plasticity and adaptation, but, its role in behavioral sensitization is still known. The MAP kinase family consists of two major subgroups, the extracellular signal-regulated kinase (ERK), which induces cell growth and proliferation (classical MAPK cascade), and the stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) and p38, which induce apoptosis, the stress reaction, and gene transcription (novel MAPK cascade). MAP kinases are activated and phosphorylated by MAPKK and MAPKKK and selectively inactivated and dephosphoryrated by mitogen-activated protein phosphatases (MKPs). Many kinds of MKPs are known, MKP-1 and MKP-3 were examined because of their abundant distribution in the brain[52,53]. Rheb, a ras homologue, which is abundant in the brain and activates MAPK cascade[54], was also examined.

MKP-1 mRNA is abundantly distributed in the cerebral cortex and thalamus of the naive rat, whereas MKP-3 mRNA is restricted to the hippocampus[55] (fig 2). MKP-1 mRNA significantly increased about 60-300% in the several areas of the cortex, striatum, and thalamus, 0.5-1 h in response to acute and chronic methamphetamine administration (fig 3 and 4). MKP-3 mRNA had increased about 50% in the cortex, striatum and hippocampus 1 h after acute methamphetamine administration, but only in the hippocampus CA1 after chronic methamphetamine administration. Pretreatment with the D1 dopamine antagonist SCH 23390 completely abolished the increase in MKP-1 and MKP-3 mRNA levels induced by acute methamphetamine administration, but it was only partially abolished by the NMDA antagonist MK-801. The MKP-1 protein level also increased in the cortex 3 h after acute and chronic methamphetamine administration. Rheb mRNA was unchanged after acute and chronic methamphetamine adminsitration. MKPs are inactivators of MAPK[56,57], but they are directly activated by activation of MAPK. MKP-1 is selectively activated by SAPK/JNK and p38, and MKP-3 by ERK[58,59]. Therefore, the increase in MKP-1 and MKP-3 mRNAs observed after methamphetamine administration must mean activation of SAPK/JNK and p38, and ERK, respectively. Taken together, these findings indicate that acute methamphetamine administration may activate both the classical and novel MAPK cascades in several areas of cortex and striatum, the novel MAPK cascade in the thalamus, and the classical MAPK cascade in the hippocapmus. By contrast, chronic methamphetamine may active selected MAPKs in restricted regions, the novel MAPK cascade in the frontal cortex and the classical MAPK cascade in the hippocampus.

6. Conclusion

The differences in regulation of several genes related to morphological plasticity after acute and subchronic methamphetamine are quite significant, and they are summarized in Table 1. The behavioral sensitization phenomenon in response to psychostimulants is considered to

consist of two major distinct processes: an early induction process and a later maintenance or expression process. Induction of sensitization has been shown to occur after administration of several doses of psychostimulants. The neurochemical changes in increased dopamine efflux and morphological changes in dendrites and spines in the accumbens and frontal cortex were demonstrated after chronic amphetamine administration. However, recent studies have shown that even a single exposure to amphetamine also induced long-term behavioral and neurochemical sensitization[60]. The neural adaptation during persistent sensitization, such as synaptogenesis and neuritogenesis, must begin with the first psychostimulant exposure, and thus the changes in gene expressional seen after a single dose of methamphetamine and during chronic administration should correspond to the molecular mechanisms of the induction and maintenance processes of sensitization, respectively. During the induction process of sensitization, various processes involved in neural adaptation, synaptogenesis, sprouting and elongation processes, and activation of MAPK cascades begin abruptly throughout almost the entire brain, including the striatum, accumbens, frontal and several areas of cortex, and hippocampus. By contrast, no additional synaptogenesis or sprouting seemed to be required during the maintenance process, because synaptophysin and stathmin transcription returned to their basal levels. Neurite elongation indicated by the increased arc mRNA may persist in those brain regions in response to every methamphetamine exposure, and this sustained neurite elongation during repeated methamphetamine administration may contribute to re-arrangement of the neural networks for behavioral sensitization. Activation of the novel MAPK kinase cascade in the frontal cortex and classical MAPK cascade in the hippocampus as indicated by the increase in MKP-1 and MKP-3 mRNAs, respectively, also persisted during the maintenance process of sensitization. This may imply occurrence of apoptosis in the frontal cortex and neurogenesis in the hippocampus, which has not been demonstrated in the sensitization phenomenon. The actions and roles of many other plasticity-related genes should be investigated to further clarify the molecular mechanisms in very-long lasting neural adaptation during sensitization.

