systematically integrated to generate many biological functions.

ii. Activity-Dependent Expression of Neurotrophins

Among neurotrophins, BDNF shows the most ubiquitous expression in the developing and adult mammalian brain. BDNF expression levels are increased dramatically during the first few weeks of postnatal development. Expression of neurotrophins in neurons is linked to neuronal activity. BDNF and NGF mRNA levels are rapidly increased by seizure activity in the hippocampus and the cerebral cortex [23-25]. In contrast, blockade of visual input causes rapid down-regulation of BDNF mRNA in the rat visual cortex of dark-reared animals [26]. A similar phenomenon has been found in cultured neurons. The introduction of glutamate with high concentration potassium-induced depolarization increase levels of BDNF and NGF mRNA, while blockade of neuronal activity with γ-aminobutyric acid (GABA) decreases such levels [27,28].

iii. Processes of BDNF Secretion

BDNF is synthesized as a 32 kDa precursor protein (proBDNF) and proteolytically cleaved to generate the mature BDNF (13 kDa). The synthesis of the pro-BDNF occurs at the rough endoplasmic reticulum (ER). Following this, pro-BDNF is transported to the Golgi apparatus and concentrated in membrane stacks of the *trans*-Golgi network (TGN). Finally, BDNF-containing vesicles bud off the TGN to eventually transport to the releasing sites. Recent studies clarified some of the details of BDNF vesicular sorting. Specifically, the pro-region of BDNF has been implicated as a regulator of BDNF sorting to secretory vesicles [29]. Moreover, fusing the pro-region of BDNF to NT-4, which is rarely sorted into secretory vesicles, allowed NT-4 to sort more efficiently into specific vesicles [30]. These data support the importance of the BDNF pro-region as a potential target to help guide secretory granules. Furthermore, binding of BDNF to the lipid-raft-associated sorting receptor carboxypeptidase E (CPE) in the TGN is also important for sorting into secretory vesicles of the regulated pathway [31]. Sortilin, a trans-membrane protein, has also been implicated in the sorting of BDNF to secretory glanules. Sortilin is expressed in secretory granules and interacts specifically with the pro-region of BDNF. Interestingly, the truncated form of sortilin results in missorting of BDNF to the secretory vesicles [32].

It is still controversial as to where and how pro-neurotrophins are processed into mature neurotrophins in the CNS. Originally, it had been thought that pro-neurotrophins are prototypically cleaved by furin and pro-protein convertases (PCs) in the TGN or in secretory granules before secretion [33]. However, recent studies have indicated that a considerable amount of BDNF is secreted in the pro-form from neurons. Released pro-BDNF is subsequently processed to mature BDNF extracellularly by proteases such as plasmin or matrix metalloproteinases [34,35]. More recently, however, it was shown that pro-BDNF is rapidly converted into mature BDNF intracellularly and almost all BDNF was secreted as the mature form from hippocampal neurons [36].

iv. Constitutive and Regulated Secretion

Secretion of neurotrophins is classified into "constitutive" and "regulated" pathways, depending on whether the secretion occurs spontaneously or in response to neuronal activity, respectively. In hippocampal neurons, BDNF appears to be sorted primarily into the regulated pathway [37-39]. In the regulated pathway, BDNF-containing vesicles are transported into either presynaptic axon terminals or post-synaptic dendrites along microtubules for activity-dependent secretion [40-43]. Recently, Lessmann and colleagues conducted an elegant study that provided the long-awaited understanding of BDNF secretion. The activity-dependent postsynaptic secretion of neurotrophins critically depends on Ca^{2+} influx *via* ionotropic glutamate receptors or voltage-gated Ca^{2+} channels, Ca^{2+} release from internal stores, activation of Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), and intact protein kinase A (PKA) signaling. Trk signaling and activation of Na^{+} channels, on the other hand, are not required for BDNF secretion [44-46]. Furthermore, recent reports suggest that the Golgi apparatus exists in dendrites as well as the cell soma, and have gone so far as to identify a local BDNF secretory pathway in neuronal dendrites [47,48]. Future works may reveal more details concerning the secretory systems of neurotrophins at the subcellular level and that may be more complex and dynamic than we can presently imagine.

II. IMPAIRED SECRETION OF BDNF AND NEUROPSYCHIATRIC DISEASES

i. Huntington's Disease

Huntington's disease (HD) is a fatal, dominantly inherited, neurodegenerative disease that usually presents during midlife. It is characterized by relatively selective degeneration of striatal neurons which lead to psychiatric, cognitive and motor dysfunction. Polyglutamine expansion (polyQ) in the protein huntingtin (htt) is thought to be the principal mechanism for the neuronal toxicity in HD. Recently, evidence has indicated the possible link between HD and BDNF. Wild type htt plays a role as a transcription factor and facilitates expression of BDNF [49]. Furthermore, htt has been implicated in BDNF-containing vesicle transport. Mutant (PolyQ)-htt perturbs post-Golgi trafficking of BDNF in the regulated secretory pathway, though it does not influence the constitutive pathway [50,51]. Conversely, the exogenously transfected BDNF gene generated increased BDNF levels and TrkB signaling in the striatum, which resulted in improved symptoms in HD model mice [52]. These findings suggest that mutation of htt reduces levels of BDNF in the striatum by inhibiting gene expression and perturbing anterograde transport of BDNF-containing vesicles from cortex to striatum. Therefore, the development of therapies focused on the reduction of BDNF release should be important for future studies.

ii. Rett Syndrome

Rett syndrome (RTT) is an X-linked disorder characterized by arrested neurological development and subsequent cognitive decline. Methylation of DNA in vertebrates occurs preferentially on cytosine residues of dinucleotides in which the cytosine is followed by a guanine residue (CpGs). Meth-

ylated CpGs bind a variety of proteins. One of these proteins, methyl-CpG binding protein 2 (MeCP2), has been implicated in the long-term silencing of gene expression. Inactivating mutations in MeCP2 is caused in the majority of cases with Rett syndrome. Chen *et al.* showed that MeCP2 selectively binds to the BDNF promoter III and represses expression of BDNF [53]. Membrane depolarization triggers the calcium-dependent phosphorylation and release of MeCP2 from the BDNF promoter III, thereby facilitating transcription [54]. A conditional BDNF transgene increased BDNF expression in the MeCP2 mutant brain, which resulted in rescue of locomotor defects, recovery of electrophysiological deficits, and extension of lifespan in MeCP2 mutant animals [55]. Although MeCP2 null mice exhibited a slightly decreased content of BDNF in some brain areas, mutant neurons demonstrated equivalent secretion levels of BDNF compared to wild-type in response to high-frequency electrical stimulation [56]. Furthermore, BDNF expression in MeCP2 null neurons was significantly improved by chronic ampakine treatment, which was administered to facilitate AMPA receptor activation [57]. These results suggest that the expression of BDNF is still plastic in the MeCP2 null condition and manipulating the BDNF level or the BDNF signaling pathways may provide therapeutic opportunities for RTT patients.

iii. Autism

Autism is a severe neurodevelopmental disorder with a childhood onset, characterized by profound disturbances in socialization, language skills, communicative, and behavioral functions. BDNF is expressed abnormally in individuals with autism and, as a result, may be involved in the pathogenesis of autism [58,59]. Elevated levels of BDNF and NT4/5 measured by archived neonatal blood samples of autistic patients were reported [60]. Elevation of BDNF was also reported in a study of 18 Japanese children with autism compared with controls [61]. These findings suggest that excess BDNF during childhood may be involved in the neurobiological abnormalities observed in autism. The specific molecular mechanisms involving BDNF and autism remain unknown, though one report suggests that genetic changes in autistic individuals may account for altered neurotrophin levels [62]. Ca^{2+}-dependent activator protein for secretion 2 (CAPS2/CADPS2) is a secretory granule-associated protein that is abundant at the parallel fiber terminals of granule cells in the mouse cerebellum and is involved in the release of BDNF and NT-3. The human CAPS2/CADPS2 gene is located on chromosome 7q31.32 within a critical autism susceptibility locus 1 (AUTS1). CAPS2 knock-out mice demonstrate autistic-like behavioral phenotypes and deficient release of BDNF and NT-3. Moreover, phosphorylation of Trk receptors is decreased in the cerebellum, which may play a role in the pronounced impairment of cerebellar development and function, including neuronal survival, differentiation and migration of postmitotic granule cells, that these mice exhibit [63]. Although there have been few reports suggesting the relation between autism and BDNF secretion, further investigation may result in novel insights.

