Table 1
Affinities and pharmacology of neurosteroids for sigma-1 receptor.

Neurosteroids	KI (nM)	Pharmacology
Progesterone (PROG)	268[a] or 36[b]	Antagonist
Testosterone	1014[a] or 201[b]	Antagonist
DHEA	706[b]	Agonist
Deoxycorticosterone	938[a]	?
Estradiol	>1000[b]	No effect
11β-hydroxyprogesterone	1535[a]	?
Pregnenolone sulfate (PREG-S)	3196[a]	Agonist
Corticosterone	4074[a]	?

From Su et al. (1988) and Waterhouse et al. (2007).
[a] Su et al. (1988).
[b] Waterhouse et al. (2007).

changes for cognition in the PREG-treated group were not significantly different from the placebo group. Furthermore, administration of PREG increased serum levels of ALLO in patients with schizophrenia, suggesting that PREG may be metabolized to ALLO (Marx et al., 2009, 2011). Subsequent studies showed that PREG may be therapeutically useful in patients with schizophrenia (Ritsner et al., 2010; Ritsner, 2011). Considering that PREG is a potent sigma-1 receptor agonist, it is likely that the sigma-1 receptor is part of the cascade which mediates therapeutic effects in schizophrenia (Hashimoto, 2010b; Niitsu et al., 2012; Yoshida et al., 2012c). As mentioned previously, ALLO exerts an antidepressant effect in an animal model of depression (Shirayama et al., 2011), implying that PREG may possess antidepressant activity as a precursor of ALLO. Interestingly, a randomized placebo-controlled study revealed that the PREG group showed trends toward greater improvement relative to placebo, on depression and mania scores. A post hoc analysis of completers found a statistically significant reduction in Hamilton rating scale for depression (HRSD) scores, for PREG treated patients compared with placebo, suggesting that PREG use may be associated with improvements in manic and depressive symptoms (Osuji et al., 2010). Given that cognitive impairment is also a feature of MDD (Porter et al., 2003; Hindmarch and Hashimoto, 2010; Marazziti et al., 2010), it is likely that PREG may show beneficial effects in this cohort.

Trilostane is an inhibitor of 3β-HSD, which converts PREG to PROG. Interestingly, trilostane shows antidepressant properties in mice, and dampens a stress-induced increase in plasma levels of corticosterone and adrenocorticotropic hormone (ACTH), via direct action on the HPA axis (Espallergues et al., 2009). Furthermore, systemic administration of trilostane directly affected peripheral and brain levels of neurosteroids as well as monoamine turnover, resulting in antidepressant activity (Espallergues et al., 2012). It would be reasonable to propose that 3β-HSD inhibitors may serve as potential therapeutic drugs for MDD.

Accumulating evidence suggests that gonadal hormones (e.g. testosterone) contribute to the pathophysiology of MDD, since testosterone confers mood-enhancing and antidepressant effects in men (Kanayama et al., 2007). It is shown that men with MDD have an increased incidence of hypogonadism, and that testosterone replacement is effective in improving mood within this cohort (Cunningham et al., 1989). A recent animal study suggests that testosterone exerts antidepressant effects in adult male rats via extracellular signal-regulated kinase 2 (ERK2), located downstream of the BDNF-TrkB pathway (Carrier and Kabbaj, 2012).

2.2.4. Neurosteroids in animal models of CVD

Sigma-1 receptor ligands influence cardiovascular function through receptors in the heart (Bhuiyan and Fukunaga, 2011). Among the endogenous neurosteroids, levels of DHEA-S in the blood are approximately 20-fold higher than those of any other neurosteroid (Parker and Odell, 1980). The age-dependent decline in DHEA and DHEA-S levels may well be part of the causative changes seen in the onset of age-related illnesses, including CVD. Chronic administration of DHEA significantly increased sigma-1 receptor expression in the LV, protected against LV hypertrophy, and promoted functional recovery of LV contractions (Bhuiyan and Fukunaga, 2009). Furthermore, DHEA treatment significantly inhibited the reduction of sigma-1 receptor expression in the thoracic aorta, following pressure overload (PO). Treatment also significantly restored PO-induced impairment of Akt phosphorylation and stimulated endothelial nitric oxide synthase (eNOS) protein expression with a concomitant increase in Akt-mediated eNOS phosphorylation (Bhuiyan et al., 2011a). These studies advocate a role for sigma-1 receptors in the cardioprotective action of DHEA (Bhuiyan and Fukunaga, 2011).

2.2.5. Neurosteroids in patients with CVD

Multiple studies have showed an association between low DHEA-S levels and an increased risk of CVD, ischemic heart disease, and all-cause mortality, although these results have not been consistently replicated, particularly in women (Barrett-Connor et al., 1986; LaCroix et al., 1992; Trivedi and Khaw, 2001; Tchernof and Labrie, 2004). Decreased plasma levels of DHEA-S conjugates show an inverse relationship to the severity of heart failure (Moriyama et al., 2000), suggesting a potential role for DHEA-S in the pathophysiology of CVD. It is also well known that neurosteroids, such as DHEA and DHEA-S, play a role in the pathophysiology of MDD (Eser et al., 2006; van Broekhoven and Verkes, 2003). These findings link DHEA and DHEA-S to the pathophysiology of both CVD and MDD, suggesting a common pathway for these two diseases.

Women develop CVD at a later age than men (Kannel et al., 1976). This gender difference has in the past been attributed to the loss of female sex hormones at the time of menopause, however, the explanations may well be more complex (Mendelsohn and Karas, 2005). Furthermore, it has been suggested that the loss of PROG may contribute to a decreased neuroprotective activity, since PROG exerts protective effects in a variety of experimental models (Singh and Su, in press). It is also well known that PROG levels decrease in women following menopause. The physiological role of sigma-1 receptors in the heart is to date very poorly understood. PROG has a moderate affinity at sigma-1 receptors, but it is still unclear how these receptors contribute to the relationship between the incidence of CVD and reduced levels of PROG in women after menopause (Table 1).

Serum levels of testosterone decrease with advancing age in men (Muller et al., 2003a). There is also considerable evidence that low testosterone levels are associated with an increased risk of CVD in men, and that testosterone replacement therapy may reduce cardiovascular mortality (Simon et al., 1997; Muller et al., 2003b; Corona et al., 2011; Cattabiani et al., 2012; Hackett, 2012). Like PROG, testosterone has a moderate affinity at sigma-1 receptors and again, it is unclear how the receptors contribute to the relationship between testosterone and CVD development (Table 1). To provide these answers, further detailed studies on the role of sigma-1 receptors and these neurosteriods will be necessary.

2.3. Selective serotonin reuptake inhibitors (SSRIs) and sigma-1 receptors

Depression after myocardial infarction is associated with higher morbidity and mortality. Selective serotonin reuptake inhibitors (SSRIs), including sertraline, paroxetine, fluoxetine, citalopram, are safe for use in the patients with CVD and even reduce post-myocardial infarction morbidity and mortality (Taylor et al., 2005). However, the molecular mechanisms

Table 2
Affinities of SSRIs and tricyclic antidepressants for sigma-1 receptor in the brain.

Antidepressants	Ki (nM)	Pharmacology
Fluvoxamine	36	Agonist
Sertraline	57	Antagonist?
Fluoxetine	240	Agonist
Citalopram	292	Agonist
Imipramine	343	Agonist
Paroxetine	1893	None
Desipramine	1987	None

From Narita et al. (1996), Hashimoto (2009a,b,c) and Bonnin et al. (2012).

fundamental to the beneficial effects of SSRIs on CVD are unknown (Bhuiyan and Fukunaga, 2011).

Previously, we reported that SSRIs and tricyclic antidepressants possessed moderate to high affinity at sigma-1 receptors in the brain. The potency of these drugs at the receptors was as follows: fluvoxamine > sertraline > fluoxetine > citalopram > imipramine > paroxparoxetine > desipramine (Table 2) (Narita et al., 1996). Of the antidepressants, fluvoxamine showed the greatest potency at sigma-1 receptors, indicating that its action may be mediated through this receptor (Narita et al., 1996; Hashimoto, 2009a). Furthermore, we reported that fluvoxamine, but not sertraline or paroxetine, potentiated nerve growth factor (NGF)-induced neurite outgrowth in PC12 cells, and that the selective sigma-1 receptor antagonist NE-100 blocked it's potentiating effects on NGF-induced neurite outgrowth (Nishimura et al., 2008). We also found that fluvoxamine, but not sertraline or paroxetine, attenuated cognitive deficits in mice after repeated administration of PCP, and that co-administration of NE-100 antagonized these effects (Hashimoto et al., 2007a; Ishima et al., 2009). These results promote the idea that fluvoxamine and sertraline may exert agonistic and antagonistic effects respectively, at sigma-1 receptors (Table 2) (Hashimoto, 2009a; Nishimura et al., 2008; Hashimoto et al., 2007a; Ishima et al., 2009). A recent study shows that the effects of citalopram on fetal thalamic axon responsiveness to netrin-1 were blocked by the sigma-1 receptor antagonist BD1047, suggesting that citalopram promotes sigma-1 receptor agonism (Table 2) (Bonnin et al., 2012).

2.4. Sigma-1 receptors and CVD

As mentioned before, SSRIs are potentially safe for use in patients with CVD (Taylor et al., 2005). However, a recent Cochrane review showed only a small beneficial effect for SSRIs on depression outcomes in patients with CVD (Baumeister et al., 2011). Although all SSRIs act by blocking serotonin transporters, leading to elevated serotonin levels throughout the brain, it is well known that their pharmacological action is quite heterogeneous (Hashimoto, 2009a; Stahl, 1998; Nemeroff and Owens, 2004; Ishikawa et al., 2007). Of the SSRIs, fluvoxamine showed the greatest potency as a sigma-1 receptor agonist, while paroxetine had no affinity at the sigma-1 receptor, and sertraline may be a receptor antagonist (Table 2) (Hashimoto, 2009a). Thus, it is likely that the heterogeneous action of SSRIs at sigma-1 receptors may partly contribute to the small effect of SSRIs observed in CVD studies. It would be, of great interest to examine individually, the beneficial effects of SSRIs, based on whether they act as agonists, antagonists or have no affinity for the sigma-1 receptors, on morbidity and mortality in depressed patients with CVD.

As with DHEA, increased expression of sigma-1 receptors induced by fluvoxamine, promoted cardioprotection that could be blocked by the sigma-1 receptor antagonist NE-100, indicating a role of sigma-1 receptor in the action of fluvoxamine (Tagashira et al., 2010). In contrast, paroxetine, another SSRI with very low affinity at the sigma-1 receptor (Table 1), was ineffective in this model (Tagashira et al., 2010). Fluvoxamine significantly attenuated PO-induced myocardial hypertrophy with a concomitant increase in sigma-1 receptor expression in the LV. In addition, fluvoxamine also decreased hypertrophy-induced LV impairment, and these cardioprotective effects of fluvoxamine could be blocked by co-administration of NE-100 (Bhuiyan et al., 2010). Fluvoxamine conferred a protective effect by attenuating hypertrophy-induced vascular injury in ovariectomized rats, and as before, these effects were antagonized by co-administration of NE-100 (Bhuiyan et al., 2011b).