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自殺を惹起する精神疾患における Chromogranin B 遺伝子の関連解析

（分担研究者　稲田俊也　名古屋大学大学院医学系研究科精神生物学分野助教授）

研究要旨：自殺を惹起する精神疾患としては，統合失調症，覚醒剤精神病，躁うつ病などがある。統合失調症を対象としたDNAマイクロサテライトマーカーを用いたゲノムスキャンにおいて有意な差がみられた20番染色体上のマーカーD20S95の最も近傍に存在するChromogranin B遺伝子の変異検索を行い，5'側調節領域において5つの変異とExon4内に11個のアミノ酸置換を伴う変異を見いだした。これらの遺伝子変異について統合失調症患者187名と健常対照者192名を対象として症例対照群間比較を行った結果，Exon4内の4つ変異において，互いの連鎖不平衡，および統合失調症との関連が認められた。このほか，双極性障害および覚醒剤精神病についても健常対照者との症例群間比較を行ったが有意な関連は見いだせなかった。

研究協力者：飯嶋良味[1)]，有波忠雄[2)]，大槻露華[2)]，氏家 寛[3)]，尾崎紀夫[4)]，前田貴記[5)]，山内惟光[6)]，岩下 覚[6)]，坂元 薫[7)]，福永貴子[8)]，伊豫雅臣[9)]，関根吉統[10)]，原野陸正[11)]，小宮山徳太郎[12)]，山田光彦[13)]，曽良一郎[14)]，中平 進[15)]，樋口輝彦[16)]

研究協力者所属施設：1)国立精神・神経センター精神保健研究所，2)筑波大学基礎医学系遺伝医学，3) 岡山大学大学院医歯学総合研究科精神神経病態学分野，4)藤田保健衛生大学医学部精神医学教室，5)慶應義塾大学医学部精神神経科，6)社会福祉法人桜ヶ丘記念病院，7)東京女子医科大学神経精神科，8)東京女子医科大学第二病院心の医療科，9)千葉大学大学院医学研究院精神医学講座，10)浜松医科大学精神科，11) 久留米大学医学部精神神経科，12)国立精神・神経センター武蔵病院，13)昭和大学附属烏山病院精神神経科，14)東北大学大学院医学系研究科精神神経生物学分野　15)東京高尾病院，16)国立精神・神経センター国府台病院

A．研究目的

本研究の目的は統合失調症，覚醒剤精神病，躁うつ病などの自殺を惹起する精神疾患とクロモグラニンB遺伝子との間に関連がみられるかどうかを検討することである。臨床遺伝学的研究から，統合失調症や双極性障害などの精神疾患には遺伝要因の関与が示されているが，全ゲノムスキャンによるそれら精神疾患の遺伝子連鎖研究では，最近急速にその報告数が増加しているにもかかわらず，一貫して連鎖陽性を示す所見が得られないことから，多くの集団に共通する Major gene は存在しないことが示

唆されている。我々は，統合失調症の発症脆弱性に関連する遺伝子座位の系統的なスクリーニング解析を行い，20 番染色体上のマーカー D20S95 において症例・対照間に有意な差を見いだした(Kitao Y *et al., Psychiatr Genet* 2000; 10: 139-143)。この D20S95 の最も近傍の位置的候補遺伝子は Chromogranin B 遺伝子である。Chromogranin familly (A, B, C)は可溶性分泌タンパクで，種々の神経細胞や脳脊髄液中に分布し，シナプスからの伝達物質の放出をコントロールしていると考えられている。Chromogranin A および B が，統合失調症患者脳脊髄液中で有意に減少しているとの報告や(Landen M *et al., Eur Neuropsychopharmacol* 1999; 9: 311-315)，reserpine, clozapine, haloperidol などの抗精神病薬に反応して，脳内における mRNA の発現増加や発現の局在変化が観察されることから(Mahata SK *et al., Brain Res Mol Brain Res* 1993; 19: 83-92, Kroesen S *et al. Neuroscience* 1995; 69: 881-891)，薬物反応性のマーカーとして，さらには精神疾患病態に関与する機能的候補遺伝子として重要な役割を担っていると考えられる。われわれは，統合失調症において Chromogranin B 遺伝子の変異検索および関連解析を行い，Exon4 に位置する 4 つ変異において，統合失調症との強い関連を見いだした。さらに，中国人グループからも Chromogranin B 遺伝子 Exon4 中の別の変異において，統合失調症との関連が報告された(Zhang B *et al., Neurosci Lett* 2002; 323: 229-233)。また，Chromogranin B 遺伝子の位置する染色体 20pter～20p12 領域は，双極性障害においても，米国 NIMH の家系を用いた研究で，パラメトリック連鎖解析によってロッド値 1 以上が示された領域である(Detera-Wadleigh SD *et al., Am J Med Genet.* 1997; 74: 254-262)。今回われわれは，双極性障害および統合失調症と類似の精神症状を示す覚醒剤精神病について，Chromogranin B 遺伝子がそれら疾患と関連するかを検討した。