iv. Epilepsy

Epilepsy is a neurological disorder characterized by recurrent and unpredictable seizures. Various studies have shown that BDNF increases neuronal excitability and is up-regulated in areas implicated in epileptogenesis. Seizure activity increases expression of BDNF mRNA and protein, and recent studies have shown that interfering with BDNF signal transduction inhibits the development of the epileptic state *in vivo* [64]. Half of all drug-resistant individuals experience seizure control with dietary manipulation, such as isocaloric substitution of carbohydrates with fats and protein referred to as the 'ketogenic diet'. Daley *et al.* reported that an inhibitor of glycolysis is shown to have antiepileptic effects in the rat kindling model, which may be related to NADH-dependent regulation of BDNF expression [65]. This result may explain how the 'ketogenic diet' treatment works. Although it is unclear whether the up-regulation of BDNF is the cause or the consequence of epilepsy, the reduction of BDNF expression or BDNF signaling can be a useful tool for the treatment of epilepsy.

v. Psychiatric Disorders

Mood and anxiety disorders are the most common psychiatric diseases. BDNF has been implicated in these disorders, because decreased levels of BDNF in the hippocampus are correlated with stress-induced depressive behaviors [66]. Other studies also showed decreased plasma levels of BDNF in patients with major depression [67]. Many classes of antidepressants, including selective serotonin reuptake inhibitors, significantly increase BDNF mRNA expression in the hippocampus and prefrontal cortex [68,69]. The time course of such increase is consistent with the slow onset of therapeutic effects of antidepressants. More recently, striking evidence for the involvement of TrkB-dependent neurogenesis in the antidepressant effect has been reported. Mice lacking TrkB in the hippocampal neuron progenitor cells had impaired neurogenesis and proliferation induced by antidepressant treatment. These mice also demonstrated increased anxiety-like behavior and decreased sensitivity to antidepressants [70,71]. Taken together, BDNF may play a key role in the brains of recovering patients during antidepressant treatment [72,73].

Many reports have isolated the possible association between BDNF levels and schizophrenia in several brain regions [74]. However, results from these studies are contradictory in that some demonstrate decreased BDNF levels in the postmortem brain or serum, while others report that the BDNF level in patients was not significantly different from that in normal controls [75]. Moreover, samples used in each experiment differ in age, species (rodents, primates, human) and regions (i.e., hippocampus, frontal cortex, CSF and blood) [76]. Although there have been many studies examining the possible role of BDNF in schizophrenia, integrated knowledge concerning this has not been produced. Despite this, the neurobiological vulnerability paradigm remains an attractive concept, supporting that increased susceptibility may be a consequence of reduced expression of BDNF (neurotrophins) at a certain point of life [77].

In the region encoding BDNF's pro-region, a SNP was identified at amino acid 66 (Val66Met). Egan and colleagues reported that the met allele was associated with decreased episodic memory and abnormal hippocampal activation as assayed with fMRI in human subjects [29]. Furthermore, neurons transfected with met-BDNF-GFP showed lower

depolarization-induced secretion, while constitutive secretion was unchanged. Met-BDNF-GFP failed to localize to secretory granules or synapses [29]. Following this, a number of association studies of this polymorphism with psychiatric disorders have been done. Unexpectedly, the Met66 allele, which reduces BDNF release, has been suggested to be protective against developing bipolar disorder [78], although this association was not confirmed by large-scale studies [79,80]. The Met66 allele has also been implicated in other disorders like anorexia [81]. Future research is required to assess how the Val66Met is associated with particular psychiatric disorders.

CONCLUSIONS

The biological mechanisms of neurotrophins are critically important for neuronal functions that affect brain functions and behavior. Growing evidence has implicated BDNF in the pathophysiology of many neuropsychiatric diseases. Genetic variations leading to deranged expression or secretion due to altered transcription, vesicular sorting, vesicular trafficking and secretion of BDNF seem to play an important role in several neuropsychiatric diseases and related behavioral phenotypes. In order to develop treatment strategies for these diseases through targeting neurotrophins and their receptors, however, clarification of more detailed mechanisms is needed. Studies that reveal not only an increase/decrease in expression of neurotrophins, but also accurate spatiotemporal secretion profiles of neurotrophins are necessary.

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TGFBR2 gene expression and genetic association with schizophrenia

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Abstract

TGFBR2 gene is a tumor suppressor gene located at chromosome 3p22, and the locus is reported to be linked with schizophrenia susceptibility. According to the previous studies, a reduced incidence of cancer is observed in schizophrenic patients compared with the general population and tumor suppressor genes may be associated with schizophrenia. We measured the mRNA expression of TGFBR2 gene in the peripheral leukocytes from 19 medication-free schizophrenics and 25 medication-free major depressive patients compared with age- and sex-matched control subjects using a quantitative real-time PCR method. We also followed up the TGFBR2 mRNA expression levels from 13 schizophrenics after several weeks – antipsychotic treatments. The TGFBR2 mRNA levels of medication free schizophrenics were significantly higher than those of control subjects and decreased to almost the same level as controls after antipsychotic treatment. On the other hand, the TGFBR2 mRNA levels of medication-free major depressive patients were not significantly different from controls. In genetic studies, we failed to find any association between the TGFBR2 gene and schizophrenia with 10 SNPs of TGFBR2 gene in Japanese subjects (279 subjects each) and there was no significant difference with haplotype analysis, either. Our results suggest that the TGFBR2 gene itself does not link to schizophrenia but that the TGFBR2 mRNA levels in the peripheral leukocytes may be a potential state marker for schizophrenia.

Keywords: TGFBR2; Gene expression; Leukocytes; Association analysis; Schizophrenia

1. Introduction

Schizophrenia is a complex psychiatric disorder that afflicts approximately 1% of the population throughout the world and has high heritability (Craddock et al., 2005). According to the previous studies, a reduced incidence of cancer is observed in schizophrenic patients compared with the general population (Catts and Catts, 2000; Grinshpoon et al., 2005). The possibility is explored to understand that alteration of the expression of oncogenes and/or tumor suppressor genes may account for tumor resistance associated with schizophrenia. Cui et al. reported that the tumor suppressor adenomatous polyposis coli (APC), which is involved in cell adhesion, was associated with schizophrenia and its expression levels were significantly increased in the leukocytes of schizophrenics no

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matter how taking or not taking antipsychotic medications (Cui et al., 2005). There are several studies that the tumor suppressor gene p53 (TP53), which is a key element in maintaining genomic stability and cell apoptosis, is associated with schizophrenia (Yang et al. 2004; Ni et al., 2005).

