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Brief report

# IQ decline and memory impairment in Japanese patients with chronic schizophrenia

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**Abstract**

The extent of IQ decline due to the development of illness in patients with chronic schizophrenia and the degree of memory impairment relative to such IQ decline still remain unclear. Our results suggest that schizophrenia patients experience marked IQ decline due to the development of illness and their wide-ranging memory impairments are even more severe than the IQ decline.


*Keywords:* Schizophrenia; IQ; Memory

## 1. Introduction

Cognitive impairment is a core feature of schizophrenia, with a great impact on patients' daily lives. Those therapies that have the potential to improve cognitive deficits of patients with schizophrenia, including cognitive remediation therapy (Medalia et al., 1998; Wykes et al., 2003), as well as the favorable effects of atypical antipsychotic drugs on cognition (Bilder et al., 2002; Harvey et al., 2006; Keefe et al., 2006), have been attracting increasing attention from researchers and clinicians. From this viewpoint, the precise delineation of cognitive impairments in schizophrenia patients is essential.

Intellectual deficits in patients with chronic schizophrenia have been reliably identified (Heinrichs and Zakzanis, 1998; Dickinson et al., 2004) with some ongoing debate as to "whether it is possible to be schizophrenic yet neuropsychologically normal" (Palmer et al., 1997; Kremen et al., 2000; Wilk et al., 2005); however, the extent of IQ decline caused by the development of schizophrenia remains unclear because the premorbid IQ scores of persons who later develop schizophrenia are lower than

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those of their peers (Fuller et al., 2002; Reichenberg et al., 2005). Impairments in memory, working memory, and attention in patients with schizophrenia are well documented (Aleman et al., 1999; Silver et al., 2003; Hori et al., 2006), but the relationship of these cognitive deficits to the possible decline in IQ has not been established. Here we assessed cognitive functions including intellectual and wide-ranging memory functioning in patients with chronic schizophrenia in relation to age- and premorbid IQ-matched healthy controls.

## 2. Materials and methods

Eighty-two patients who met the DSM-IV criteria (American Psychiatric Association, 1994) for schizophrenia participated in this study. All patients were receiving antipsychotic drugs at the National Center of Neurology and Psychiatry (NCNP), Musashi Hospital and were clinically stable at the time of the neuropsychological tests. Eighty-two age- and premorbid IQ-matched healthy volunteers were recruited from hospital staff and their associates and also from the community. Healthy participants were interviewed by a research psychiatrist using the Japanese version of the Mini-International Neuropsychiatric Interview (MINI, Shechan et al., 1998) to confirm the absence of any psychiatric illnesses. A portion of the subjects were from our previous sample (Hori et al., 2006). Written informed consent was obtained from all subjects prior to their inclusion in the study. The study was approved by the ethics committee of the NCNP.

Premorbid IQ was estimated with the Japanese Adult Reading Test (JART, Matsuoka et al., 2002; 2006), a Japanese version of the National Adult Reading Test (NART, Nelson and Wilson, 1991). This test is considered to provide an estimate of premorbid IQ in schizophrenia patients (Uetsuki et al., 2006), which is consistent with the original NART (Crawford et al., 1992; O'Carroll et al., 1992). In this test, subjects were required to read out 100 idioms of Han-Chinese characters (Japanese kanji characters). JART-estimated premorbid IQ was calculated for each subject according to previous reports (Matsuoka et al., 2002, 2006). The full version of the Wechsler Memory Scale-Revised (WMS-R, Wechsler, 1987; Sugishita, 2001) was administered to all participants. Outcome measures of the WMS-R were verbal memory, visual memory, delayed recall, auditory attention, visual attention, verbal working memory, and visual working memory. To precisely assess subjects' current intellectual function, a full version of the Wechsler Adult Intelligence Scale-Revised (WAIS-R, Wechsler, 1981; Shinagawa et al., 1990) was administered, yielding age-corrected indices of verbal, performance, and full-scale IQs.

Schizophrenic symptoms were assessed by an experienced research psychiatrist in 46 of the 82 patients using the Positive and Negative Syndrome Scale (PANSS, Kay et al., 1987). Daily doses of antipsychotics and anticholinergic antiparkinsonian drugs were converted to chlorpromazine equivalents (CPZeq) and biperiden equivalents (BPDeq), respectively, using published guidelines (American Psychiatric Association, 1997; Inagaki et al., 1999; Minzenberg et al., 2004).

