFPS significantly improved dizocilpine-induced cognitive deficits in the social recognition test. These findings suggest that GlyT-1 inhibitors could potentially serve as therapeutic drugs to treat the cognitive deficits associated with schizophrenia.

SARCOSINE-DERIVED GlyT-1 INHIBITORS

Org 24461, *R,S*-(+/−)*N*-methyl-*N*-[(4-trifluoromethyl)phenoxy]-3-phenyl-propylglycine (Fig. **5**), and NFPS inhibited the uptake of [^{3}H]glycine in hippocampal synaptosomal preparation with IC_{50} values of 2.5 and 0.022 μM, respectively. These compounds did not alter apomorphine-induced climbing and stereotypy in a dose of 10 mg/kg in mice and did not induce catalepsy in a dose of 10 mg/kg in rats. The ID_{50} values of Org24461 for inhibition of PCP- and D-amphetamine-induced hypermotility in mice were 2.5 and 8.6 mg/kg, respectively [57]. Both NFPS and Org24461 (1-10 mg/kg) reversed PCP-induced changes in EEG power spectra in conscious rats. Recently, it has been reported that co-administration of Org24461 and antipsychotic drug risperidone caused a decrease of extracellular dopamine levels accompanied with sustained elevation of extracellular glycine levels [58]. Interestingly, the extracellular levels of glutamate were also enhanced. This study suggests that co-administration of an antipsychotic drug with a GlyT-1 inhibitor could normalize hypofunctional NMDA receptor-mediated glutamatergic neurotransmission with reduced dopaminergic side effects characteristic for antipsychotic medication [58].

Org24598, *R*-(-) isomer of Org24461, is more potent than Org24461 for GlyT-1 [59]. Org24598 (10 mg/kg) significantly reversed neonatal ventral hippocampal lesion-induced prepulse inhibition deficits in rats, which are animal models of schizophrenia



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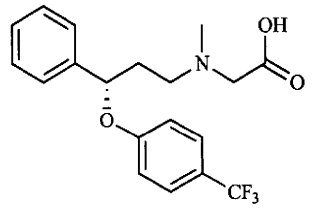
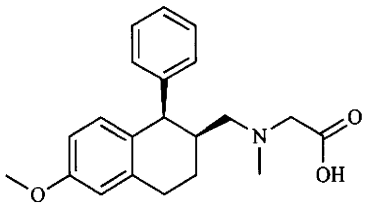


ORG24598 (R isomer of ORG24461)



ORG 25935

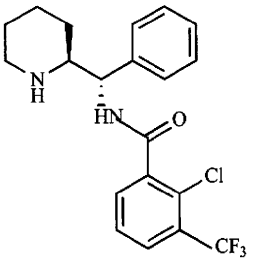
Fig. (5). Chemical structure of Org24461, Org24598, and Org25935.

[60]. Furthermore, Org25935, (now SCH900435) cis-N-methyl-N-(6-methoxy-1-phenyl-1,2,3,4-tetrahydronaphthalen-2-ylmethyl) amino-methyl-carboxylic acid hydrochloride (Figure 5), was also a GlyT-1 inhibitor that easily crosses the blood-brain barrier. Org25935 (6 mg/kg, i.p.) was shown to increase striatal extracellular glycine levels by ~50-80% for ~2.5 hours [61].

NON-SARCOSINE-DERIVED GlyT-1 INHIBITORS

Depoortere *et al.* [62] reported the detailed neuropharmacological profile of SSR504734 (Fig. **6**) as part of a biochemical approach to developing a selective and reversible GlyT-1 inhibitor (IC_{50} = 18 nM for human GlyT-1). SSR504734, a selective and reversible inhibitor of GlyT-1, blocked the *ex vivo* uptake of glycine in a rapid, reversible, and long-term manner. In animal models of schizophrenia, SSR504734 normalized spontaneous PPI deficits in DBA/2 mice, and reversed both amphetamine-induced locomotor hyperactivity as well as selective attention deficits in adult rats treated neonatally with PCP. Furthermore, SSR504734 did not induce catalepsy nor increase prolactin. These findings suggest that SSR504734 is a potent and selective GlyT-1 inhibitor that exhibits ameliorative effects in animal models of schizophrenia; this compound may therefore be efficacious not only in treating positive, but also negative symptoms (i.e., cognitive deficits) of schizophrenia [62]. In addition, it should be noted that SSR504734 exerts antidepressant and anxiolytic effects in animal models [62].

It has been reported that SSR504734 (10 mg/kg) enhanced the facilitatory influence of glutamatergic afferents on dopamine neurotransmission in the nucleus accumbens, and this synergistic effect was found to be dependent on glutamatergic tone [63]. Furthermore, SSR504734 (1-10 mg/kg) significantly attenuated social novelity discrimination deficits induced neonatal PCP treatment [64]. Moreover, SSR504734 was effective in the PCP-induced



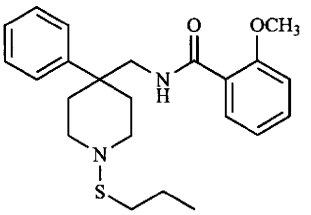
SSR 504734

Fig. (6). Chemical structure of SSR504734.

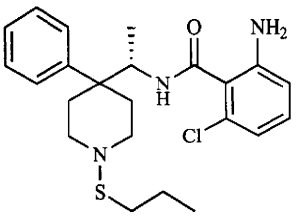
functional activation in the cortico-limbo-thalamic circuits [65] and working memory deficits [66]. In addition, SSR504734 attenuated PCP-induced hyperlocomotion in mice, but potentiated the motor stimulant and motor depressant effects of amphetamine and apomorphine, respectively [67]. A recent study showed that SSR-504734 attenuated hyperlocomotion in mice after administration of L-687,414 (50 mg/kg, s.c.), the antagonist of NMDA receptor glycine modulatory site [68].

Subsequently, Boulay *et al.* [69] reported the detailed neuropharmacological profile of SSR103800 (IC_{50} = 1.9 nM for human GlyT-1), a novel selective and reversible GlyT-1 inhibitor. SSR-103800 decreased dizocilpine- and PCP-induced locomotor hyperactivity in rodents. SSR103800 was shown to attenuate social recognition deficit in adult rats induced by neonatal PCP treatment and the deficit in short-term visual episodic-like memory induced by a low challenge dose of PCP, in PCP-sensitized rats. Furthermore, SSR103800 improved the PPI deficits in DBA/1J mice, and SSR103800 elevated glycine levels in the prefrontal cortex. Moreover, SSR103800 was also effective in the forced-swimming model of depression. Taken together, SSR103800 exhibits potential therapeutic activity in animal models considered representative of the positive, cognitive, and depressive symptoms observed in patients with schizophrenia [70]. Black *et al.* [66] reported that SSR103800 (1 and 3 mg/kg) and SSR504734 (1 and 10 mg/kg) potentiated LI under conditions where LI was not present in non-treated controls, and that SSR103800 (1 mg/kg) reversed amphetamine-induced disrupted LI while not affecting LI on its own. Additionally, SSR103800 (1 and 3 mg/kg) and SSR504734 (3 and 10 mg/kg) reversed abnormally persistent LI induced by dizocilpine. In the neurodevelopmental model, SSR504734 (3 and 10 mg/kg) reverted the LI back to control (normal) levels [66]. Recently, it has been reported that SSR103800 (10-30 mg/kg) blocked dizocilpine-induced hyperactivity and partially reversed spontaneous hyperactivity of NMDA $Nr1^{neo-/-}$ mice [70]. In contrast, SSR103800 failed to affect hyperactivity induced by amphetamine or naturally observed in dopamine transporter $DAT^{-/-}$ knockout mice (10-30 mg/kg). Together these findings suggest that SSR103800 produces antipsychotic-like effects, which differ from those observed with compounds primarily targeting the dopaminergic system [70].