Transforming growth factor-β receptor 2 (TGFBR2) gene is a putative tumor suppressor gene implicated in several malignancies (e.g. colon cancer, gastric cancer, gliomas, etc.) (Markowitz et al., 1995; Myeroff et al., 1995; Izumoto et al., 1997), and recently has been to be associated with Marfan syndrome (Mizuguchi et al., 2004). There have been several reports of Marfan syndrome cosegregating with schizophrenia within families (Romano and Linares, 1987; Sirota et al., 1990), which suggest that some genetic resemblances may be shared between schizophrenia and Marfan syndrome. The TGFBR2 gene consists of seven exons and encodes the human TGF-β receptor, type II. This receptor belongs to the serine-threonine kinase family of cell surface receptors, which regulates several cellular processes, including proliferation, cell cycle arrest, apoptosis, differentiation and formation of extra cellular matrix (Annes et al., 2003; ten Dijke and Hill, 2004). TGFBR2 is expressed in the brain as well as other tissues and its locus lies at chromosome 3p22, which has been previously reported to be linked with schizophrenia (Lewis et al., 2003). These above findings imply that TGFBR2 gene may be involved in the pathogenesis of schizophrenia.

To investigate the pathological role of TGFBR2 gene to schizophrenia, we measured the TGFBR2 mRNA expression levels in the peripheral leukocytes of medication-free 19 schizophrenic patients, 25 major depressive patients and age- and sex-matched control subjects using a quantitative real time PCR method. In addition, we conducted a genetic case-control study of the TGFBR2 gene with schizophrenia in Japanese subjects (schizophrenics; $n = 279$, control subjects; $n = 279$).

2. Materials and methods

2.1. Subjects for analysis

All patients and control subjects were biologically unrelated Japanese. The diagnosis of schizophrenia and major depression was made by at least two experienced psychiatrists according to DSM-IV criteria (American Psychiatric Association, 1994). Clinical symptoms were evaluated by the Brief Psychiatric Rating Scale scores (BPRS) (Overall and Gorham, 1962) in schizophrenic patients when blood samples were taken. Age- and sex-matched controls were in good physical health without a history of any psychiatric or serious somatic diseases and taking any medication during the sample collection period. Probands who had first-degree relatives with psychiatric disorders were excluded from the control subjects.

Table 1a
Demographic data for medication-free schizophrenic patients studied in TGFBR2 mRNA expression analysis ($N = 19$)

	Age (y.o)	Gender	Age at onset (years)	BPRS score	Family history of Schizophrenia in first-degree relative
S1	25	M	22	64	+
S2	24	M	24	42	–
S3	24	M	24	31	–
S4	27	M	24	37	–
S5	36	M	36	34	–
S6	39	M	38	59	–
S7	27	M	26	58	–
S8	20	F	19	46	–
S9	23	F	23	48	–
S10	34	F	31	36	–
S11	47	F	47	30	–
S12	15	F	13	30	+
S13	26	F	21	100	–
S14	23	M	23	31	–
S15	28	M	25	63	–
S16	47	F	47	37	–
S17	37	F	21	36	–
S18	30	F	25	41	–
S19	45	F	43	36	+

The age (years old: y.o) represent the age of the subject when the leukocytes were drawn. M = male, F = female, + indicates that at least one of the first-degree relatives has schizophrenia.

For the measurement of expression levels of the TGFBR2 mRNA, the subjects consisted of 19 medication-free patients with schizophrenia (subject number S1–S19, Tables 1a and 1b) (14 first-episode and drug-naïve schizophrenic patients, 5 schizophrenic patients without antipsychotic treatment for at least two months; 9 males and 10 females, mean age: 30.4 ± 9.3), 19 age- and sex-matched controls for schizophrenic patients (9 males and 10 females, mean age: 30.6 ± 8.6), 25 medication-free patients with major depression (17 first-episode and drug-naïve depressive patients, 8 depressive patients without antidepressant treatment for at least two months; 9 males and 16 females, mean age: 39.8 ± 13.2) and 25 age- and sex-matched controls for depressive patients (9 males and 16 females, mean age: 40.9 ± 13.1). In addition, The TGFBR2 mRNA levels after antipsychotic treatment for several weeks were investigated in 13 out of 19 subjects (subject number S1–S13, Tables 1a and 1b, 7 males and 6 females, mean age: 28.2 ± 8.6) who were able to be followed up and compared with 13 age- and sex-matched controls (7 males and 6 females, mean age: 28.6 ± 7.5).

For the genetic studies, we used genomic DNA samples from 279 in-patients (189 male and 90 female; mean age: 51.3 ± 13.7 years) with schizophrenia from eleven psychiatric hospitals in the neighboring area of Tokushima Prefecture in Japan (population: about 820,000). Age- and sex-matched controls were selected from volunteers after assessing psychiatric problems (189 male and 90 female; mean age: 51.4 ± 12.0) for the association and haplotype-based case-control studies.

Table 1b
TGFBR2 mRNA expression in medication-free schizophrenic ($N = 19$) and control subjects ($N = 19$)

		Male ($N = 9$)	Female ($N = 10$)	Total ($N = 19$)
Schizophrenia (S1–S19)	Age	28.1 ± 5.6	32.4 ± 11.5	30.4 ± 9.3
	The TGFBR2 mRNA expression before treatment	Isoform A + isoform B		
		0.99 ± 0.23	1.11 ± 0.18	1.05 ± 0.20*
		Isoform B		
		1.00 ± 0.24	1.19 ± 0.34	1.11 ± 0.30*
Control	Age	27.6 ± 4.8	33.4 ± 10.4	30.6 ± 8.6
	The TGFBR2 mRNA expression	Isoform A + isoform B		
		0.79 ± 0.17	0.83 ± 0.16	0.81 ± 0.16
		Isoform B		
		0.78 ± 0.12	0.88 ± 0.16	0.83 ± 0.15

The mean TGPBR2 mRNA levels of medication-free schizophrenia patients were significantly higher than those of age-and sex-matched controls (isoform A + isoform B, $P < 0.001$, isoform B; $P = 0.003$, paired T-test). No correlation between TGFBR2 mRNA levels and baseline BPRS scores were observed (isoform A + isoform B; $P = 0.23$, isoform B; $P = 0.97$, Spearman's correlation efficient).

* $P < 0.01$, compared with the control group.

All subjects signed written informed consent to participate in the expression and genetic association studies approved by the institutional ethics committees.

3. Quantitative real-time PCR

Total RNA was extracted from the peripheral leukocytes using the PAXgene Blood RNA kit (Qiagen, Tokyo, Japan) according to the manufacturer's recommendations. One microgram of total RNA was used for cDNA synthesis by QuantiTect Reverse Transcription Kit (Qiagen, Japan) after assessing RNA quality and quantity with NanoDrop (NanoDrop Technologies, DE, USA). Expression of the TGFBR2 gene transcript was quantified by real-time PCR with the TaqMan Gene Expression Assay (Applied Biosystems, CA, USA). TGFBR2 gene has two splicing variants (isoform A, isoform B) (Lin et al., 1992; Nikawa, 1994). Suzuki et al. indicated that both isoforms of TGFBR2 gene mouse homolog are expressed in all tissues studied (Suzuki et al., 1994) and Hirai et al. showed that the isoform B is a major type of human TGFBR2 mRNA determined by RT-PCR (Hirai and Fujita, 1996). We measured the expression levels of isoform B separately as well as the transcript combinations of isoform A + isoform B using ABI probe/primers (Hs00559661_m1, Hs00947893_m1). GAPDH gene expression was used as an internal control and measurement of threshold cycle (Ct) was performed in triplicate. Data were collected and analyzed with Sequence Detector Software version 2.1 (Applied Biosystems) and the standard curve method. Relative gene expression was calculated as the ratio of TGFBR2 to GAPDH gene and the mean of the three replicate measures was assigned to each individual. Chronbach's alpha coefficient of three replicate measures was 0.980 and standard error of measurement was 0.122. The expression of the TGFBR2 mRNA in the peripheral leukocytes was not changed among blood samples collected at several points during the day time or over several weeks in the same control subject.