Results are reported as mean±standard deviation (S.D.). Demographic characteristics and cognitive test results were compared between groups. We used *t*-test or analysis of variance (ANOVA) to compare mean scores and the $\chi^2$ tests to compare categorical variables. Analysis of covariance (ANCOVA) was used to compare means between groups, controlling for confounding variables. Statistical significance was set at two-tailed $P<0.05$. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Japan, Tokyo).

## 3. Results

Male/female ratios of patients and controls were 48/34 and 25/57, respectively, indicating that the patient group had a greater representation of males ($\chi^2(1)=13.06$, $P<0.001$). The mean ages of the patients and controls were 44.3±13.8 and 44.2±14.9, respectively ($t=0.05$, $df=162$, $P=0.96$). The mean years of education of the patients and controls were 13.4±2.5 and 14.1±2.2, respectively ($t=1.88$, $df=162$, $P=0.06$). The JART-predicted premorbid IQ scores of patients and controls were 102.2±11.6 and 102.3±7.4, respectively ($t=0.46$, $df=137.8$, $P=0.96$). Of the 82 patients, 56 were outpatients and 26 were inpatients. The mean age of illness onset was 24.7±8.8. Illness duration was 19.6±13.7 years, demonstrating that our patients were in the chronic phase of schizophrenia. CPZeq and BPDeq were 781.7±710.1 and 2.2±2.0, respectively. PANSS positive, negative, and total scores were 13.9±6.7, 19.1±7.1, and 62.1±17.9, respectively.

Verbal, performance, and full-scale IQs of patients with schizophrenia and healthy controls are presented in Supplementary Table 1. ANOVA showed that these three IQ indices in patients were significantly lower than those in controls (all $P<0.001$). The VIQ/PIQ ratios of patients and controls were 1.08±0.18 and 0.95±0.11, respectively ($F=22.5$, $df=1,160$, $P<0.001$, by ANCOVA with gender as a covariate). Scores of 13 subscales of the WMS-R in patients and controls are also shown in Supplementary Table 1. Patients performed significantly

more poorly than controls on all these cognitive domains (all $P<0.001$), except for auditory attention ($P=0.15$). Fig. 1(a) shows mean scores of the patients and controls on JART-estimated IQ, WAIS-R full-scale IQ, and the main three memory indices of the WMS-R. Dips of current IQ and all memory domains in patients are apparent, although the two groups are matched for the JART-estimated premorbid IQ.

To control for the current IQ and gender effects on these test results, ANCOVA was used with full-scale IQ and gender as covariates. It revealed that patients performed significantly more poorly than controls on verbal memory, visual memory, delayed recall, visual attention, and verbal working memory, even after controlling for full-scale IQ and gender (Supplementary Table 1). To confirm these results, additional comparisons were made between patients whose current IQ scores were within normal limit (IQ-WNL patients, defined as WAIS-R full-scale IQ ≥ equal to or greater than 85; $n=46$) and total controls ($n=82$). Fig. 1(b) summarizes the results, showing that there was no difference in current IQ between IQ-WNL patients (mean IQ: 98.85±8.55) and controls (mean IQ: 101.95±11.30), while these patients still showed significantly lower scores on all three memory indices compared with controls. On the other hand, the JART-estimated premorbid IQ of IQ-WNL patients was significantly higher than that of controls.