B. 研究方法

対象は東京近郊の精神科治療施設に通院または入院中の患者で，文書と口頭で本研究の目的と意義についての説明をおこない，書面での同意が得られた統合失調症患者 187 名，DSM-IV 診断基準で双極 I 型障害または双極 II 型障害と診断された 178 名および Japanese Genetics Initiative for Drug Abuse [JGIDA]（代表：氏家　寛）の各サンプル収集施設において収集された覚醒剤依存症患者 143 名である。健常対照者は，精神疾患に関する遺伝子解析研究に自発的意志により参加を表明し，年齢・性別のマッチした 192 名である。さらに覚醒剤依存症サンプルの解析については，症例対照間の地域格差の問題をなくすため，疾患群と地域・性別・年齢をマッチさせた健常対照者を加えている。各対象者から採血した血液より DNA を抽出し，Chromogranin B 遺伝子の各変異部位を PCR-Direct Sequence 法にて増幅，ABI 3100 genetic analyzer にて遺伝子型の判定を行った。

両群の遺伝子型出現頻度をそれぞれ集計し，2X2，2X3 の χ^2 検定を行った。有意水準は $p<0.05$ とした。なお，本研究は名古屋大学医学部および国立精神・神経センター国府台地区における倫理委員会の審査で承認を得て行っている。

C. 研究結果

統合失調症患者 24 名を用いて Chromogranin B 遺伝子の変異検索をおこなったところ，5'側調節領域において 5 つの変異，さらに Exon4 内に 11 個のアミノ酸置換を伴う変異を見いだした。このうち，Exon4 内の 8 個の変異については既に dbSNP データベースに登録されているものであった。また，我々の見つけた変異のうち 12 個は中国人集団でも確認された。その他の 4 個は我々が新規に同定したものであった。統合失調症患者 187 名，健常対照者約 192 名について関連解析を行った結果，全ての変異の出現頻度は Hardy-weinberg 平衡に矛盾しなかった。中国人集団において，統合失調症との関連の見られた 433G/A，533A/G について，本研究で用いた日本人集団における，統合失調症との関連は確認できなかった（表 1）。Exon4 内の 4 つの変異 1058G/C，1104G/A，1238C/T，1250G/A，において，アリル頻度，遺伝子型頻度ともに症例対照間で有意な差が見られた（表 2）。1058G/C と 1104G/A は完全に連鎖しており，これら 4 つの変異の間では強い連鎖不平衡が見られた。また，マイクロサテライトマーカー D20S95 とこれら変異との間にも連鎖不平衡が確認された。日本人集団において統合失調症との関連が認められた 1058G/C，1104G/A，1238C/T，1250G/A 変異について，双極性障害および覚醒剤依存症との関連解析をおこなった結果、それら疾患との関連は認められなかった。

D. 考察

Chromogranin は，種々の神経細胞に分布している可溶性分泌蛋白であり，褐色細胞種や神経内分泌腫瘍においては，カテコールアミン等と共に過剰に分泌されるため，診断の有用なマーカーであると考えられているが，生理的意義についてはまだ不明な点が多い。最近，統合失調症患者において，脳脊髄液中における Chromogranin A および B の有意な減少（Landen ら,1999）や海馬歯状回における Chromogranin A 陽性細胞数の有意な減少（Shibata ら，2000）が報告されており，今回のわれわれの結果も，Chromogranin B 遺伝子と統合失調症との間に有意な関連を示した。Chromogranin B 遺伝子は自殺を惹起する精神疾患の中でも統合失調症の病態生理に関連する有力な候補遺伝子の一つであると考えられ，現在さらに例数を増やして解析を進めていく予定である。双極性障害および覚醒剤依存症については、症例を増やして再検討するとともに，症例をサブタイプに分けて解析をおこなったり，別の部位についての検討も行っていく予定である。