Researchers at Merck Research Laboratories reported the pharmacological profile of a class of novel GlyT-1 inhibitors related to 4,4-disubstituted piperidines, including 2-methoxy-*N*-{1-[4-phenyl-1-(propylsulfonyl)piperidin-4-yl]methyl}benzamide (compound 1: Fig. 7) and 2-amino-6-chloro-*N*-{(1*S*)-1-[4-phenyl-1-(propylsulfonyl)piperidin-4- yl]ethyl}benzamide (compound 2: Fig. 7) [71,72]. A rapid and sustained increase in the extracellular levels of glycine in the prefrontal cortex of freely moving rats was observed at all three experimental doses (1, 3, 10 mg/kg) of this compound. Furthermore, the compound 1 was found to significantly enhance PPI at three different doses (3, 30, 100 mg/kg) in DBA/2J



Compound 1



Compound 2

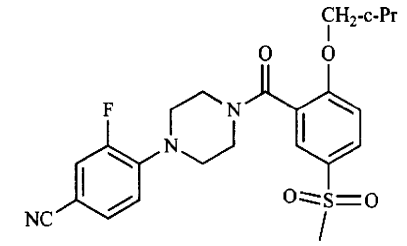
Fig. (7). Chemical structure of compound 1 (2-methoxy-*N*-{[4-phenyl-1-(propylthio)piperidin-4-yl]methyl}benzamide) and compound 2 (2-amino-6-chloro-*N*-{(1*S*)-1-[4-phenyl-1-(propylthio)piperidin-4-yl]ethyl}benzamide)

mice. Brain levels of the compound 1 ranged from 400 nM to 2300 nM during the time course of the PPI experiments. By selectively increasing extracellular glycine levels in the prefrontal cortex via the inhibition of GlyT-1, this compound significantly enhanced performance in a behavioral animal model of sensorimotor gating [71].

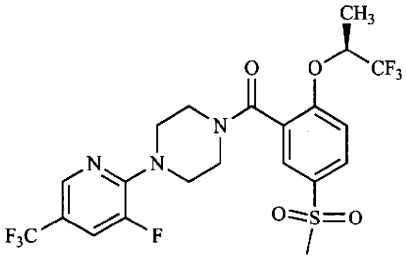
Researchers at F. Hoffmann-La Roche, Ltd. reported a novel series of *N*-(2-aryl-cyclohexyl) substituted spiropiperidines as highly selective GlyT-1 inhibitors [73-77]. Furthermore, a potent and selective GlyT-1 inhibitor 4-(4-{[2-(cyclopropylmethoxy)-5-(methylsulfonyl)phenyl]carbonyl}piperazin-1-yl)-3-fluorobenzonitrile (RO4840700: EC_{50} = 16 nM for GlyT-1) (Fig. **8**) increased extracellular glycine levels in the mouse striatum after oral administration [78]. A recent study showed that oral administration of RO4840700 attenuated hyperlocomotion in mice after administration of L-687,414 (50 mg/kg, s.c.), the antagonist of NMDA receptor glycine modulatory site [68].

Recently, Pinard *et al.* [79] reported that the selective and potent GlyT-1 inhibitor RG1678, (*S*)-4-(3-Fluoro-5-trifluoromethyl-pyridin-2-yl)piperazin-1-yl]- [5-methanesulfonyl-2-(2,2,2-trifluoro-1-methylethoxy)phenyl]-methanone (Fig. **8**), was effective *in vivo* efficacy in the L-687,414 (a selective and brain-penetrating antagonist at glycine site on the NMDA receptors)-induced hyperlocomotion assay. *In vivo* pharmacokinetic studies revealed that RG1678 had a low plasma clearance, an intermediate volume of distribution, a good oral bioavailability, and a favorable terminal half-life in rat and monkey. Finally, RG1678 did not significantly inhibit the major drug metabolizing CYP450 enzymes 3A4, 2D6, 2C9, 1A2 and was without activity in genotoxicity assays. An oral administration of RG1678 (10 mg/kg) produced a robust and sustained increase of extracellular glycine levels in rat striatum [79]. These findings indicate that RG1678 would be a potential therapeutic drug for schizophrenia. Clinical study of RG1678 in patients with schizophrenia is currently underway.

Researchers at H. Lundbeck A/S developed the compound (*R*)-4-[5-chloro-2-(4-methoxy-phenylsulfanyl)-phenyl]-2-methyl-piperazin-1-yl-acetic acid (compound 3: IC_{50} = 150 nM) (Fig. **9**), which was shown to elevate extracellular glycine levels in the rat ventral hippocampus, as measured by *in vivo* microdialysis at doses of 1.2-4.6 mg/kg (s.c.) [80]. Furthermore, the same group reported

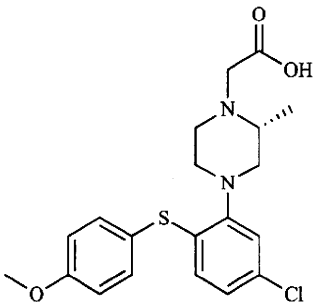


RO4840700

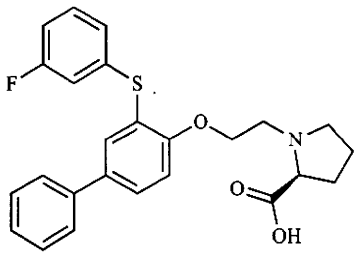


RG1678

Fig. (8). Chemical structure of RO4840700 and RG1678.



Compound 3

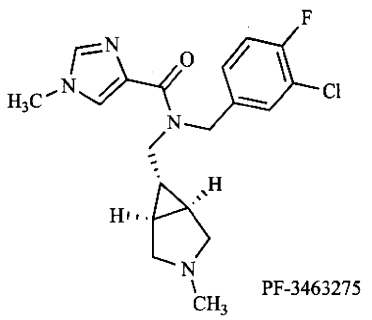


Compound 4

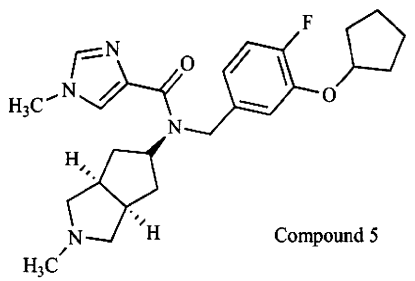
Fig. (9). Chemical structure of compound 3, (*R*)-4-[5-chloro-2-(4-methoxy-phenylsulfanyl)-phenyl]-2-methyl-piperazin-1-yl-acetic acid and compound 4, (S)-1-{2-[3-(3-fluoro-phenylsulfanyl)biphenyl-4-yloxy]ethyl}pyrrolidine-2-carboxylic acid.

the new compound (*S*)-1-{2-[3-(3-fluoro-phenylsulfanyl)biphenyl-4-yloxy]ethyl}pyrrolidine-2-carboxylic acid (compound 4: IC_{50} = 59 nM) [81] (Fig. **9**). *In vitro* and *in vivo* assessments revealed that the CNS utility of this class of compounds might be diminished due to active efflux transporter activity [81].