Very recently, Itoh et al. (2012) reported that decreased levels of sigma-1 receptors in the brain play a crucial role in the link between heart failure and depression. Mice with impaired cardiac function induced by aortic banding and high salt intake, exhibited decreased levels of sigma-1 receptors in the brain, and depression-like behavior. Intracerebroventricular (ICV) infusion of the sigma-1 receptor agonist PRE084, increased brain sigma-1 receptor expression, lowered sympathetic activity, and improved cardiac function and depression-like behavior in this model (Itoh et al., 2012). In contrast, ICV infusion of the sigma-1 receptor antagonist BD1063 increased sympathetic activity and decreased cardiac function in sham mice. In addition, oral administration of fluvoxamine attenuated the sympathetic hyperactivation in addition to improving depression-like behavior in mice with aortic banding (Itoh et al., 2012). Taken all together, it is likely that sigma-1 receptor agonists mediate potent cardioprotection, through the stimulation of sigma-1 receptors in the brain and heart (Itoh et al., 2012; Tagashira and Fukunaga, 2012).

Ifenprodil, an antagonist at the *N*-methyl-D-aspartate (NMDA) receptor, with high affinity for the NR2B subunit, is a widely used cerebral vasodilator (Williams, 2001). As well as binding to the α1 adrenergic receptor and NMDA receptor antagonists, ifenprodil also binds to the sigma-1 receptor with high affinity, suggesting that some of its action may be mediated through this receptor (Hashimoto and London, 1993, 1995; Hashimoto et al., 1994; Hashimoto and Ishiwata, 2006). Recently, we reported that ifenprodil potentiated NGF-induced neurite outgrowth in PC12 cells, and that its effects were antagonized by treatment with NE-100, suggesting that ifenprodil is an agonist at sigma-1 receptors (Ishima and Hashimoto, 2012). Preclinical and clinical studies show that ifenprodil can induce cardiovascular changes (Monassier et al., 1999, 2001; Catelli et al., 2000). Very recently, we reported that ifenprodil was effective in treating emotional incontinence in patients with vascular dementia (Kishimoto et al., in press), and flashbacks in female patients with a history of childhood sexual abuse (Kishimoto et al., 2012a), although the precise mechanisms underlying this therapeutic effect are currently unknown. Given the role of sigma-1 receptor in the pathophysiology of CVD, it is possible that this receptor in part, plays a role in the therapeutic mechanisms of ifenprodil.

3. Brain-derived neurotrophic factor (BDNF)

Brain-derived neurotrophic factor (BDNF), is initially synthesized as the precursor protein, preproBDNF, in the ER. Following cleavage of the signal peptide, proBDNF is transported to the Golgi for sorting into either constitutive or regulated secretory vesicles. ProBDNF is converted to mature BDNF by extracellular proteases, such as matrix metalloproteinase-9 (MMP-9) and plasmin (Lu, 2003; Lu et al., 2005; Hashimoto, 2007, 2010a; Hwang et al., 2005; Ethell and Ethel, 2007; Yoshida et al., 2012a) (Fig. 4). It was initially thought that only secreted, mature BDNF was biologically active, and that proBDNF, localized intracellularly, serving as an inactive precursor. However, recent studies show that proBDNF and mature BDNF elicit opposing effects via the $p75^{NTR}$ and TrkB receptors,

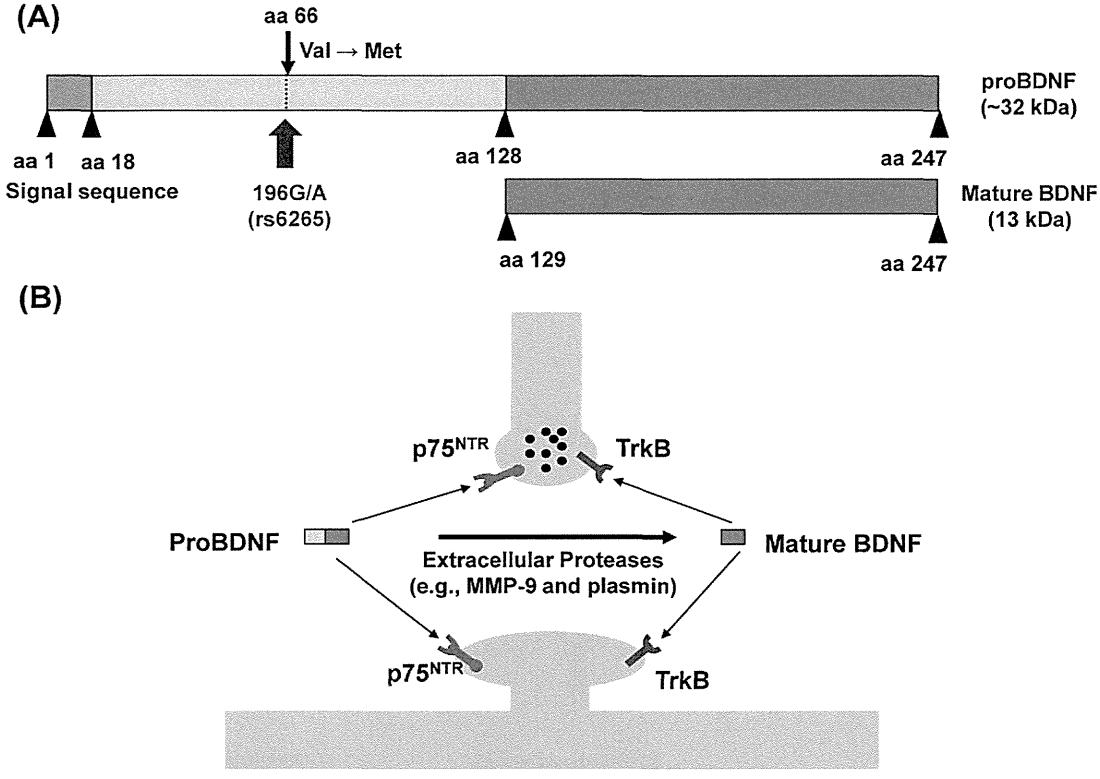

Fig. 4. Structure of proBDNF and mature BDNF, and proBDNF-p75^{NTR} and mature BDNF-TrkB pathways.
(A) Structure of proBDNF and mature BDNF. Arrowheads indicate known protease cleavage sites involved in processing to mature BDNF. Position of the single nucleotide polymorphism (rs6265, Val66Met) in the human *BDNF* gene is indicated by an arrow. (B) Extrasynaptic cleavage of proBDNF to mature BDNF. ProBDNF preferentially binds to p75^{NTR}. ProBDNF is cleaved by extracellular proteases (e.g., MMP-9 and plasmin) at synapses and is converted to mature BDNF. Mature BDNF preferentially binds the TrkB receptor. This figure is a modified version of previously published figures (Hashimoto, 2007, 2010a; Yoshida et al., 2012a).

respectively, and that both proBDNF and mature BDNF play important roles in several physiological functions (Lu et al., 2005; Hashimoto, 2010a) (Fig. 4). Multiple lines of evidence suggest that BDNF plays an important role in the pathophysiology of MDD, as well as in the mechanisms of action of antidepressants (Hashimoto, 2010a; Duman et al., 1997; Altar, 1999; Nestler et al., 2002; Coyle and Duman, 2003; Hashimoto et al., 2004; Duman and Monteggia, 2006; Martinowich et al., 2007). Given the opposite physiological roles of proBDNF and mature BDNF, proBDNF also may play a role in the pathophysiology of MDD (Hashimoto, 2010a).

3.1. *BDNF in animal models of depression*

BDNF confers antidepressant effects in animal models of depression (Hashimoto et al., 2004; Hashimoto, 2010a). Specifically, infusion of BDNF into the midbrain has an antidepressant-like influence on learned helplessness and the forced swim test, two animal models of depression (Russo-Neustadt et al., 2001). Furthermore, Shirayama et al. (2002) reported that a single bilateral infusion of BDNF into the dentate gyrus (DG) of the hippocampus relieved depression in both the learned helplessness and forced swim test paradigms. These effects were observed as early as 3 days after a single infusion of BDNF, and lasted for at least 10 days (Shirayama et al., 2002). In addition, infusions of the broad-spectrum tyrosine kinase (Trk) inhibitor K252a, or the selective extracellular signal-regulated protein kinase (ERK) inhibitor U0126 blocked the antidepressant effects of BDNF, implying that the TrkB/mitogen-activated protein (MAP) kinase cascade plays a role in the therapeutic action of antidepressants (Shirayama et al., 2002).

It has been demonstrated that intra-ventral tegmental area (VTA) infusions of BDNF resulted in 57% shorter latency of immobility relative to control animals (a depression-like effect), and that rats given intra-nucleus accumbens (NAc) injections of a virus expressing a truncated version of the BDNF receptor had an almost 5-fold longer latency of 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). Interestingly, using a viral-mediated, mesolimbic dopamine pathway-specific knockdown of BDNF, Berton et al. (2006) highlighted that BDNF is required for the development of experience-dependent social aversion, in an animal social defeat stress model, which measures depression. Additionally, they found that gene profiling in the NAc, indicated that local knockdown of BDNF obliterates most of the effects of repeated aggression exposure on gene expression within this circuit. This effect was similar to that produced by chronic treatment with the SSRI, fluoxetine (Berton et al., 2006). These findings suggest that BDNF acts within the VTA-NAc pathway, resulting in a depressive-like phenotype (Eisch et al., 2003; Berton et al., 2006; Nestler and Carlezon, 2006). This profile is opposite to the antidepressant-like effects of BDNF in the hippocampus (Shirayama et al., 2002; Duman, 2004; Nestler et al., 2002). Interestingly, the novel TrkB antagonist ANA-12, N2-(2-{[(2-oxoazepan-3-yl)amino]carbonyl}-phenyl)benzo[b]thiophene-2-carboxamide (Fig. 5), showed antidepressant-like effects in the forced swim test and tail-suspension test, suggesting that inhibition of the BDNF-TrkB pathway in the reward system, alleviates depression (Cazorla et al., 2011). Further detailed studies of the TrkB system in animal models of depression are needed to fully understand the relevant mechanisms.