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表 1　統合失調症とクロモグラニンB遺伝子多型（433G/A および 533A/G）との関連

433G/A	GG	GA	AA	n	Gratio	Aratio	95% CI	Odds ratio	GTvsHW	AF freq	GT freq
Control (Chinese)	71	70	29	170	62.0%	38.0%	(vs Cont)		0.110	(vs Cont)	
(Japanese)	73	90	24	187	63.1%	36.9%			0.963		
Schizo (Chinese)	103	74	16	193	73.0%	27.0%	1.170 - 2.180	1.600	0.600	0.0044	0.016
(Japanese)	77	85	23	185	64.6%	35.4%	0.695 - 1.264	0.937	1.000	0.672	0.878
533A/G	AA	AG	GG	n	Aratio	Gratio	95% CI	Odds ratio	GTvsHW	AF freq	GT freq
Control (Chinese)	51	85	34	170	55.0%	45.0%	(vs Cont)		0.900	(vs Cont)	
(Japanese)	51	90	47	188	51.1%	48.9%			0.918		
Schizo (Chinese)	94	74	25	193	68.0%	32.0%	1.280 - 2.340	1.730	0.093	0.0017	0.005
(Japanese)	41	100	45	186	48.9%	51.1%	0.689 - 1.223	0.918	0.738	0.559	0.439

表2　統合失調症とクロモグラニンB遺伝子多型（1058G/C, 1104G/A, 1238C/T, 1250G/A および1499G/A）との関連

1058G/C	GG	GC	CC	n	Gratio	Cratio	95% CI	Odds ratio	GTvsHW	AF freq	GT freq
Control	54	89	49	192	51.3%	48.7%	(vs Cont)		0.801	(vs Cont)	
Schizophrenia	87	82	21	190	67.4%	32.6%	0.381 - 0.684	0.510	0.973	0.000	0.000
Bipolar	51	84	43	178	52.2%	47.8%	0.721 - 1.285	0.963	0.892	0.797	0.955
Control for MAP	69	127	65	261	50.8%	49.2%	(vs Cont)		0.954	(vs Cont)	
MAP user	37	72	43	152	48.0%	52.0%	0.841 - 1.481	1.116	0.900	0.448	0.734
1104G/A	GG	GA	AA	n	Gratio	Aratio	95% CI	Odds ratio	GTvsHW	AF freq	GT freq
Control	54	89	49	192	51.3%	48.7%	(vs Cont)		0.801	(vs Cont)	
Schizophrenia	91	76	20	187	69.0%	31.0%	0.352 - 0.637	0.474	0.891	0.000	0.000
Bipolar	51	84	43	178	52.2%	47.8%	0.721 - 1.285	0.963	0.892	0.797	0.955
Control for MAP	71	126	65	262	51.1%	48.9%	(vs Cont)		0.925	(vs Cont)	
MAP user	38	72	43	153	48.4%	51.6%	0.843 - 1.482	1.118	0.900	0.440	0.736
1238C/T	CC	CT	TT	n	Cratio	Tratio	95% CI	Odds ratio	GTvsHW	AF freq	GT freq
Control	155	36	1	192	90.1%	9.9%	(vs Cont)		0.821	(vs Cont)	
Schizophrenia	147	38	2	187	88.8%	11.2%	0.724 - 1.832	1.152	0.995	0.550	0.766
Bipolar	142	35	1	178	89.6%	10.4%	0.655 - 1.703	1.056	0.821	0.823	0.974
Control for MAP	212	50	1	263	90.1%	9.9%	(vs Cont)		0.577	(vs Cont)	
MAP user	119	33	1	153	88.6%	11.4%	0.748 - 1.853	1.177	0.819	0.481	0.754
1250G/A	GG	GA	AA	n	Gratio	Aratio	95% CI	Odds ratio	GTvsHW	AF freq	GT freq
Control	71	84	37	192	58.9%	41.1%	(vs Cont)		0.670	(vs Cont)	