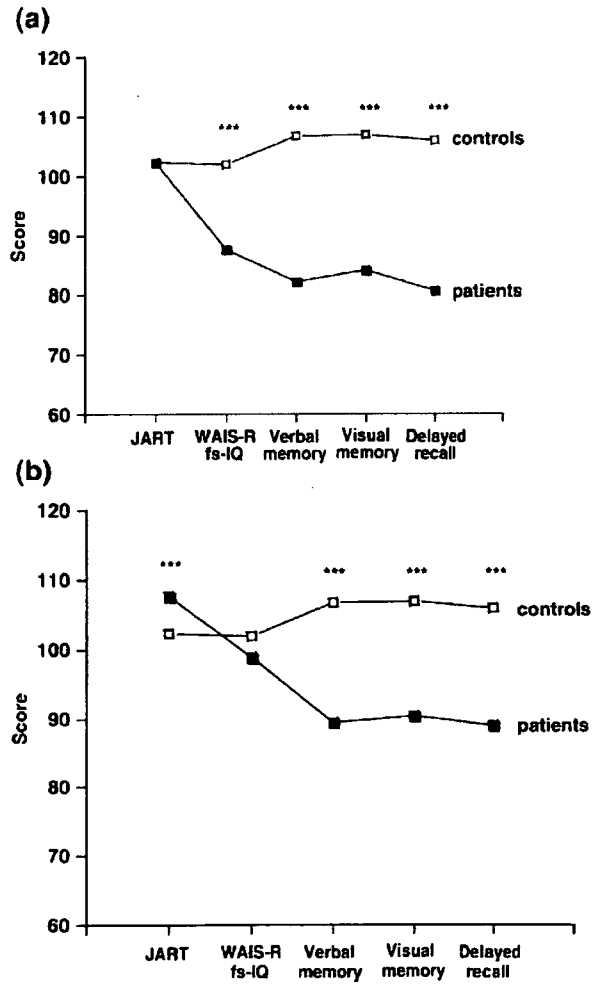


Fig. 1. Mean scores of patients and controls on JART IQ, WAIS-R full-scale IQ, Verbal memory, Visual memory, and Delayed recall indices (WMS-R). (a) total patients ($n=82$) vs. total controls ($n=82$) and (b) IQ-WNL patients (defined as WAIS-R full-scale IQ ≥ 85, $n=46$) vs. total controls ($n=82$).***$P<0.001$.

## 4. Discussion

In the present study we examined intellectual and memory functions in patients with chronic schizophrenia relative to age- and premorbid IQ-matched healthy controls. Our results confirmed that patients with chronic schizophrenia have wide-ranging cognitive impairments, consistent with the literature on schizophrenia.

The relationship of the development of schizophrenia to declining IQ scores has been confounded by findings that premorbid intelligence itself is likely to be lower in persons who later develop schizophrenia than in their peers (Fuller et al., 2002; Reichenberg et al., 2005). To address this issue, we employed a premorbid IQ-matched case-control sample. Although the cross-sectional nature of the present study does not allow any definite conclusions to be drawn concerning the time when the IQ decline actually occurred (i.e., during the prodromal stage, immediately after illness onset, or during the chronic course of illness), the observed differences in current IQs between patients and controls provide evidence for marked IQ decline due to the development of schizophrenia. Means of estimated premorbid IQ and current full-scale IQ in patients were 102.20 and 87.68, respectively, suggesting an approximate 1 S.D. decline in IQ score related to the development of illness. On the other hand, the subgroup of patients whose current IQ was within normal limits (and thus similar to that of controls) showed significantly higher premorbid IQ as estimated by the JART than controls (Fig. 1(b)), which favors the view that even neuropsychologically normal patients with chronic schizophrenia have compromised cognitive functioning relative to their presumed premorbid level of intellectual function (Kremen et al., 2000). Furthermore, in the present study performance IQ of the patients was more severely impaired than verbal IQ, congruent with prior reports (Heinrichs and Zakzanis, 1998).

Pervasive memory impairment in patients with schizophrenia relative to premorbid IQ-matched controls was

found, and most deficits remained significant even after current IQ was controlled for, supporting that memory impairment is a core feature of schizophrenia (Saykin et al., 1991; Heinrichs and Zakzanis, 1998; Aleman et al., 1999). The marked impairment in verbal memory is consistent with numerous studies (e.g., Saykin et al., 1991; Heinrichs and Zakzanis, 1998). Although visual memory deficits in schizophrenia have attracted less attention from researchers than verbal memory, several studies have reported substantial impairment of visual memory (Saykin et al., 1991; Aleman et al., 1999), consistent with the present study. The pronounced impairment in delayed recall observed here is also in line with prior reports (Aleman et al., 1999; Dickinson et al., 2004). Deficits of verbal and spatial working memory in schizophrenia tapped by the Wechsler digit span backward and spatial span backward subtests, respectively, are fairly consistent findings (Conklin et al., 2000; Silver et al., 2003; Dickinson et al., 2004), which were replicated in the current study. Previous studies have reported that the performance on the forward digit span task of schizophrenia patients is significantly poorer than that of healthy people, indicating impaired attentional function in schizophrenia (Conklin et al., 2000; Silver et al., 2003). The findings of the present study, by contrast, suggest that auditory attention as measured by the forward digit span subtest is preserved in schizophrenia. The discrepant findings regarding auditory attention in the present study relative to previous ones might be due in part to the distinct matching status between patients and controls regarding education and premorbid IQ.

In conclusion, our results suggest that patients with chronic schizophrenia have substantially lower intellectual function relative to their presumed premorbid level and that their memory impairment is even more severe than the IQ decline. To definitively delineate the lifetime course of cognitive decline in schizophrenia, longitudinal studies that range from childhood to the chronic phase are needed.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2007.11.002.