Monteggia et al. (2007) showed that mice with a conditional BDNF knock-out also display increased depression-like behavior, as measured in the forced swim and sucrose preference tests, suggesting that low BDNF production may precipitate depressive disorders. In a subsequent study, Adachi et al. (2008) used a viral-mediated gene

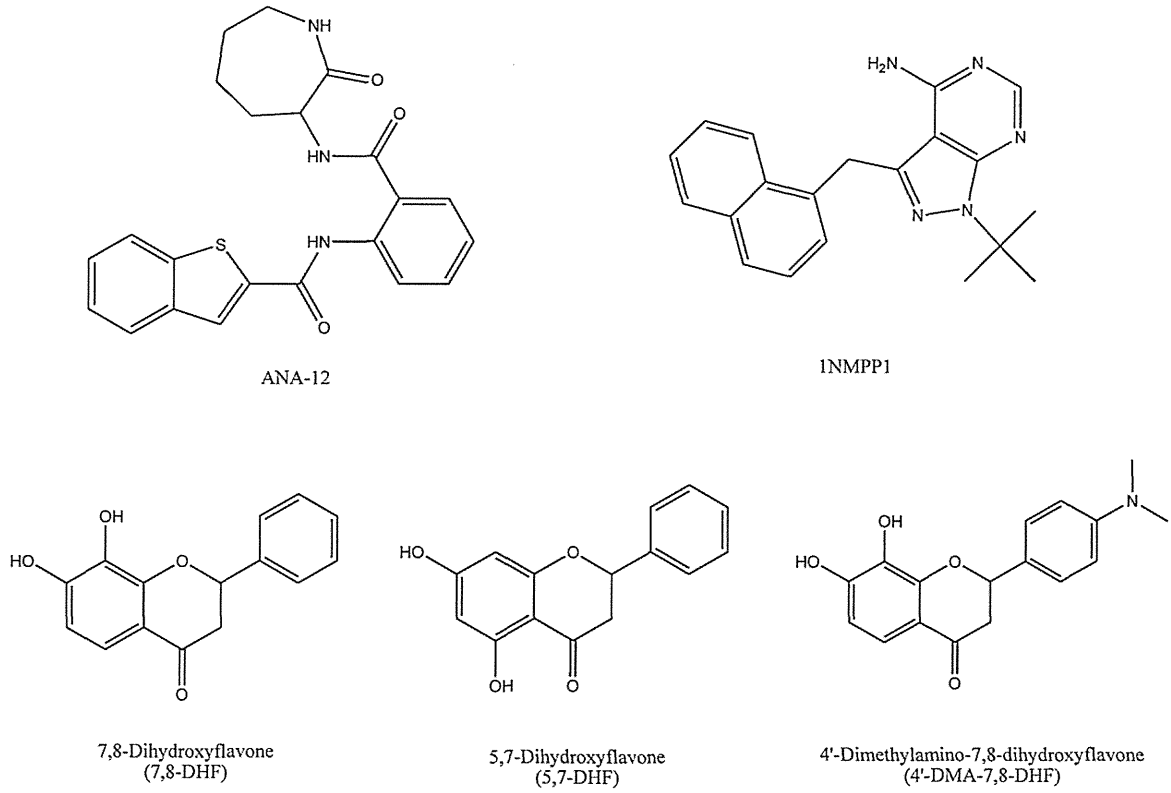

Fig. 5. Chemical structures of ANA-12, 1NMPP1, 7,8-dihydroxyflavone (7,8-DHF), 5,7-dihydroxyflavone (5,7-DHF), and 4′-dimethyl-7,8-dihydroxyflavone (4′-DMA-7,8-DHF). ANA-12 and 1NMPP1 are TrkB antagonists. 7,8-DHF and 4′-DMA-7,8-DHF are TrkB agonists. 5,7-DHF is an inactive ligand at TrkB.

transfer approach to assess the role of BDNF in subregions of the hippocampus. The loss of BDNF in CA1 or the DG of the hippocampus did not alter locomotor activity, anxiety-like behavior, fear conditioning, or depression-related behavior. However, selective loss of BDNF in the DG attenuated the actions of antidepressants in the forced swim test. These data highlight a role for BDNF in mediating the therapeutic effect of antidepressants via the DG (Adachi et al., 2008).

3.2. Role of BDNF in the antidepressant effects of ketamine

Accumulating evidence suggests a crucial role for glutamate in the pathophysiology of MDD (Sanacora et al., 2008; Skolnick et al., 2009; Hashimoto, 2009c, 2011a,b; Hashimoto et al., 2007b; Zarate et al., 2010; Aan Het Rot et al., 2012; Tokita et al., 2012). Randomized, placebo-controlled studies demonstrate that the NMDA receptor antagonist, ketamine, has confers rapid antidepressant effects in patients with treatment resistant MDD and bipolar depression (Zarate et al., 2006, 2012; Diazgranados et al., 2010). This makes ketamine an attractive therapeutic drug for refractory depression (Krystal, 2007, 2010; Machado-Vieira et al., 2009; Hashimoto, 2011a; Bunney and Bunney, 2012; Mathew et al., 2012; Murrough, 2012; Aan Het Rot et al., 2012).

A single nucleotide Val66Met polymorphism (rs6265) (Fig. 4) located within proBDNF is associated with cognition (e.g., episodic memory and extinction learning) and hippocampal volume in humans (Egan et al., 2003; Soliman et al., 2010). Chen et al. (2006) generated a variant proBDNF (66Met/Met) mouse that produces the phenotypic hallmarks seen in humans with a variant allele, and, when placed in stressed settings, proBDNF (66Met/Met) mice exhibit increased anxiety-related behaviors that are not attenuated by fluoxetine. This study highlights the impact of the proBDNF Met substitution on anxiety-related behavior (Chen et al., 2006; Hashimoto, 2007). Using knock-in mice with the BDNF Val66Met mutation, Liu et al. (2012) studied the effects of this polymorphism on synaptogenic and antidepressant responses to ketamine in mice. The authors found that mice carrying the Met allele displayed constitutive atrophy of distal apical dendrites, and decrements in apically targeted, excitatory postsynaptic currents, in layer V pyramidal cells of the prefrontal cortex. These same mice showed decreased spine density and diameter, as well as impaired synaptic formation/maturation (synaptogenesis). Interestingly, ketamine did not induce antidepressant or synaptogenic effects in Met/Met mice. These findings imply that expression of the BDNF Met allele results in basal synaptic deficits, which block the synaptogenic and antidepressant actions of ketamine. Therefore, it may be of interest to examine whether patients who are resistant to the rapid antidepressant effects of ketamine, carry the Met allele (Hashimoto, 2012).

Very recently, Laje et al. (in press) reported an association between BDNF Val66Met polymorphism status and ketamine treatment response. The mean percent change in Hamilton Depression Rating Scale (HAM-D) scores (improvement) was 24% for Met allele, and 41% for Val allele carriers. In the Caucasian only group, mean changes for the Met allele and Val allele were 20 and 40%, respectively. These findings suggest that ketamine is more likely to elicit an increased antidepressant response in MDD patients carrying the Val allele, compared with carriers of the Met allele (Laje et al., in press). The Met allele occurs at a frequency of 40–50% within the Asian population, which is significantly higher than the 20–30% frequency seen in Caucasian populations (Shimizu et al., 2004; Kim et al., 2007), indicating an ethnic-based difference in the distribution of this polymorphism (Hashimoto, 2012). Therefore, it is unlikely that ketamine would produce antidepressant effects in MDD patients who carry the Met allele, a cohort that represents the majority of the Asian population.

A recent study using conditional BDNF knock-out mice showed a causal association between ketamine's rapid antidepressant

effects and increased activity-dependent BDNF in mice (Autry et al., 2011), suggesting a crucial role for BDNF-TrkB signaling in the action of ketamine. Duncan et al. (in press) more recently reported that plasma levels of BDNF and early sleep slow wave activity (SWA) in patients with refractory MDD ($n = 30$), increased after ketamine infusion. These findings suggest that sleep SWA parameters and blood BDNF levels, could serve as non-invasive indices for testing the efficacy of newly developed antidepressant therapies that target the glutamatergic system (Duncan et al., in press).

3.3. Effects of TrkB agonists in animal models of depression

As described above, the BDNF-mediated TrkB signaling pathway is pivotal in the action of antidepressants (Hashimoto, 2010a; Duman et al., 1997; Altar, 1999; Nestler et al., 2002; Coyle and Duman, 2003; Hashimoto et al., 2004; Duman and Monteggia, 2006; Martinowich et al., 2007). Recently, Jang et al. (2010) discovered a selective TrkB agonist, 7,8-dihydroxyflavone (7,8-DHF) (Fig. 5), with potent neurotrophic activity. Interestingly, chronic ICV administration of 7,8-DHF dampens the onset of depressive-like behavior (e.g., lack of HPA hyperactivity, anhedonia, or helplessness behavior) and changes of hippocampal parameters, such as hippocampal volume, cell proliferation, apical dendritic length and spine density, in rats after a priming, stressful event (Blugeot et al., 2011). Subsequent to these experiments, the same group developed a new compound, 4′-dimethylamino-7,8-dihydroxyflavone (4′-DMA-7,8-DHF) (Fig. 5). This compound displays higher TrkB agonistic activity than 7,8-DHF (Liu et al., 2010). Both 7,8-DHF and 4′-DMA-7,8-DHF show antidepressant effects in wild-type mice, but not TrkB F616A knock-in mice (Liu et al., 2010). In addition, the antidepressant effects of these compounds were reversed by the TrkB antagonist, 1-(1,1-dimethylethyl)-3-(1-naphthalenylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1NMPP1; Fig. 5) (Liu et al., 2010). Very recently, we found that systemic administration of 7,8-DHF showed antidepressant effects in lipopolysaccharide (LPS)-induced depressive behavior in mice, and that 5,7-DHF (Fig. 5), an inactive stereoisomer of 7,8-DHF, had no effect (Zhang et al., 2012). These findings imply that 7,8-DHF and 4′-DMA-7,8-DHF act through the TrkB receptor signaling cascade. This would make TrkB agonists attractive, potential therapeutic drugs for MDD (Jang et al., 2010; Monteggia, 2011; Blugeot et al., 2011).

3.4. Blood levels of BDNF and its precursor proBDNF in patients with depression

Previously, we reported that serum levels of BDNF in antidepressant naïve patients with MDD were significantly lower than those of normal controls, and that decreased serum BDNF levels in these patients recovered to normal levels with an associated recovery of depressive symptoms, after treatment with antidepressants (Shimizu et al., 2003). These findings were confirmed by three meta-analysis studies (Sen et al., 2008; Brunoni et al., 2008; Bocchio-Chiavetto et al., 2010) and a large cohort study (Molendijk et al., 2011). Therefore, accurate measurement of blood BDNF levels could serve as a potential biomarker for MDD (Hashimoto, 2010a). Although BDNF levels in human blood can be measured using commercially available Enzyme-Linked Immuno Sorbent Assay (ELISA) kits, accurate readings are sometimes hampered by the limited specificity of the BDNF antibody, as early kits were unable to distinguish between proBDNF and mature BDNF (Yoshida et al., 2012a). Recently, we reported on the measurement of serum levels of proBDNF and mature BDNF in healthy subjects, using newly available human proBDNF and BDNF ELISA kits (Yoshida et al., 2012a). Using these kits, we found that in patients with MDD, serum levels of mature BDNF, but not proBDNF, are significantly lower than those of healthy controls (Yoshida et al., 2012b).