Schizophrenia	42	98	47	187	48.7%	51.3%	1.132 - 2.011	1.509	0.897	0.005	0.008
Bipolar	60	88	29	177	58.8%	41.2%	0.749 - 1.346	1.004	0.976	0.979	0.502
Control for MAP	95	123	45	263	59.5%	40.5%	(vs Cont)		0.937	(vs Cont)	
MAP user	60	73	20	153	63.1%	36.9%	0.644 - 1.150	0.860	0.970	0.310	0.528
1499G/A	GG	GA	AA	n	Gratio	Aratio	95% CI	Odds ratio	GTvsHW	AF freq	GT freq
Control	160	29	2	191	91.4%	8.6%	(vs Cont)		0.839	(vs Cont)	
Schizophrenia	166	18	2	186	94.1%	5.9%	0.380 - 1.163	0.665	0.754	0.150	0.270
Bipolar	141	25	1	167	91.9%	8.1%	0.547 - 1.582	0.930	1.000	0.789	0.895
Control for MAP	225	34	3	262	92.4%	7.6%	(vs Cont)		0.849	(vs Cont)	
MAP user	131	20	1	152	92.8%	7.2%	0.550 - 1.621	0.944	1.000	0.843	0.887

Relationship between catechol-O-methyltransferase polymorphism and treatment-resistant schizophrenia

Toshiya Inada[1)], Ataru Nakamura[2)], Yoshimi Iijima[3)]

1) Department of Psychiatry and Psychobiology, Nagoya University, Graduate School of Medicine, Nagoya, Japan; 2) Inokashira Hospital, Mitaka-shi, Tokyo, Japan; 3) National Institute of Mental Health, National Center of Neurology and Psychiatry, Chiba, Japan

Abstract: Catechol-O-methyltransferase (COMT) plays a crucial role in the regulation of central dopaminergic systems. We examined the allelic association of a functional polymorphism of the COMT gene with the clinical manifestations and the response to antipsychotics of 100 schizophrenic patients and 201 healthy controls from the general Japanese population. No statistically significant difference was observed in the allele and genotype frequencies between the schizophrenic patients and the healthy controls. The daily neuroleptic dosage that patients received during their maintenance therapy was significantly higher in patients with the L/L genotype than in the other patients ($p<0.05$). The present results suggest that the presence of the COMT genotype does not help in evaluating the susceptibility to the development of schizophrenia, but that it may help in the estimation of treatment-resistant features of schizophrenia.

Key words: COMT, dopamine, antipsychotic drug, extrapyramidal symptom, gene

INTRODUCTION

Polymorphisms at gene loci related to central dopaminergic systems have been extensively examined as candidate loci for schizophrenia because antipsychotic drugs, which act as dopamine antagonists, are effective in the treatment of psychiatric symptoms seen in schizophrenic patients.

Although the results are still controversial, a number of studies have shown a positive association between schizophrenia as a whole or subgroups of this disease with some specific polymorphic sites of the gene loci related to central dopaminergic transmission. However, whether specific mutations in the dopamine-related genes can contribute significantly to the clinical features of schizophrenia still remains to be demonstrated.

Catechol-O-methyltransferase (COMT) metabolizes catecholamines such as dopamine, and catechol drugs such as L-DOPA. The COMT gene, which lies on the cytogenetic band of 22q11.2 on chromosome 22, has been considered to be one of the candidate genes for schizophrenia because it inactivates dopamine. There is an amino acid alteration that determines the activity of the COMT enzyme (Lotta et al., 1995; Lachman et al., 1996; Sander et al., 1997). It has been shown that a G–A transition exists at codon 158 of the COMT gene that results in a valine-to-methionine substitution. The association with this functional polymorphism has been examined extensively in the general population of schizophrenic patients or in subgroups with various clinical features. For example, relationships with this polymorphism have been reported in schizophrenia (Ohmori et al., 1998), violence in patients with schizophrenia or schizoaffective disorders (Lachman et al., 1998), homicidal schizophrenia (Kolter et al., 1999), suicidal behavior in patients with schizophrenia (Nolan et al., 2000) and aggressive behavior in

patients with schizophrenia (Jones et al., 2001). This polymorphism is reportedly associated with specific subgroups of various psychiatric diseases such as type-I alcoholism (Tiihonen et al., 1999), higher alcohol consumption (Kauhanen et al., 2000), delirium tremens in alcoholics (Nakamura et al., 2001), polysubstance abuse (Vandenbergh et al., 1997), obsessive-compulsive disorder (Karayiorgou et al., 1997), rapid-cycling mood disorder (Papolos et al., 1998), female bipolar disorder (Mynett-Johnson et al., 1998), and depressive disorder (Ohara et al., 1998). However, there are also some reports of a lack of significant association with schizophrenia (Daniels et al., 1996; Strous et al., 1997; Wei et al., 1999; de Chaldee et al., 2001; Liou et al., 2001), affective disorders (Kunugi et al., 1997) and alcoholics (Ishiguro et al., 1991), suggesting that the exact role of COMT polymorphism in determining the phenotype in terms of clinical psychiatry and human behavior is not yet fully elucidated.