4. Genotyping

Genotyping was performed using commercially available TaqMan probes for TGFBR2 gene (C_29354774_10, C_29354775_10, C_27491740_10, C_1612565_10, C_11565984_20, C_1612508_10, C_11566050_10, C_8778140_10, C_25809090_10, C_15882489_10) with Applied Biosystems 7500 Fast Real Time PCR System according to the protocol recommended by the manufacturer (Applied Biosystems, CA, USA). We selected these 10 single nucleotide polymorphic (SNP) markers for genotyping from the public databases (dbSNP Home page) according to International Hap Map Project (http://www.hapmap.org/index.html.en). The heterozygocities of these 10 SNPs, rs7625858 (C/T), rs7648606 (C/T), rs3087465 (A/G), rs4522809 (C/T), rs12487185 (A/G), rs1864615 (A/G), rs3773652 (A/G), rs1367609 (A/C), rs3773663 (A/G) and rs2276767 (A/C) in Japanese population are reported as 0.23, 0.10, 0.18, 0.38, 0.37, 0.45, 0.48, 0.49, 0.42 and 0.09, respectively.

5. Statistical analysis

Statistical calculations were carried out using the SPSS Statistical Software Package 11.5 (SPSS, Tokyo, Japan). Expressional differences between patients and age-and sex-matched control subjects were calculated using the paired T-test after checking equal variances by Kolmogorov–Smirnov test. Changes before and after treatment were also analyzed with the paired T-test. Spearman correlation coefficients were used to evaluate the correlations between TGFBR2 mRNA levels and BPRS scores. Analysis of covariance (ANCOVA) was performed to determine the independent and combined effect of sex, diagnosis and age with the expression of TGFBR2 between groups. All significance levels were two-tailed. Allele and genotype frequencies of patients and control subjects were compared using Fisher's exact test. The SNPAlyze 3.2Pro software (DYNACOM, Japan) was used to estimate haplotype

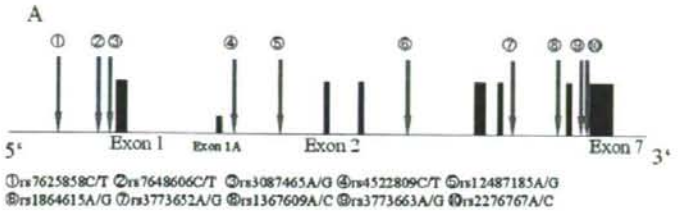

Fig. 1. Graphic representation of the TGFBR2 gene and the SNPs analyzed in the present study. Isoform B is major spliced variant without exon 1A. The amino acid sequence of isoform A contains an inset of 26 amino acids after Ser31, replacing Val132 of TGFBR2 isoform B.

frequencies, LD, and permutation P values. Pair-wise linkage disequilibrium (LD) indices, D' and r2, were calculated in the control subjects. The criterion for significance was set at $P < 0.05$ for all tests. Data are presented as mean ± SD. Our sample size had a post hoc power of 0.81 to detect an effect size of $w = 0.22$ at the 0.05 significance level, as calculated by software program G Power (Erdfelder et al., 1996) (see Fig. 1).

6. Results

6.1. TGFBR2 mRNA expression in medication free schizophrenic and control subjects (Tables 1a and 1b)

Relative expression levels of TGFBR2 mRNA (isoform A + isoform B) in 19 medication-free patients were 1.05 ± 0.20, while 0.81 ± 0.16 in healthy volunteers, showing a statistical difference (paired T-test: $P < 0.001$, Kolmogorov–Smirnov test: $P = 0.200$, Fig. 2). No correlation between TGFBR2 mRNA levels and baseline BPRS scores were observed (Spearman's correlation efficient: $P = 0.23$). The same result was also obtained in the mRNA expression levels of TGFBR2 isoform B (data shown in Tables 1a and 1b).

6.2. TGFBR2 mRNA expression in schizophrenia after several weeks antipsychotic treatment (Tables 2a and 2b)

The TGFBR2 mRNA levels after antipsychotic treatment for several weeks were investigated in 13 subjects who were able to be followed up among 19 medication-free patients. Mean chlorpromazine-equivalent doses were 490.4 ± 510.1 mg/day and mean duration of treatment was 68.6 ± 23.9 days. BPRS scores were significantly improved after antipsychotic treatment for several weeks (at baseline: 43.3 ± 19.6, after treatment: 35.1 ± 13.4; paired T-test: $P = 0.002$, Kolmogorov–Smirnov test: $P = 0.200$) and the mean TGFBR2 mRNA levels (isoform A + isoform B) also showed a significant decrease toward healthy control levels after antipsychotic treatment (at baseline: 1.04 ± 0.18, after treatment: 0.88 ± 0.23; paired T-test: $P = 0.027$, Kolmogorov–Smirnov test: $P = 0.200$). The TGFBR2 mRNA levels after treatment were not different from controls' (paired T-test: $P = 0.14$). No correlation between TGFBR2 mRNA levels and BPRS scores after treatment were observed (Spearman's correlation efficient: $P = 0.37$). The changes of BPRS scores did not show significant correlation with the change of the mRNA levels (Spearman correlation efficient: $P = 0.86$).

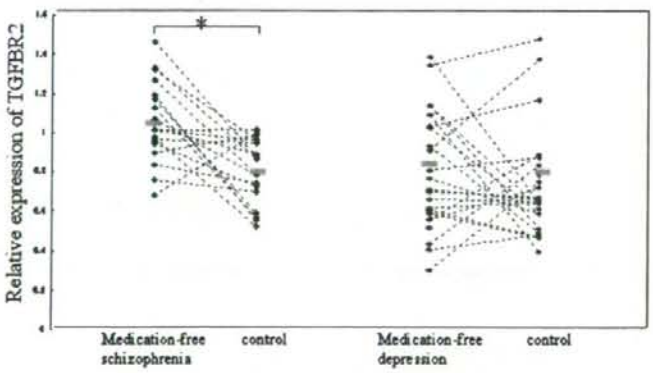

Fig. 2. Compared with the normal control group, the mean TGFBR2 mRNA level (isoform A + isoform B) in the leukocytes of medicaction-free schizophrenic patients ($N = 19$) was significantly higher than that of age- and sex-matched controls (patients: 1.05 ± 0.20, controls: 0.81 ± 0.16, paired T-test: $P < 0.001$). The mean TGFRBR2 mRNA level (isoform A + isoform B) in the leukocytes of medication-free major depressive patients ($N = 25$) showed no significant difference compared with sex- and age-matched controls (patients: 0.89 ± 0.31, controls: 0.84 ± 0.28, paired T-test: $P = 0.452$). *$P < 0.01$, compared with the control group.

The same result was also obtained in the mRNA expression levels of TGFBR2 isoform B (data shown in Tables 2a and 2b).

6.3. TGFBR2 mRNA expression in medication free major depression and control subjects

Relative expression levels of TGFBR2 mRNA (isoform A + isoform B) in 25 medication-free major depressive patients were 0.89 ± 0.31, while 0.84 ± 0.28 in healthy volunteers, showing no significant statistical difference (paired T-test: $P = 0.452$, Fig. 2). TGFBR2 mRNA expression levels of isoform B also showed the same result.

7. Genetic association analysis (Tables 3 and 4)

There were no significant deviations in all 10 SNPs from Hardy–Weinberg equilibrium in either patients or control subjects. Allele and genotype frequencies of the eight SNPs are shown in Table 4. There were no associations between these SNPs and schizophrenia neither in the allelic frequencies nor in the genotypic distributions. Permutation test of rs7625858–rs7648606 ($D' = 0.895$), rs7648606–rs3087465 ($D' = 0.866$) and rs3773663–rs2276767 ($D' = 0.945$) showed no significant difference in estimated frequencies of these haplotypes between the controls and patients (permutation $P = 0.19, 0.27, 0.96$, each).