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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 co-segregating 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

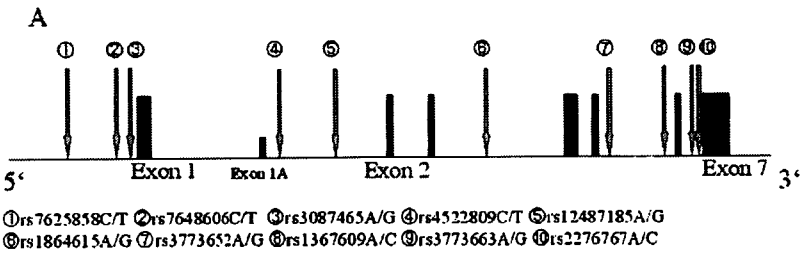


Fig. 1. Graphic representation of the TGFBR2 gene and the SNPs analyzed in the present study. Isoform B is major spliced variant without exon 1A. The amino acid sequence of isoform A contains an inset of 26 amino acids after Ser31, replacing Val132 of TGFBR2 isoform B.

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: $P = 0.86$).

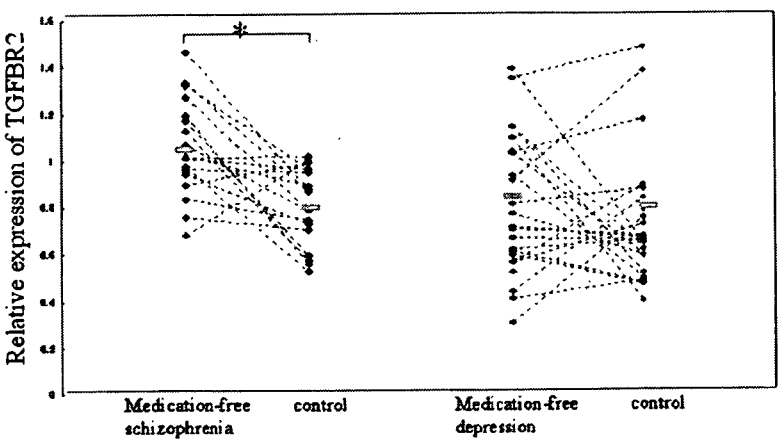


Fig. 2. Compared with the normal control group, the mean TGFBR2 mRNA level (isoform A + isoform B) in the leukocytes of medicaction-free schizophrenic patients ($N = 19$) was significantly higher than that of age- and sex-matched controls (patients: 1.05 ± 0.20, controls: 0.81 ± 0.16, paired $T$-test: $P < 0.001$). 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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# A Case of Schizophrenia with Chromosomal Microdeletion of 17p11.2 Containing a Myelin-Related Gene *PMP22*

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**Abstract:** We report a patient with schizophrenia who had a chromosomal deletion of 17p11.2 containing a myelin-related gene *PMP22* by using comparative genomic hybridization (CGH) array and quantitative PCR. Since genetic linkage to 17p11, reduced expression of PMP22, and alterations in myelination have previously been reported, this report further suggests an etiological role of *PMP22* in schizophrenia.

**Keywords:** Schizophrenia, PMP22, 17p11.2.

## INTRODUCTION

Chromosomal aberrations found in patients with schizophrenia have provided possibly important insights into the molecular mechanism of the illness [1,2], although such aberrations are rare [3]. For example, 22q11 deletion syndrome (22q11DS) is a well known risk factor for schizophrenia [4]. DISC1, which is one of promising candidate genes of schizophrenia, was found as a disrupted gene by chromosomal translocation [5,6]. In this context, we have been screening chromosomal abnormalities by using the array comparative genomic hybridization (CGH) in 42 schizophrenia subjects and found a patient who had a chromosomal microdeletion of 17p11.2 containing the myelin-related gene, *PMP22*. *PMP22* is causal to Charcot-Marie-Tooth neuropathy type 1 A (CMT1A) [7] and hereditary neuropathy with liability to pressure palsies (HNPP) [8]. Three copies of *PMP22* (duplication) result in CMT1A, while one copy (deletion) in HNPP. Patients with CMT1A have reduced nerve conduction velocities [9]. HNPP is characterized by diverse sensory or motor nerve palsies which are often precipitated by minor trauma.

