between COMT and schizophrenia in a French population. Psychiatry Res 102: 87–90.

Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D, Weinberger DR. 2001. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci U S A 98: 6917–6922.

Herken H, Erdal ME. 2001. Catechol-O-methyltransferase gene polymorphism in schizophrenia: evidence for association between symptomatology and prognosis. Psychiatr Genet 11: 105–109.

Inada T, Dobashi I, Sugita T, Inagaki A, Kitao Y, Matsuda G, Kato S, Takano T, Yagi G, Asai M. 1997. Search for a susceptibility locus to tardive dyskinesia. Hum Psychopharmacol 12: 35–39.

Inada T, Yagi G, Miura S. 2002. Extrapyramidal symptom profiles in Japanese patients with schizophrenia treated with olanzapine or haloperidol. Schizophr Res 57: 227-238.

Inagaki A, Inada T, Fujii Y, Yagi G. 1998. Dose equivalence of psychotropic drugs. Part IV. Dose equivalence of orally administered neuroleptics (4). (In Japanese) *Rinsyo Seishin Yakuri (Jpn J Clin Psychopharmacol)* 1: 443-448.

Ishiguro H, Shibuya H, Toru M, Saito T, Arinami T 1999. Association Study between high and low activity polymorphism of catechol-O-methyltransferase gene and alcoholism. Psychiatr Genet 9: 135-138.

Jones G, Zammit S, Norton N, Hamshere ML, Jones SJ, Milham C, Sanders RD, McCarthy GM, Jones LA, Cardno AG, Gray M, Murphy KC, Owen MJ. 2001. Aggressive behaviour in patients with schizophrenia is associated with catechol-O-methyltransferase genotype. Br J Psychiatry 179: 351–355.

Kane J, Honigfeld G, Singer J, Meltzer H. 1988. Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. Arch Gen Psychiatry 45: 789-96.

Karayiorgou M, Altemus M, Galke BL, Goldman D, Murphy DL, Ott J, Gogos JA. 1997. Genotype determining low catechol-O-methyltransferase activity as a risk factor for obsessive-compulsive disorder. Proc Natl Acad Sci USA 94: 4572-4575.

Kauhanen J, Hallikainen T, Tuomainen TP, Koulu M, Karvonen MK, Salonen JT, Tiihonen J. 2000. Association between the functional polymorphism of catechol-O-methyltransferase gene and alcohol consumption among social drinkers. Alcohol Clin Exp Res 24: 135–139.

Kunugi H, Vallada HP, Hoda F, Kirov G, Gill M, Aitchison KJ, Ball D, Arranz MJ, Murray RM, Collier DA. 1997. No evidence for an association of affective disorders with high- or low- activity allele of catechol-O-methyltransferase gene. Biol Psychiatry 42: 282-285.

Kotler M, Barak P, Cohen H, Averbuch IE, Grinshpoon A, Gritsenko I, Nemanov L, Ebstein RP. 1999. Homicidal behavior in schizophrenia associated with a genetic polymorphism determining low catechol-O-methyltransferase (COMT) activity. Am J Med Genet 88: 628–633.

Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski C, Weinshilboum RM. 1996. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 6: 243–250.

Lachman HM, Nolan KA, Mohr P, Saito T, Volavka J. 1998. Association between catechol-O-methyltransferase genotype and violence in schizophrenia and schizoaffective disorder. Am J Psychiatry 155: 835–837.

Liou YJ, Tsai SJ, Hong CJ, Wang YC, Lai IC. 2001. Association analysis of a functional catechol-o-methyltransferase gene polymorphism in schizophrenic patients in Taiwan. Neuropsychobiology 43: 11–14.

Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melen K, Julkunen I, Taskinen J. 1995. Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. Biochemistry 34: 4202–4210.

Mynett-Johnson LA, Murphy VE, Claffey E, Shields DC, McKeon P. 1998. Preliminary evidence of an association between bipolar disorder in females and the catechol-O-methyltransferase gene. Psychiatr Genet 8: 221–225.

Nakamura A, Inada T, Kitao Y, Katayama Y. 2001. Association between catechol-O-methyltransferase (COMT) polymorphism and severe alcoholic withdrawal symptoms in male Japanese alcoholics. Addict Biol 6: 233–238.

Nolan KA, Volavka J, Czobor P, Cseh A, Lachman H, Saito T, Tiihonen J, Putkonen A, Hallikainen T, Kotilainen I, Rasanen P, Isohanni

M, Jarvelin MR, Karvonen MK. 2000. Suicidal behavior in patients with schizophrenia is related to COMT polymorphism. Psychiatr Genet 10:117-24.