Table 2a
Demographic data for schizophrenic patients after short-term antipsychotic treatment studied ill TGFBR2 mRNA expression analysis ($N = 13$)

	Age (y.o)	Gender	Duration of treatment (day)	Medication (at second point)	BPRS score
S1	25	M	90	Olz 10 mg	34
S2	24	M	134	Ris 3 mg	37
S3	24	M	54	Ris 3 mg	20
S4	27	M	55	Sulpiride 100 mg	27
S5	36	M	57	Olz 20 mg	23
S6	39	M	74	Olz 20 mg	36
S7	27	M	59	Olz 5 mg	47
S8	20	F	57	Ris3 mg, Lp25 mg	36
S9	23	F	71	Ris 2 mg	34
S10	34	F	85	Ris 2 mg	20
S11	47	F	47	Olz 15 mg	40
S12	15	F	44	Ris 2 mg	31
S13	26	F	65	Olz 20 mg, Ris 12 mg	71

Thirteen subjects (S1–S13) in Tables 2a and 2b were samples who were able to be followed up among 19 medication-free patients in Tables 1a and 1b. The age (years old: y.o) represent the age of the subject when the leukocytes were drawn. M = male, F = female, Olz: olanzapine, Ris: risperidone, LP: levomepromazine.

Table 2b
TGFBR2 mRNA expression in schizophrenics before treatment and after several weeks antipsychotic treatment ($N = 13$) and control subjects ($N = 13$)

		Male ($N = 7$)	Female ($N = 6$)	Total ($N = 13$)
Schizophrenia (S1–S13)	Age	28.9 ± 6.1	27.5 ± 11.5	28.2 ± 8.6
	The TGFBR2 mRNA expression before treatment	Isoform A + isoform B		
		1.00 ± 0.20	1.08 ± 0.16	$1.04 \pm 0.18^{*}$
		Isoform B		
		0.97 ± 0.21	1.13 ± 0.39	$1.04 \pm 0.30^{*}$
	The TGFBR2 mRNA expression after treatment	Isoform A + isoform B		
		0.75 ± 0.23	1.03 ± 0.10	0.88 ± 0.23
		Isoform B		
		0.61 ± 0.19	0.86 ± 0.17	0.72 ± 0.22
Control	Age	28.1 ± 5.2	29.2 ± 10.0	28.6 ± 7.5
	The TGFBR2 mRNA expression	Isoform A + isoform B		
		0.76 ± 0.18	0.77 ± 0.17	0.77 ± 0.17
		Isoform B		
		0.78 ± 0.14	0.82 ± 0.13	0.80 ± 0.13

BPRS scores were significantly improved after antipsychotic treatment for several weeks (at baseline: 43.3 ± 19.6, after treatment: 35.1 ± 13.4; paired T-test: $P = 0.002$).

The mean TGFBR2 mRNA levels showed a significant decrease toward healthy control levels after antipsychotic treatment (isoform A + isoform B; $P = 0.027$, isoform B; $P = 0.003$, paired T-test).

The TGFBR2 mRNA levels after treatment were not different from controls' (isoform A + isoform B; $P = 0.14$, isoform B; $P = 0.20$, paired T-test).

* $P < 0.05$, compared with the control group.

Table 3
Linkage disequilibrium (LD) indices (lower left are r2, upper right are D')

	rs 7625858	rs 7648606	rs 3087465	rs 4522809	rs 12487185	rs 1864615	rs 3773652	rs 1367609	rs 3773663	rs 2276767
rs 7625858	–	0.89465	0.58411	0.39018	0.38766	0.11919	0.00178	0.08145	0.06098	0.00053
rs 7648606	0.24556	–	0.8664	0 35761	0.31141	0.419	0.25127	0.18866	0.10999	0.59183
rs 3087465	0.30864	0.25458	–	0.0239	0.06625	0.00499	0.06456	0.03747	0.12032	0.03609
rs 4522809	0.01817	0.02333	0.00006	–	0.79095	0.69391	0.1183	0.18935	0.03031	0.81976
rs 12487185	0.02622	0.0121	0.00069	0.42727	–	0.76359	0.06257	0.1694	0.0435	0.49541
rs 1864615	0.00601	0.00907	0	0.13551	0.23876	–	0.04822	0.09866	0.07076	0.40741
rs 3773652	0	0.0034	0.00066	0.00943	0.00386	0.00224	–	0.14808	0.08763	0.43636
rs 1367609	0.00191	0.00315	0.00037	0.01748	0.02032	0.00568	0.01578	–	0.40153	0.87012
rs 3773663	0.00081	0.00081	0.00429	0.00034	0.0015	0.00259	0.00413	0.12234	–	0.94548
rs 2276767	0	0.00281	0.00053	0.02974	0.01588	0.02651	0.01238	0.08098	0.10863	–

Table 4
Genetic studies of TGFBR2 with schizophrenia in ease-control samples

Snp	Group	Genotype			n	Hardy–Weinberg P-value		Allele		P-value
rs7625858		T/T	C/T	C/C				T	C	
	sch	166	94	16	276	0.702	0.732	426	126	0.469
	cont	177	87	15	279	0.420		441	117	
rs7648606		T/T	C/T	C/C				T	C	
	sch	227	45	4	276	0.508	0.465	499	53	0.238
	cont	239	38	2	279	0.944		516	42	
rs3087465		A/A	A/G	G/G				A	G	
	sch	16	98	163	277	0.933	0.224	130	424	0.095
	cont	13	82	184	279	0.432		108	450	
rs4522809		T/T	T/C	C/C				T	C	
	sch	123	122	31	276	0.964	0.649	368	184	0.403
	cont	131	122	25	278	0.757		384	172	
rs12487185		A/A	A/G	G/G				A	G	
	sch	57	126	94	277	0.269	0.476	240	314	0.223
	cont	48	124	106	278	0.319		220	336	
rs1864615		A/A	A/G	G/G				A	G	
	sch	36	123	117	276	0.780	0.385	195	357	0.260
	cont	47	117	108	272	0.154		211	333	
rs3773652		A/A	A/G	G/G				A	G	
	sch	44	142	92	278	0.447	0.466	230	326	0.626
	cont	47	128	104	279	0.559		222	336	
rs1367609		A/A	C/A	C/C				A	C	
	sch	75	133	70	278	0.552	0.192	283	273	0.338
	cont	58	151	69	278	0.114		267	289	
rs3773663		A/A	A/G	G/G				A	G	
	sch	58	132	85	275	0.699	0.588	248	302	1.0
	cont	52	145	80	277	0.401		249	305	
rs2276767		A/A	A/C	C/C				A	C	
	sch	3	43	232	278	0.799	1.0	49	507	1.0
	cont	4	42	233	279	0.355		50	508	

sch, schizophrenia; cont, control subjects. P-values are calculated by Fisher's exact test.
There were no associations between these SNPs and schizophrenia neither in the allelic frequency not in the genotypic distributions.

8. Discussion

In the present study, relative expression levels of the TGFBR2 mRNA (isoform A + isoform B, isoform B) in both medication-free schizophrenic patients and major depressive patients were investigated. In addition, the association between 10 polymorphisms in the TGFBR2 locus and schizophrenia was investigated. To the best of our knowledge, this is the first study to investigate the role of TGFBR2 in the pathogenesis of schizophrenia.

