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機能的脳画像の最近の展開

音楽表出の機能的脳画像

山﨑　貴男[1]、吉川　宏起[2]、飛松　省三[1]、杉下　守弘[3]

【要旨】　機能的脳画像法（PET, fMRI）の進歩により、音楽の脳内処理基盤はかなり解明されてきたが、受容面に比べ表出面に関する研究は非常に少なく、未だ不明な点が多い。本稿では表出性失音楽に関する先行研究の結果をまとめた後、我々が行った fMRI の成績を紹介する。最後に研究上の問題点、今後の課題について述べた。

はじめに

音楽を歌う、演奏する、聴いて楽しむ等の能力は、ヒトにおける最も高次な脳機能の一つである。音楽の脳内処理基盤に関する研究は古くから脳損傷例の臨床的観察研究を主体に行われてきたが、近年の機能的脳画像法（PET, fMRI）の進歩により飛躍的にその脳内基盤は明らかになってきている。しかしながら、今までの研究は音楽受容（聴く）に関する研究が多く、表出面（歌う、ハミングする）に関する研究は非常に少ない。

本稿では、まず失音楽の定義、分類、責任病巣などについて述べた後、音楽表出（歌唱）の脳内基盤に関する先行研究の知見をまとめる。さらに我々が行った音楽表出（ハミング課題）に関する fMRI の研究を紹介し、最後に研究上の問題点や今後の課題について述べる。

I.　失音楽とは
失音楽の定義と分類

「後天的な脳損傷により生じた音楽能力の障害もしくは喪失」を失音楽（amusia）という[1]。失音楽は表出面の障害である表出性失音楽（expressive or motor amusia）と受容面の障害である受容性失音楽（receptive or sensory amusia）に大きく分けられる[2]。さらに表出性失音楽は声音性失音楽（vocal motor amusia）と演奏性失音楽（instrumental motor amusia）に分けられる[1]。

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声音性失音楽の定義

声音性失音楽は「歌唱、ハミング、口笛による音楽表出能力の障害」をいう[1]。メロディー表出が障害されるものを失メロディー症（amelodia）、リズム表出が障害されるものを失リズム症（arrhythmia）と呼ぶこともある[3]。

声音性失音楽の責任病巣

声音性失音楽を伴わない失語を呈する症例や、失語を伴わない声音性失音楽の症例が存在することから、言語と音楽は少なくとも部分的に異なった脳内処理が行われると考えられる。特に失語を伴わない声音性失音楽の症例は右半球損傷例がほとんどであり[4]、音楽表出（歌唱）には右半球がより関与すると考える研究者が多い。右半球の中では、側頭葉、前頭葉病変の報告が多い[4]。しかしながら、左半球損傷でも声音性失音楽を生じる[5,6]。また右半球損傷ではメロディー表出、左半球損傷ではリズム表出が障害されることが多い[4-6]。右半球だけでなく左半球も失音楽に関与するのは確実と思われるが、左半球損傷では失語症を生じることが多いために失音楽の評価が非常に困難であり、どの程度左半球が失音楽に関与しているかは未だ不明である。

なお非音楽家では脳損傷後に音楽能力の障害が生じても、病前の音楽能力が個々人によって異なるため、障害の有無、程度の評価は難しい。従って、過去の声音性失音楽の報告はほとんどが音楽家を対象にしており[4]、非音楽家においても同部位の障害で失音楽を生じるか否かは不明である。

II. 音楽表出に関する先行研究

Wada テスト

Gordon と Bogen[7] は、右利き非音楽家を対象に、左右の頸動脈にアモバルビタールを注入したところ、右側注入では歌唱（メロディー表出）の障害が認められたが、発話は保たれた。一方、左側注入では歌唱、発話とも障害されたが、その後の回復は歌唱が発話に比べ速く現れ、回復時間も速かった。従って、右半球が歌唱により関連していると述べている。

機能的脳画像

Perry ら[8] の PET 研究では、非音楽家を対象とし、歌唱時（メロディーの無い音を音節 /ä/ で歌う）の局所脳血流量（rCBF）を、受動的に音を聴いている時の rCBF と比較している。その結果、右側の補足運動野、左側の前部帯状回、両側の島前部、中心前回、小脳、右側の聴覚野が活性化した。Jeffries ら[9] の PET 研究では、よく知っている曲（ハッピーバースデー）を歌う時の rCBF を、発話（曲の歌詞を言う）時の rCBF と比較している。その結果、歌唱時は右側の上・中側頭回、島、上側頭溝、前頭前野、中心溝、小脳がより活性化し、逆に発話時は左側の上側頭回、縁状回、前頭弁蓋部がより活性化した。Riecker ら[10]、Ackermann ら[11] の fMRI 研究では、非音楽家を対象に、歌唱時（よく知っている曲（歌詞は無い）を音節 /La/ で歌う）と、発話時（1 月、2 月、……と月の名前を繰り返し言う）の BOLD 変化を比較している。歌唱時は右運動皮質、島前部、左小脳がより活性化し、逆に発話時は左運動皮質、島前部、右小脳が活性化した。また Zarate と Zatorre[12] は fMRI を用いて非音楽家と音楽家間での歌唱（発声－聴覚フィードバック機構）の脳内基盤の違いを検討している。その結果、両群とも両側の聴覚野、一次運動野、補足運動野、前部帯状回、視床、島、小脳の活性化を認めた。音楽家では非音楽家に比べ前部帯状回と島の活性化が強かった。

以上のように、各研究により課題や比較課題が異なるため結果も様々であるが、少なくとも歌唱と発話では脳内処理基盤は異なり、歌唱では発話に比べると右半球が優位に働くようである[9-11]。しかし発話課題との比較でなければ[8,12]両半球の活性化が認められ、左半球の働きも重要であると考えられる。ただし、後述するが歌唱課題では言語的な要素が含まれるため、メロディー表出と

左半球との関連性は検討しにくい。

III. 歌唱とハミングの違い

前述の如く声音性失音楽は「歌唱、ハミング、口笛による音楽表出能力の障害」と定義される[1]。我々は fMRI 研究を行う上で、この定義中の歌唱とハミングの違いについて注目している。歌唱では構音器官（口唇、舌、口蓋など）の働きを必要とする。また、ある曲を歌詞あるいは音節 /la/、/da/ などで歌うので、ある程度言語的な要素が含まれる。一方、ハミングでは構音器官を用いない。また歌詞がない曲であれば言語は表出されない。従って、ハミングは歌唱に比べてより非言語的であるといえる。

以上より、fMRI 研究を行う上で、より非言語的な音楽表出（ハミング）課題を用いることで、言語による左半球の活性化を減らすことができ、音楽表出に関連する脳内処理基盤（特に左半球の関与の程度）を検討しやすいと考えられる。

IV. fMRI 実験

我々は歌唱課題に比べてより非言語的であるハミング課題を用いた fMRI により、健常成人における音楽表出（メロディー表出）の脳内処理基盤の検討を行った。対象は音楽教育を受けた経験のない右利き健常成人で、言語の影響をできる限り少なくするために課題曲（エーデルワイス）のメロディーは知っているが、歌詞を知らない者を対象とした。課題は Song humming：エーデルワイスをハミングする（非言語性のメロディーのある表出）と Monotonous humming：「ンー」とうなる（メロディーのない表出）を用いた。機械雑音を減らす目的でイヤホンを装着しているため、両課題とも自分の声は主に骨導を通してフィードバックされる。撮影は 1.5 T GE SIGNA を使用し、課題 40 秒、課題間に Rest（40 秒）を挟むブロックデザインとした。解析は SPM2 を用いた。Conjunction 解析（Song humming vs. Rest と Monotonous humming vs. Rest の共通活性化部位の解析）の結果、メロディーの有無に関わらず非言語性表出に関連する脳部位は、両側の下前頭回、島、中心前回、前部帯状回、上・中側頭回、下頭頂小葉であることがわかった（図1）。さらに Song humming vs. Monotonous humming の結果、メロディー表出で特異的に活性化する脳部位は、
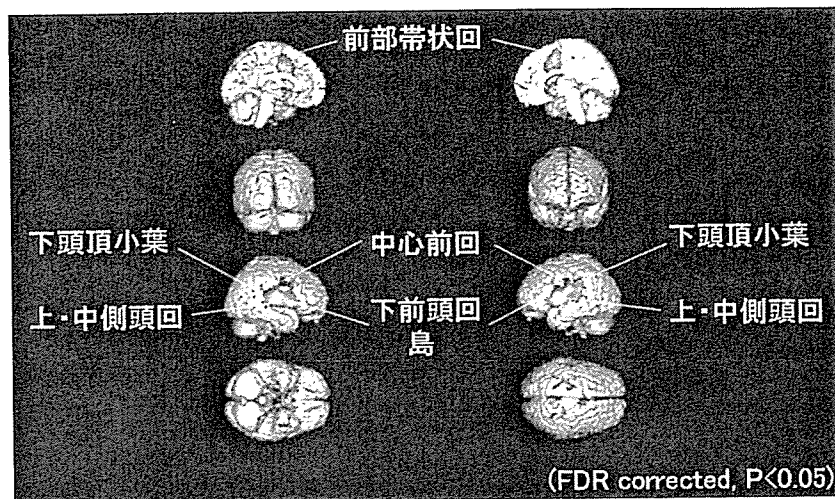
図 1. Conjunction 解析　(Song humming vs. Rest と Monotonous humming vs. Rest の共通活性化部位の解析)
における活性化脳部位
両側の下前頭回、島、中心前回、前部帯状回、上・中側頭回、下頭頂小葉の活性化を認める (FDR corrected, P＜0.05)。
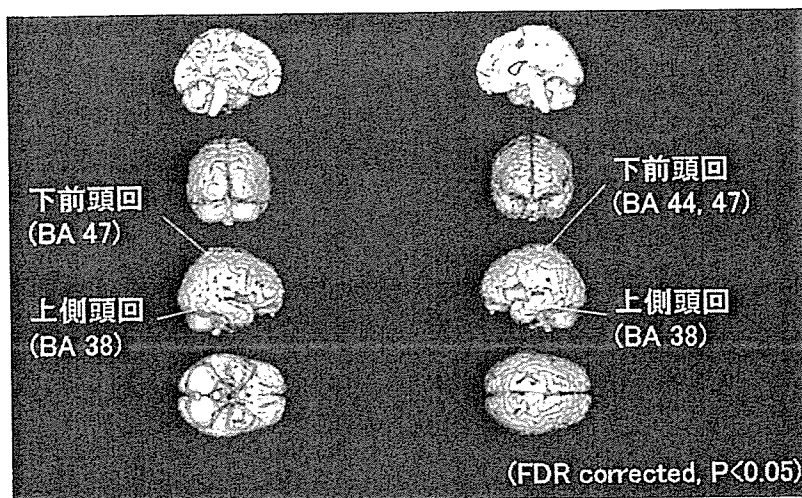
図 2. Song humming vs. Monotonous humming における活性化脳部位
左下前頭回 (Brodmann (BA) 44)、両下前頭回 (BA 47)、両上側頭回 (BA 38) の活性化を認める (FDR corrected, P＜0.05)。