The present study examined the relationship between COMT and schizophrenia in the Japanese population, focusing on the allelic association of COMT polymorphism with the clinical manifestations and antipsychotic features of schizophrenia.

METHODS

Ethical considerations: This study had the approval of the ethics committee of the Kohnodai Area, National Center of Neurology and Psychiatry, Chiba, Japan. Written informed consent was obtained from all subjects who participated.

Subjects: The subjects for the present study were chronic inpatients who met the criteria of the DSM-IIIR diagnosis (American Psychiatric Association, 1987) for schizophrenia, who had been hospitalized and had been receiving antipsychotic therapy for at least one year during their current hospitalization. Volunteer control subjects were mostly medical staff with no history of psychosis or substance abuse, or of receiving antipsychotic medication. All subjects were Japanese and had Japanese parents.

Status of extrapyramidal symptoms: Extrapyramidal symptoms (EPS) induced by antipsychotic medication were basically evaluated using DIEPSS (Inada et al., 2002): we assumed that acute EPS had been present within 3 months of the initial neuroleptic therapy if the clinical records clearly described EPS and a subsequent reduction in the neuroleptic dosage or a subsequent addition of antiparkinsonian drugs. Patients who showed no signs of acute EPS in spite of receiving neuroleptic therapy for more than 6 months were regarded as not having acute EPS. Tardive dyskinesia (TD) was assessed with the Japanese version of the Abnormal Involuntary Movement Scale (AIMS). The diagnosis of TD was made according to the criteria of Schooler and Kane (1982). The inclusion criteria used to subclassify patients with or without TD were those reported by Inada et al. (1997). Briefly, patients who had been suffering from TD with at least one item persistently rated 3 on the AIMS for more than 1 year or patients who had been suffering from persistent TD for more than 1 year that had developed within 5 years after the first neuroleptic exposure were classified as having TD. Patients who had never developed TD despite receiving neuroleptics for more than 10 years were regarded as not having TD. In rating EPS and TD, suspected cases were videotaped for later evaluation. When information about the presence of acute EPS in the initial stage of neuroleptic therapy for the first episode of schizophrenia was incomplete, the patient was excluded from the acute EPS study. When the TD status could not be obtained, the patient was omitted from the TD study.

Neuroleptic dosage: Information about the neuroleptic therapy that schizophrenic patients had been receiving was obtained from their clinical records. The daily neuroleptic dosage was calculated from the most recent 1-year neuroleptic prescription history. The chlorpromazine-equivalent daily dose administered to each patient was calculated from a table developed specifically for Japanese patients (Inagaki et al., 1998).

Definition of treatment-resistant schizophrenia: Schizophrenic patients were diagnosed as having treatment-resistant schizophrenia (TRS) when they had been hospitalized for more than 1 year and had been receiving antipsychotic therapy at dosages of at least 1,000 mg/day chlorpromazine equivalents for more than 1 year.

Experimental procedure: Genomic DNA was extracted from samples of whole blood obtained from the subjects. The COMT polymorphism is generated by the presence of a G or A encoding a valine or methionine at codon 158. The 210-bp target segment was amplified by the polymerase chain reaction (PCR) method according to the standard protocol (Daniels et al., 1996), using the primers 5'-ACT GTG GCT ACT CAG CTG TG and 5'-CCT TTT TCC AGG TCT GAC AA. The

Table 1. Genotype and allele frequencies of catechol-O-methyltransferase H/L polymorphism in schizophrenic patients and controls