Lowe *et al.* [82] developed the novel selective GlyT-1 inhibitor PF-3463275 (Fig. **10**). The Ki value of PF-3463275 for GlyT-1 is



PF-3463275

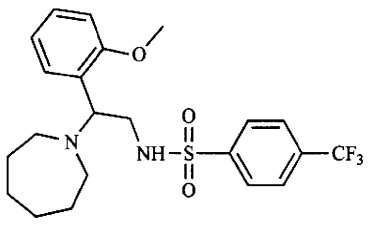


Compound 5

Fig. (10). Chemical structures of PF-3463275 and compound 5.

11.6 nM. The dose that doubles extracellular baseline levels of glycine was 3.5 mg/kg. Thus, PF-3463275 provides a balance of *in vitro* and *in vivo* potency with favorable pharmacokinetic properties, suggesting the potential for good oral bioavailability in humans [82]. PF-3463275 (0.01 - 0.17 mg/kg) alleviated the deficits in spatial working memory induced by ketamine in Rhesus monkeys, suggesting that this drug would be a potential therapeutic drug for cognitive deficits in schizophrenia [83]. However, PF-3463275 was not effective ketamine-induced positive symptom-like behavior (hallucinatory-like behavior)[83]. A clinical study to evaluate the safety, tolerability, and efficacy of two dose regimens of PF-3463275 compared with placebo added to ongoing atypical antipsychotic therapy for cognitive deficits in subjects with chronic symptoms of schizophrenia has been performed. However, clinical trial with PF-03463275 has been terminated although the reason has not been disclosed [84]. Subsequently, Lowe *et al.* [85] also reported the novel potent, selective GlyT-1 inhibitor compound 5 (Ki=3.76 nM for GlyT-1) (Fig. **10**).

Researchers at AsreaZeneka reported *N*-(2-(acepan-1-yl)-2-phenylethyl)-benzenesulfonamides as novel GlyT-1 inhibitors [86]. Brain-plasma ratio of compound 6 (IC_{50} = 37 nM for GlyT-1, IC_{50} > 20 μM for GlyT-2) (Fig. **11**), *N*-(2-(acepan-1-yl)-2-(2-methoxy-phenylethyl)-4-trifluoromethylbenzenesulfonamide, was 2.1, indicating good partitioning across the blood-brain barrier and distribution into brain tissue.

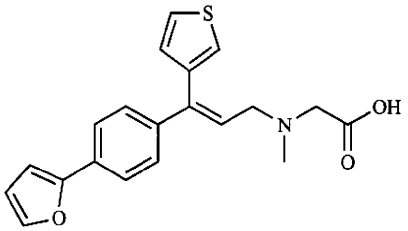


Compound 6

Fig. (11). Chemical structure of compound 6.

CLINICAL STUDY OF GlyT-1 INHIBITORS

Recently, Liem-Moolenaar *et al.* [87] reported the effects of the sarcosine-derived GlyT-1 inhibitor R231857 (Fig. **12**) on the CNS and on scopolamine-induced impairments in cognitive and psychomotor function in healthy subjects. R231857 had some small effects on scopolamine-induced CNS-impairment, which were also not clearly dependent on dose. Scopolamine proved to be an accurate, reproducible and safe model for the induction of CNS impairment by an anticholinergic mechanism. R231857 lacked consistent dose-related effects in this study, probably because the CNS concentrations were too low to produce significant/reproducible CNS effects or to affect the scopolamine challenge in healthy volunteers. The effects of higher doses in healthy volunteers and the clinical efficacy in patients remain to be established. To date, clinical findings with GlyT-1 inhibitors are limited because of the early stage of development of the most high-affinity compounds.



R231857

Fig. (12). Chemical structure of R231857.

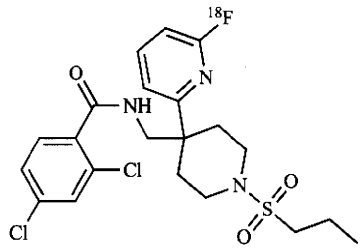
On November 10, 2009, Roche reported the results of a phase II proof-of-concept study of RG1678 (Fig. **8**) in patients (n=320) with schizophrenia. RG1678 was given as an add-on treatment to patients who were stable on antipsychotic therapy and suffered mainly from negative or disorganized thought symptoms. The compound was well tolerated at all doses tested. The analysis showed that RG1678 has a robust and clinically meaningful effect in patients with schizophrenia. Improvements were observed on negative symptoms, overall physician's assessment (clinical global impression: CGI) and patient's personal and social performance (PSP) in a prospectively defined intent to treat population. On December 6, 2010, Roche reported a phase IIb multicentre, randomized, double-blind study of RG1678 in patients (n=323) with schizophrenia [89]. Changes in PANSS negative symptom factor score demonstrated a statistically significant improvement in negative symptoms in patients taking RG1678 (10 and 30 mg) compared to placebo. Differences in CGI improvement in negative symptoms were statistically significant between RG1678 (10 mg) and placebo. Furthermore, there was also a trend towards functional improvement as assessed by the PSP scale in the RG1678 (10 mg) group compared to placebo [89]. Therefore, RG1678 would be a potential therapeutic drug for both negative symptoms and personal and social functioning in patients with schizophrenia [88,89].

It is known that the sarcosine-derived GlyT-1 inhibitors suffer from a range of serious side effects including ataxia, hypoactivity, and decreased respiratory activity, the latter often leading to death in preclinical species upon chronic dosing [90]. Thus, these observations led to a backlash against GlyT-1 mechanisms as a toxic and undrugable target in the early 2000s. In the mid-2000s, both the *in vitro* and *in vivo* pharmacology of the non-sarcosine-derived GlyT-1 inhibitors as well as toxicity profiles, were fundamentally different than the sarcosine-derived GlyT-1 inhibitors [71,90]. Therefore, in the near future, the non-sarcosine-derived GlyT-1 inhibitors such as RG1678 would be potential therapeutic drugs for schizophrenia.

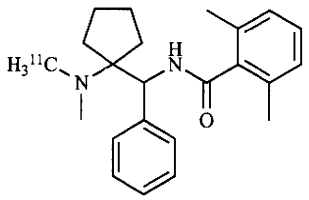
POSITRON EMISSION TOMOGRAPHY STUDY OF GlyT-1 IN THE BRAIN

The distribution, density, and activity of GlyT-1 in the living human brain can be visualized non-invasively by the specific radioligands and positron emission tomography (PET), and the receptor binding can be quantified by appropriate tracer kinetic models, which can be modified and simplified for particular applications. Therefore, *in vivo* PET imaging of GlyT-1 in the human brain provides a method for quantitative study of the GlyT-1-related pathophysiology in schizophrenia.