## CASE REPORT

### Case History

The proband was 32-year-old man who was the first child of reportedly unrelated parents. He had a surgical operation for inguinal herniation when he was 2 months old. According to his mother, the development of his verbal communication was delayed. When he was 11 years old, he lost consciousness for a few minutes with no apparent external event inducing such loss of consciousness; however, no abnormality was detected with electroencephalography or the computed tomography (CT) scan of the head immediately after the loss of consciousness. The CT film is not available now because the brain CT scan was carried out more than 20 years ago in another hospital. He entered ordinary elementary and junior high schools without receiving any special education. Although he went on to enter a high school, he dropped out at the age of 17 years. When he was 19 years old, auditory hallucination and delusion of persecution developed. Six months later, he started antipsychotic treatment at a psychiatric clinic. Then he was introduced to our hospital to control his psychotic symptoms and adverse effects (tremor) induced by the antipsychotics with a clinical diagnosis of schizophrenia and mental retardation. Finally, his psychiatric symptoms and tremor were controlled by 100mg floropipamide, 2mg trihexyphenidyl, and 1mg biperiden. He had never showed motor paralysis or sensory disturbance. His intelligence quotient (IQ) was 42 by the Wechsler adult intelligence scale-revised (WAIS-R) [10] at the age of 29 years. Laboratory tests for blood and urine did not show any abnormality. His final diagnoses were schizophrenia and mental retardation, according to the structured interview of DSM-IV [11, 12].

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This study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Tokyo, Japan. Written informed consent was obtained from the proband and his parents to perform chromosomal examination, and publish this case report.

### Family History

His father was a professor of a university and his mother was a housewife with normal social function. Structural interview by Mini-International Neuropsychiatric Interview [13,14] did not indicate any present or past psychiatric diagnosis in his parents. The proband had a younger sister who had no current or past history of psychiatric illness according to the parents.

### Array CGH Findings

The proband participated in our ongoing screening of chromosomal abnormalities in a series of patients with schizophrenia. Chromosomal abnormalities were assessed with the array CGH method developed by Miyake *et al.* [15] using a newly developed 4.2K microarray with 4,235 FISHed BAC clones. A typical 1.4-Mb microdeletion at 17p11.2, containing *PMP22,* was identified. The deletion was also confirmed by fluorescence *in situ* hybridization (FISH) analysis (Fig. **1**).

### Quantitative PCR for Copy Number Variation

We examined copy number of *PMP22* for the proband and his parents by the quantitative PCR of genomic DNA. TaqMan probes were chosen, and delta-delta Ct method was applied, according to a previous study [16]. Quantitative PCR was done with the ABI prism 7900 (Applied Biosystems, Foster city, CA, USA). A Delta-delta Ct value around 0.5 indicates that the copy number of the gene is one and the value around 1 means two copies. The delta-delta Ct values of the proband, his father, and his mother were 0.65, 0.50, and 1.06, respectively, suggesting that the microdeletion of the proband was transmitted from his father.

### Neurological Assessment

A clinical neurologist assessed neurological symptoms of the proband. However, no symptom of HNPP was apparent including abnormality of deep tendon reflex. A nerve conduction study was carried out for the proband; however, the result was within normal limit except for the distal motor latency of right tibial nerve. He does not show *pes cavus* or hammer toes. These findings are incongruent with the phenotype of *PMP22* deletion described by Mouton *et al.* [17]. His father did not report any neurological symptoms, either, although a nerve conduction study was not carried out for him.

## DISCUSSION

We report a case of schizophrenia who had a microdeletion of chromosome 17p11.2 containing a myelin-related gene, *PMP22.* To our knowledge, this is the first report of such a case. Chromosomal microdeletion was confirmed by FISH and RT-PCR in addition to the initial array CGH method. Thus it is unlikely that the detected deletion was an artifact, although the case we report here was asymptomatic with respect to HNPP and had no clear family history of HNPP. Mouton *et al.* [17] investigated 99 individuals with the 17p11.2 deletion in 22 families and found that fourteen individuals showed no symptom. Thus asymptomatic individuals like our case seem to be not rare.