左下前頭回 (Brodmann (BA) 44)、両下前頭回 (BA 47)、両上側頭回 (BA 38) であった (図 2)。

　これらの結果から、メロディーの有無に関わらず、非言語性表出では、運動コントロール (下前頭回、島、中心前回、前部帯状回)、聴覚性自己フィードバック (上・中側頭回)、運動ー聴覚情報統合 (下頭頂小葉) を行う脳部位が両半球性に活性化することがわかった。さらに、両下前頭回 (BA 47) は音楽構造の処理[13]、両上側頭回 (BA 38) は複雑なメロディー処理[14] に関与し、左下前頭

回 (BA 44) にはミラーニューロンが存在するとされていることから[15]、メロディーのある表出では運動ー聴覚コントロール機構に加えて、音楽構造やメロディーの処理、ミラーニューロンの活動に関連する脳部位が働くことが示唆された。

　以上のように、言語を出来る限り除去した音楽表出課題により左半球の活性化も認められたことから、音楽表出では右半球だけでなく、左半球の働きも重要であり、両半球性の脳内ネットワークが働くことが示唆された。

V. 問題点および今後の課題

　音楽表出の研究において、症例研究では音楽家での検
討がほとんどである。一方、機能的脳画像研究では非音
楽家での検討がほとんどである。従って、両手法により
音楽家と非音楽家間での脳内処理基盤の違いについて
検討していく必要があると思われる。

　また機能的脳画像法は空間分解能に優れるが時間分
解能が悪い。従って、事象関連電位など時間分解能に優
れた手法の併用や、時間分解能に優れた機能的脳画像法
の開発も望まれる。

おわりに

　音楽表出には従来考えられてきた以上に左半球の働
きも重要であり、両半球性のネットワークが働くと考え
られる。本稿で挙げた問題点や今後の課題について検討
することにより、音楽表出の脳内基盤がより明らかに
なっていくと思われる。

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ELSEVIER

Genetic structure of the dopamine receptor D4 gene (*DRD4*) and lack of association with schizophrenia in Japanese patients

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Abstract

In order to investigate the contribution of genetic variation in the human dopamine receptor D4 gene (*DRD4*) to the risk of developing schizophrenia, we carried out a genetic analysis of 27 polymorphisms in 216 schizophrenic patients and 243 healthy controls from the Kyushu region of Japan. Twenty-two single nucleotide polymorphisms (SNPs) and five insertion/deletion polymorphisms were analyzed in this study, including four novel SNPs and a novel mononucleotide repeats. Linkage disequilibrium (LD) and haplotype analyses reveal weak LD across the *DRD4* gene. In univariate analysis female individuals with allele −521C had a higher risk for schizophrenia. However, this finding was not significant after correction for multiple hypothesis testing. No other polymorphisms or haplotypes differed between schizophrenic patients and controls. Likewise, multivariate analyses did not reveal any statistically significant associations.
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Keywords: Schizophrenia; Genetic association; Polymorphism; Dopamine receptor D4; Linkage disequilibrium; Haplotype

1. Introduction

Schizophrenia is the most prevalent of the major psychotic disorders with 1% of the population affected worldwide. Although family, twin and adoption studies strongly suggest that genetic variation contributes to the etiology of schizophrenia (Gottesman, 1991; Kendler and Diehl, 1993), the underlying molecular basis and pathophysiological mechanisms leading to the development of schizophrenia are still unclear. Several lines of clinical and pharmacological evidence suggest the possible involvement of dopaminergic neurotransmission systems in the pathogenesis of schizophrenia (reviewed by Willner, 1997). The "dopamine hypothesis" is supported by the observation that dopamine receptor antagonists modulate the symptoms of schizophrenia and the observation of altered dopamine levels in the striatum, prefrontal cortex and limbic system of schizophrenic patients. Accordingly dopamine receptors have been a focus of genetic studies aimed at finding abnormalities associated with schizophrenia. In particular, the *DRD4* gene, a member of the D2-like dopamine receptor family, has been considered a strong candidate gene for schizophrenia. This is partly based on the finding that the atypical antipsychotic drug, clozapine, has a relatively high affinity for DRD4 (Van Tol et al., 1991), and

that elevated levels of DRD4 protein and mRNA were found postmortem in the brains of schizophrenia patients (Seeman et al., 1993; Stefanis et al., 1998). The DRD4 gene has also been investigated in attention deficit hyperactivity disorder (ADHD) and in relation to personality traits such as novelty seeking.

The *DRD4* locus is highly polymorphic (Cichon et al., 1995; Mitsuyasu et al., 2001; Mitsuyasu et al., 1999; Okuyama et al., 2000; Paterson et al., 1996; Seaman et al., 1999; Van Tol et al., 1992; Wang et al., 2004; Wong et al., 2000).

Association between various polymorphisms and schizophrenia have been reported by some investigators, however, efforts to replicate those results have generally been unsuccessful. Only three studies reported positive association results (Okuyama et al., 1999; Weiss et al., 1996; Xing et al., 2003). Among the polymorphisms analyzed for association with schizophrenia, the −521T/C polymorphism is one of the most extensively studied, not only in relation to schizophrenia (Jonsson et al., 2001; Jonsson et al., 2003; Mitsuyasu et al., 2001; Okuyama et al., 1999; Xing et al., 2003), but also ADHD (Bellgrove et al., 2005; Kirley et al., 2004; Lowe et al., 2004; Mill et al., 2003) and personality traits (Bookman et al., 2002; Ekelund et al., 2001; Joyce et al., 2003; Lakatos et al., 2002; Lee et al., 2003; Mitsuyasu et al., 2001; Okuyama et al., 2000; Ronai et al., 2001; Strobel et al., 2002; Strobel et al., 2003). However, although several studies suggest that the 48-base pair (bp) variable number of tandem repeat (VNTR) polymorphism in exon 3 of *DRD4* is associated with ADHD and personality traits (Faraone et al., 2005; Jonsson et al., 2003; Savitz and Ramesar, 2004; Schinka et al., 2002), the overall results of these extensive investigations are inconsistent.

Previously, we reported nine novel polymorphisms in the upstream region of the *DRD4* gene in the Japanese population (Mitsuyasu et al., 1999). Our analysis of five single nucleotide polymorphisms (SNPs), including −521T/C, in 208 schizophrenia patients and 210 normal controls revealed no significant association (Mitsuyasu et al., 2001).

In this report, we describe a more exhaustive analysis of polymorphism in the *DRD4* gene by carrying out LD and haplotype analyses with a total of 27 polymorphisms including the polymorphic 120-bp tandem repeat (TR) in the 5' UTR and the 48-bp VNTR in exon 3. Both SNP and haplotype based association analyses, using uni- and multivariate statistical methods, were carried out to clarify the relationship between schizophrenia and polymorphisms of *DRD4*.