Investigators at Merck also developed the novel PET ligand [^{18}F]2,4-dichloro-*N*- ((1-(propylsulfonyl)-4-(6-fluoropyridin-2-yl) piperidin-4-yl)methyl)benzamide (CFPyPB) (Fig. **13**) for GlyT-1 in the human brain [91]. Recently, Passchier *et al.* [92] developed the novel PET ligand [^{11}C]GSK931145 (pIC_{50} = 8.4 for GlyT-1, Fig. **13**) for GlyT-1 in the brain. Following intravenous administration of [^{11}C]GSK931145 in the pig showed good brain penetration and a heterogeneous distribution in agreement with reported GlyT-1 localization. Pretreatment with GlyT-1 inhibitor GSK565710 (0.5 mg/kg) reduced the uptake of radioactivity in the brain after injection of [^{11}C]GSK931145. The use of [^{11}C]GSK931145 for GlyT-1 imaging in humans results in an effective human radiation dose, which is within the conventional range of [^{11}C]tracer radiation burden [93]. These studies suggest that [^{11}C]GSK931145 would be a useful PET ligand for studying GlyT-1 in the human brain. In addition, we have developed some potential PET ligands for *in vivo* labeling GlyT-1 in the brain (Fig. **14**) [94]. Taken together, [^{18}F]CFPyPB and [^{11}C]GSK931145 would be potential PET ligands for *in vivo* visualization of GlyT-1 in the living human brain with PET. These PET ligands represent a new tool for the evaluation of glutamatergic neurotransmission in the pathophysiology of schizophrenia.



[^{18}F]CFPyPB



[^{11}C]GSK931145

Fig. (13). Chemical structures of [^{18}F]CFPyPB and [^{11}C]GSK 931145, potential PET ligands for GlyT-1.

CONCLUDING REMARKS

Approach to increase synaptic glycine levels by GlyT-1 inhibitors is the most attractive target for developing potential therapeutic drugs for the treatment of schizophrenia. At present, a number of pharmaceutical companies are developing novel and selective GlyT-1 inhibitors for the treatment of schizophrenia. Gaining a better understanding of the role of GlyT-1 in the neuron-glia com-

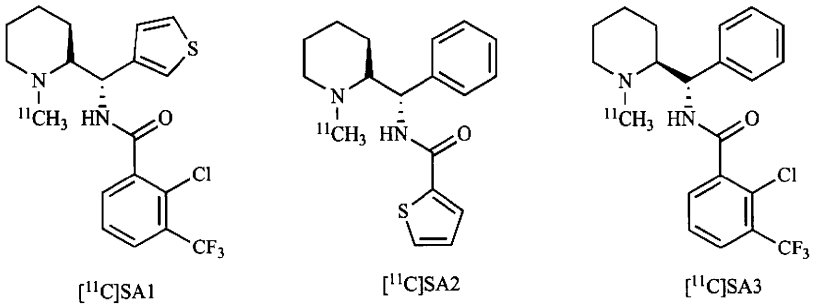


Fig. (14). Chemical structures of [^{11}C]SA1, [^{11}C]SA2 and [^{11}C]SA3, potential PET ligands for GlyT-1.

munication in the pathophysiology of schizophrenia will be expected to provide new perspectives for treating this disorder. Finally, the pharmacological modulation of glycine modulatory sites on NMDA receptors by non-sarcosine-derived GlyT-1 inhibitors would be beneficial in the treatment of the cognitive deficits, negative symptoms and psychosis associated with several psychiatric diseases, including schizophrenia.

CONFLICT OF INTEREST

The authors have no conflict of interest.

ACKNOWLEDGEMENT

This study was supported in part by a grant from the Minister of Education, Culture, Sports, Science, and Technology of Japan (to K.H.), and by a grant from the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation of Japan (to K.H.). I would also like to thank my collaborators who are listed as the co-authors of our papers in the reference list.

ABBREVIATIONS

CGI	=	Clinical Global Impression
CNS	=	Central nervous system
EEG	=	Electroenphalogram
GAF	=	Global assessment of functioning
GlyT-1	=	Glycine transporter-1
LI	=	Latent inhibition
NMDA	=	*N*-Methyl-D-aspartate
PANSS	=	Positive and Negative Syndrome Scale
PCP	=	Phencyclidine
PET	=	Positron emission tomography
PPI	=	Prepulse inhibition
PSP	=	Personal and Social Performance
QOL	=	Quality of life
SAM	=	S-Adenosylmethionine

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Received: January 26, 2011 **Accepted: February 4, 2011**

Psychiatry and Clinical Neurosciences

PCN Frontier Review

Brain-derived neurotrophic factor as a biomarker for mood disorders: An historical overview and future directions

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Mood disorders, such as major depressive disorder (MDD) and bipolar disorder (BPD), are the most prevalent psychiatric conditions, and are also among the most severe and debilitating. However, the precise neurobiology underlying these disorders is currently unknown. One way to combat these disorders is to discover novel biomarkers for them. The development of such biomarkers will aid both in the diagnosis of mood disorders and in the development of effective psychiatric medications to treat them. A number of preclinical studies have suggested that the brain-derived neurotrophic factor (BDNF) plays an important role in the pathophysiology of MDD. In 2003, we reported that serum levels of BDNF in antidepressant-naive patients with MDD were significantly lower than those of patients medicated with antidepressants and normal controls, and that serum BDNF levels were negatively correlated with the severity of depression. Additionally, we found that decreased serum levels of BDNF in antidepressant-naive patients recovered to normal levels associated with the recovery of depression after treatment with antidepressant medication. This review article will provide an historical overview of the role played by BDNF in the pathophysiology of mood disorders and in the mechanism of action of therapeutic agents. Particular focus will be given to the potential use of BDNF as a biomarker for mood disorders. BDNF is initially synthesized as a precursor protein proBDNF, and then proBDNF is proteolytically cleaved to the mature BDNF. Finally, future perspectives on the use of proBDNF as a novel biomarker for mood disorders will be discussed.

Key words: biomarker, brain-derived neurotrophic factor, gene, mood disorders, post-mortem brain.

MOOD DISORDERS ARE among the most prevalent, recurrent, and disabling of mental illnesses. Major depressive disorder (MDD) is a serious disorder that affects approximately 17% of the population at some point in life, resulting in major social and economic consequences.[1-3] According to the DSM-IV, MDD is a heterogeneous disorder that manifests with symptoms at the psychological, behavioral, and physiological levels. There is still very little known about the neurobiological alterations that underlie the pathophysiology or treatment of MDD. Several lines of evidence suggest that depression in most people is caused by interactions between a genetic predisposition and some environmental factors.[3-7]

Bipolar disorder (BPD), also known as manic-depressive illness, is a brain disorder that causes unusual shifts in a person's mood, energy, and ability to function. More than 2 million American adults, or

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Accepted 16 April 2010.

about 1 percent of the population aged 18 and older in any given year, have BPD.[2,8–10] BPD typically develops in late adolescence or early adulthood. However, some people have their first symptoms during childhood, and some develop them later in life. BPD is often not recognized as an illness, and people may suffer for years before it is properly diagnosed and treated. Like diabetes or heart disease, BPD is a long-term illness that must be carefully managed throughout a person's lifetime.[2,4,5,9,10]

The precise neurobiology underlying these mood disorders is currently unknown. One way to combat these disorders would be to discover novel biomarkers for them, which potentially could revolutionize their recognition and management.[11–14] Identification of biomarkers would aid both in the diagnosis of these disorders, and in the development of effective psychiatric medications to treat them. In addition, biomarkers could provide the basis for early intervention and prevention efforts targeting at-risk individuals. Currently, there are no diagnostic biomarkers available for mood disorders, although easily accessible bodily fluids, including blood, urine, and cerebral spinal fluid (CSF), are potential sources for the identification of biomarkers.

This article provides a review of the recent findings on the role of brain-derived neurotrophic factor (BDNF) in the pathophysiology of mood disorders such as MDD and BPD, and discusses the potential use of BDNF as a biomarker for these disorders.