It is possible that coexistence of schizophrenia and the deletion of 17p11.2 in the proband may have occurred by simple coincidence. The absence of psychiatric history in the proband's father, who had the same deletion, further supports such a possibility. However, it is also possible that incomplete penetrance of the genetic effect of the deletion may explain the discrepancy in psychiatric condition between the proband and his father. Indeed, several lines of evidence in addition to our case have suggested that *PMP22* may play a role in the pathogenesis of schizophrenia. Dracheva *et al.* [18] reported that mRNA of *PMP22* was reduced in the hippocampus and anterior cingulate cortex in post mortem brains of schizophrenia. *PMP22* is highly expressed and plays a critical role in functions of oligodendrocytes, which accords with previous studies indicating the oligodendrocyte dysfunction in schizophrenia [19,20,21]. Decreased number of perineuronal oligodendrocytes was reported in the prefrontal cortex of patients with schizophrenia [22]. Transgenic mice which have oligodendrocyte dysfunction have increased levels of dopamine receptors and transporters [23]. Then defects in white matter can cause hyper-dopaminergic symptoms (e.g. delusion and hallucination). Reduced fraction anisotropy in white matter of patients with schizophrenia by a diffusion tensor imaging study [24] may due to oligodendrocyte dysfunction. Oligodendrocytes produce trophic factors such as brain derived neurotrophic factor (BDNF) [25] and neuregulins (NRGs) [26]. BDNF and NRG1 are believed in playing an important role in the etiology of schizophrenia. *PMP22* dysfunction may decrease the function of BDNF [27] and NRG1 [28]. Finally, some genome wide linkage studies provide evidence for linkage to 17p11.2-q25.1 in schizophrenic pedigrees [29,30,31]. To further elucidate the possible role of PMP22 in schizophrenia, molecular genetic studies and psychiatric examination on individuals with CMT1A and HNPP are warranted.

## CONCLUSIONS

In conclusion, we found a patient with schizophrenia who had a chromosomal microdeletion of 17p11.2 containing *PMP22,* a gene critical to oligodendrocyte functions. Since reduced expression of *PMP22,* alterations in oligodendrocyte functions, and genetic linkage to 17p11 have previously been reported, our case further supports an etiological role of *PMP22* in schizophrenia.

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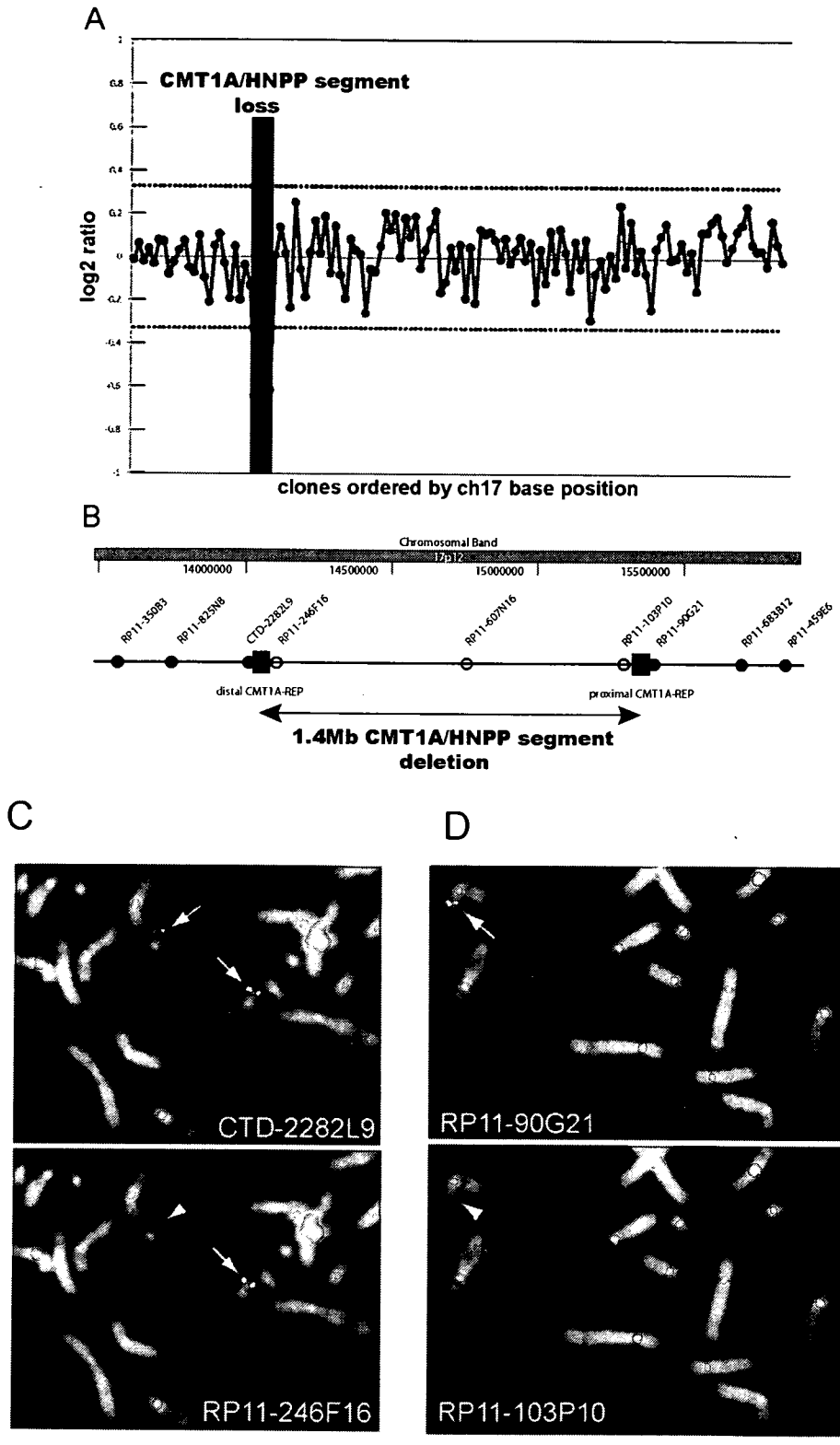