3.5. Role of BDNF in animal models of CVD

Accumulating evidence suggests that BDNF is also involved in the developing cardiovascular system (Chaldakov et al., 2004). Deficient expression of BDNF impairs the survival of endothelial cells in intramyocardial arteries and capillaries in the early postnatal period. Additionally, BDNF deficiency results in reduced endothelial cell–cell contacts and eventual endothelial cell apoptosis, leading to intraventricular wall hemorrhage, depressed cardiac contractility and early postnatal death (Donovan et al., 2000). Moreover, BDNF acts on endothelial cells and promotes neovascularization in response to hypoxic stimuli, via the Akt pathway (Kim et al., 2004; Nakamura et al., 2006). The transcriptional activation of TrkB is crucial to the development of coronary vessels (Wagner et al., 2005). Taken together, it is likely that BDNF-TrkB signaling plays an essential role in the pathophysiology of CVD.

In a patent application, Daimon et al. (2004) reported increases in LV weight, infarction size, LV diastolic diameter, as well as a decrease in LV fractional shortening, after myocardial infarction in heterozygous BDNF knock-out mice (approximately 50% of BDNF of wild-type mice) compared with wild-type mice. Very recently, Okada et al. (2012) reported on BDNF-induced CNS-mediated mechanisms that protect against cardiac remodeling after myocardial infarction. Compared with controls, cardiac size and cardiomyocyte death were markedly increased in cardiomyocyte-specific conditional BDNF knock-out mice, 2 weeks after myocardial infarction. Deletion of TrkB from heart tissue, also led to exacerbation of cardiac dysfunction after myocardial infarction. After an infarction, plasma levels of BDNF were markedly elevated, and this increase was associated with up-regulation of BDNF expression in the brain, but not heart. Furthermore, peripheral administration of BDNF significantly restored the cardiac phenotype of neuronal BDNF-deficient mice. These intriguing findings suggest that the cardioprotective effect of BDNF is mediated through a CNS dependent pathway (Okada et al., 2012). These combined findings highlight the importance of BDNF-TrkB signaling in cardiac dysfunction. It would therefore be reasonable to propose that BDNF or TrkB agonists such as 5,7-DHF or 4′-DMA-7,8-DHF could offer novel therapeutic options for treatment after myocardial infarction.

3.6. Blood levels of BDNF in patients with CVD

In the patent application, we found that serum levels of BDNF in the ischemic heart disease risk group were significantly lower than those of normal controls (Daimon et al., 2004), suggesting that measurement of blood BDNF is useful as a biological marker for an ischemic heart disease risk group. Furthermore, Manni et al. (2005) reported reduced plasma levels of BDNF in patients with acute coronary syndromes. Moreover, differences in BDNF levels between the coronary sinus and aorta were significantly greater in the unstable angina group compared with stable effort angina and non-coronary artery disease groups (Ejiri et al., 2005). Golden et al. (2010) reported that plasma BDNF significantly correlates with multiple risk factors (body mass index, fat mass, diastolic blood pressure, total cholesterol, low-density lipoprotein cholesterol, triglycerides, free triiodo-thyronine, testosterone, sex-hormone binding globulin, adiponectin) for metabolic syndrome and cardiovascular dysfunction. A recent study showed that plasma levels of BDNF in patients with angina pectoris were inversely associated with triglycerides levels and low-density

lipoprotein cholesterol, the presence of diabetes mellitus, fibrinogen levels, male sex and age, and positively associated with high-density lipoprotein cholesterol levels and platelet counts (Jiang et al., 2011), suggesting that low BDNF plasma levels may be associated with future coronary events and mortality in these patients. These findings strongly suggest a crucial role for BDNF in the pathogenesis of CVD. In addition, considering the important role that both proBDNF and mature BDNF play in physiological functions (Hashimoto, 2010a), it would be informative to measure levels of proBDNF and mature BDNF in the blood of patients with CVD.

3.7. *Effects of sigma-1 receptor agonists on BDNF expression*

Chronic administration of antidepressants, including SSRIs, increased the levels of BDNF protein in the brain, supporting the hypothesis of BDNF-TrkB signaling pathway on MDD (Hashimoto, 2010a; Duman et al., 1997; Nestler et al., 2002; Hashimoto et al., 2004). In addition, chronic administration of cutamesine (SA4503), a novel and potent sigma-1 receptor agonist which has been tested in a completed Phase II clinical trial in MDD, significantly increased the levels of BDNF protein in rat hippocampus (Kikuchi-Utsumi and Nakaki, 2008). Very recently, Fujimoto et al. (2012) reported that cutamesine increased sigma-1 receptor protein and concomitantly increased the secretion of endogenous BDNF, suggesting that BDNF is regulated by sigma-1 receptor stimulation. Overexpression of sigma-1 receptors potentiated conversion of the precursor proBDNF, to mature BDNF and enhanced the secretion of mature BDNF into extracellular spaces (Fujimoto et al., 2012). These pieces of evidence indicate that sigma-1 receptor activation promotes chaperone activity, which in turn, regulates the secretion of BDNF, and also may inhibit the aggregation of proteins (e.g., BDNF) induced by ER stress, which play a role in the pathogenesis of CVD and psychiatric diseases (Fig. 2). Therefore, it seems that increased secretion of BDNF by stimulation of sigma-1 receptors could also produce beneficial effects on cardiovascular function. Nonetheless, further studies on the close relationship between sigma-1 receptors and BDNF-TrkB signaling will be needed.

4. Role of ER stress in the pathophysiology of CVD and depression

The ER is a cellular organelle within which protein folding, calcium homeostasis, and lipid biosynthesis occur. Stimuli such as oxidative stress, ischemic insults, disturbances in calcium homeostasis, and enhanced expression of normal and/or folding-defective proteins, lead to the accumulation of unfolded proteins, a condition referred to as ER stress (Minamino and Kitakaze, 2010; Minamino et al., 2010). ER stress triggers the unfolded protein response (UPR) in order to maintain ER homeostasis. The UPR constitutes a group of signal transduction pathways that ameliorate the accumulation of unfolded protein, by increasing ER-resident molecular chaperones, inhibiting protein translation and accelerating the degradation of unfolded proteins (Minamino and Kitakaze, 2010; Minamino et al., 2010).

Accumulating evidence suggests a crucial role for ER stress and misfolded proteins in the pathophysiology of CVD as well as in MDD (Fig. 2) (Dickhout et al., 2011; Groenendyk et al., 2010; Hayashi et al., 2011; Minamino and Kitakaze, 2010; Minamino et al., 2010; Su et al., 2010). For example, markedly increased expression of BiP was found in patients with heart failure (Sawada et al., 2010), suggesting that UPR activation is associated with the pathophysiology of heart failure in humans (Minamino and Kitakaze, 2010; Minamino et al., 2010). Furthermore, the levels of ER stress proteins such as BiP, GRP94, and calreticulin were increased in the temporal cortex of depressed patients (Bown et al., 2000), highlighting a potential role for ER stress in depression. It should be noted that BiP is a binding partner of sigma-1 receptors (Hayashi and Su, 2007). Considering the function of sigma-1 receptors as molecular chaperones that regulate protein folding and degradation at the ER, activation of sigma-1 receptors could prevent the misfolding of proteins which promote the development of CVD and MDD (Fig. 2).

5. Conclusion

The role of ER stress in the pathophysiology of CVD suggests that targeting the UPR could be an effective therapeutic approach for treating CVD. Pharmacological agents that directly activate or deactivate UPR components may be of greatest potential use. For example, the activation of sigma-1 receptors by agonists, and the subsequent secretion of BDNF might bestow beneficial effects in patients with CVD, although further detailed studies on the relationship between sigma-1 receptors and BDNF-TrkB signaling are necessary (Fig. 2). This proposal is supported by two recent animal studies which demonstrate a role for sigma-1 receptor and BDNF-TrkB signaling in the CNS on the pathophysiology of CVD (Itoh et al., 2012; Okada et al., 2012). Finally, understanding the precise molecular mechanisms of the sigma-1 receptor and proBDNF–mature BDNF–TrkB signaling pathway in CVD will provide new targets for drug discovery and therapeutic intervention.

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Reduced white matter fractional anisotropy and clinical symptoms in schizophrenia: A voxel-based diffusion tensor imaging study

Kazue Nakamura [a,*], Yasuhiro Kawasaki [a,b,d], Tsutomu Takahashi [a,b], Atsushi Furuichi [a], Kyo Noguchi [c], Hikaru Seto [c], Michio Suzuki [a,b]

[a] Department of Neuropsychiatry, University of Toyama Graduate School of Medicine and Pharmaceutical Sciences, 2630 Sugitani, Toyama 930-0194, Japan
[b] Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Tokyo, Japan
[c] Department of Radiology, University of Toyama Graduate School of Medicine and Pharmaceutical Sciences, 2630 Sugitani, Toyama 930-0194, Japan
[d] Department of Neuropsychiatry, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa, 920-0293, Japan

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ABSTRACT

Although not consistently replicated, diffusion tensor imaging (DTI) studies in schizophrenia have revealed lower fractional anisotropy (FA) in various white matter regions, a finding consistent with the disruption of white matter integrity. In this study, we used voxel-based DTI to investigate possible whole-brain differences in the white matter FA values between 58 schizophrenia patients and 58 healthy controls. We also explored the association between FA values and clinical symptoms in schizophrenia. Compared with the controls, the schizophrenia patients showed significant FA reductions in bilateral superior longitudinal fasciculus, bilateral inferior fronto-occipital fasciculus, and genu of right internal capsule. Furthermore, in the patient group, the FA value of the anterior part of the corpus callosum was negatively correlated with the avolition score on the Scale for the Assessment of Negative Symptoms. These findings suggest widespread disruption of white matter integrity in schizophrenia, which could partly explain the severity of negative symptomatology.

1. Introduction

Accumulating evidence suggests regional disconnectivity and white matter pathology in the brains of schizophrenia patients (Friston and Frith, 1995; Stephan et al., 2006; Walterfang et al., 2006). Standard magnetic resonance imaging (MRI) analyses have generally reported subtle volume reductions in total as well as regional (e.g., fronto-temporal and callosal regions) white matter areas (Suzuki et al., 2002; Zhou et al., 2003; Walterfang et al., 2006; Makris et al., 2010). Neuropathological and genetic findings have implicated the pathology of oligodendrocytes in schizophrenia, which could be the result of excitotoxicity during critical periods of myelination (Walterfang et al., 2006). In contrast, a longitudinal MRI finding of periventricular white matter reduction only in the schizophrenia patients who responded to antipsychotic medication (Christensen et al., 2004) suggests that white matter changes may represent, at least in part, state-related abnormalities after onset. Different imaging modalities that enable the examination of the white matter microstructure and connectivity are required to further investigate the white matter pathology of schizophrenia.