Group	Genotype distribution			*p* value	Allele frequency		*p* value
	H/H	H/L	L/L	(vs controls)	H	L	(vs controls)
Controls (*n*=201)	43.8%	49.8%	6.5%	-----	68.7%	31.3%	-----
Schizophrenia (*n*=100)	41.0%	51.0%	8.0%	0.88	66.5%	33.5%	0.59
Delusion and hallucination (*n*=64)	51.6%	43.8%	4.7%	0.53	73.4%	26.6%	0.31
Bizarre behavior (*n*=70)	50.0%	44.3%	5.7%	0.67	72.1%	27.9%	0.44
Disorganization (*n*=72)	44.4%	51.4%	4.2%	0.77	70.1%	29.9%	0.74
Negative symptoms (*n*=54)	55.6%	38.9%	5.6%	0.30	75.0%	25.0%	0.20
Positive first-degree family history (*n*=42)	47.6%	45.2%	7.1%	0.87	70.2%	29.8%	0.78
Onset at 21 years or younger (*n*=45)	44.4%	48.9%	6.7%	0.99	68.9%	31.1%	0.97

For comparison between controls and schizophrenic patients as a whole or their subgroups listed in this table, two-tailed chi-squared tests for 2×2 and 2×3 contingency tables were performed.

PCR products were digested with 5 units of Nla III for 2 h at 37°C, and electrophoresed using automated and manual staining of DNA separated on polyacrylamide gels with a DNA silver staining kit (Pharmacia Biotech, Tokyo, Japan).

Statistics: Allele and genotype frequencies were compared by using the chi-squared test for 2×2 and 2×3 contingency tables. The association between neuroleptic-induced EPS (the status of TD and acute EPS) and the polymorphism was also assessed by the chi-squared test. Comparison of the daily neuroleptic dosage between the L/L and the other genotypic subgroups was performed using the Mann–Whitney U test. The probability values are listed in the tables. The computer package SPSS for Windows (release 11.0J, SPSS Japan, Tokyo, Japan) was used for the statistical analyses.

RESULTS

The COMT genotype (H/H, H/L, L/L) and allele frequency in schizophrenic subjects are shown in Table 1. No significant differences in allele frequencies were observed between the healthy controls and whole schizophrenic patients or any of the subgroups classified according to the psychiatric symptoms seen at the patient's first episode.

Table 2 shows the genotype and allele frequencies of the COMT H/L polymorphism and the status of TD and acute EPS in schizophrenic patients. No significant differences were observed in the susceptibility to neuroleptic-induced extrapyramidal side effects among three subgroups of the COMT genotype.

The characteristics of neuroleptic treatment among the three subgroups showing COMT H/L polymorphism are shown in Table 3. The daily neuroleptic dosage received during maintenance therapy was significantly higher in patients with the L/L genotype than in the other patients (Mann–Whitney U test, $z = -2.248$, $p = 0.0246$). The rate of TRS tended to be higher in patients with the COMT L/L genotype than in the other patients, although this is a marginal difference statistically expressed as a significant trend level (chi-squared = 3.782, df = 1, $p = 0.052$). The odds ratio of L/L for TRS was 4.392 (95% confidence interval: 0.894–21.588).

DISCUSSION

In the present study, no significant association was observed between COMT polymorphism and schizophrenic patients as a whole or any of the subgroups classified according to the psychiatric symptoms seen at the

Table 2. Genotype and allele frequencies of Catechol-O-Methyltransferase H/L polymorphism and the status of TD and acute EPS in schizophrenic patients

Group	Genotype			*p* value	Allele frequency		*p* value
	H/H	H/L	L/L		H	L	
Status of TD							
Patients with TD (*n*=33)	42.4%	51.5%	6.1%	0.79	68.2%	31.8%	0.60
Patients without TD (*n*=46)	34.8%	58.7%	6.5%		64.1%	35.9%	
Status of acute EPS							
Acute EPS present (*n*=30)	43.3%	50.0%	6.7%	0.67	68.3%	31.7%	0.44
Acute EPS absent (*n*=45)	33.3%	57.8%	8.9%		62.2%	37.8%	

For comparison of the status of TD and acute EPS, two-tailed chi-squared tests for 2×2 and 2×3 contingency tables were performed. Abbreviations: TD, tardive dyskinesia; EPS, extrapyramidal symptom.

patient's first episode, suggesting that this polymorphism is unlikely to play an essential role in the development of schizophrenia. The lack of association with the general population of schizophrenic subjects is consistent with previous reports of French (de Chaldee et al., 2001), Taiwanese (Liou et al., 2001), Turkish (Herken and Erdal, 2001), and Caucasian (Wei et al., 1999) schizophrenic populations, but contradictory to the report of Ohmori et al. (1998). Although most of the studies failed to detect significant relationships between this polymorphism and schizophrenic subjects as a whole (Chen et al., 1999; Semwal et al., 2001), relationships with this polymorphism have been reported in certain subgroups of schizophrenia (Herken and Erdal, 2001; Liou et al., 2001). Recently, Egan et al. (2001) reported that the COMT low-activity genotype slightly increases the risk for schizophrenia by its effect of increasing prefrontal dopamine catabolism.