**Fig. (1).** (A) Microarray CGH analysis showing deletions at CMT1A/HNPP locus. (B) Schematic presentation of BAC clones delineating the CMT1A/HNPP deletion in the patient. Open circle: BAC clone deleted in the patient, closed circle: BAC clone not deleted in the patient. Closed square: low copy repeat which may have mediated genomic rearrangements. A common 1.4-Mb deletion of HNPP occurs between proximal CMT1A-REP and distal CMT1A-REP. (C,D) BAC FISH analysis in the proband. Arrows show intact signals in 17p11.2 region. Arrow heads show the loss of signal in 17p11.2 region. Clone positions are indicated in Fig. (**1B**).

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ORIGINAL ARTICLE

# Pituitary adenylate cyclase-activating polypeptide is associated with schizophrenia

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**Pituitary adenylate cyclase-activating polypeptide (PACAP, ADCYAP1: adenylate cyclase-activating polypeptide 1), a neuropeptide with neurotransmission modulating activity, is a promising schizophrenia candidate gene. Here, we provide evidence that genetic variants of the genes encoding PACAP and its receptor, PAC1, are associated with schizophrenia. We studied the effects of the associated polymorphism in the PACAP gene on neurobiological traits related to risk for schizophrenia. This allele of the PACAP gene, which is overrepresented in schizophrenia patients, was associated with reduced hippocampal volume and poorer memory performance. Abnormal behaviors in PACAP knockout mice, including elevated locomotor activity and deficits in prepulse inhibition of the startle response, were reversed by treatment with an atypical antipsychotic, risperidone. These convergent data suggest that alterations in PACAP signaling might contribute to the pathogenesis of schizophrenia.**
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**Keywords:** schizophrenia; PACAP; SNP; hippocampus; memory; PPI

## Introduction

Schizophrenia is a common neuropsychiatric disorder affecting 0.5–1% of the general population worldwide. This disease is characterized by psychosis and profound disturbances of cognition, emotion and social functioning. The pathophysiology of schizophrenia is still unclear; however, this disease is highly heritable[1] and several intermediate phenotypes such as neurocognitive dysfunction, abnormal brain morphology and deficits in prepulse inhibition (PPI) of the startle response are known to be useful to identify susceptibility genes for schizophrenia.[2,3]

The adenylate cyclase-activating polypeptide 1 (ADCYAP1) gene encodes pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide, which is a member of the vasoactive intestinal peptide (VIP)/secretin/glucagon family. It exerts multiple activities as a neurotransmitter or neuromodulator via three heptahelical G-protein-linked receptors, one PACAP-specific (PAC1) receptor and two receptors that are shared with VIP (VPAC1 and VPAC2).[4–6] PACAP induces cyclic AMP accumulation through activation of these receptors.[4–6] We generated mice lacking the PACAP gene ($PACAP^{-/-}$); these mice had profound behavioral abnormalities including hyperactivity and explosive jumping in an open field, increased novelty-seeking behavior and deficits in PPI.[7,8] In addition, the PACAP gene is located on 18p11, which linkage studies have suggested as a locus for schizophrenia and bipolar disorder.[9] Although previous studies indicated that the PACAP gene could be a good candidate gene for schizophrenia, only one preliminary study has examined a

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possible association with schizophrenia and reported negative results.[10] Here, we present data demonstrating a possible association between PACAP-PAC1 signaling and schizophrenia, using a multidisciplinary approach in both humans and rodents.