Diffusion tensor imaging (DTI), which measures the mobility of water molecules *in vivo*, examines the organization of fibers of white matter tracts (Beaulieu, 2002). Specifically, fractional anisotropy (FA) is a quantitative measure of directionally averaged diffusion, with the areas of sparse, poorly myelinated, or divergent fibers having lower FA values (Le Bihan et al., 2001; Beaulieu, 2002). Early DTI studies using region of interest (ROI)-based FA analysis have shown mixed findings in schizophrenia, but with the strongest data suggesting subtle FA reductions in frontal white matter, corpus callosum (CC), and cingulate bundle (CB) (reviewed by Kanaan et al., 2005; Walterfang et al., 2006; Kubicki et al., 2007; Kyriakopoulos et al., 2008). While these studies cannot address the question of whether these FA changes are localized to specific ROIs, more recent voxel-based studies of whole-brain FA alteration suggest widespread white matter pathology [e.g., fronto-temporal areas and multiple tracts such as the CC, arcuate fasciculus (AF), CB, and internal capsule] in schizophrenia (Kyriakopoulos et al., 2008). A recent meta-analysis of 15 voxel-based DTI studies, which included a total of 407 schizophrenia patients, demonstrated FA reduction especially in left fronto-temporal areas (Ellison-Wright and Bullmore, 2009). On the other hand, previous studies on symptom correlates of DTI findings in schizophrenia have yielded inconsistent results. While negative symptoms were correlated with low FA in several tract regions (Wolkin et al., 2003; Kubicki et al., 2008; Szeszko et al., 2008), positive symptoms such as auditory hallucinations were

* Corresponding author. Tel.: +81 76 434 7323; fax: +81 76 434 5030.
E-mail address: krnaka@med.u-toyama.ac.jp (K. Nakamura).

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reported to be both *positively* (Hubl et al., 2004; Fujiwara et al., 2007; Seok et al., 2007) and *negatively* (Skelly et al., 2008) correlated with FA in various brain regions. These discrepancies could be partly due to the small sample, limited brain regions investigated, different sample characteristics, and other confounding factors (e.g., illness chronicity, medication) in these previous studies.

In this voxel-based DTI study, we aimed to clarify the whole-brain FA alteration and its relationship to clinical variables, especially symptom severity, in a relatively large sample of schizophrenia patients. As the regional disconnectivities might be responsible for a range of clinical symptoms in schizophrenia (Walterfang et al., 2006), we predicted that 1) the schizophrenia patients would have lower FA values, especially in fronto-temporal and various tract regions, than age- and gender-matched healthy controls and that 2) these FA differences would be partly related to the severity of clinical symptoms.

2. Methods

2.1. Participants

Table 1 shows demographic and clinical data of the subjects in this study. Fifty-eight patients with schizophrenia (38 males and 20 females), who met the ICD-10 research criteria (World Health Organization, 1993), were recruited from the inpatient and outpatient clinics of the Department of Neuropsychiatry, Toyama University Hospital. Diagnoses were made following structured clinical interviews by experienced psychiatrists with the Comprehensive Assessment of Symptoms and History (CASH; Andreasen et al., 1992). Clinical symptoms were assessed with the Scale for the Assessment of Negative Symptoms (SANS; Andreasen, 1984a) and the Scale for the Assessment of Positive Symptoms (SAPS; Andreasen, 1984b) at the time of scanning.

All but five patients were receiving neuroleptic medication at the time of scanning; five patients were treated with typical neuroleptics, 31 were receiving atypical neuroleptics, and 17 were taking both typical and atypical neuroleptics. Five patients were neuroleptic-free. They were also receiving anticholinergic drugs ($N=23$), benzodiazepines ($N=31$), antidepressants ($N=4$), carbamazepine ($N=1$), and/or lithium carbonate ($N=3$).

The control subjects consisted of 58 healthy volunteers (38 males and 20 females) recruited from members of the community, hospital staff, and university students. They were given a questionnaire consisting of 15 items concerning their personal (13 items; e.g., a history of obstetric complications, substantial head injury, seizures, neurological or psychiatric diseases, impaired thyroid function, hypertension, diabetes, and substance use) and family (2 items) histories of illness. They did not have any personal or family history of psychiatric illness in their first-degree relatives. All the control candidates were administered the Minnesota Multiphasic Personality Inventory (MMPI) by experienced psychologists to obtain a rather homogeneous control group without eccentric profiles on the MMPI, and were excluded if they had an abnormal profile with any T-score for the validity scales or the clinical scales exceeding 70. However, assessment of IQ was not comprehensively undertaken in this sample. This study was approved by the ethics committee of Toyama University. Written informed consent was obtained from all subjects prior to study participation.

Table 1
Clinical and demographic characteristics[a].

Characteristic	Patients ($n=58$)	Comparison subjects ($n=58$)
Gender (male/female)	38/20	38/20
Age (years)	27.6 (6.9)	26.4 (3.1)
Educational level (years)[b]	13.2 (2.1)	17.1 (2.2)
Parental educational level (years)[c]	12.9 (2.1)	14.0 (2.1)
Age at onset	23.3 (5.4)	N/A
Duration of illness (months)	51.2 (52.4)	N/A
Duration of medication (months)	36.3 (51.9)	N/A
Drug (mg/day, haloperidol equivalent)[d]	10.1(8.8)	N/A
Total SAPS score	26.9 (20.7)	N/A
Total SANS score	52.1 (23.9)	N/A

[a] Values given as mean (S.D.).
[b] Significant difference between groups ($t=-9.9$, d.f.$=114$, $p<0.001$).
[c] Significant difference between groups ($t=2.6$, d.f.$=114$, $p=0.01$).
[d] The different typical and atypical neuroleptic dosages were converted into haloperidol equivalents using the guidelines of Toru (2001).

2.2. Acquisition of images

Data were acquired on a 1.5-Tesla Magnetom Vision scanner (Siemens Medical Systems, Inc., Erlangen, Germany), with actively shielded magnetic field gradients (maximum amplitude $=25$ mT m^{-1}). Each diffusion tensor image was acquired with a pulsed gradient, double spine echo, echo planar imaging (EPI) sequence in the axial direction parallel to the anterior commissure-posterior commissure line. The acquisition parameters were as follows: echo time (TE) $=100$ ms; repetition time (TR) $=4000$ ms; number of excitations $=4$; $b=0$ and 1000 s/mm^2 along six noncollinear directions; acquisition matrix $=128\times128$, native voxel dimensions $=1.88\times1.88\times5$ mm (reconstruction matrix $=256\times256$, voxel resolution $=0.94\times0.94\times5$ mm); field of view (FOV) $=240$ mm; 20 contiguous 5-mm slices without gap. Movement-related artifacts were minimized by stabilization of the participant's head with foam cushions and an elastic forehead strap, but images with robust motion artifacts were removed from the analysis.

2.3. Analysis of images

Using the software package Dr View/LINUX (AJS, Tokyo, Japan), correction for distortion due to eddy currents was carried out. Estimation of the diffusion tensor in each voxel was performed and FA maps were calculated for all subjects using dTV II and VOLUME-ONE software (Image Computing and Analysis Laboratory, Department of Radiology, The University of Tokyo Hospital, Tokyo, Japan; available at: http: //www.ut-radiology.umin.jp/people/masutani/dTV.htm) with dimensions of voxel $=0.94\times0.94\times0.94$ mm. FA maps were reoriented in SPM2 (London, Wellcome Department of Imaging Neuroscience, Functional Imaging Laboratory; available at: http://www.fil.ion.ucl.ac.uk/spm/software/spm2/) and trilinear interpolations of FA maps were performed with final dimensions of voxel $=2\times2\times2$ mm. FA maps were normalized following the iterative approach. The $b=0$ images of all subjects were first normalized to the SPM2 EPI template with T_2* weighting (the Montreal Neurological Institute space) for better correction for the distortions in the $b=0$ images, applying a 12-parameter affine registration. After the affine registration, the nonlinear normalization was performed using the following parameters: nonlinear frequency cut-off $=25$ mm, medium regularization, and number of nonlinear iterations $=16$ (Rosario et al., 2008). The derived normalizing parameters were applied to the corresponding FA map of each subject (Shergill et al., 2007). All resulting FA maps were then averaged to create an initial FA template. The original FA map was again normalized to the initial template and then averaged together to form a study-specific FA template (Skelly et al., 2008). Finally, original FA maps were normalized to a study-specific FA template. Normalized FA maps were smoothed with a 6-mm full width at half maximum Gaussian kernel.

2.4. Statistical analysis

The FA values were compared between the schizophrenia and control subjects, using a parametric two-sample *t*-test of SPM2 in the PET model, with age, duration of medication, dosage of antipsychotic drugs, duration of illness, and parental education level as covariates. The threshold of FA cutoff was set at FA >0.18 in order to reduce the partial volume effect from the cerebrospinal fluid (CSF) and gray matter (Kunimatsu et al., 2008). Voxels surviving a family-wise error (FWE) with corrected threshold of $P<0.05$ were considered significant.

The association between FA of white matter and clinical symptoms in patients with schizophrenia was investigated by a series of single regression correlations (Pearson) in SPM2, with the total and each of the subscale scores of SANS/SAPS as the covariates (11 variables). FWE correction was used to correct for multiple comparisons in these analyses and the voxels with a corrected threshold of $p<0.0045$ (0.05/11, Bonferroni correction) were considered significant. In all analyses, the voxels with significant changes and the clusters including significant voxels were superimposed on the study-specific average normalized FA template. By means of meticulous comparison to a DTI color map atlas of the human brain (Mori et al., 2005), significant voxels were identified by specific white matter tract and verified in three dimensions by two independent raters (KN and YK).

3. Results

3.1. Clinical and demographic characteristics

The sample characteristics of the participants are shown in Table 1. Groups were matched for age, gender, and handedness. However, educational level showed a significant group difference, with the controls having a higher educational level than the schizophrenia patients ($p<0.001$). Parental educational level also showed a significant group difference, with the controls having a higher parental educational level than the schizophrenia patients ($p=0.001$).

3.2. Group comparison of FA value

Compared with the controls, schizophrenia patients showed significant FA reductions in the left ($p<0.001$) and right ($p=0.011$) superior longitudinal fasciculus (SLF), left ($p=0.004$) and right ($p=0.013$) inferior fronto-occipital fasciculus (IFO), and genu of right internal capsule (IC) ($p=0.001$) (Fig. 1 and Table 2). There were no voxels that demonstrated significantly lower FA in the controls compared with those of the schizophrenia patients.

3.3. Association between clinical symptoms and FA in patients with schizophrenia

Correlational analyses showed a negative correlation between the FA value in the anterior part of the corpus callosum and the SANS avolition score (Fig. 2 and Table 3), which remained significant even when we used age, illness duration, and medication (dose, duration) as controlling factors (Table 4). Total or other subscale scores of the SANS/SAPS did not correlate with the FA values for any brain regions. There was no significant correlation between the clinical symptoms and FA values extracted from the regions with significant group difference (bilateral SLF, IFO, and right IC). No significant correlations were found between the FA values and other demographic and clinical variables (age, illness duration, and dose and duration of antipsychotic medication).