2. Materials and methods

2.1. Study population

Two hundred sixteen schizophrenic patients fulfilling the DSM-IV diagnostic criteria for schizophrenia (121 male and 95 female), aged 18–82 (mean 51.5 ± 13.7, male

50.5 ± 14.0, female 52.7 ± 13.3), were recruited from nine hospitals in the northern area of Kyushu. 243 controls (138 male and 105 female), aged 30–71 years (mean 50.2 ± 4.6, male 52.1 ± 1.2, female 47.7 ± 6.1), were recruited from the personnel of the Japanese Self-Defense Forces and the staff of three hospitals in Fukuoka prefecture, Kyushu. All patients and controls were ethnically Japanese. There are no significant differences between the ages of the schizophrenic and control populations, or between male schizophrenics and controls, total female and total male populations or female and male schizophrenic populations. In contrast, there are significant age differences between female and male control populations and between female patients vs. controls: the average female control is 4.3 years younger than the average male control ($p < 0.0001$) and 5.0 years younger than the average female schizophrenic ($p = 0.001$).

The controls were selected based on information acquired from a questionnaire that interrogated various aspects of socio-economic, physical and mental status, as well as neuro-psychiatric and psychological characteristics. This questionnaire provides information similar to that obtained from batteries such as the Temperament and Character Inventory (Cloninger et al., 1993; Kijima et al., 1996), the Beck Depression Inventory (Beck et al., 1961), the State-Trait Anxiety Inventory (Spielberger et al., 1970), the Maudsley Obsessive-Compulsive Inventory (Hodgson and Rachman, 1977), and the Kurihama Alcoholism Screening Test (Saito and Ikegami, 1978). The inclusion criteria for controls were: (1) over 30 years old, (2) no physical or psychiatric history, (3) good social adjustment with occupation, and (4) no intellectual deficit. All control subjects were assessed for mental and physical illness by administering the Japanese edition of the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Sheehan and Lecrubier, 1998).

All subjects gave informed consent. This study was approved by and performed in accordance with the guidelines of the Ethics Committee of the Graduate School of Medical Sciences, Kyushu University.

2.2. Genotyping methods

Genomic DNA was purified from peripheral blood leukocytes as previously described (Lahiri and Nurnberger, 1991; Mitsuyasu et al., 2001). Genotyping experiments were performed using polymerase chain reaction (PCR) and/or direct sequencing methods. The amplified fragments and primer pairs for PCR are summarized in Table 1 and Fig. 1. Both the 120-bp TR and 48-bp VNTR polymorphisms were genotyped by detecting the length of each amplified fragment. The 26 other polymorphisms were genotyped by sequencing two PCR amplified fragments.

The 120-bp TR polymorphism was genotyped using a previously reported PCR-based typing method (Seaman et al., 1999). Genotypes were read based on the presence of 429-bp and/or 549-bp fragments.

Table 1
List of primers for genomic DNA fragment amplification and sequencing reactions

Name of primer	Sequence (5'–3')	Direction	Position[c]		Product size (bp)	Purpose
D4-120F[a]	GTTGTCTGTCTTTTCTCATTGTTTCCATTG	Sense	−1726	−1697	} 429, 549	Amp[f]
D4-120R[a]	GAAGGAGCAGGCACCGTGAGC	Antisense	−1179	−1199		Amp
D4iF3	CACACCTGTCCCTGGTGCAGG	Sense	−1256	−1236	} 606	Amp, Seq[g]
D4iR3	CCCACCCGTTGCACAGTTGATC	Antisense	−651	−672		Amp, Seq
D4iiF3	TACCTAGCTCACGGTCTTGGGC	Sense	−765	−744	} 1160	Amp
D4ivR2	CTGGAAGCTCCGCACCAGAAAG	Antisense	395	374		Amp
D4iiF5	GCTGTCCGCCCAGTTTCGGAG	Sense	−706	−686		Seq
D4pos3[b]	CTCAGGTCTTTCTGCGTCTGGC	Sense	−472	−451		Seq
D4EX1F[c]	CGCCATGGGGAACCGCAG	Sense	−4	14		Seq
D4iiiR1	GTGGCCACGCTCACGCACACG	Antisense	182	162		Seq
D4iiiR2	CGCTGAGCACCGCGGACAACG	Antisense	−17	−37		Seq
D4iiR1	TCGACGCCAGCGCCATCCTAC	Antisense	−346	−366		Seq
D4neg3[a]	CAGGTCACAGGTCACCCCTCTT	Sense	−947	−926	} 792	Amp, Seq
D4neg4[a]	TTGCTCATCTTGGAATTTTGCG	Antisense	−156	−177		Amp, Seq
D4-48F[d]	AGGTGGCACGTCGCGCCAAGCTGCA	Sense	2612	2636	} 174 + (48 × N[h])	Amp
D4-48R[d]	TCTGCGGTGGAGTCTGGGGTGGGAG	Antisense	2929	2905		Amp

[a] Seaman et al. (1999).
[b] Mitsuyasu et al. (1999, 2001).
[c] Catalano et al. (1993).
[d] Nanko et al. (1993).
[e] Relative position to the first nucleotide of initiation codon of the genomic sequence (GenBank Accession No. AC021663).
[f] Amp, these primers were used for PCR amplification.
[g] Seq, these primers were used for direct sequencing.
[h] N, number of repeats of the 48-bp sequence in exon 3.

The 48-bp VNTR was genotyped according to published methods (Nanko et al., 1993; Van Tol et al., 1992). PCR products were electrophoresed on an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., USA). The size of the amplified fragments was 174 bp plus 48 bp multiplied by the repeat number.

DNA sequencing was used to genotype 26 polymorphisms. First DNA sequencing templates were generated by PCR amplification of two DNA fragments (606-bp and 1160-bp) from genomic DNA of each individual (Fig. 1). PCR primers (Table 1) were designed based on GenBank Accession No. AC021663. The 606-bp fragment was amplified in a 10 μl reaction mixture that contained 1 μM of each primer, 0.2 mM of dNTPs (Amersham Biosciences Corporation, USA), 50 ng template DNA, 0.025 U/μl of AmpliTaq polymerase (Applied Biosystems, USA), 5.5 ng/μl of TaqStart Antibody (Clontech, USA), 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.5 mM MgCl₂ and 10% of dimethylsulfoxide (DMSO) (Wako Pure Chemical Industries, Ltd., Japan). Thermal cycling profile was 1 min at 95 °C for initial denaturation, followed by 40 cycles of 30 s at 95 °C, 30 s at 60 °C and 1 min at 72 °C, followed by a final incubation at 72 °C for 5 min. The 1160-bp fragment was amplified in 1 μM of each primer, 0.2 mM of dNTPs, 50 ng template DNA, 0.025 U/μl of KOD Dash polymerase (Toyobo, Japan), KOD Dash PCR buffer supplied by the manufacturer and 10% of DMSO in a total volume of 20 μl. The thermal cycling profile was 1 min at 96 °C for the initial denaturation, followed by 33 cycles of 30 s at 95 °C, 2 s at 63 °C and 30 s at 74 °C followed by a final incubation at 74 °C for 5 min.

These two DNA fragments were then used for 26 minisequencing reactions. First the template fragments were treated with two units of shrimp alkaline phosphatase (Roche Diagnostics Corporation, USA) and exonuclease I (New England Biolabs, USA) at 37 °C for 1 h. Both enzymes were heat inactivated at 80 °C for 15 min. Cycle sequencing was carried out by BigDye Terminator Cycle Sequencing Ready Reaction Kit ver 2.0 (Applied Biosystems, USA) according to the manufacturer's instructions. Depending on the fragments and primers used (Table 1 and Fig. 1), the protocols were slightly modified. Extension products were purified by Multiscreen 96-Well Filter Plates (Millipore, USA). Sample electrophoresis and data analysis were performed on the ABI PRISM 3100 and/or 3700 DNA Analyzer (Applied Biosystems, USA). Duplicate genotypes were generated from 133 individuals using as sequencing template a 792-bp fragment located between position −947 and −156, as previously described (Mitsuyasu et al., 2001). This fragment contains 12 polymorphisms (−713C/T, −616G/C, −615A/G, −603del/T, −600G/C, −598G/T, −597(G)₂₋₅, −521T/C, −376C/T, −364A/G, −291C/T and −234C/A) (Table 1 and Fig. 1) and was used to confirm results generated from the 1160-bp fragment.