BDNF AND ITS PRECURSOR, proBDNF

Mammalian neurotrophins are homodimeric proteins that include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3, and NT-4/5. Most functions of neurotrophins are mediated by the tropomyosin receptor kinase (Trk) family of tyrosine kinase receptors. The interaction of neurotrophins with the Trk receptors is specific: NGF binds with TrkA; BDNF and NT-4 bind with TrkB; and NT-3 binds with TrkC.[15–19] BDNF is found in the central nervous system and periphery. BDNF is a 27-kDa polypeptide that is recognized as playing an important role in the survival, differentiation, and outgrowth of select peripheral and central neurons during development and in adulthood.[15,16] It is well known that BDNF participates in use-dependent plasticity mechanisms such as long-term potentiation, learning, and memory.[15–17,20,21]

The BDNF gene encodes a precursor peptide, proBDNF. BDNF is initially synthesized as a precursor protein (preproBDNF) in the endoplasmic reticulum. Following cleavage of the signal peptide, proBDNF is transported to the Golgi for sorting into either constitutive or regulated secretory vesicles. ProBDNF may be converted into mature BDNF intracellularly in the *trans*-Golgi by members of the subtilisin–kexin family of endoproteases, such as furin, or in the immature secretory granules by proprotein convertases (Fig. 1).[17,22–25] ProBDNF could be converted to mature BDNF by extracellular proteases, such as plasmin and matrix mettaloproteinase-7 (Fig. 1).[26,27] It has long been thought that only secreted mature BDNF is biologically active, and that proBDNF is exclusively localized intracellularly, serving as an inactive precursor. However, recent accumulating evidence suggests that proBDNF may also be biologically active.[25,27,28] It has been reported that proBDNF induces neuronal apoptosis via activation of the $p75^{NTR}$ receptor.[29] Taken together, these findings suggest that proBDNF and BDNF elicit opposite effects via the $p75^{NTR}$ and TrkB receptors, respectively (Fig. 1), and that both proBDNF and BDNF play an important role in several physiological functions.[25,30–32]

PRECLINICAL STUDIES

Antidepressant effects of BDNF

BDNF has been shown to have antidepressant effects in animal models of depression. Specifically, infusion of BDNF into the midbrain has an antidepressant-like influence on two animal models of depression, i.e. learned helplessness following exposure to inescapable shock, and learned helplessness following exposure to inescapable shock as well as in response to the forced-swim test.[33] Furthermore, it has been reported that forced swimming decreased BDNF mRNA in particular regions (CA1, CA3, and the dentate gyrus) of the hippocampus, and that a combination of physical activity and antidepressant treatment increased the level of hippocampal BDNF mRNA to well above the baseline value as well as enhanced swimming time in an animal model,[34] supporting the possibility that the upregulation of BDNF expression may be important in the clinical response to antidepressant treatment. Moreover, Shirayama *et al.*[35] reported that a single bilateral infusion of BDNF into the dentate gyrus of the hippocampus

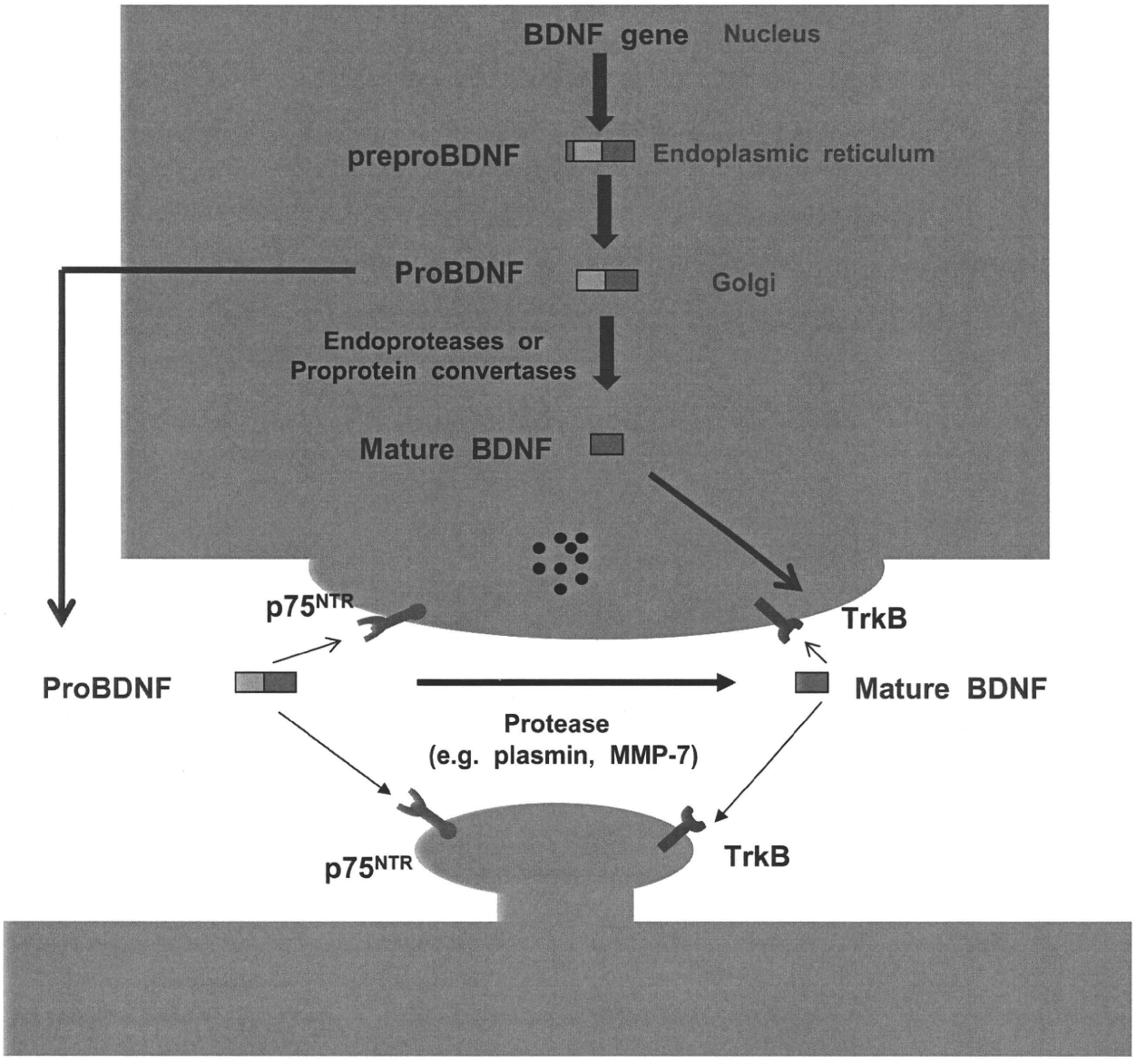

Figure 1. The synthesis of brain-derived neurotrophic factor (BDNF) from proBDNF. The BDNF gene produced precursor protein BDNF (preproBDNF) in the endoplasmic reticulum. ProBDNF binds to intracellular sortilin in the Golgi to facilitate proper folding of the mature domain. ProBDNF preferentially binds $p75^{NTR}$ receptors. ProBDNF is cleaved by proteases (e.g. plasmin, matrix metalloproteinase [MMP]-7) at the synapses and converted to mature BDNF. Mature BDNF preferentially binds tropomyosin receptor kinase (Trk) B receptor. This figure is a slight modification of that from the article by Hashimoto.[31]

produced an antidepressant effect in both the learned helplessness and forced-swim test paradigms. It should be noted that these effects were observed as early as 3 days after a single infusion of BDNF, and these effects lasted for at least 10 days.[35] In addition, infusions of a broad-spectrum Trk tyrosine kinase inhibitor, K252a, or of a selective extracellular signal-regulated protein kinase (ERK) inhibitor, U0126, blocked the antidepressant effects of BDNF, suggesting that the TrkB/mitogen-activated protein (MAP) kinase cascade plays a role in the therapeutic action of antidepressants.[35] Interestingly, it has been demonstrated that intra-ventral tegmental area (VTA) infusions of BDNF resulted in 57% shorter latency to

immobility relative to control animals (a depression-like effect), and that rats given intra-nucleus accumbens (NAc) injections of a virus expressing the truncated version of the BDNF receptor had almost fivefold longer latency to immobility relative to rats that received a vehicle injection or a virus expressing the full-length version of the BDNF receptor (an antidepressant-like effect). These findings suggest that the action of BDNF in the VTA-NAc pathway might be related to development of a depressive-like phenotype.[36]