First, our data showed that the mRNA expression level of TGFBR2 gene in the peripheral leukocytes was significantly higher in medication-free schizophrenics but not in medication-free depression. The results suggest that the expressional change of TGFBR2 gene in schizophrenia may be disease-specific and not due to non-specific stress from psychiatric condition. The BPRS scores were significantly improved after several week-antipsychotic treatment and the mean TGFBR2 mRNA levels showed a significant decrease toward healthy control levels after treatment. The

decrease of the TGFBR2 mRNA expression after treatment may be a consequence of pharmacological effects of antipsychotics or clinical improvement. These results suggest that altered expression of TGFBR2 mRNA in the peripheral leukocytes from schizophrenic patients may not be trait-oriented but state-related change. Be contrary to our anticipation, the mRNA expression level of TGFBR2 gene was not up-regulated in schizophrenia who took antipsychotic medications. TGFBR2 may be associated with reportedly low susceptibility to cancer in unmedicated but not medicated schizophrenia. Other tumor suppressor genes or oncogenes may have strong influence on tumor resistance associated with schizophrenia. In spite of the limited number of medication-free schizophrenic samples, the fact that altered mRNA expression of TGFBR2 gene in schizophrenia before treatment may have pathophysiological significance because peripheral lymphocytes could reflect the metabolism of brain cells (Gladkevich et al., 2004). Further expression study using human brain tissue is needed in order to reveal the pathological role of TGFBR2 gene to schizophrenia.

Second, we investigated the genetic association between TGFBR2 gene and schizophrenia in Japanese population. The TGFBR2 gene is located at 3p22, which has been previously reported to be linked with schizophrenia. However we did not find any association of 10 SNPs in TGFBR2 gene (rs7625858, rs7648606, rs3087465, rs4522809, rs12487185, rs1864615, rs3773652, rs1367609, rs3773663 and rs2276767) with schizophrenia. Haplotype analyses in the TGFBR2 gene did not reveal any significance, either. Further studies with denser polymorphisms and a larger sample set will be needed although our sample sizes were suitable for genetic comparison (power > 0.8).

In conclusion, our investigation revealed that the mean TGFBR2 mRNA levels (isoform A + isoform B, isoform B) in medication-free schizophrenic patients were significantly higher than those of age- and sex-matched controls and showed a significant decrease toward healthy control levels after antipsychotic treatment. There were no associations between the TGFBR2 gene and schizophrenia. We conclude that the TGFBR2 gene itself does not link to schizophrenia but that the TGFBR2 mRNA levels in the peripheral leukocytes may be a potential state marker for schizophrenia.

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ORIGINAL ARTICLE

Microarray comparative genomic hybridization analysis of 59 patients with schizophrenia

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Abstract Schizophrenia is a common psychiatric disorder with a strong genetic contribution. Disease-associated chromosomal abnormalities in this condition may provide important clues, such as *DISC1*. In this study, 59 schizophrenia patients were analyzed by microarray comparative genomic hybridization (CGH) using custom bacterial artificial chromosome (BAC) microarray (4,219 BACs with 0.7-Mb resolution). Chromosomal abnormalities were found in six patients (10%): 46,XY,der(13)t(12;13)(p12.1;p11).ish del(5)(p11p12); 46,XY, ish del(17)(p12p12); 46,XX.ish dup(11)(p13p13); and 46,X,idic(Y)(q11.2); and in two cases, mos 45,X/46XX. Autosomal abnormalities in three cases are likely to be pathogenic, and sex chromosome abnormalities in three follow previous findings. It is noteworthy that 10% of patients with schizophrenia have (sub)microscopic chromosomal abnormalities, indicating that genome-wide copy number survey should be considered in genetic studies of schizophrenia.

Keywords Schizophrenia · Chromosomal abnormality · Array comparative genomic hybridization · Copy number variation

Introduction

Schizophrenia is a common psychiatric disorder involving approximately 1% of the population worldwide. Family, twin, and adoption studies suggest genetic factors contribute to this illness (Lang et al. 2007; McGuffin et al. 1995). Meta-analysis including 18 genome scans revealed strong evidence at chromosomal regions 22q, 8p, and 13q

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as the susceptibility loci (Badner and Gershon 2002), and another meta-analysis of 20 genome-wide scans suggested regions of chromosomes 2q, 5q, 3p, 11q, 6p, 1q, 22q, 8p, 20q, and 14p as the significant loci (Lewis et al. 2003). Chromosomal abnormalities in patients with schizophrenia may provide useful information regarding the susceptible loci (Bassett et al. 2000). Disrupted in schizophrenia 1 (*DISC1*) gene isolated from a large Scottish family with t(1;11)(q42.1;q14.3) and high risk of schizophrenia in velo-cardio-facial syndrome (VCFS) with a 22q11 deletion are good examples (Arinami 2006; Millar et al. 2000; Murphy 2002). Some linkage and association studies support that schizophrenia could be associated with *DISC1* and genes at 22q11 (Chubb et al. 2008; Liu et al. 2002; O'Donovan et al. 2003; Shifman et al. 2002).

Microarray technologies have now become practical tools for detection of submicroscopic copy number changes. Using custom bacterial artificial chromosome (BAC) microarray (4,219 BACs at 0.7-Mb resolution), we analyzed 59 patients with schizophrenia. Chromosomal abnormalities found in this study are presented.

Materials and methods

Subjects

A total of 59 subjects (31 men and 28 women) with schizophrenia were recruited in this study. Forty-one had family history. Diagnosis was made for each patient according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV) criteria on the basis of unstructured interviews and information from medical records. Participants were excluded if they had organic brain diseases, including head injury and infection, or if they met criteria for alcohol/drug dependence. After written informed consent, genomic deoxyribonucleic acid (DNA) from lymphoblastoid cell line (LCL) of all patients was isolated using DNA isolation systems [Quick Gene-800 (Fujifilm, Tokyo, Japan) and/or NA-3000 (Kurabo, Osaka, Japan)]. Micorarray comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH) analysis were performed using materials from LCL. Peripheral blood lymphocytes were reevaluated in ID394, MZ102, and MZ127, but could not be obtained for reexamination in ID67, ID345, or ID391. Only parents of ID345 subjects were available for familial analysis. Other parents or sibs could not be evaluated. Experimental protocols were approved by the Committee for Ethical Issues at Yokohama City University School of Medicine.

Microarray CGH analysis

Comparative genomic hybridization analysis was performed using our custom BAC microarray containing 4,219 BAC clones, as previously described (Saitsu et al. 2008). In brief, after complete digestion using *Dpn*II, subject's DNA was labeled with Cy-5 dCTP (Amersham Biosciences, Piscataway, NJ), and reference DNA was labeled with Cy-3 deoxycytidine triphosphate (dCTP) (Amersham Biosciences) using the DNA random primer Kit (Invitrogen). Prehybridization, probe hybridization, washing, and drying steps for arrays were preformed on a Tecan hybridization station HS400 (Tecan Japan, Kawasaki, Japan). Arrays were scanned by GenePix 4000B (Axon Instruments, Union City, CA, USA) and analyzed using GenePix Pro 6.0 (Axon Instruments). The signal intensity ratio between patient and control DNA was calculated from the data of the single-slide experiment using the ratio of means formula (F635 mean − B635 median/F532 mean − B532 median) according to GenePix Pro. 6.0. The standard deviation was calculated from the data of all clones. We regarded the signal ratio as abnormal if it ranged out of ±3 standard deviations (SD). Clones showing abnormal copy number were checked to see whether they were in the position of previously registered copy number variations using the Human Genome Variation Database (http://www.hgvbase.org/) (Iafrate et al. 2004). Unregistered changes were considered for further confirmation. Genome position was based on the UCSC genome browser Human Mar. 2006 (hg18) assembly.

Fluorescence in situ hybridization

To confirm status of clones with a possibly abnormal copy number, FISH was performed, as previously described (Shimokawa et al. 2005). BAC DNA was labeled with SpectrumGreen™-11-deoxyuridine triphosphate (dUTP) or SpectrumOrange™-11-dUTP (Vysis, Downers Grove, IL, USA) by nick translation and denatured at 70°C for 10 min. Probe-hybridization mixtures (15 µl) were applied on chromosomes, incubated at 37°C for 16–72 h, then washed and mounted in antifade solution (Vector, Burlingame, CA, USA) containing 4'-6'-diamidino-2-phenylindole (DAPI). Photographs were taken on an AxioCam MR CCD fitted to Axioplan2 fluorescence microscope (Carl Zeiss, Oberkochen, Germany). In ID394 and MZ102, we counted 100 interphase nuclei to validate the number of cells with X aneuploidy, as well as 30 metaphases.