## Materials and methods

*Subjects*

Subjects for the clinical association study were 804 patients with schizophrenia (51.1% males with a mean age of 44.2 years (s.d. 14.5) and a mean age of onset of 24.8 years (s.d. 8.8)) and 967 healthy controls (47.7% males with a mean age of 40.4 years (s.d. 16.1)). All the subjects were biologically unrelated Japanese. Three hundred and fifty-one patients with schizophrenia and 518 controls were from Tokyo Metropolitan (the east part of Japan), and 453 patients with schizophrenia and 449 controls were from Aichi prefecture (the central part of Japan). Patients were recruited at the National Center Hospital of Mental, Nervous, and Muscular Disorders; Nagoya University Hospital; Showa University Hospital and hospitals related to Department of Psychiatry, Nagoya University Graduate School of Medicine or Department of Psychiatry, Showa University School of Medicine. Healthy controls, including hospital and institutional staff, were recruited from local advertisements in Tokyo and Aichi. Magnetic resonance (MR) measurements and neurocognitive tests were performed only on some subjects (MR measurements: 81 patients with schizophrenia and 201 healthy controls; neurocognitive tests: 62 patients with schizophrenia and 139 healthy controls), all of whom were recruited at National Center of Neurology and Psychiatry. Demographic information for the subjects receiving MR measurements and neurocognitive tests is shown in detail in Supplementary Table 1 and Figure 1b. Consensus diagnosis was made for each patient by at least two trained psychiatrists, according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria, based on clinical interview and other available information including medical records and other research assessments. No patient was diagnosed by medical records alone. Controls were healthy volunteers who had no current or past contact to psychiatric services. After a description of the study, written informed consent was obtained from every subject. The study protocol was approved by institutional ethics committees.

*Genetic analysis*

Venous blood was drawn from subjects and genomic DNA was extracted from whole blood according to standard procedures. Seven single nucleotide polymorphisms (SNPs) in the PACAP gene and three SNPs in the PAC1, VPAC1 and VPAC2 genes were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay, as described previously.[11,12] Primers and probes for the detection of the SNPs are available on request. Statistical analysis of genetic association studies was performed using SNPAlyse (DYNACOM, Yokohama, Japan). The presence of Hardy–Weinberg equilibrium was examined by using the $\chi^2$ test for goodness of fit. Allele distributions between patients and controls were analyzed by the $\chi^2$ test for independence. All *P*-values reported are two-tailed. Statistical significance was defined as $P<0.05$.

*Neuroimaging analysis*

All MR studies were performed on a 1.5 T Siemens Magnetom Vision plus system (Siemens, Erlangen, Germany). A three-dimensional volumetric acquisition of a T1-weighted gradient echo sequence produced a gapless series of 144 sagittal sections using an MPRage sequence (TE/TR, 4.4/11.4 ms; flip angle, 15°; acquisition matrix, 256 × 256; 1NEX, field of view, 31.5 cm; slice thickness, 1.23 mm).

Data were analyzed with Statistical Parametric Mapping 2 (SPM2) running on MATLAB 6.5. MR images were processed using optimized voxel-based morphometry (VBM) in SPM2 as described in detail previously.[13,14] Normalized segmented images were modulated by multiplication with Jacobian determinants of the spatial normalization to encode the deformation field for each subject as tissue density changes in normal space. Following modulation, images were smoothed using a 12 mm full-width half-maximum of isotropic Gaussian kernel, because previous studies had proved that this should be a reasonable filter.[13,15,16] In addition, we confirmed that the results of statistical analyses with three different smoothing filters (6, 8 and 12 mm Gaussian kernels) were essentially the same.

Statistical analyses were performed with SPM2, which implemented a general linear model. A hypothesis-driven regions of interest (ROIs) approach was used to investigate the hippocampus, using an ROI from the Wake Forest University PickAtlas.[17] Our hypothesis is that the PACAP genotype related to the risk of developing schizophrenia is associated with hippocampal volume, because PACAP is associated with hippocampal function in rodents, and hippocampal volume is reported to be reduced in schizophrenia. The genotype and diagnostic effects on hippocampal gray matter volume change were assessed statistically using a single-subject condition and covariate model with a significance level set to 0.05 (corrected for multiple comparisons within the ROI). Age and gender were included in the model to control for confounds. Anatomic localization was according to both MNI coordinates and Talairach coordinates, obtained from M. Brett's transformations (http://www.mrccbu.cam.ac.uk/Imaging/Common/mnispace.shtml) and presented as Talairach coordinates.

*Neurocognitive tests*

Several memory tests, subscales of the Wechsler Memory Scale revised version (logical memory I, logical memory II, visual reproduction I, visual reproduction II, verbal paired associates I (VPAI),