BDNF gene knock-out (or knock-in) mice

Based on the putative role of BDNF in depression, it is of interest to evaluate heterozygous BDNF knock-out ($BDNF^{\pm}$) mice as a potential animal model of the disease. Two studies using mice with a genetic deletion of the BDNF gene have demonstrated that BDNF play a critical role in neuronal differentiation and survival.[37,38] Heterozygous BDNF knock-out ($BDNF^{\pm}$) mice that do not show abnormal mortality have been widely used because BDNF null mutant ($BDNF^{-/-}$) mice die during the first few weeks after birth.[37,38] Heterozygous BDNF knock-out ($BDNF^{\pm}$) mice have forebrain BDNF mRNA and protein levels that are 50% of the levels in wild-type mice.[39–41] In addition to abnormalities in serotonergic neurotransmission, these mice develop premature age-associated decrements in forebrain serotonin levels and fiber density, and exhibit enhanced intermale aggressiveness,[42,43] as well as abnormal eating behaviors.[43–45] However, MacQueen *et al.*[46] have reported that heterozygous BDNF knock-out ($BDNF^{\pm}$) mice and wild-type mice did not differ in measures of activity, exploration, or hedonic sensitivity, and also did not differ in response to the forced-swim test. Although heterozygous $BDNF^{\pm}$mice were slower to escape after training than wild-type mice in the learned helplessness paradigm, this effect may have been due to a reduced sensitivity to centrally mediated pain.[46] In the forced-swim test, the immobility time of imipramine-treated heterozygous $BDNF^{\pm}$mice was not significantly different from that of the saline-treated (wild-type or heterozygous BDNF knock-out) mice.[47] At the present time, therefore, it seems unlikely that heterozygous $BDNF^{\pm}$mice will provide an effective model of genetic vulnerability to depression, although further detailed studies will be needed to confirm this.[48]

The development of a conditional targeting strategy for the BDNF gene will help to discriminate between the roles of BDNF during development and adulthood, as well as between central and peripheral contributions to impaired function in the absence of BDNF. It would also be of interest to evaluate conditional BDNF knock-out mice as an animal model of depression. Monteggia *et al.*[49] showed that conditional BDNF knockout mice also display an increase in depression-like behavior in the forced-swim and sucrose preference tests, suggesting that low production of BDNF may precipitate depressive disorder. Subsequently, Adachi *et al.*[50] used a viral-mediated gene transfer approach to assess the role of BDNF in subregions of the hippocampus. The loss of BDNF in either the CA1 or the dentate gyrus of the hippocampus did not alter locomotor activity, anxiety-like behavior, fear conditioning, or depression-related behaviors. However, the selective loss of BDNF in the dentate gyrus attenuated the actions of antidepressants (e.g. desipramine and citalopram) in the forced-swim test. These data suggest that BDNF in the DG might be essential in mediating the therapeutic effect of antidepressants.[50]

Chen *et al.*[51] generated a variant proBDNF (66Met/Met) mouse that produces the phenotypic hallmarks in humans with a variant allele, and, when placed in stressed settings, proBDNF (66Met/Met) mice exhibited increased anxiety-related behaviors that were not attenuated by fluoxetine. This study suggests the impact of Met substitution of proBDNF on anxiety-related behaviors.[31]

Expression of BDNF by antidepressants

Several lines of evidence suggest that the expression of BDNF may be a downstream target of a variety of antidepressant treatments; BDNF might therefore be an important target for therapeutic recovery from depression, and it might also provide protection against stress-induced neuronal damage.[6,7,52–58] The molecular elements known to regulate neuronal plasticity in models of learning and memory are also involved in the actions of drugs used for the treatment of MDD and BPD.[6,7,52–58] Administration of antidepressants increased the expression of BDNF mRNA in limbic structures in response to chronic, but not acute, treatment; these results are consistent with the time course typically required for the therapeutic action of antidepressants to take effect.[59–61] Subsequently, a number of studies reported an

increase of BDNF protein levels in the brain after chronic treatment with antidepressants.[57] In addition, the expression of BDNF has been shown to be decreased by exposure to stress,[62–64] suggesting the possibility that BDNF might also be involved in the pathophysiology of stress-related mood disorders.

Electroconvulsive therapy

Electroconvulsive therapy (ECT) can be effectively used in the treatment of MDD.[65,66] Electroconvulsive seizures increase the levels of both BDNF mRNA and its receptor TrkB mRNA in the rat hippocampus, and chronic administration of electroconvulsive seizures blocked the downregulation of BDNF mRNA in the hippocampus in response to restraint-induced stress.[59,67] Electroconvulsive stimulus applied for 8 days increased the levels of BDNF protein in the hippocampus, striatum, and occipital cortex of rats.[68] Furthermore, ten consecutive daily exposures to electroconvulsive seizures increased BDNF protein in the parietal cortex (219%), entorhinal cortex (153%), hippocampus (132%), frontal cortex (94%), neostriatum (67%), and septum (29%) of rats.[39] Increases of BDNF protein peaked at 15 h after the last electroconvulsive seizures and lasted at least 3 days thereafter.[39] Moreover, chronic treatment with electroconvulsive seizures induces sprouting of the granule cell mossy fiber pathway in the hippocampus, and electroconvulsive seizure-induced sprouting is significantly diminished in heterozygous $BDNF^{\pm}$mice, suggesting that BDNF contributes to mossy fiber sprouting.[69] Thus, the sprouting of the mossy fiber pathway would appear to oppose the mechanism of the action of stress, and could thereby contribute to the therapeutic effects of ECT; however, the functional consequences of ECT remain unclear at this time.

Gersner *et al.*[70] reported that repeated subconvulsive electrical stimulation of either the nucleus accumbens or the ventral but not the dorsal prelimbic cortex reversed the main behavioral deficit and the reduction of BDNF levels in the hippocampus that were induced by chronic mild stress. ECT was more effective because it also normalized a behavioral deficit associated with anxiety but produced a learning and memory impairment. This study suggests that local intermittent subconvulsive electrical stimulation can induce an antidepressant effect similar to that of ECT, without the cognitive impairment caused by the convulsive treatment.

Repetitive transcranial magnetic stimulation (rTMS)

Repetitive transcranial magnetic stimulation (rTMS) has been increasingly used as a therapeutic tool for the treatment of psychiatric diseases such as MDD.[65,71–73] It has been reported that rTMS increased BDNF mRNA and BDNF protein in the hippocampus as well as in the parietal and piriform cortex.[74] Such findings are similar to those observed after antidepressant drug treatment and electroconvulsive stimuli; it is therefore likely that rTMS and antidepressant treatment strategies share a common molecular mechanism of action.