Results and discussion

Six patients showed chromosomal abnormalities (10%, 6/59) (Table 1). As we could not obtain materials from most

Table 1 Summary of six patients with (sub)microscopic chromosomal rearrangements

Patient	Gender	FH	Karyotype	Size of imbalance
ID67	M	No	46,XY,der(13)t(12;13)(p12.1; p11).ish del(5)(p11p12)	1.7 Mb deletion (chr.5) 23.1 Mb gain (chr.12)
MZ127	F	Yes	46,XX.ish dup(11)(p13p13)	430 bp (?) gain (chr.11)
ID345	M	No	46,XY, ish del(17)(p12p12)	1.3 Mb deletion (chr.17)
MZ102	F	Yes	Mos45,X/46,XX	Whole X loss (mosaic)
ID394	F	Yes	Mos45,X/46,XX	Whole X loss (mosaic)
ID391	M	Yes	46,X,idic(Y)(q11.2)	Yq12-qter deletion Yq11.23-Yq12 gain

FH family history of schizophrenia and/or other psychiatric disorders

of their parents and sibs, heritability of the abnormalities could not fully be investigated. According to our experiences of microarray CGH analysis of more than 200 Japanese patients associated with mental-retardation-related disorders, all chromosomal abnormalities described here were never detected. Thus, it is less likely that the changes are polymorphisms.

In ID67, arr cgh 5p12p12(RP11-1037A10 → RP11-929P16) × 1, 12pterp12.1(GS-124K20 → RP11-12D15) × 3 was found. A 23.2-Mb copy number gain from 12pter to 12p12.1 (chr12: 0–23,176,547 bp) was detected (Fig. 1a). G-banded chromosomal analysis revealed that 12pter-12p12.1 was translocated to 13p11 (Fig. 1a). The 12p12.1 translocation breakpoint was localized between two BAC clones, RP11-35A22 and RP11-349E13, by FISH (chr12: 23,176,547–23,861,227 bp) (data not shown). Additionally, a 1.7-Mb submicroscopic deletion at 5p12 from RP11-1037A10 to centromeric sequence gap (chr5: 44,778 009–46,437 323 bp) was also found in this patient (Fig. 1a). The 12p trisomy is recognized as multiple congenital anomalies/mental retardation (MCA/MR) syndrome characterized by dysmorphic face, heavy birth weight, foot deformities, hypotonia, and mental retardation (Allen et al. 1996). A previous study suggested that partial duplication of 12pter-p13.2 is sufficient for recognizable phenotype of 12p trisomy (Rauch et al. 1996). The 23.1-Mb duplicated region contained at least 229 genes. Dysmorphic facial features of 12p trisomy (Rauch et al. 1996) were not recognized in this patient. It is interesting that ID67 also had a 1.7-Mb deletion at 5p12, containing two genes, *MRPS30* (the mitochondrial ribosomal protein S30 gene) and *HCN1* (the hyperpolarization-activated cyclic nucleotide-gated potassium channel 1 gene). It is worth noting linkage findings within the vicinity of this region in Costa Rican schizophrenia samples (Cooper-Casey et al. 2005). *HCN1* is an intriguing candidate gene. The general *Hcn*1 loss in mice led to a defect in the learning of motor tasks, and specific deletion of the gene in forebrain neurons resulted in an unexpected enhancement of spatial learning and memory (Herrmann et al. 2007; Nolan et al. 2003). ID67 (a 72-year-old male) developed psychotic symptoms (delusions, hallucinations, and psychomotor excitement) at age 20 years. He had received electroconvulsive therapy many times and continuous sleep therapy until antipsychotic medication (chlorpromazine) was introduced at age 23 years. Since the onset of the illness, he has spent most of his life in psychiatric hospitals because of exacerbations of psychotic episodes and marked deterioration of social functions. Intelligent quotient (IQ) at 72 years was 72. He had no family history of major psychosis within the first-degree relatives.

In MZ127, arr cgh 11p13p13(RP11-51J14) × 3 was recognized. Duplication of RP11-51J14 at 11p13 (chr11: 33,302,231–33,302,660 bp) was confirmed by FISH using LCL and peripheral blood lymphocytes (Fig. 1b). According to the genome browser, the size of RP11-51J14 is 430 bp, indicating that the reference sequence is somehow odd and may contain a deletion overlapping with RP11-51J14 as FISH signals of RP11-51J14 are strong enough to detect on a microscope, suggesting that its size is at least >10 kb. *HIPK3* (the homeodomain interactive protein kinase 3 gene) was corresponding to this clone. HIPK3 is a Fas-associated death-domain (FADD)-interacting kinase involved in apotosis (Curtin and Cotter 2003), remaining unknown in relation to schizophrenia. MZ127 (42-year-old woman) presented with epilepsy at age 12 years and has had recurrent depression and slight mania since age 29 years. She began to exhibit auditory hallucination, not synchronizing with mood swing, and was diagnosed as schizophrenia at 40 years. Her mother and sister suffered from major depression and schizophrenia, respectively. Her father committed suicide induced by depression.

In ID345, arr cgh 17p12p12(RP11-78J16 → RP11-103P10) × 1 was found, as previously described (Ozeki et al. 2008). The deletion from RP11-246F16 to RP11-103P10 (chr17: 14,061,460–15,374,745 bp) is 1.4 Mb, compatible with the common deletion found in approximately 85% of hereditary neuropathy with liability to pressure palsies (HNPP; OMIM #162500) (Stogbauer et al.

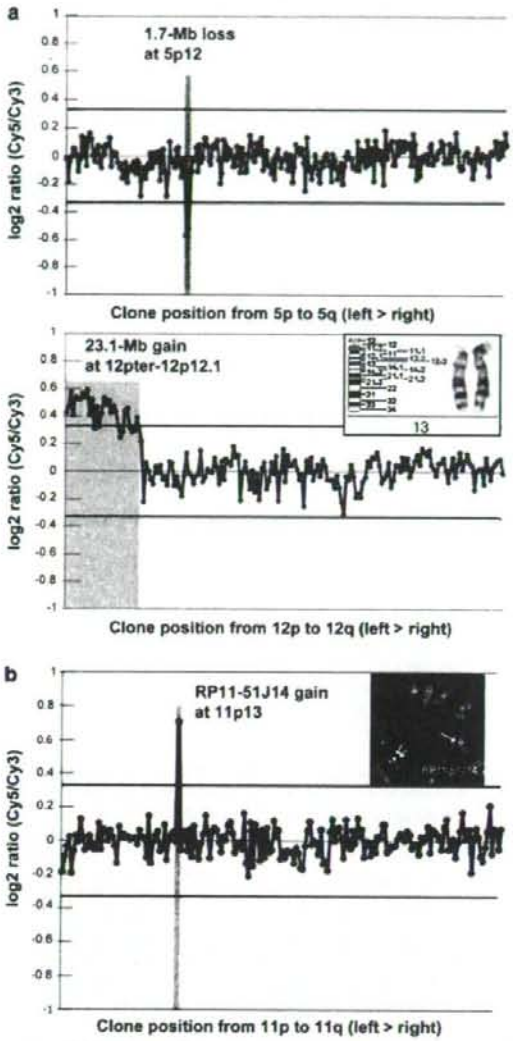

Fig. 1 Results of microarray comparative genomic hybridization (*CGH*) in ID67 (**a**) and MZ127 (**b**). Chromosomes 5 (*upper*) and 12 (*lower*) are displayed (**a**). The karyotype is arr cgh 5p12p12(RP11-1037A10 → RP11-929P16) × 1, 12pterp12.1(GS-124K20 → RP11-12D15) × 3. Partial karyotype clearly shows a 12pter-p12.1 segment is translocated to 13p11. Chromosome 11 is presented (**b**) . The karyotype is arr cgh 11p13p13(RP11-51J14) × 3. RP11-51J14 at 11p13 is duplicated

2000). The deletion was also identified in his father's chromosomes from peripheral blood lymphocytes. He suffered from auditory hallucination and delusion of persecution and received antipsychotic treatment at age 19. Neurological examination did not reveal any manifestations of HNPP (Ozeki et al. 2008). Pareyson et al. (1996) reported that about 25% of individuals with HNPP deletion are asymptomatic. The peripheral myelin protein 22 gene (*PMP22*) may be a candidate that is not only expressed in the peripheral nervous system but also in the central nervous system (Ohsawa et al. 2006), this being supported by linkage studies of psychotic bipolar disorder (Park et al. 2004) and schizophrenia (Owen et al. 2004). No family history regarding psychiatric disorders was observed in ID345.

