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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	Isoform A + isoform B		
	before treatment	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

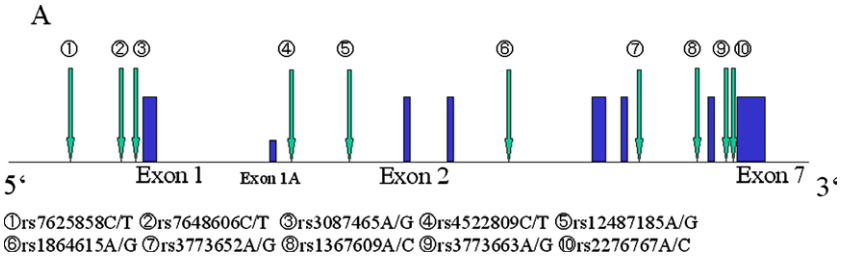

Fig. 1. Graphic representation of the TGFBR2 gene and the SNPs analyzed in the present study. Isoform B is major spliced variant without exon 1A. The amino acid sequence of isoform A contains an inset of 26 amino acids after Ser31, replacing Val132 of TGFBR2 isoform B.

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: $P = 0.86$).

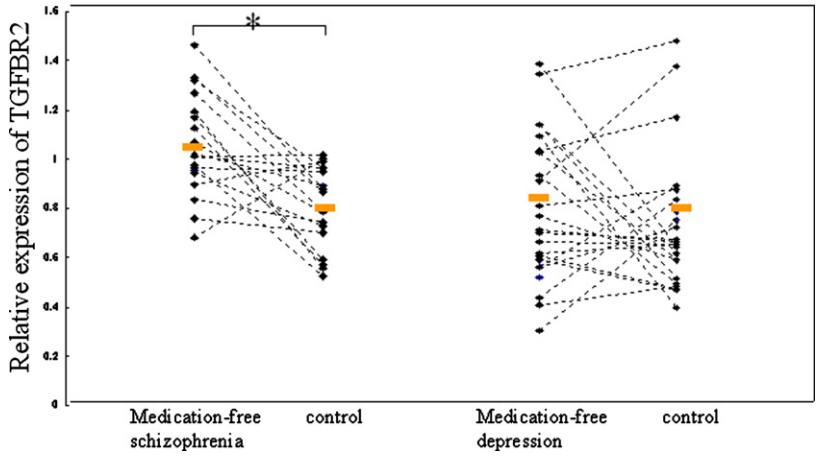

Fig. 2. Compared with the normal control group, the mean TGFBR2 mRNA level (isoform A + isoform B) in the leukocytes of medicaction-free schizophrenic patients ($N = 19$) was significantly higher than that of age- and sex-matched controls (patients: 1.05 ± 0.20, controls: 0.81 ± 0.16, paired T-test: $P < 0.001$). 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 ± 0.18*
		Isoform B		
		0.97 ± 0.21	1.13 ± 0.39	1.04 ± 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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