Mood stabilizers

Lithium and valproate are widely prescribed mood stabilizers that effectively treat acute mood disorders, in addition to providing long-term prophylactic treatment of BPD. Although the mechanisms underlying the therapeutic effects of these drugs remain poorly understood, recent studies have implicated certain critical signal transduction pathways as being integral to the pathophysiology and treatment of BPD.[9,54,55,75–77]

Chronic administration of lithium or valproate has been shown to increase the expression of BDNF in the rat brain, suggesting that these mood stabilizers may produce a neurotrophic effect mediated by the upregulation of BDNF in the brain.[78] Furthermore, pretreatment with either lithium or BDNF protected rat cortical neurons from glutamate excitotoxicity, and a BDNF-neutralizing antibody as well as K252a (an inhibitor of Trk tyrosine kinase) were both able to suppress the neuroprotective effects of lithium.[79] Moreover, subchronic administration of another mood stabilizer, lamotrigine (30 mg/kg, twice-daily for 7 days), increased the expression of BDNF in the frontal cortex and hippocampus in both naive and stressed rats, and restored the stress-induced reduction of BDNF expression.[80] All these findings suggest that the BDNF/TrkB pathway could play a role in mediating the neuroprotective effects of mood stabilizers.[81]

CLINICAL STUDIES

BDNF gene polymorphisms and brain functions

The human BDNF gene maps to human chromosome 11p13 and is composed of six 5′ exons that are

differentially spliced to a single 3′ terminal exon (exon 7) that encodes the entire sequence of mature BDNF.[82] The BDNF 196G/A gene polymorphism converts a valine (Val) to methionine (Met) at codon 66 in the 5′ pro-region of the human BDNF gene (Fig. 2). Neurons transfected with GFP-labeled BDNF-66Met showed lower depolarization-induced secretion, while constitutive secretion was unchanged. Furthermore, GFP-labeled BDNF-66Met failed to localize to secretory granules or synapses.[83] Interestingly, it has been demonstrated that the BDNF 196G/A gene polymorphism is associated with episodic memory, hippocampal activation (as assayed by functional magnetic resonance imaging [fMRI]), and decreased hippocampal *N*-acetyl aspartate (NAA) levels (as measured by magnetic resonance spectroscopy [MRS]), indicating that the BDNF gene plays a role in human memory and hippocampal function.[83] In addition, a study using blood oxygenation level-dependent fMRI and a declarative memory task in healthy individuals has demonstrated an association between the BDNF gene 196G/A polymorphism and hippocampal activity during episodic memory processing.[84]

Whereas this BDNF 196G/A gene polymorphism does not affect mature BDNF protein function, it has been shown to dramatically alter the intracellular trafficking and packaging of proBDNF.[30,31,83] These findings suggest that the BDNF gene is involved in human episodic memory by virtue of its effects on hippocampal neuronal function, and that the BDNF gene might contribute to the pathogenesis of neuropsychiatric diseases such as mood disorders.

BDNF gene analysis in MDD

Mood disorders are common psychiatric disorders with complex causes that likely involve multiple genes in addition to non-genetic influences.[85–87] Given the role of BDNF in the pathophysiology of MDD, it is of interest to examine the BDNF gene as potentially associated with the risk of developing MDD. However, in studies in a Chinese population, the genotype and/or allele frequencies of the BDNF 196G/A gene polymorphism were not significantly different among subjects in the MDD and control groups, suggesting that the BDNF 196G/A gene polymorphism plays no major role in the pathogenesis of MDD in the Chinese population.[88,89] Furthermore, it has been reported that the BDNF 196G/A polymorphism was not related to the development of MDD but was related to the clinical features of MDD in a Japanese population[90] and an Italian population.[91] In contrast, an association has been demonstrated between the BDNF 196G/A polymorphism and geriatric depression in a Chinese population[92] and depression associated with Alzheimer's disease in an Italian population.[93]

Schumacher *et al.*[94] reported that haplotype analysis of the marker combination rs988748-(GT)n-rs6265 of the BDNF gene produced nominally significant associations for all investigated phenotypes (MDD, $P = 0.00006$; BPD, $P = 0.0057$). Association with MDD was the most robust finding and could be replicated in a second German sample of MDD patients and control subjects ($P = 0.0092$), suggesting that BDNF may be a susceptibility gene for MDD in the German population.[94]

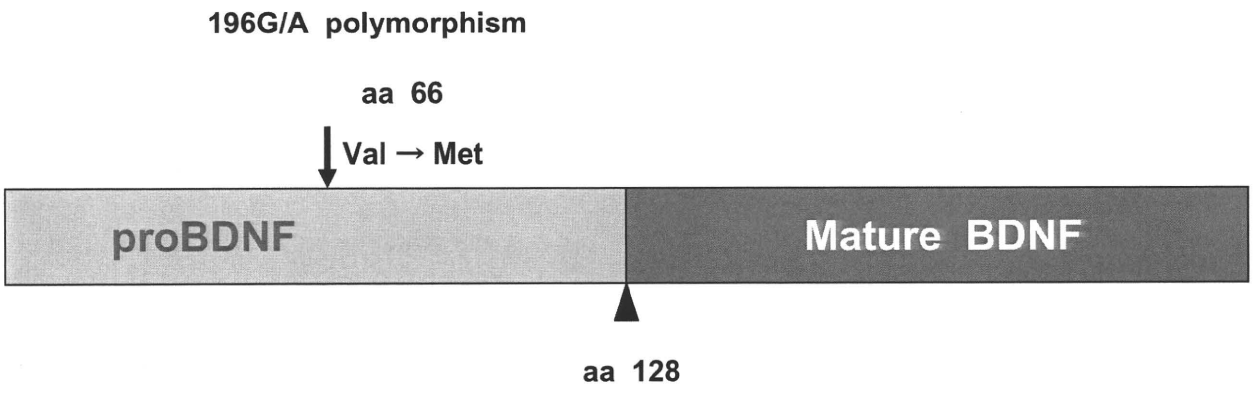

Figure 2. Structure of pro-brain-derived neurotrophic factor (BDNF) protein. Arrowheads indicate known protease cleavage sites involved in processing of the mature BDNF form, as well as of the secreted proBDNF. An arrow indicates the position of the single nucleotide polymorphism (SNP: 196G/A, Val66Met) of the BDNF gene. Met, methionine; Val, valine.

Licinio *et al.*[95] reported that six single-nucleotide polymorphisms on the BDNF gene (rs12273539, rs11030103, rs6265, rs28722151, rs41282918, and rs11030101) were associated with MDD and that two haplotypes in different blocks were significantly associated with MDD. Interestingly, a 5′ untranslated region polymorphism (rs61888800) was associated with antidepressant response after adjusting for age, sex, medication, and baseline score on the 21-item Hamilton Depression Rating Scale. However, there was no major impact of the BDNF gene on antidepressant treatment response.[96] A recent meta-analysis study demonstrated an association between BDNF Val66Met polymorphism and the treatment response in patients with MDD, with Val66Met heterozygous patients showing a better response rate than the Val/Val homozygotes, especially in Asian populations.[97] Further gene–gene and gene–environment interaction studies based on larger sample sizes and stratified by ethnicity will be necessary. Very recently, it was reported that a combination of several independent risk alleles within the TrkB (or NTRK2) gene were associated with suicide attempts among patients with MDD, suggesting that the BDNF-TrkB pathway plays a role in the pathophysiology of suicide.[98]

In vivo brain imaging studies have demonstrated reduced hippocampal volumes in patients with MDD.[99–103] Interestingly, significantly smaller hippocampal volumes were observed for MDD patients and for controls carrying the BDNF-66Met allele compared with subjects homozygous for the BDNF-66Val allele in a German population,[104] suggesting that BDNF-66Met allele carriers might be at risk of developing smaller hippocampal volumes and may be susceptible to MDD.[104,105] We reported that the frequency of healthy individuals who carried the G/G (Val/Val) genotype was significantly lower in Japanese subjects than in Italians or in Americans (Table 1), suggesting an ethnic difference in the frequency of the BDNF 196G/A polymorphism.[106] Given this possibility of an ethnic difference, further detailed studies using other ethnic samples will be needed to confirm the association between the BDNF Val66Met polymorphism and the reduction of hippocampal volume.