2.3. Population genetic analyses

Hardy–Weinberg equilibrium of each bi-allelic polymorphism was assessed by χ^2 test. Pairwise LD statistic D' and r^2 were calculated with unphased genotype data by Haploview 3.2 software (Barrett et al., 2005). LD calculations were done for a total of 17 polymorphisms including 14
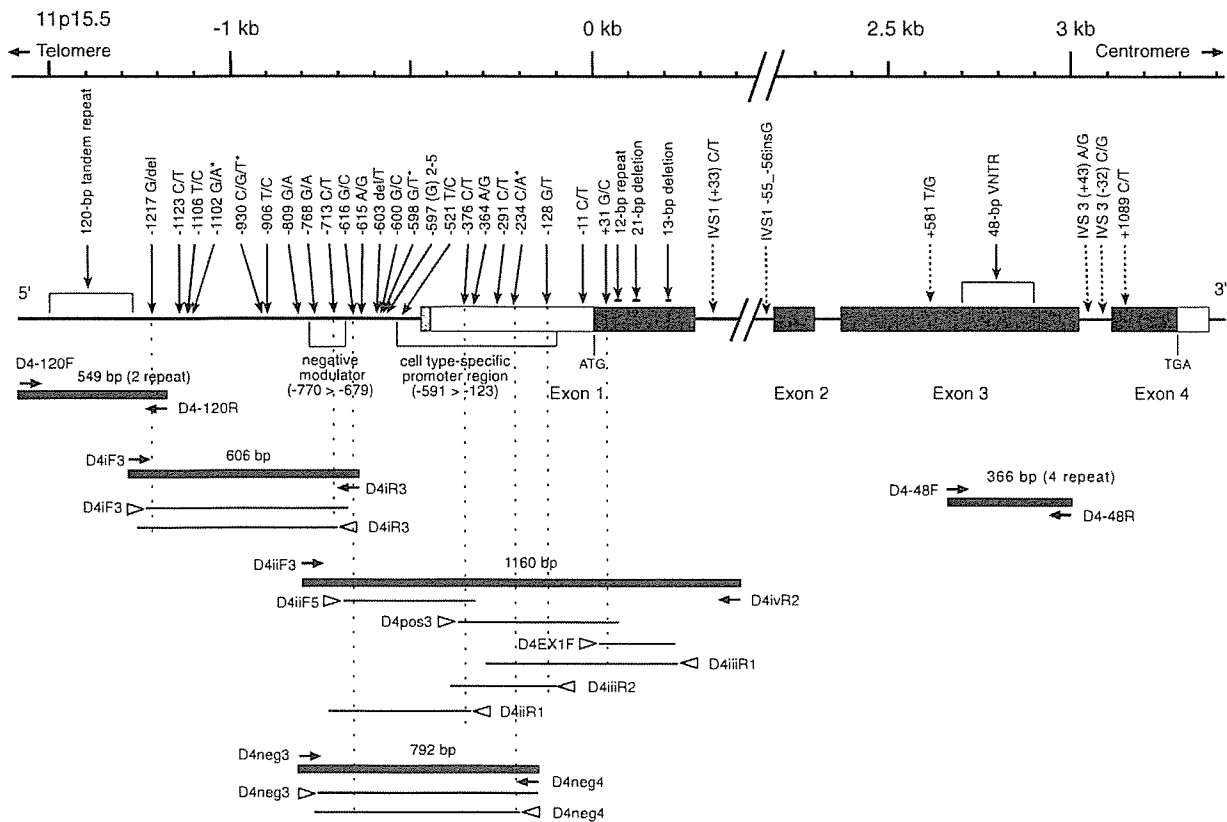
Fig. 1. Schematic representation of polymorphisms of the *DRD4* gene. The *DRD4* gene spans approximately 3.9 kbp consisting of four exons (black boxes: coding regions; white boxes: untranslated regions; hatched box: multiple transcription start sites). The region between position −1217 and +31 nucleotide (the numbering is relative to the first nucleotide of the initiation codon (ATG)) was extensively searched for novel or published SNPs. In total 34 polymorphisms (arrows) were collected from databases (dbSNP; Sherry et al.. 1999) and JSNP (Hirakawa et al.. 2002), published resources (PubMed) and our experiments. 28 polymorphisms (closed arrows) out of 34 were genotyped, including four novel polymorphisms (asterisks) first reported in this study. For genotyping, five fragments (bold lines) (549-bp, 606-bp, 1160-bp, 792-bp and 366-bp in length) were amplified by five primer sets (closed arrows; details are shown in Table 1) and sequenced by primers as indicated (open arrowheads). Thin lines next to open arrows indicate sequenced regions and orientation of primers. Exact positions of genotyped markers on each sequenced fragment are shown by longitudinal dotted lines. The reference sequence was AC021663 (GenBank).

biallelic polymorphic markers (120-bp TR, −1217G/del, −1106T/C, −906T/C, −809G/A, −768G/A, −713C/T, −616G/C, −603del/T, −600G/C, −521T/C, −376C/T, −291C/T and 12-bp repeat) for which minor allele frequencies exceeded 0.01, and three multi-allelic polymorphisms (−930C/G/T, −597(G)$_{2-5}$ and 48-bp VNTR). Since the Haploview software can analyze only bi-allelic data, we excluded individuals with allele T for −930C/G/T, and individuals with allele (G)$_2$ or (G)$_5$ for −597(G)$_{2-5}$. For the same reason, only individuals with genotype 4/4, 2/4, or 2/2 at the 48-bp VNTR were included. LD blocks were defined according to the confidence intervals described by Gabriel et al. (2002). Haploview LD analysis was carried out by selecting confidence intervals as specified in the software.

LD blocks in the 4.4-kb region of the *DRD4* gene were investigated and tag-SNPs (haplotype tagging markers) selected using Tagger software in Haploview. Markers whose r^2 values were more than 0.8 were selected by Tagger as part of an LD block.

Tag-markers selected using Tagger were used for haplotype estimation by PHASE ver 2.1 software (Stephens and Donnelly. 2003: Stephens et al.. 2001). The distribution of the predicted haplotypes was compared between: (i) all schizophrenic patients vs. all controls, (ii) female schizophrenic patients vs. female controls, and (iii) male schizophrenic patients vs. male controls by χ^2 test.

We also carried out a sliding window haplotype analysis using the HTR (Haplotype Trend Regression) program (http://statgen.ncsu.edu/zaykin/htr.html) (Zaykin et al.. 2002). This program estimates haplotype frequencies and performs a sliding window mode of haplotype association analysis between cases and controls. In this study, window size was set to be from 2 to 6 markers.

2.4. Statistical methods

Genotype frequencies of 17 polymorphic markers were compared between: (i) all schizophrenic patients vs. all controls, (ii) female schizophrenic patients vs. female controls,

and (iii) male schizophrenic patients vs. male controls by χ^2 test. When the expected number of any cell in a contingency table was less than 5, we employed Fisher's exact test. The significance level (α) for all statistical tests was two sided 0.05. Odds ratios (ORs) were calculated with 95% confidence intervals (CIs).

Following univariate analysis, stepwise logistic regression analyses were carried out using gender, age and the 17 polymorphic markers as independent variables. The binary dependent variable was "schizophrenia affected" = 1 or "control" = 0.

A modified Bonferroni procedure was used to correct for multiple hypothesis testing. According to Bonferroni, since multiple tests were performed, the α level of 0.05 should be divided by the number of tests. However, this correction is almost certainly too strict because of the existence of LD between some of the polymorphisms. Therefore we also used a modified Bonferroni correction (Nyholt, 2004). According to this method, an effective number of independent marker loci is calculated and used in the denominator of the Bonferroni correction. Another adjustment to Bonferroni's method (Li and Ji, 2005) was also used to calculate an effective number of polymorphisms.

Statistical calculations were performed using BMDP statistical software (BMDP Statistical Software, Inc., USA) and SPSS 13.0J software (SPSS Japan Inc., Japan). StatXact (Cytel Software Corporation, USA) was used to compute Fisher's exact test, except for 2 × 2 contingency tables.

Our sample size had a post-hoc power of 0.848 to detect an effect size of $w = 0.10$ (weak) at the 0.05 significance level (two-tailed), as calculated by software program G*Power (http://www.psycho.uni-duesseldorf.de/aap/projects/gpower/how_to_use_gpower.html) (Erdfelder et al., 1996).

3. Results

3.1. Polymorphism detection and genotyping

Fig. 1 shows the structure of the *DRD4* gene and the locations of all reported polymorphisms (see also Table 2). We collected data on 34 polymorphisms including 28 SNPs and six insertion/deletions within an approximately 4.9 kbp region. The data was obtained from dbSNP (Sherry et al., 1999) (http://www.ncbi.nlm.nih.gov/SNP/snp_summary.cgi), JSNP (Haga et al., 2002; Hirakawa et al., 2002), other published reports and our experiments. As shown in Fig. 1, there are 27 polymorphisms, including 22 SNPs, in the 1.8 kbp region starting 1.5 kbp upstream of the 3' end of exon 1. This is a much higher SNP density (12.2 SNPs/kbp) than the genome-wide average SNP density [reported to be 0.827 SNPs/kbp in dbSNP or 1.91 kbp/SNP by Sachidanandam et al. (2001)]. Table 2 summarizes data on 27 *DRD4* polymorphisms genotyped in this study, including four novel SNPs (−1102G/A, −930C/G/T, −598G/T and −234C/A) and one novel mononucleotide repeat polymorphism: −597(G)$_{2-5}$. The −597(G)$_{2-5}$ polymorphism was previously reported in the database as either −602G/del or −602(G)$_{8-9}$ (Mitsuyasu et al., 2001; Mitsuyasu et al., 1999; Okuyama et al., 2000). The −602(G)$_7$ and −602(G)$_{10}$ alleles were also identified in our experiments. In addition, a novel SNP (−598G/T) was found within the mononucleotide repeat of −602(G)$_{7-10}$. Thus, the −602(G)$_{8-9}$ polymorphism appears to be a combination of a guanine mononucleotide repeat with 2–5 units (−597(G)$_{2-5}$), together with a SNP at −598G/T and an invariant four guanine nucleotide repeat immediately upstream. Thus we suggest a designation of −597(G)$_{2-5}$ for this polymorphism instead of −602G/del or −602(G)$_{8-9}$. The −598G/T SNP was registered as −598G/A/del in the dbSNP database, however, our study showed only the −598G and T genotypes. For this reason, we classified this SNP as novel.