Entire X chromosome copy number aberration was suspected in two patients, ID394 and MZ102 (data not shown). FISH analysis using RP11-65B15 at Xq23 revealed mosaic monosomy of chromosome X: mos45,X[41]/46,XX[59] in ID394 and mos45,X[84]/46,XX[16] in MZ102. X aneuploidy is well known to be seen in elderly normal females (Stone and Sandberg 1995). ID394 and MZ102 were 67 and 38 years old, respectively. The fraction of cells with X monosomy was very high (84% and 41%) in lymphoblastoid cell lines of these patients. Reevaluation of peripheral blood lymphocytes showed mos 45,X[7]/46,XX[98] in ID394 and mos 45,X[4]/46,XX[96] in MZ102. These findings may support involvement of X-chromosomal abnormalities in schizophrenia (Kumra et al. 1998; Kunugi et al. 1999), but mosaic X monosomy is also found in age-matched normal controls (Toyota et al. 2001). ID394 (a 67-year-old woman) developed psychotic symptoms (paranoid delusion and hallucinations) at age 31 years when she delivered her second child. Since then, she had been admitted to a psychiatric hospital three times (each for a few months). She quit her job as a pharmacist after the onset of the illness and has lived as a housewife. She has been managed by antipsychotic medications without major exacerbation for the past decade. The second child developed schizophrenia-like symptoms, including social withdrawal and lack of volition. MZ102 (a 38-year-old woman) exhibited psychomotor excitement and was diagnosed as having schizophrenia at age 23 years. Her father showed psychotic disorder, and her uncle had schizophrenia. In ID391, arr cgh Ypterq11.23(GS-98C4 → RP11-214M24) × 3, Yq11.23qter(RP11-263C17 → RP11-80F8) × 1 was identified. FISH analysis using BACs, RP11-74L17 at PAR1, RP11-375P13 at Yp11.2, RP11-655E20 at Yq11.2, and RP11-80F8 at Yq12 revealed the isodicentric Y chromosome [46,X,idic(Y)(q11.2)] (data not shown). Previously, two cases of idic(Yp) were reported in schizophrenia, although idic(Yp) is one of the most common rearrangements in the Y chromosome (Nanko et al. 1993; Yoshitsugu et al. 2003). ID391 (a 29-year-old man) developed hallucinations and abnormal sense of self at age 21 years, when he was admitted to a psychiatric hospital for 3 months. Since then, his illness has been well controlled by antipsychotic medication. He quit university after the onset

of illness and has not obtained a job, suggesting deterioration of functioning. His younger sister (apparently without the Y chromosome) has schizophrenia. Thus, contribution of sex chromosomal abnormalities found in this study is less likely.

Four microarray CGH studies of schizophrenia were reported: 1,440 BAC microarray for 30 patients, 2,460 BAC microarray for 35 patients, a tiling-path microarray consisting of ~36,000 BACs for 93 patients, and high-resolution microarrays (85,000–2,100,000 oligos) for 150 patients (Kirov et al. 2008; Moon et al. 2006; Walsh et al. 2008; Wilson et al. 2006). We could not replicate any similar abnormalities, though microarray platforms were all different in terms of clones and genome coverage. In this study, (sub)microscopic rearrangements were detected in 10% of patients. Similarly, 15% of patients analyzed by high-resolution microarrays were found to possess submicroscopic chromosomal changes (Walsh et al. 2008). Various kinds of recurrent and unique submicroscopic changes were found in 10–17% of idiopathic mental retardation and 7% of autism by microarray CGH analysis (Miyake et al. 2006; Sebat et al. 2007; Zahir and Friedman 2007). Importantly, a 22q13 deletion (in autism) involving Sh3 and multiple ankyrin repeat domains 3 (*SHANK3*), whose point mutation was related to autism (Durand et al. 2007), strongly supports this approach as one of the most powerful and straightforward strategies in neuropsychiatric disorders.

In conclusion, microarray technologies could provide good opportunity to identify chromosomal copy number changes in relation to mental and psychiatric disorders, and genome-wide copy number survey should be considered in genetic studies of these disorders.

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Relationship between three serotonin receptor subtypes (*HTR3A, HTR2A* and *HTR4*) and treatment-resistant schizophrenia in the Japanese population

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Abstract

The proportion of treatment-resistant schizophrenia (TRS) has been estimated as 20–40% in the schizophrenic patients. Genetic factors are considered to be involved in the development of this condition. Serotonin subtypes are hypothesized to be the candidate genes. In the present study, single marker and haplotype analyses between several mutations of serotonin receptor subtypes (*HTR2A*, *HTR3A* and *HTR4*) and TRS (TRS = 101, NON-TRS = 239) were performed to determine a possible relationship with the development of TRS. Additionally, we also compared the daily neuroleptic dosage among each genotype. No significant association was observed between TRS and each allele, genotype, and haplotype. However, the daily neuroleptic dosage that patients had been receiving during their maintenance therapy was significantly higher in patients with the T/T genotype of *HTR3A* polymorphism (rs1062613, $p = 0.041$). The present results support further research to examine the relationship between *HTR3A* polymorphism and the development of TRS in the Japanese population.

Keywords: *HTR*; Serotonin; Antipsychotic drug; Refractory; Gene; Haplotype

The proportion of treatment-resistant schizophrenia (TRS) has been estimated as 20–40% in the schizophrenic patients, and this unfortunate situation in the clinical psychiatric field still remain unchanged even after the introduction of several atypical antipsychotic agents [27]. Among the atypical antipsychotics, only clozapine has been reported to be effective for 30–60% of schizophrenic patients refractory to typical and atypical antipsychotics [20,28]. Clozapine is known to provide antipsychotic effects through binding to the several serotonin receptor subtypes (5-HT) [3] although the actual mechanism of clozapine for TRS has not been elucidated yet. In order to clarify this mechanism several researches investigated the predictable genetic factors for the clinical response to clozapine, as a result a significant association with the 5-HT receptor subtypes has been reported in a number of studies as follows.

Clozapine has a high affinity for 5-HT2A receptor [21] and produces a significant downregulation of cortical 5-HT2A receptor in the radioligand binding studies [2]. In addition, two PET studies have shown that the systemic administration of clozapine to schizophrenic patients produces an 84–90% occupation of cortical 5-HT2A receptor [23,6]. A couple of researches have reported the association between 5-HT2A receptor gene (*HTR2A*) polymorphism and TRS [13,7] or response to clozapine [17], although no association study has been reported in the Japanese subjects with TRS.

Since the 5-HT3A receptor has been reported to have potential anxiolytic and antipsychotic properties from animal studies, 5-HT3A receptor antagonists are being explored as therapeutic agents for a variety of behavioral disorders [5]. Additionally, 5-HT3A receptor gene (*HTR3*) is located on 11q23.1, where linkage with schizophrenia has been suggested in several studies [19,16]. These results suggest that *HTR3* may be related to

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