BDNF gene analysis in BPD

The family-based association studies have suggested that the BDNF gene is a potential risk locus for the development of BPD.[107,108] The dinucleotide repeat (GT)n polymorphism at position −1040 bp on the BDNF gene showed that allele A3 was preferentially transmitted to affected individuals ($P = 0.04$), and that the BDNF 196G/A (val66met) polymorphism showed a significant association with BPD ($P = 0.0006$).[107] Furthermore, an association study of the relationship between 76 candidate genes and BPD suggested that BDNF is a potential risk gene for the disease.[108] It is of note that of the 76 candidate genes involving a variety of neurobiological systems, only the BDNF gene emerged as a potential risk locus after genotyping and haplotyping studies were carried out using the original trios and in a replication sample.[108] Subsequent association studies replicated an association between the BDNF gene and BPD.[109] However, no evidence has been found for an allelic or genotypic association of the two polymorphisms (-1360C/T and 196G/A) of the BDNF gene in Japanese (or Chinese) patients with BPD,[89,110] suggesting that the BDNF gene is unlikely to confer susceptibility to BPD in the Asian population. As mentioned above, an ethnic difference in the frequency of the BDNF 196G/A polymorphism has been suggested.[106] This phenomenon should be taken into consideration when verifying the role of certain variations in the BDNF gene as risk factors for BPD.

Table 1. Allele and genotype frequencies of the brain-derived neurotrophic factor gene polymorphism at position 196

	Japan	Italy	USA
Allele			
Allele A (Met)	41.1%	29.7%	18.0%
Allele G (Val)	58.9%	70.3%	82.0%
Genotype			
A/A (Met/Met)	15.9%	8.1%	4.5%
G/A (Val/Met)	50.3%	43.2%	27.1%
G/G (Val/Val)	33.8%	48.7%	68.4%

A slight modification of the table by Shimizu *et al.*[106] reproduced with permission.
Met, methionine; Val, valine.

Post-mortem human brain sample studies

Several studies using post-mortem brain samples have suggested that BDNF plays a role in the pathophysiology of mood disorders. A post-mortem study of patients with MDD and BPD demonstrated evidence of the specific loss of neuronal and glial cells in mood disorders.[111] Three patterns of morphometric cellular changes were noted: cell loss (subgenual

prefrontal cortex), cell atrophy (dorsolateral prefrontal cortex and orbitofrontal cortex), and increased numbers of cells (hypothalamus, dorsal raphe nucleus). Thus, cellular changes in patients with mood disorders are suggested to play a role in stress and prolonged prefrontal cortex development, and neurotrophic/neuroprotective factors are also suggested to be involved in these disorders. The precise anatomic localization of dysfunctional neurons and glia in mood disorders may reveal novel therapeutic targets for mood disorders, including MDD and BPD.[58]

Chen *et al.*[112] observed increased levels of BDNF immunoreactivity in post-mortem hippocampal tissue obtained from subjects who were being treated with antidepressant medications at the time of death, compared with the BDNF levels observed in samples from antidepressant-untreated subjects. Interestingly, Dunham *et al.*[113] reported that reductions in proBDNF were seen in all layers of the right but not the left hippocampus, with no changes in the dentate gyrus of the brains of MDD patients from the Stanley consortium. The pattern was similar but less marked for BPD. In addition, BPD, but not MDD patients, had bilateral reductions in $p75^{NTR}$ in hippocampal layers, but not in the dentate gyrus. These findings suggest that both MDD and BPD may be associated with impairment in proBDNF expression,[113] although further detailed studies on proBDNF will be needed to confirm this.

Suicide is a major public health problem that is at least partly related to mood disorders.[114,115] Dwivedi *et al.*[116] reported that the mRNA levels of BDNF and its receptor TrkB were significantly reduced in both the prefrontal cortex and hippocampus of suicide subjects, as compared with those in control subjects. These reductions were associated with significant decreases in the protein levels of BDNF and of full-length TrkB. These findings suggest that the BDNF-TrkB pathway may play an important role in the pathophysiological aspects of suicidal behavior.[116] Furthermore, Karege *et al.*[117] reported a significant decrease in BDNF and NT-3 levels in the hippocampus and prefrontal cortex (only BDNF), but not in the entorhinal cortex, of suicide victims who were drug-free compared with non-suicide controls. In drug-treated suicide victims, neurotrophin levels were not significantly different from those in non-suicide controls.[117] Moreover, BDNF protein was significantly decreased in the prefrontal cortex, but not the hippocampus, of teenage suicide victims compared with normal control subjects.[118] In addition, a decrease of the T1 splice variant of TrkB has been detected in the frontal cortex of suicide completers.[119]

Very recently, it has been reported that post-mortem brain samples from suicide subjects showed a statistically significant increase in DNA methylation at specific CpG sites in the BDNF promoter/exon IV compared with non-suicide control subjects, suggesting a novel link between epigenetic alteration in the brain and suicidal behavior.[120] All these findings support a role of the BDNF-TrkB pathway in the suicidal behavior associated with pathogenesis of mood disorders.

Blood levels of BDNF in patients with MDD

BDNF is also present in human blood, although it is more highly concentrated in brain tissue.[121,122] Previously, it was reported that BDNF could cross the blood–brain barrier,[123] and that BDNF levels in the brain and serum underwent similar changes during the maturation and aging processes in rats,[124] suggesting that serum BDNF levels may reflect BDNF levels in the brain. Karege *et al.*[125] reported that serum BDNF levels were significantly decreased in antidepressant-free patients with MDD, and that serum BDNF levels were negatively correlated with the severity of depression. We reported that serum BDNF levels in antidepressant-naive patients with MDD were significantly lower than those of patients medicated with antidepressants and normal controls; we also demonstrated that serum BDNF levels were negatively correlated with the severity of depression.[126] Interestingly, we reported a preliminary finding showing that decreased serum BDNF levels in antidepressant-naive patients recovered to normal levels associated with lower Hamilton Depression Rating Scale scores after treatment with antidepressant medication.[126] Subsequently, a number of reports have shown decreased BDNF levels in patients with MDD and increased BDNF levels in patients treated with antidepressants.[127–136] Two meta-analysis studies on blood levels in MDD have been reported. One meta-analysis study demonstrated strong evidence that BDNF levels were lower in depressed subjects than healthy control subjects ($P < 6.8 \times 10^{-8}$), and that BDNF levels were significantly ($P = 0.003$) increased after antidepressant treatment.[137] As shown in Figure 3, serum BDNF levels in patients with MDD were lower than those of