In order to understand the relationship between these polymorphisms, including the four novel SNPs, and the well studied 120-bp TR and 48-bp VNTR polymorphisms we include data on the latter in this study. The 120-bp TR is located approximately 0.8 kb upstream of the 5' end of exon 1. The 48-bp VNTR is in exon 3. It has been reported that two adjacent intronic SNPs (IVS3(+43)A/G and IVS3(−32)C/G) are in strong LD with the 48-bp VNTR 4 repeat allele (Ding et al., 2002). Based on that data we typed the 48-bp VNTR polymorphism as a representative marker for variation in the 3' region of the gene.

Twenty-seven polymorphisms were genotyped. (The 13-bp deletion in exon 1 could not be analyzed for technical reasons.) Twenty-one were biallelic SNPs (19 substitution, two insertion/deletion), one triallelic. Five SNPs (−1123C/T, −615A/G, −364A/G, −11C/T and +31G/C) were monomorphic in the study population, as was the 21-bp deletion (Table 2). Four markers (−1102G/A, −598G/T, −234C/A and, −128G/T) were singletons. These polymorphisms were not analyzed for disease association. The seven repeat allele of the 48-bp VNTR was rare; only four heterozygous genotypes (4/7) were found.

The genotype distribution of each biallelic polymorphism was consistent with Hardy–Weinberg equilibrium (data not shown).

3.2. Association with schizophrenia

Uni- and multivariate analyses were carried out with 17 polymorphisms to assess the effect of polymorphism on risk of developing schizophrenia. Specifically, 12 known SNPs (−1217G/del, −1106T/C, −906T/C, −809G/A, −768G/A, −713C/T, −616G/C, −603del/T, −600G/C, −521T/C, −376C/T, and −291C/T), three repeat polymorphisms (120-bp TR, 12-bp repeat, and 48-bp VNTR) and two novel polymorphisms (−930C/G/T and −597(G)$_{2-5}$) were analyzed. Results from univariate statistical analyses are shown in Table 2.

No polymorphisms differed in frequency between the schizophrenic patients and the controls, even before adjusting for multiple hypothesis testing (Table 2). Comparing the female schizophrenic patients with the female controls, we

Table 2
Comparison of genotype frequencies of polymorphisms of the *DRD4* between schizophrenic patients and controls in Japanese population

Polymorphism[a]		All			Female			Male			db SNP[c]	JSNP[d]	References
		Control	Schizophrenia	p[b]	Control	Schizophrenia	p	Control	Schizophrenia	p			
120-bp tandem repeat (−1480 to −1240)	n[f]	239	214	0.827	105	95	0.369	134	119	0.798	—	—	Paterson et al. (1996), Seaman et al. (1999)
	2/2	0.582	0.603		0.524	0.621		0.627	0.588				
	2/1	0.364	0.350		0.429	0.347		0.313	0.353				
	1/1	0.054	0.047		0.048	0.032		0.060	0.059				
−1217G/del[e]	n	238	209	0.713[g]	105	93	0.566[g]	133	116	0.497[g]	rs12720364	—	Okuyama et al. (2000), Wang et al. (2004)
	G/G	0.685	0.718		0.695	0.688		0.677	0.741				
	G/del	0.294	0.258		0.295	0.280		0.293	0.241				
	del/del	0.021	0.024		0.010	0.032		0.030	0.017				
−1123C/T	n	241	215		105	94		136	121		—	—	Okuyama et al. (2000)
	C/C	1.000	1.000		1.000	1.000		1.000	1.000				
	C/T	0.000	0.000		0.000	0.000		0.000	0.000				
	T/T	0.000	0.000		0.000	0.000		0.000	0.000				
−1106T/C	n	242	215	1.000[g]	105	94	0.226[g]	137	121	0.488[g]	rs936460	IMS-JST111981	Wang et al. (2004)
	T/T	0.798	0.800		0.838	0.777		0.766	0.818				
	T/C	0.190	0.186		0.162	0.202		0.212	0.174				
	C/C	0.012	0.014		0.000	0.021		0.022	0.008				
−1102G/A	n	239	214		103	94		136	120		—	—	Present study
	G/G	0.996	1.000		0.990	1.000		1.000	1.000				
	G/A	0.004	0.000		0.010	0.000		0.000	0.000				
	A/A	0.000	0.000		0.000	0.000		0.000	0.000				
−930C/G/T	n	240	214	0.396[g]	104	94	0.793[g]	136	120	0.365[g]	—	—	Present study
	C/C	0.979	0.967		0.981	0.979		0.978	0.958				
	C/G	0.017	0.023		0.019	0.011		0.015	0.033				
	C/T	0.000	0.009		0.000	0.011		0.000	0.008				
	G/G	0.004	0.000		0.000	0		0.007	0				
−906T/C	n	239	214	0.714	104	94	0.801[g]	135	120	0.289	rs3758653	IMS-JST111982	Wang et al. (2004)
	T/T	0.669	0.645		0.654	0.670		0.681	0.625				
	T/C	0.280	0.313		0.317	0.287		0.252	0.333				
	C/C	0.050	0.042		0.029	0.043		0.067	0.042				
−809G/A	n	241	215	0.486	104	94	0.097[g]	137	121	0.867	rs936461	IMS-JST111983	Mitsuyasu et al. (1999), Okuyama et al. (2000), Mitsuyasu et al. (2001)
	G/G	0.643	0.605		0.654	0.606		0.635	0.603				
	G/A	0.299	0.349		0.308	0.394		0.292	0.314				
	A/A	0.058	0.047		0.038	0.000		0.073	0.083				
−768G/A	n	240	215	0.544	104	94	0.449[g]	136	121	1.000[g]	rs4987058	—	Mitsuyasu et al. (1999), Mitsuyasu et al. (2001)
	G/G	0.963	0.977		0.952	0.979		0.971	0.975				
	G/A	0.038	0.023		0.048	0.021		0.029	0.025				
	A/A	0.000	0.000		0.000	0.000		0.000	0.000				
−713C/T	n	240	215	0.105[g]	104	94	0.049[g]	136	121	1.000[g]	rs11246224	—	Present study
	C/C	0.996	0.977		1.000	0.957		0.993	0.992				
	C/T	0.004	0.023		0.000	0.043		0.007	0.008				
	T/T	0.000	0.000		0.000	0.000		0.000	0.000				
−616G/C	n	240	210	0.503	103	94	0.486	137	116	0.876	rs747302	—	Mitsuyasu et al. (1999), Okuyama et al. (2000), Mitsuyasu et al. (2001)
	G/G	0.467	0.486		0.515	0.543		0.431	0.440				
	G/C	0.408	0.424		0.359	0.383		0.445	0.457				
	C/C	0.125	0.090		0.126	0.074		0.124	0.103				