Here we have used a definition of TRS that is modified from that proposed by Kane et al. (1988), which was defined based on observations of monotherapy antipsychotic treatment. The main reason for modifying the definition of TRS is that Japanese psychiatrists generally prefer polypharmacy in routine clinical practice: if patients relapse during their antipsychotic maintenance therapy, Japanese psychiatrists often prescribe another antipsychotic agent in addition to the original one, instead of switching to it, so that it is quite rare for patients in Japanese routine psychiatric practice to be diagnosed as having TRS as proposed by Kane et al. (1988), even when the schizophrenia is considered treatment-resistant. In the present study, a significantly higher daily neuroleptic dosage was observed in patients with the L/L genotype (average: 1,226 mg/day) than in those with the other genotypes. In addition, the rate of TRS tended to be higher in patients with the COMT L/L genotype than in the other patients. These findings suggest that the low-activity COMT genotype is more common in TRS. This is consistent with the results of Herken and Erdal (2001), who reported that Turkish schizophrenics with the L/L genotype may have clinical signs that are much more severe. Here we assumed that the patients with persistent and severe condition of psychoses usually receive high doses of antipsychotics for the long period. Base on this assumption, we did not examine the direct observation of 'current and persistent positive symptoms of psychosis and at least moderate overall severity of current illness' in the present study, although they are included in the Kane's criteria of TRS. Therefore, this is a limitation of this study: some of the patients who were identified as TRS in this study might contain the

Table 3. Characteristics of neuroleptic treatment among three subgroups showing catechol-O-methyltransferase H/L polymorphism

	Catechol-O-methyltransferase H/L polymorphism		
	H/H	H/L	L/L
Daily neuroleptic dosage (mg / day)	728 ± 655	685 ± 621	1,226 ± 1,069*
(n=78)	(*n*=32)	(*n*=40)	(*n*=6)
Rate of treatment-resistant cases	25.0%	22.0%	57.1%

Data are expressed as mean ± standard deviation. Daily neuroleptic dosage was calculated from the most-recent 1-year neuroleptic therapy as chlorpromazine equivalent dose using the table of Inagaki et al (1998). *$p<0.05$ when compared to the (H/H + H/L) subgroups (Mann-Whitney U tests).

mere chronic schizophrenic patients with poor positive symptoms (Conley et al., 1997), although they had been hospitalized for more than 1 year and had been receiving high doses of antipsychotic therapy for more than 1 year.

As for the susceptibility to neuroleptic-induced EPS, no significant differences were observed among three subgroups of the COMT genotype. If the low-activity COMT genotype contributed to the high dopamine concentration in the synaptic cleft due to the decrease in COMT activity, then EPS would be less likely to develop in patients with the COMT L/L genotype. On the other hand, the patients with the COMT L/L genotype are observed to receive significantly higher doses of antipsychotics in the present study, which suggests that EPS would be more likely to develop in these patients. Our present results of no differential characteristics of neuroleptic-induced EPS among three subgroups of the COMT genotype may be the consequence of offset of these two factors discussed above. To clarify the exact role of the COMT H/L polymorphism for the susceptibility to neuroleptic-induced EPS, the comparison should be done under the condition that the neuroleptic dosage patients receive is strictly controlled.

Since the COMT H/L polymorphism examined in the present study has been demonstrated to alter enzyme activity (Lotta et al., 1995; Lachman et al., 1996; Sander et al., 1997), the polymorphism is considered to affect the clinical manifestations related to central dopaminergic systems. However, it should be noted that the present conclusion was drawn mainly from the findings in only eight patients with the COMT L/L genotype, which represents a small sample size. Further research using a larger sample set is required to clarify the exact role of this polymorphism; that is, whether alteration of enzyme activity could modify the phenotype of various psychiatric diseases and human behavior, including the characteristics of TRS suggested in this study.

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