4. Discussion

In the present DTI study in a relatively large cohort of schizophrenia patients, we employed an automated voxel-based method to investigate the whole-brain white matter FA alteration as well as its relationship to clinical variables (e.g., medication, illness duration, severity of clinical symptoms). We demonstrated the widespread FA reduction in plural neural fiber tracts in schizophrenia patients compared with that of healthy controls. We found no correlations between the FA values and age, illness duration, or antipsychotic medication, but lower FA in the anterior part of the CC was associated with more severe negative symptoms in schizophrenia. These findings support the notion of regional disconnectivity in the brain in schizophrenia (Friston and Frith, 1995; Stephan et al., 2006) and further suggest its role in the neurobiology of clinical symptoms of the illness.

Consistent with previous DTI studies in schizophrenia (e.g., Walterfang et al., 2006; Kubicki et al., 2007; Mitelman et al., 2007; Shergill et al., 2007; Kyriakopoulos et al., 2008), the present whole-brain FA analysis demonstrated a global neural disconnectivity (interhemispheric connections, corticosubcortical pathways, and networks interconnecting fronto-temporal and fronto-occipital regions) in schizophrenia. Among the widespread FA changes, we found the most robust FA reduction in left SLF, a major fronto-temporal association tract related to language processing, partly supporting the notion of language pathway abnormalities in schizophrenia (reviewed by Li et al., 2009). DTI findings in first-episode patients are likely to be less robust than in chronic patients (reviewed by Ellison-Wright and Bullmore, 2009; Peters et al., 2010), but a recent DTI study on first-episode psychosis (Pérez-Iglesias et al., 2010a) also demonstrated FA reduction predominantly in this tract, which might underlie poor executive functioning (Pérez-Iglesias et al., 2010b) or working memory deficits (Karlsgodt et al., 2008) observed in these patients. Although we cannot clearly address the laterality issue, our finding of FA reduction of the right internal capsule (IC) in schizophrenia might be in accordance with a recent DTI study by Levitt et al. (2010), who found a right-sided relationship between the FA value of the IC and cognitive functioning in schizophrenia. Given that cognitive dysfunction has been identified as a core feature of schizophrenia (Keefe, 2008), in combination with functional and structural MRI investigations suggesting abnormal connectivity between the frontal and temporal cortices in schizophrenia (Meyer-Lindenberg et al., 2001, 2005; Mitelman et al., 2005), our and other DTI findings support the role of the disruption of white matter integrity, especially fronto-temporal connectivity, in the core pathophysiology of schizophrenia (Frith et al., 2000; Davis et al., 2003; Frith, 2005).

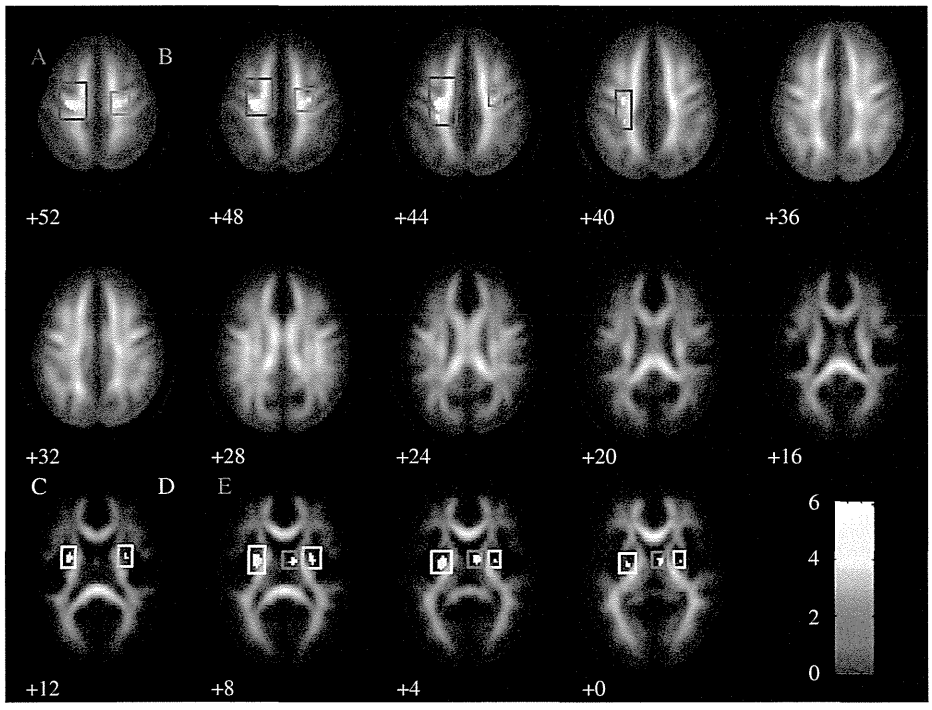

Fig. 1. Group difference of the fractional anisotropy (FA) values between 58 schizophrenia and 58 healthy participants. The brain regions where the schizophrenia patients had lower FA values (corrected $P<0.05$) are displayed on the study-specific average normalized FA template. A, superior longitudinal fasciculus; B, superior longitudinal fasciculus; C, inferior fronto-occipital fasciculus; D, inferior fronto-occipital fasciculus; E, genu of internal capsule.

Table 2
White matter regions with significant group differences in DTI FA values.

Anatomical region	Talairach and Tournoux Coordinates (x, y and z)			p^a
	x	*y*	*z*	
Left superior longitudinal fiscicu1us	−26	−18	48	<0.001
	−16	−8	46	0.005
	−26	−32	44	0.027
Genu of right internal capsule	6	−6	4	0.001
Left inferior fronto-occipital fasciculus	−26	−4	8	0.004
Right superior longitudinal fasciculus	20	−20	52	0.011
	28	−14	48	0.017
Right inferior fronto-occipital fasciculus	28	−2	10	0.013

[a] The statistical significance level was thresholded for correction of multiple comparisons by an FWE ($p<0.05$).

Table 3
Correlation between the FA and SANS avolition score before controlling for possible confounding factors.

Anatomical region	Talairach and Tournoux Coordinates (x, y and z)			*t*	p^a
	x	*y*	*z*		
The anterior part of the corpus callosum	2	16	24	5.89	0.003

[a] The statistical significance level was thresholded for correction of multiple comparisons by an FWE ($p<0.05$) as well as a Bonferroni correction ($p<0.0045$).

Regarding the association with clinical features of schizophrenia, we found a negative correlation between the FA value of the anterior corpus callosum and the SANS avolition score. However, our results do not necessarily mean that the reduced FA in the corpus collosum is specifically related to avolition-apathy, since negative symptoms are often interrelated and, in fact, the avolition score was significantly correlated with four other SANS negative symptom scores in the present study ($r=0.38$–0.59, all $p\leq0.005$). The present findings support a recent DTI study by Kubicki et al. (2008) in showing that FA changes in the anterior part of the CC, a tract of interhemispheric brain connectivity in frontal regions (Miyata et al., 2007), relate to the severity of negative symptoms. On the other hand, Mitelman et al. (2007) reported that the FA in the truncus region of the CC was related to the severity of negative symptoms, with that in the anterior CC being related to positive symptomatology. Other DTI studies in schizophrenia found no relationship to symptom scores (Foong et al., 2001; Minami et al., 2003) or association between the FA changes in other tract regions and the severity of a range of clinical symptoms (Wolkin et al., 2003; Shin et al., 2006; Skelly et al., 2008). For example, auditory hallucinations were *positively* related to FA in the CC, CB, SLF, and other regions (Hubl et al., 2004; Fujiwara et al., 2007; Seok et al., 2007) and *negatively* with plural regions (Skelly et al., 2008). Data on the relationship between DTI measures and clinical variables in first-episode or recent onset schizophrenia are sparse (Peters et al., 2010), but several studies found positive correlations between FA and positive symptoms (Karlsgodt et al., 2008; Szeszko et al., 2008; Cheung et al., 2011). Despite these inconsistencies between reports potentially due to the heterogeneity of the illness and/or methodological differences (Peters et al., 2010), our findings are in line with anatomical, pharmacological, and behavioral evidence for a prominent prefrontal involvement in negative symptoms of schizophrenia (Goldman-Rakic and Selemon, 1997). Several studies with other imaging modalities in schizophrenia have also implicated the involvement of anterior callosal disconnection, which was demonstrated as structural changes (Woodruff et al., 1997; Hulshoff Pol et al., 2004; Takahashi et al., 2009) and altered metabolic (MR spectroscopy studies; Aydin et al., 2007, 2008) or macromolecular structural (magnetization transfer imaging study; Foong et al., 2001) integrity in the symptom severity, especially for negative symptoms. As the associations between these different neuroimaging measures (e.g., volume, FA, and magnetization transfer ratio) have not been well documented (Rotarska-Jagiela et al., 2008), future research using a combination of DTI with other modalities that highlight white matter pathologies in the same group of patients is required for better understanding of callosal integrity abnormalities in schizophrenia.

Although our relatively large sample provided sufficient power to examine subtle group differences in FA, several confounding factors of the present study should be taken into account. First, the current DTI study used a relatively low MR field strength system (1.5 T) with only six direction acquisitions and rather thick original slices, which could cause a low signal to noise ratio, high sensitivity to motion artifacts, and the possibility of misregistration and inadequate findings of decreased FA by normal conditions (e.g., fiber crossing). Thus, the present DTI findings need replication using a more technologically advanced methodology. Second, as most patients in this study were not experiencing their first episode and were on antipsychotic medication (mean illness duration = 51.2 months), our FA findings might have been biased by chronicity of the illness and/or the effect of medication (Mori et al., 2007; Kanaan et al., 2009). Discrepant findings between the tract regions with FA reduction and those associated with symptom severity might be partly related to this issue. However, we found no association between these clinical variables (age, illness duration, dosage and

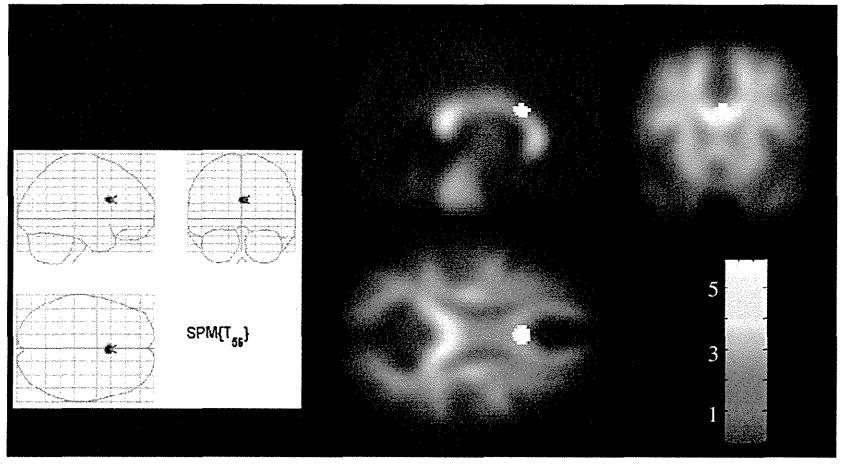

Fig. 2. Correlation between the FA and SANS avolition score. Anterior part of the corpus callosum showed a significant negative correlation ($t=5.89$, $p=0.003$).