SNP	Genotype	(239)	(197)	p	(103)	(94)	p	(137)	(116)	p	rs	IMS-JST	Reference
−615A/G	n	239	197		103	94		137	116		rs936462	—	Mitsuyasu et al. (1999), Okuyama et al. (2000), Mitsuyasu et al. (2001)
	A/A	1.000	1.000		1.000	1.000		1.000	1.000				
	A/G	0.000	0.000		0.000	0.000		0.000	0.000				
	G/G	0.000	0.000		0.000	0.000		0.000	0.000				
−603del/T	n	240	210	0.517	103	94	0.542	137	116	0.110	rs747303	—	Mitsuyasu et al. (1999)
	del/del	0.500	0.529		0.553	0.564		0.460	0.500				
	del/T	0.388	0.390		0.388	0.340		0.387	0.431				
	T/T	0.113	0.081		0.058	0.096		0.153	0.069				
−600G/C	n	240	210	0.107	103	94	0.499[a]	137	116	0.186[a]	rs10902180	—	Mitsuyasu et al. (1999)
	G/G	0.963	0.990		0.981	1.000		0.949	0.983				
	G/C	0.038	0.010		0.019	0.000		0.051	0.017				
	C/C	0.000	0.000		0.000	0.000		0.000	0.000				
−598G/T	n	238	207		100	93		138	114		—	—	Present study
	G/G	1.000	0.995		1.000	0.989		1.000	1.000				
	G/T	0.000	0.005		0.000	0.011		0.000	0.000				
	T/T	0.000	0.000		0.000	0.000		0.000	0.000				
−597(G)₂₋₅	n	239	210	0.347[a]	103	94	0.616[a]	136	116	0.302[a]	(rs3842250)	(IMS-JST186019)	Present study
	G2/G3	0.004	0.000		0.010	0.000		0.000	0.000				
	G3/G3	0.151	0.124		0.097	0.117		0.191	0.129				
	G3/G4	0.481	0.438		0.553	0.479		0.426	0.405				
	G3/G5	0.004	0.010		0.010	0.011		0.000	0.009				
	G4/G4	0.360	0.419		0.330	0.372		0.382	0.457				
	G4/G5	0.000	0.010		0.000	0.021		0.000	0.000				
−521T/C	n	239	206	0.330	102	93	0.046	137	113	0.930	rs1800955	IMS-JST186020	Mitsuyasu et al. (1999), Okuyama et al. (2000), Mitsuyasu et al. (2001)
	T/T	0.389	0.325		0.402	0.237		0.380	0.398				
	T/C	0.481	0.515		0.441	0.548		0.511	0.487				
	C/C	0.130	0.160		0.157	0.215		0.109	0.115				
−376C/T	n	237	212	0.808[a]	102	95	0.922[a]	135	117	0.452	rs916455	IMS-JST186021	Mitsuyasu et al. (1999), Mitsuyasu et al. (2001)
	C/C	0.814	0.835		0.843	0.832		0.793	0.838				
	C/T	0.181	0.160		0.147	0.158		0.207	0.162				
	T/T	0.004	0.005		0.010	0.011		0.000	0.000				
−364A/G	n	237	212		102	95		135	117		rs916456	—	
	A/A	1.000	1.000		1.000	1.000		1.000	1.000				
	A/G	0.000	0.000		0.000	0.000		0.000	0.000				
	G/G	0.000	0.000		0.000	0.000		0.000	0.000				
−291C/T	n	234	214	0.380	102	95	0.412[a]	132	119	0.501[a]	rs916457	IMS-JST186022	Mitsuyasu et al. (1999), Mitsuyasu et al. (2001)
	C/C	0.752	0.743		0.735	0.789		0.765	0.706				
	C/T	0.209	0.238		0.206	0.189		0.212	0.277				
	T/T	0.038	0.019		0.059	0.021		0.023	0.017				
−234C/A	n	237	212		101	95		136	117		—	—	Present study
	C/C	0.996	1.000		1.000	1.000		0.993	1.000				
	C/A	0.004	0.000		0.000	0.000		0.007	0.000				
	A/A	0.000	0.000		0.000	0.000		0.000	0.000				
−128G/T	n	237	214		102	95		135	119		—	—	Mitsuyasu et al. (1999)
	G/G	0.996	1.000		1.000	1.000		0.993	1.000				
	G/T	0.004	0.000		0.000	0.000		0.007	0.000				
	T/T	0.000	0.000		0.000	0.000		0.000	0.000				
−11C/T	n	239	197		93	88		131	109		—	—	Cichon et al. (1995)
	C/C	1.000	1.000		1.000	1.000		1.000	1.000				
	C/T	0.000	0.000		0.000	0.000		0.000	0.000				
	T/T	0.000	0.000		0.000	0.000		0.000	0.000				

Table 2 (*continued*)

Polymorphism[a]		Genotype frequency									db SNP[c]	JSNP[d]	References
		All			Female			Male					
		Control	Schizophrenia	p[b]	Control	Schizophrenia	p	Control	Schizophrenia	p			
+31G/C	n[f]	239	197		92	86		130	106		–	–	Cichon et al. (1995)
	G/G	1.000	1.000		1.000	1.000		1.000	1.000				
	G/C	0.000	0.000		0.000	0.000		0.000	0.000				
	C/C	0.000	0.000		0.000	0.000		0.000	0.000				
12-bp repeat (+64 to +87)	n	239	197	0.240	104	89	0.797[g]	135	108	0.205[g]	–	–	
	2/2	0.736	0.690		0.721	0.708		0.748	0.676				
	2/1	0.222	0.284		0.231	0.258		0.215	0.306				
	1/1	0.042	0.025		0.048	0.034		0.037	0.019				
21-bp deletion (+106 to +126)	n	239	197		102	88		134	106		–	–	
	+/+	1.000	1.000		1.000	1.000		1.000	1.000				
	+/–	0.000	0.000		0.000	0.000		0.000	0.000				
	–/–	0.000	0.000		0.000	0.000		0.000	0.000				
48-bp VNTR (+2689 to +2880)	n	237	212	0.618	102	95	0.507	135	117	0.170	–	–	Van Tol et al. (1992)
	4/4	0.696	0.736		0.716	0.726		0.681	0.750				
	4/2	0.186	0.160		0.167	0.158		0.200	0.164				
	4/5	0.055	0.047		0.059	0.063		0.052	0.034				
	4/3	0.013	0.014		0.029	0.000		0.000	0.026				
	4/6	0.017	0.009		0.000	0.011		0.030	0.009				
	4/7	0.013	0.005		0.000	0.000		0.022	0.009				
	2/2	0.004	0.009		0.010	0.021		0.000	0.000				
	5/5	0.008	0.005		0.000	0.011		0.015	0.000				
	3/3	0.000	0.005		0.000	0.011		0.000	0.000				
	5/2	0.000	0.005		0.000	0.000		0.000	0.009				
	5/3	0.004	0.000		0.010	0.000		0.000	0.000				
	5/6	0.004	0.000		0.010	0.000		0.000	0.000				

[a] Polymorphism names of each SNP or the number below names stand for nucleotide variation and relative position to the first nucleotide of the initiation codon of reference sequence AC021663 (141798 = +1).

[b] p values of χ^2 test (with Yates' correction for 2×2 table) were not corrected for multiple testing. There was no statistical significance after correction. Detailed statistical method was described in the text.

[c] dbSNP, a database of single nucleotide polymorphisms at National Center for Biotechnology Information.

[d] JSNP, a database of common gene variations in the Japanese population (Hirakawa et al., 2002).

[e] del, insertion/deletion polymorphism.

[f] n, the number of subject genotyped at each polymorphism.

[g] p values of Fisher's exact test were not corrected for multiple testing. There was no statistical significance after correction. Detailed statistical method was described in the text.

found significant differences – before correction for multiple hypothesis testing – in the distribution of both −713C/T ($p = 0.049$) and −521T/C ($p = 0.046$, Table 2). In the case of −713C/T, the minor allele frequency was very low (0.02 in female schizophrenic patients, 0 in controls). There were four heterozygous schizophrenic patients (no rare homozygotes) compared to zero in the female controls. The −521C allele was more frequent in the female schizophrenic patients than the female controls ($p = 0.034$, OR:1.58, 95% CI: 1.06–2.37). When comparing the OR for each genotype using genotype T/T as the referent in the female group, the OR for T/C was 2.11 (95% CI: 1.10–4.07) while the OR for C/C was 2.33 (95% CI: 1.01–5.38). If the −521C allele behaved as a dominant, the OR for the combined C/C and T/C female group would be 2.17 (95% CI: 1.17–4.04, $p = 0.021$) relative to the T/T female group. However, when either the Bonferroni correction, or a less conservative modified Bonferroni that accounts for LD (Li and Ji, 2005; Nyholt, 2004) was applied these results were no longer significant.

There were no significant differences between patients and controls in the male subgroup, even before multiple hypothesis correction (Table 2). Likewise, stepwise logistic regression analyses failed to detect any significant association between polymorphisms and schizophrenia.

Having failed to detect any influence of individual polymorphisms on risk of schizophrenia, we next sought to determine whether *DRD4* haplotypes might influence schizophrenia risk. Before using software to predict haplotypes it is efficient to first remove polymorphisms that are in strong LD with other polymorphisms. Accordingly, we determined the LD coefficient D' and the correlation r^2 between all pairs of 17 polymorphisms (Fig. 2 and Table 3).

The International HapMap Project (http://www.hapmap.org) includes data on only five DRD4 SNPs that are polymorphic in Japanese: rs3758653 (5′ flanking region), rs3889692 (exon 3), rs11246226, rs936465 and rs4331145 (3′ flanking region). These SNPs were analyzed by Haploview. Only one LD block was formed, comprising the three downstream SNPs. Two HapMap SNPs, rs3758653 (−906T/C in this study) and rs3889692 (not genotyped in this study) were not correlated with each other or the other four SNPs (r^2 values 0.024–0.061). These results indicate low LD across the *DRD4* gene.

We used our genotype data to analyze LD in the 4.4-kb region of the *DRD4* gene and select tag-markers using
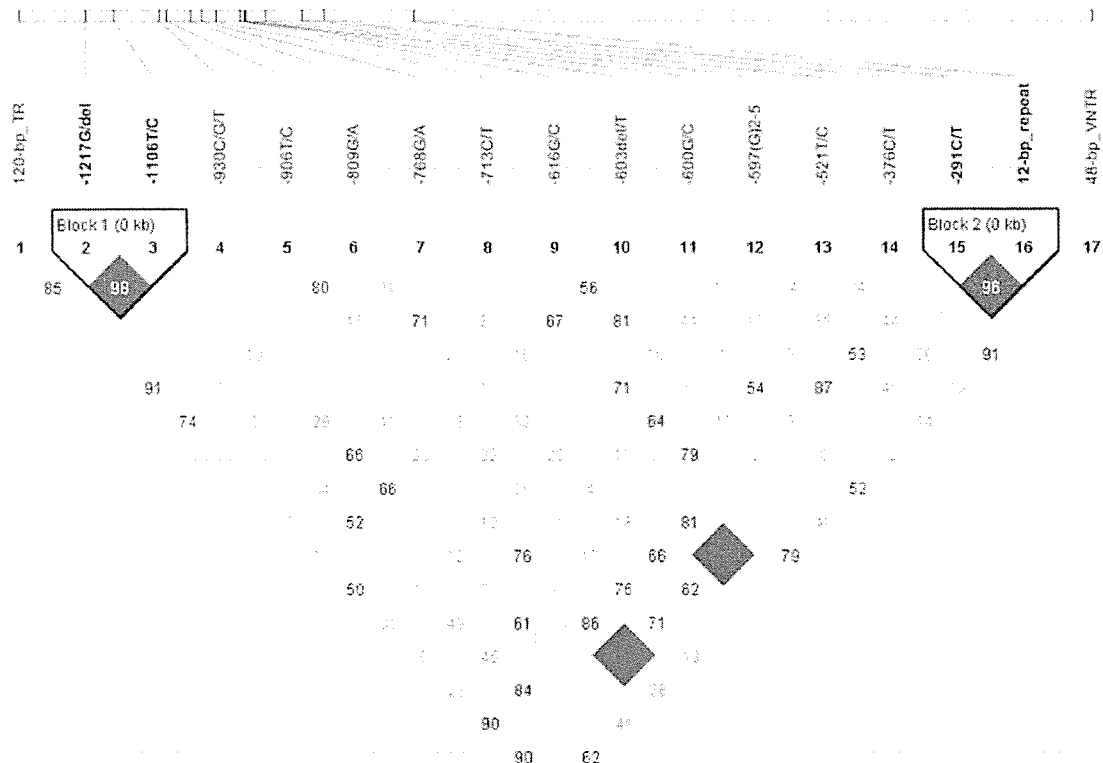

Fig. 2. LD coefficient D' representation of polymorphisms of the *DRD4* gene. The values in the boxes represent D' between pairs of markers. The LD display is from Haploview software. The boxes without values indicate complete LD ($D' = 1.0$). The dark grey boxes indicate strong LD. The light grey boxes indicate uninformative variant pairs. The white boxes indicate low LD. LD blocks were defined according to the algorithm in Haploview (Gabriel et al., 2002).

Tagger software in Haploview. Two small LD blocks were detected, one between −1217G/del and −1106T/C ($D' = 0.98$; $r^2 = 0.59$), the other between −291C/T and the 12-bp repeat ($D' = 0.96$; $r^2 = 0.86$). Other polymorphisms were only very weakly correlated, if at all ($r^2 < 0.80$). However, −376C/T and the 48-bp VNTR indicated relatively high correlation value ($r^2 = 0.78$). Based on this analysis 16 markers were selected for haplotype analysis. Thus, as a result of the low LD across the DRD4 gene, we were only able to decrease the independent polymorphism number from 17 to 16 tag-markers.

The 48-bp VNTR polymorphism is in strong LD with −376C/T ($D' = 0.91$, $r^2 = 0.78$). However, it did not exhibit a high r^2 value with any other polymorphism in the region between the 120-bp TR and the 12-bp repeat of DRD4 (Table 3). D' values between 120-bp TR and −906T/C ($D' = 0.91$) and −291C/T ($D' = 0.90$) were ⩾0.90. However, since corresponding r^2 values were less than 0.80, these polymorphisms could not be dropped based on our criteria for removing certain polymorphisms as described in Section 2.

Using 16 markers, a total of 136 haplotypes were estimated by PHASE. We compared the distribution of a total of 20 haplotypes with allele frequencies >0.01 between schizophrenics and controls in: (i) all subjects, (ii) female subgroup, and (iii) male subgroup. When the difference in haplotype frequencies was analyzed by the χ^2 test no significant differences were observed. Using 16 tag-markers, p values of sliding window haplotype analysis with window size 2 and 6 showed no statistically significant difference between schizophrenic patients and controls before adjustment for multiple hypothesis testing. Fig. 3 indicates the results of this analysis only for window sizes 2 and 3 (Fig. 3).

4. Discussion

In order to clarify the structure of genetic variation in the DRD4 gene and to further explore potential genetic influences on schizophrenia, we genotyped 216 Japanese schizophrenics and 243 healthy controls at 27 polymorphic sites, including four novel SNPs.

Not surprisingly, we found the allele frequencies of some polymorphisms to be different in the Japanese population compared to European or other populations: −615A/G is polymorphic in Caucasians (Ronai et al., 2004), however, it was monomorphic in our study. The same phenomenon was observed with −364A/G, −11C/T and +31G/C (Cichon et al., 1995) and with a 21-bp deletion reported in a single individual suffering from obsessive-compulsive disorder and panic disorder (Cichon et al., 1995). Other polymorphisms (−1102A, −930T, −713T, −598T, −597(G)2, −597(G)5, −234A and −128T) had very low allele frequencies in the Japanese population (Table 2).

In order to assess the relationship between schizophrenia and DRD4 polymorphisms, we carried out association analyses between Japanese schizophrenic patients and healthy controls. Univariate analyses indicated that none
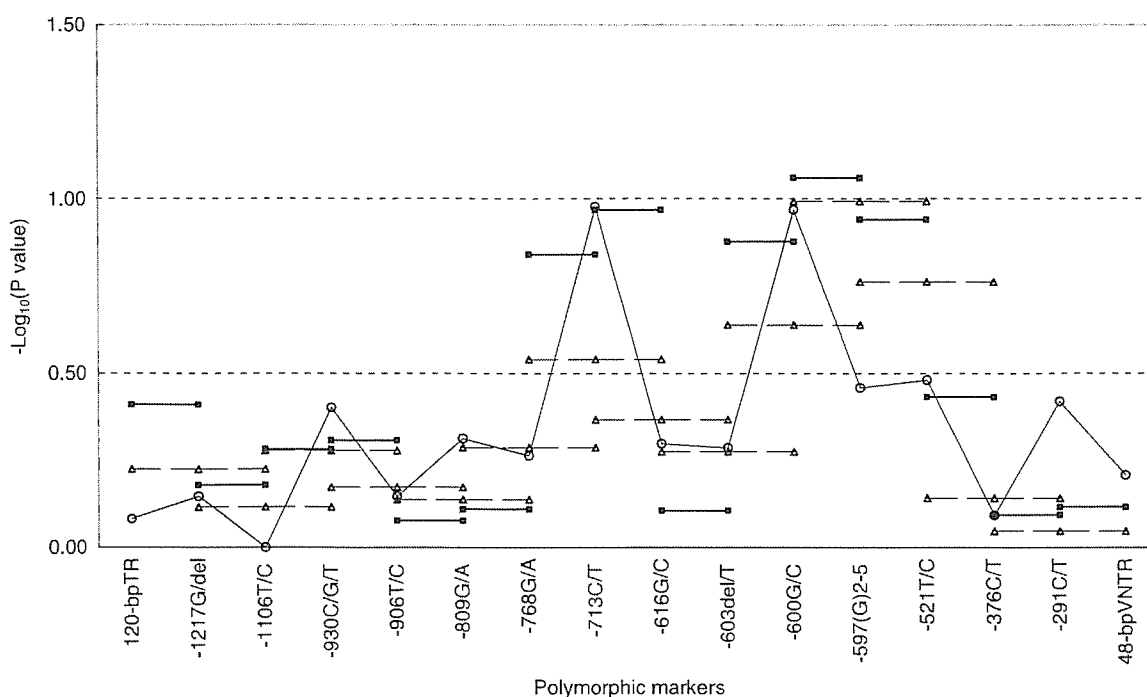
Table 3
Pairwise LD measure (r^2) of polymorphisms of the DRD4

	120-bp TR	−1217G/del	−1106T/C	−930C/G/T	−906T/C	−809G/A	−768G/A	−713C/T	−616G/C	−603del/T	−600G/C	−597(G)₂₋₅	−521T/C	−376C/T	−291C/T	12-bp repeat
−1217G/del	0.04															
−1106T/C	0.03	0.59														
−930C/G/T	0.05	0.00	0.00													
−906T/C	0.66	0.04	0.03	0.06												
−809G/A	0.46	0.00	0.01	0.06	0.62											
−768G/A	0.06	0.00	0.01	0.00	0.01	0.01										
−713C/T	0.03	0.00	0.00	0.00	0.03	0.02	0.00									
−616G/C	0.00	0.01	0.02	0.00	0.01	0.03	0.00	0.02								
−603del/T	0.01	0.02	0.02	0.00	0.01	0.01	0.00	0.01	0.28							
−600G/C	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.02	0.04						
−597(G)₂₋₅	0.02	0.00	0.02	0.01	0.00	0.00	0.00	0.01	0.02	0.14	0.00					
−521T/C	0.00	0.07	0.00	0.00	0.01	0.01	0.07	0.01	0.00	0.00	0.00	0.02				
−376C/T	0.02	0.00	0.02	0.06	0.39	0.28	0.10	0.05	0.05	0.08	0.00	0.03	0.02			
−291C/T	0.43	0.02	0.02	0.08	0.37	0.27	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.02		
12-bp repeat	0.47	0.03	0.02	0.00	0.01	0.01	0.00	0.00	0.06	0.00	0.00	0.02	0.02	0.02	0.86	
48-bp VNTR	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.04	0.06	0.08	0.00	0.04	0.02	0.78	0.02	0.02

Pairwise LD measures (r^2) were calculated by Haploview software.