Table 4
Correlation between the FA and SANS avolition score after controlling for possible confounding factors.

Anatomical region	Talairach and Tournoux Coordinates (x, y and z)			t	p^a
	x	y	z		
The anterior part of the corpus callosum	4	16	24	5.73	0.004

Age, duration of medication, dosage of antipsychotic drugs, duration of illness, and parental education level were treated as covariates.

[a] The statistical significance level was thresholded for correction of multiple comparisons by an FWE ($p<0.05$) as well as a Bonferroni correction ($p<0.0045$).

duration of antipsychotic medication) and FA values in our sample. This result seems to be consistent with previous findings of FA reduction in antipsychotic-naïve first-episode patients (Gasparotti et al., 2009; Cheung et al., 2011) in suggesting that FA reductions in schizophrenia are not fully attributable to medication. Nevertheless, the regional disconnectivities of the brain in schizophrenia and their role in a range of clinical features should be tested further in a sample at earlier stages, ideally in medication-naïve patients.

In conclusion, our findings indicate that schizophrenic patients have lower FA values, especially in fronto-temporal and various tract regions, compared with age- and gender-matched healthy controls, supporting the disconnectivity model of schizophrenia (Friston and Frith, 1995). These FA changes are associated with the severity of negative symptoms in the anterior part of the CC, suggesting that the regional disconnectivities are, at least in part, responsible for the clinical symptomatology of schizophrenia.

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Gray matter changes in subjects at high risk for developing psychosis and first-episode schizophrenia: a voxel-based structural MRI study

Kazue Nakamura[1]*, Tsutomu Takahashi[1,2], Kiyotaka Nemoto[3], Atsushi Furuichi[1], Shimako Nishiyama[1], Yumiko Nakamura[1], Eiji Ikeda[1], Mikio Kido[1], Kyo Noguchi[4], Hikaru Seto[4] and Michio Suzuki[1,2]

[1] *Department of Neuropsychiatry, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan*
[2] *Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Tokyo, Japan*
[3] *Department of Psychiatry, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan*
[4] *Department of Radiology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan*

Edited by:
Jun Soo Kwon, Seoul National University College of Medicine, South Korea

Reviewed by:
Stefan Borgwardt, University of Basel, Switzerland
Kim Jae-Jin, Yonsei University, South Korea

***Correspondence:**
Kazue Nakamura, Department of Neuropsychiatry, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan.
e-mail: krnaka@med.u-toyama.ac.jp

Objectives: The aim of the present study was to use a voxel-based magnetic resonance imaging method to investigate the neuroanatomical characteristics in subjects at high risk of developing psychosis compared with those of healthy controls and first-episode schizophrenia patients.

Methods: This study included 14 subjects with at-risk mental state (ARMS), 34 patients with first-episode schizophrenia, and 51 healthy controls. We used voxel-based morphometry with the Diffeomorphic Anatomical Registration through Exponentiated Lie Algebra tools to investigate the whole-brain difference in gray matter volume among the three groups.

Results: Compared with the healthy controls, the schizophrenia patients showed significant gray matter reduction in the left anterior cingulate gyrus. There was no significant difference in the gray matter volume between the ARMS and other groups.

Conclusion: The present study suggests that alteration of the anterior cingulate gyrus may be associated with development of frank psychosis. Further studies with a larger ARMS subjects would be required to examine the potential role of neuroimaging methods in the prediction of future transition into psychosis.

Keywords: schizophrenia, psychosis, high risk, MRI, cingulate gyrus

INTRODUCTION

Neuroimaging studies have demonstrated subtle but widespread brain structural alterations, such as volume reduction of fronto-temporo-limbic regions as well as enlarged lateral and third ventricles, in first-episode schizophrenia (Steen et al., 2006; Vita et al., 2006; Ellison-Wright et al., 2008), which are not due to illness chronicity and antipsychotic medication. Recent prospective longitudinal magnetic resonance imaging (MRI) studies, including our own data showing progressive gray matter reduction of the temporal region (approximately 2–3% per year) (Takahashi et al., 2010, 2011), further revealed progressive brain structural change and its relationship to clinical course or outcome in first-episode schizophrenia (Andreasen et al., 2011). These longitudinal findings might be consistent with the clinical observation that a long duration of untreated psychosis (DUP), which could lead to severe brain pathological changes during the early illness stage (Lappin et al., 2006; Takahashi et al., 2007), is related to poor outcome of schizophrenia patients (Marshall et al., 2005; Perkins et al., 2005). Examining potential neurobiological markers that predate the onset of psychosis might lead to appropriate early intervention and thus prevent deterioration of social function and the progression of structural brain alterations.

It is not yet clear at which illness stage brain abnormalities occur in schizophrenia. Subjects with at-risk mental state (ARMS), who exhibit prodromal-like symptoms and have an increased risk of developing psychosis (Yung et al., 2003), might share disease vulnerability as well as brain morphological changes with patients with overt schizophrenia. Subjects with ARMS are heterogeneous on the basis of their outcome, as only about 36% of them develop psychosis during 3-year follow-up (Fusar-Poli et al., 2012). Previous MRI studies using voxel-based morphometry (VBM), which allows automated whole-brain analysis, revealed more severe gray matter reduction predominantly in the fronto-temporo-limbic regions in ARMS subjects with later transition than in those without (Pantelis et al., 2003; Borgwardt et al., 2007; Fusar-Poli et al., 2011). More specifically, Fornito et al. (2008) revealed that baseline differences in the anterior cingulate cortical thickness distinguished between ARMS with and without later transition, but they did not directly compare ARMS subjects and patients with overt psychosis.

This voxel-based MRI study aimed to investigate the nature of neuroanatomical abnormalities in high-risk subjects compared with both healthy controls and first-episode schizophrenia patients. On the basis of previous neuroimaging findings, we predicted that both first-episode schizophrenia and ARMS subjects, especially those with later transition, would show brain morphological changes in fronto-temporo-limbic regions compared with healthy subjects.

MATERIALS AND METHODS

PARTICIPANTS

Fourteen individuals (10 males and 4 females) defined as ARMS for psychosis were recruited from the Consultation Support Service in Toyama (CAST), which was launched in 2006 as a specialized clinical setting to study and treat young persons (aged 15–30 years) at risk of developing psychosis (Mizuno et al., 2009). The subjects with ARMS were diagnosed according to the Comprehensive Assessment of ARMS (CAARMS) (Yung et al., 2004); they were characterized by one or more of the following: (1) attenuated psychotic symptoms; (2) brief, limited intermittent psychotic symptoms with spontaneous resolution; or (3) family history of psychosis in first-degree relatives or a personal history of schizotypal personality disorder accompanied by a decline in general functioning. Their clinical symptoms were assessed using the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1983) and the Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen, 1984) at the time of scanning. Eleven ARMS subjects were neuroleptic-naïve at scanning, but two subjects were treated with atypical neuroleptics and one was receiving sulpiride. Their duration of medication use was shorter than 2 weeks for atypical neuroleptics and shorter than 6 months for sulpiride. They were also receiving benzodiazepines ($N = 2$), antidepressants ($N = 1$), and tandospirone ($N = 3$).

Thirty-four patients with first-episode schizophrenia (20 males and 14 females), who met the ICD-10 research criteria (World Health Organization, 1993), were recruited from the inpatient and outpatient clinics of the Department of Neuropsychiatry, Toyama University Hospital. The patients were diagnosed following structured clinical interviews by experienced psychiatrists using the Comprehensive Assessment of Symptoms and History (CASH; Andreasen et al., 1992). Their durations from manifestations of overt psychotic symptoms were shorter than 1 year. Their clinical symptoms were assessed using SANS and SAPS at the time of scanning. Thirty-three patients were receiving neuroleptic medication at the time of scanning; 2 patients were treated with typical neuroleptics, 26 were receiving atypical neuroleptics, 5 were taking both typical and atypical neuroleptics, and 1 patient was neuroleptic-free. They were also receiving anticholinergic drugs ($N = 8$), benzodiazepines ($N = 9$), antidepressants ($N = 1$), carbamazepine ($N = 1$), and lithium carbonate ($N = 3$).

Exclusion criteria for ARMS subjects and schizophrenia patients were other neurological diseases, past or present regular alcohol abuse, and/or consumption of illicit drugs as reported by the study participants and/or the patients' records, as well as past head trauma with loss of consciousness or electro-convulsive treatment.

The control subjects consisted of 51 healthy volunteers (30 males and 21 females) recruited from members of the community, hospital staff, and university students. They were given a questionnaire consisting of 15 items concerning their personal (13 items; including a history of obstetric complications, substantial head injury, seizures, neurological or psychiatric diseases, impaired thyroid function, hypertension, diabetes, and substance use) and family (2 items) histories of illness. They did not have any personal or family history of psychiatric illness in their first-degree relatives. This study was approved by the ethics committee of Toyama University. Written informed consent was obtained from all subjects prior to study participation.

MRI ACQUISITION

Magnetic resonance images were obtained by utilizing a 1.5-T Magnetom Vision (Siemens Medical System, Inc., Erlangen, Germany) with a three-dimensional gradient-echo sequence FLASH (fast low-angle shots) yielding 160–180 contiguous T1-weighted slices of 1.0-mm thickness in the sagittal plane. The imaging parameters were as follows: TR = 24 ms; TE = 5 ms; flip angle = 40°; field of view = 256 mm; and matrix size = 256 × 256 pixels. The voxel size was 1.0 mm × 1.0 mm × 1.0 mm. All scans in the patient and control groups were acquired in the same system with the same protocol.

MRI DATA PROCESSING

All T1-weighted MRI data were first converted from the Dicom format to the NIFTI format and then processed using Statistical Parametric Mapping 8 (SPM8, Wellcome Institute of Neurology, University College London, UK, http://www.fil.ion.ucl.ac.uk/spm) running under MATLAB R2008b (The MathWorks Inc., USA).

The unified segmentation model consisting of spatial normalization, bias field correction, and tissue segmentation was performed in order to improve the quality of data preprocessing (Ashburner and Friston, 2005). Tissue probability maps were registered to the subject's data, and final tissue probability maps were derived from prior maps with the use of a combination with tissue probabilities based on the voxel intensity. To make the processed data more accurate, we used the Diffeomorphic Anatomical Registration through Exponentiated Lie Algebra (DARTEL) (Ashburner, 2007; Ashburner and Friston, 2009; Klein et al., 2009) tool in SPM8. DARTEL is not integrated into the segmentation model and requires the input of gray matter tissue maps produced by unified segmentation. This algorithm records inter-subject images using diffeomorphisms, which preserve the object properties through deformations, twistings, and stretchings, and archives a more accurate inter-subject registration. Because DARTEL produces a more accurate registration, it improves the sensitivity of finding and localizing differences between groups in terms of the gray matter volume. Registered tissue maps were transformed to the stereotactic space of the Montreal Neurological Institute (MNI) and multiplied with the Jacobian determinants of the deformations in order to preserve the volume of tissue in each structure. Finally, the modulated, warped tissue maps were then written with an isotropic voxel resolution of 1.5 mm^3 and smoothed with a 10-mm Full-Width Half-Maximum (FWHM) Gaussian kernel (Salmond et al., 2002; Jones et al., 2005).