Fig. 3. Sliding window haplotype analysis of the *DRD4* gene. The *X*-axis displays each polymorphism analyzed in this study. The *Y*-axis shows $-\log_{10}(p$ value) of each marker and sliding window (window size 2 and 3) haplotype analysis. Open circles indicated the result of single marker analysis of each polymorphism (univariate analysis). Each line between two closed boxes indicates *p* value of 2-marker sliding window analysis. Each dashed line with three triangles indicates *p* value of 3-marker sliding window analysis.

of the markers was statistically significant after correction for multiple hypothesis testing.

There have been inconsistent reports regarding the −521T/C polymorphism in schizophrenia. Okuyama et al. reported that the T allele of this polymorphism reduces *DRD4* transcriptional efficiency by 40% compared with the C allele, and that, in the Japanese population, this marker is associated with schizophrenia (Okuyama et al., 1999). However, attempts to replicate these results in other populations such as Chinese and Caucasian have failed (Ambrosio et al., 2004; Jonsson et al., 2001; Xing et al., 2003). Based on these results one might speculate that there is heterogeneity in the genetics of schizophrenia. However, our negative findings regarding −521T/C in another Japanese population suggest that the result of Okuyama may reflect type I error.

We also carried out LD and haplotype analyses, however, the *DRD4* region is unusual both in terms of high SNP density and low LD. Consequently the potential power of haplotype based association methods is not much different from SNP based approaches. Only two LD blocks were formed in the *DRD4* region, each consisting of only two Polymorphisms, leaving most polymorphisms as independent variables. These results are consistent with other reports on the population genetic structure of *DRD4* (Wang et al., 2004). No statistically significant haplotype associations with schizophrenia were detected.

There are several limitations of this study that should be borne in mind. One concern is that the control population may not be perfectly matched with the schizophrenic population. Most of the male controls were Japanese Self Defense Forces personnel aged about 50 years old. There might be some characteristics of this population that differ from other healthy control populations. Ideally more detailed socioeconomic information should be collected to guide selection of a balanced control population, and for inclusion in a statistical model along with genetic variables. Also, in view of the effects of environmental factors on the development of schizophrenia, it is important to collect as much information as possible on environmental exposures.

In conclusion, we report in detail the structure of genetic variation across the *DRD4* gene in the Japanese population. LD analysis revealed two small LD blocks, however, the most notable pattern was low LD across most of the gene. Haplotype analysis using 16 tag-markers selected by LD block analysis revealed no associations with risk of schizophrenia. Despite the biological role of DRD4 in dopamine signaling, and reports of functional effects associated with polymorphisms such as the 48-bp repeat, this report contributes to the increasing body of literature suggesting that the gene does not contribute significantly to risk of schizophrenia.

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STEP-BD: 米国NIMH双極性障害の縦断的治療研究

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Key Words
STP-EP-BD，双極性障害，治療研究，lamotrigene

1　はじめに

　STEP-BD（Systematic Treatment Enhancement Program for Bipolar Disorder）とは，米国NIMH（National Institute of Mental Health）により1998年9月～2005年9月まで行われた，多施設共同による双極性障害を対象とした薬物療法および心理社会的治療を含む長期的治療効果の検証を目的とする研究である[8,14]。本研究の中から，双極性障害の患者群を用いた種々のコホート研究，再発に関する前方視的研究，合併する他の精神医学的問題についての研究，長期的な薬物療法や社会的介入の効果についてなど，非常に多面的かつ膨大なデータが現在までに報告されている[8]。双極性障害に関するこのような大規模で縦断的な治療研究は世界的にみても初めてであり，今後も引き続きその結果について報告されるであろう。当然のことながら，その知見には注目が集まっており，その意義は重要である。本稿では，STEP-BDの概要について述べるとともに，現在までに報告されている調査結果について簡単に紹介していきたい。

2　STEP-BDの目的と方法

　1998年9月～2005年9月まで行われた本治療研究では，米国における多施設（カリフォルニア，コロラド，マサチューセッツ，オハイオ，オクラホマ，オレゴン，ペンシルバニア，テキサスの各施設）から当初は5,000人の双極性障害患者群の抽出を目標とされたが，結果として，総計4,360人の双極性障害患者を対象として行なわれた[8,14]。双極性障害の治療に用いられるすべての臨床的治療において最良の選択を評価する目的で，長期のフォローを行い，どの治療法あるいは治療法の組み合わせが，患者のうつ病相・躁病相の治療や，再発予防に対して最も有効なのかについて検討された。評価の対象となった治療は，薬物療法としては，気分安定薬（lithium，valproate），抗うつ薬（bupropion，paroxetine），抗精神病薬（lamotrigene，risperidone），その他（inositol，tranylcypromine）から，心理社会的治療としては，認知行動療法，家族指向療法，社会リズム療法および心理教育までと，多岐にわたっている[8]。

　STEP-BDは，基本的にはランダマイズド・ダブルブラインド・プラシーボコントロール研究であるが，2つの治療的経路 "pathways" があり，参加者はそのどちらに参加してもよいこ

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とになっている。その2つの"pathways"というのは，"Best Practice Pathway"と"Randomized Care Pathways"である。

"Best Practice Pathway"では，参加者はSTEP-BD研究認定の医師によってフォローされ，治療法の選択は個人にあわせて行う。参加者はその担当医とともに最良の治療法を決定し，必要があれば変更してもよい。従来の治療を続けたい者は，それも可能である。15歳以上の者がこのpathwayに参加できる[8]。

18歳以上の者は，もう1つの治療経路である"Randomized Care Pathways"に参加することもできる。参加者のそれぞれの症状によって，1つあるいはそれ以上のRandomized Care Pathwaysを提案される。ただし，気分安定薬による治療は継続する。しかし，担当する医師も，どの治療戦略がこの疾患に最適であるのかを決定しづらいため，他の薬物治療や対話療法が加えられることもある[8]。

Best Practice Pathwayと違い，Randomized Care Pathwaysは，参加者にランダムに振り分けられる。いくつかの場合では，バイアスを避けるために二重盲検法を用いることもある。もちろん，以前その患者が拒んだり，悪い反応を起こしたり，担当医がその患者にあわないと判断した薬物が割り当てられることはない。割り当てられた薬の料金は無料である。治療に反応しない場合は，他のpathwayに移行することもできるし，Best Practice Pathwayに戻ることもできる。本研究では，少なくとも約1,500人が少なくとも1つのRandomized Care Pathwayに参加することが期待された[8]。

STEP-BDでは，治療の連続性を失わないように配慮されており，はじめBest Practice Pathwayに参加していた者がRandomized Care Pathwayに移行した場合も同じ担当医および治療チームが担当し，Randomized Care Pathwayの終了後は，継続的な個人にあわせた治療目的のため，Best Practice Pathwayに戻ることができるようにデザインされている[8]。

3　これまでに得られたSTEP-BDによる結果および知見について

STEP-BDにおいては，本来の研究目的である治療効果の研究結果とともに，数々の疫学的，症候学的な知見も得られている。それらの報告は，現在までに30本以上の論文において報告されている[8]。また，参加者が500人，1,000人，2,000人となった時点においての種々の横断的な疫学的研究および縦断的な治療効果についての統計解析結果が報告されるというスタイルになっており，本稿ではそれらの主な知見について報告したいと思う。

1．First 500 participants

STEP-BD参加者の最初の500人に関して，4編の論文による報告がなされている[5,6,15,16]。

Lembke Aら[5]による最初の500人の参加者における心理社会的サービスの利用に関する報告によると，①STEP-BDに参加する3カ月前にほとんどの参加者（54％）が，薬物療法とともに，少なくとも1つの心理社会的サービスに参加していた。②それらは多い順番から，心理士による治療，自助グループへの参加，ソーシャルワーカーによる治療，その他のタイプのサービス供給者による治療の順であった。③また，人格障害を合併した群，アルコールあるいはその他の薬物乱用障害を合併した群，不安障害を合併した群は，それらを合併していない群と比較するとより心理社会的サービスを利用しやすい傾向にあったという。彼らは結論として，双極性障害に罹患した外来患者の心理社会的サービスの利用は，障害の多様性と重篤度がより大きくなるにつれて強く連関するようになると述べている。

Schneck CDら[15]は，STEP-BD参加者の最初の双極性障害Ⅰ型およびⅡ型の500人を対象に，ラピッド・サイクリング群とそうでない群の人口動勢，病歴，症候学的特長を比較した。対象の20％がラピッドサイクラーであった。また，ラピッド・サイクリング群の特徴は，女性