STATISTICAL ANALYSIS

Demographic data

Group differences in age, educational level, parental educational level, and intracranial volume (ICV) were examined with one-way analysis of variance (ANOVA) and *post hoc* Scheffé's test. Group differences in terms of gender were tested with Chi-square tests. The level of statistical significance was defined as $p < 0.05$ (two-tailed). Statistical analyses were performed with Statistica, version 06J for Windows (StatSoft Japan Inc., Tokyo, Japan).

Voxel-based analysis of gray matter volume

Gray matter volume differences between the ARMS subjects, schizophrenia patients, and healthy controls were analyzed using two-sample *t*-tests implemented in the general linear model approach of SPM8 with age and ICV as nuisance covariates. We used cluster level inference (the extent of contiguous clusters of individual significant voxels) for determination of statistical significance (Meisenzahl et al., 2008). Because cluster size distribution varies according to local smoothness, the cluster sizes in this study were adjusted according to the local smoothness within the framework of the Random Field Theory (RFT) (Worsley et al., 1999; Hayasaka et al., 2004). Our statistical inference was performed at the cluster level by assessing the SPM{t} images by the non-stationary cluster extent correction (Hayasaka et al., 2004), which has been reported to be robust when MRI experiments fulfill (1) degrees of freedom > 30 and (2) image smoothness (FWHM) > 3 × voxel sampling resolution (Hayasaka et al., 2004), as in this study. The cluster-defining threshold was set to $p < 0.001$. Then, a family-wise error-corrected (FWE) cluster size threshold of $p < 0.05$ was applied to account for multiple comparisons of the results (corrected cluster sizes). Finally, cluster sizes were adjusted for smoothness non-uniformity using the VBM8 toolbox (Gaser, 2009), which implements the methodology of Hayasaka et al. (2004).

Voxel coordinates are given as an indication of location in a standardized brain. Voxels were localized in MNI space and transformed into Talairach and Tournoux coordinates (Talairach and Tournoux, 1988).

RESULTS

DEMOGRAPHIC DATA

Table 1 shows demographic and clinical data of the subjects in this study. Groups were matched for gender, parental education, and ICV. However, the controls ($p < 0.001$) and schizophrenia patients ($p < 0.001$) were older than the ARMS subjects. The controls had a higher educational level than the other two groups ($p < 0.001$) and the schizophrenia patients had a higher educational level than the ARMS subjects ($p = 0.004$).

VOXEL-BASED ANALYSIS OF GRAY MATTER VOLUME

Compared with the healthy controls, the schizophrenia patients showed significant gray matter volume reduction in the left anterior cingulate gyrus (FWE-corrected $p = 0.047$) (**Figures 1** and **2**; **Table 2**). There was no difference between the ARMS subjects and the schizophrenia patients or the healthy controls.

DISCUSSION

In this study, we performed VBM analyses using the DARTEL method to investigate gray matter change in early psychosis. In comparison to the healthy controls, first-episode schizophrenia patients showed significant gray matter reduction in the left anterior cingulate gyrus, but the ARMS subjects showed no significant difference in gray matter volume. This negative finding may be partly related to the heterogeneity of the ARMS subjects, as those with later transition to psychosis had a similar distribution of the cingulate gyrus gray matter volume to that in first-episode schizophrenia patients (**Figure 2**). These preliminary results are partly consistent with previous findings by Fornito et al. (2008), who reported that baseline differences of anterior cingulate gyrus distinguish between high-risk individuals who do and do not subsequently develop overt psychosis.

Neuroimaging studies comparing schizophrenia patients to healthy controls have shown evidence of morphological change in the anterior cingulate gyrus (Ellison-Wright et al., 2008; Shepherd et al., 2012). Gray matter volume reduction (Salgado-Pineda et al., 2003; Koo et al., 2008; Meisenzahl et al., 2008; Leung et al., 2011) and reduced cortical thickness (Schultz et al., 2010) in the anterior

Table 1 | Clinical and demographic characteristics[a].

Characteristic	ARMS (*N* = 14)	Schizophrenia (*N* = 34)	Healthy control (*N* = 51)
Gender (male/female)	10/4	20/14	30/21
Age (years)[b]	18.9 (1.4)	24.7 (5.5)	23.9 (1.8)
Educational level (years)[c]	11.6 (1.4)	13.5 (2.0)	16.0 (1.7)
Parental educational level (years)	13.7 (1.4)	13.3 (1.7)	14.1 (2.2)
Age at onset (years)	N/A	23.3 (5.4)	N/A
Duration of medication (months)	0.43 (1.6)	1.7 (1.8)	N/A
Drug (mg/day, haloperidol equivalent)[d]	0.55 (1.1)	6.3 (6.5)	N/A
Intracranial volume (cm^3)	1557.8 (130.0)	1602.1 (150.7)	1573.6 (143.0)

[a] *Values given as mean (SD).*

[b] *Significant difference between groups.*

[c] *Significant difference between groups.*

[d] *The different typical and atypical neuroleptic dosages were converted into haloperidol equivalents using the guidelines of Toru (2001).*

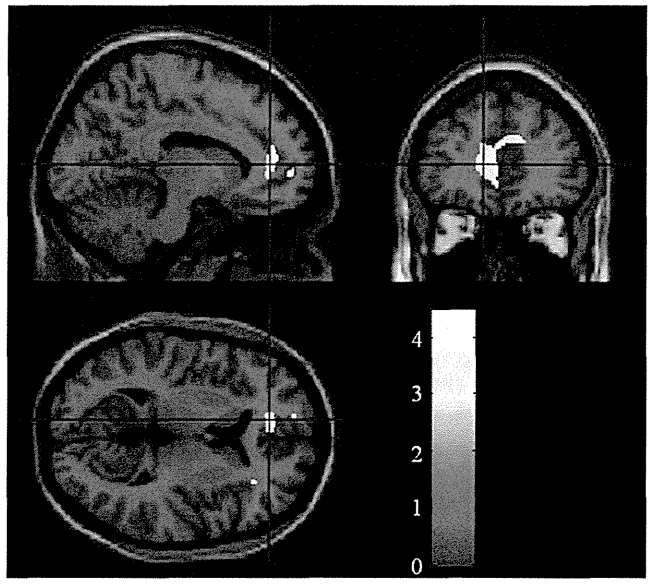

FIGURE 1 | Group difference of the gray matter between the schizophrenia patients and the healthy controls. The cluster in which the schizophrenia patients show gray matter reduction is located in the left anterior cingulate gyrus.

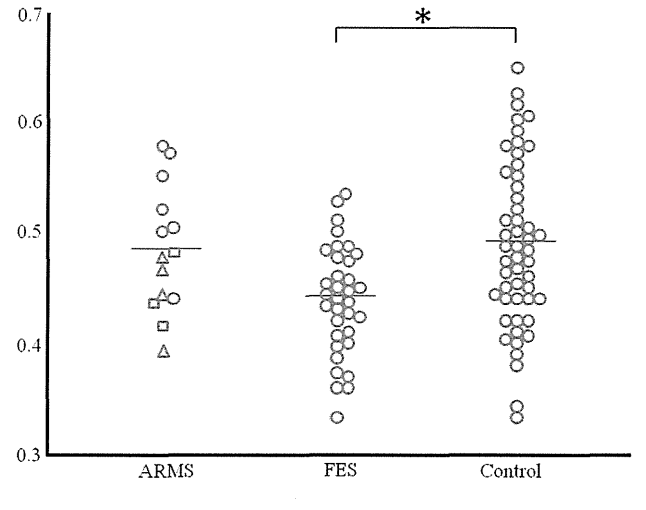

FIGURE 2 | Scatter plots of gray matter volume of the peak coordinate at the left anterior cingulate gyrus that revealed the difference between schizophrenia patients and controls. The ARMS subjects were classified into three groups according to clinical outcome (square: ARMS who developed psychosis, triangle: ARMS with unknown outcome, circle: ARMS without transition to psychosis). $*p < 0.05$.

cingulate gyrus have been revealed by MRI studies in first-episode and neuroleptic-naïve patients to minimize the influence of neuroleptic medication or chronicity of the illness. In this study, gray matter volume reduction in the left anterior cingulate gyrus in the schizophrenia patients had no relationship with any effects of medication (data not shown).

Table 2 | Talairach coordinates for regions of reduced gray matter volume in the schizophrenia patients compared to the healthy controls.

Region	Voxel	Peak coordinate			*T*	*p*
		x	*y*	*z*		
lt. anterior cingulate gyrus	631	−11	42	8	3.82	0.047

One major aim of high-risk studies for psychosis has been to identify clinical and neurobiological predictors of future transition to psychosis, which would allow specific and targeted preventive strategies (McGorry et al., 2006); indeed, previous neuroimaging studies have identified such predictive markers. The VBM study by Pantelis et al. (2003) revealed the association between later transition and gray matter reduction in temporal and frontal regions predominantly in the right hemisphere and cingulate gyrus bilaterally in clinical high-risk subjects, which was largely replicated in an independent high-risk cohort (Borgwardt et al., 2007). Recent multi-center (Mechelli et al., 2011) and meta-analytic (Smieskova et al., 2010; Fusar-Poli et al., 2011) MRI studies on large numbers of high-risk subjects generally supported the assertion that brain morphological changes in the fronto-temporo-limbic regions, including the cingulate gyrus, already exist prior to the onset of psychosis. Although our data are clearly limited by the small sample size as discussed below, the distribution of the anterior cingulate gray matter volume (**Figure 2**) implies that ARMS subjects with later transition may have morphological changes of the cingulate gyrus to the same degree as those with overt schizophrenia. There has been debate about the risk-benefit ratio of antipsychotic treatment in prodromal patients (Woods et al., 2007; Weiser, 2011). However, given the hypothesized active brain pathology in the early phases of psychosis, which could affect the subsequent course of the illness (Birchwood et al., 1998), and the potential ameliorating effects of atypical antipsychotics for brain structural abnormalities (Lieberman et al., 2005; Girgis et al., 2006), intervention before the expression of frank psychosis may reduce neurobiological deterioration as well as the transition rate to psychosis (McGorry et al., 2002; McGlashan et al., 2006), especially in subjects with neurobiological risk markers.

The sample size of the current ARMS group (especially those who later developed psychosis) was small and some individuals dropped out during clinical follow-up ($N = 4$, unknown outcome group). Significant group differences in age (ARMS < schizophrenia and controls) might also have biased our results, although we used age as a controlling factor in all imaging analyses. In contrast to our prediction, we did not find significant brain morphological changes in the ARMS subjects, potentially due to the small sample size. It was also not possible to examine the relationship between brain morphology and clinical outcome (later transition) in our ARMS subjects statistically. In addition, direct comparison between the three groups using the ANOVA model with age and ICV as covariates failed to replicate significant group difference in the cingulate gyrus gray matter volume. Thus, further study with a larger well-defined