Ohara K, Nagai M, Suzuki Y, Ohara K. 1998. Low activity allele of catechol-o-methyltransferase gene and Japanese unipolar depression. Neuroreport 9: 1305–1308.

Ohmori O, Shinkai T, Kojima H, Terao T, Suzuki T, Mita T, Abe K. 1998. Association study of a functional catechol-O-methyltransferase gene polymorphism in Japanese schizophrenics. Neurosci Lett 243: 109–112.

Papolos DF, Veit S, Faedda GL, Saito T, Lachman HMR. 1998. Ultra-ultra rapid cycling bipolar disorder is associated with the low activity catecholamine-O-methyltransferase allele. Mol Psychiatry 3: 346–349.

Sander T, Harms H, Podschus J, Finckh U, Nickel B, Rolfs A, Rommelspacher H, Schmidt LG. 1997. Allelic association of a dopamine transporter gene polymorphism in alcohol dependence with withdrawal seizures or delirium. Biol Psychiatry 41: 299–304.

Schooler NR, Kane JM. 1982. Research diagnoses for tardive dyskinesia. Arch Gen Psychiatry 39: 486-487.

Semwal P, Prasad S, Bhatia T, Deshpande SN, Wood J, Nimgaonkar VL, Thelma BK. 2001. Family-based association studies of mono-aminergic gene polymorphisms among North Indians with schizophrenia. Mol Psychiatry 6: 220–224.

Strous RD, Bark N, Woerner M, Lachman HM. 1997. Lack of association of a functional catechol-O-methyltransferase gene polymorphism in schizophrenia. Biol Psychiatry 41: 493-495.

Tiihonen J, Hallikainen T, Lachman H, Saito T, Volavka J, Kauhanen J, Salonen JT, Ryynanen O-P, Koulu M, Karvonen MK, Pohjalainen T, Syvalahti E, Hietala J. 1999. Association between the functional variant of the catechol-O-methyltransferase (COMT) gene and type 1 alcoholism. Mol Psychiatry 4: 286–289.

Vandenbergh DJ, Rodriguez LA, Miller IT, Uhl GR, Lachman HM. 1997. High-activity catechol-O-methyltransferase allele is more prevalent in polysubstance abusers. Am J Med Genet 74: 439–442.

Wei J, Hemmings GP. 1999. Lack of evidence for association between the COMT locus and schizophrenia. Psychiatr Genet 9: 183–186.

神経発達障害仮説による遺伝子解析

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研究要旨

我々は、統合失調症の候補遺伝子として、疾患の神経発達障害仮説に基づきneurotrophin-3(NT-3)およびbrain-derived neurotrophic factor(BDNF)を想定し、これらの遺伝子内のCAリピート多型と疾患との関連研究を行っている。既に、統合失調症患者男性80名、女性56名の血液試料からDNA抽出を終えた。今後、性と年齢をマッチさせた健常対照者を選択し、解析を進める予定である。また、Minor physical Anomalies(MPAs)を疾患の背景にある神経発達障害の一つの指標と仮定し、統合失調症患者群と健常対照群ともに、高MPA群、低MPA群に分けた解析も行う予定である。

A.研究目的

統合失調症は、生涯罹患率がおよそ1%と非常に高率であることが知られているが、その中でも10～15%が自殺という不幸な転帰をたどるとされている。近年、統合失調症の成因として、胎生期の形成異常など人生早期の軽微な静止的脳障害の存在を想定し、好発期までの年月のあいだの神経発達の結果、その病理的役割が触発されて発症に至るとす

る神経発達障害仮説が有力視されている。われわれは、この仮説に基づいて、brain-derived neurotrophic factor遺伝子(BDNF)とNeurotrophin -3(NT-3)遺伝子に注目し、疾患との関連を調べ、さらに、Minor physical Anomalies(MPAs)を疾患の背景にある神経発達障害の一つの指標と仮定し、統合失調症患者群と健常対照群ともに、高MPA群、低MPA群に分けた解析を行うことにした。

B.研究方法

対象者:

予定対象者は、DSM-IV精神分裂病の診断基準をみたす患者200名と、性と年齢をマッチさせた健常対照者200名である。ゲノムDNAは、末梢血リンパ球からフェノール法で抽出した。

BDNF多型について:

PCRは、Proschelらの報告にのっとり、5'-GCCACTTTATCTAATCCAGT-3'および5'-AGCACTAGCTGCCTATTCCA-3'のプライマーを用い、annealing 56℃ 60sec、extension 72℃ 60sec、denaturation 95℃ 60sec、35cyclesでおこない、PCR産物をPharmacia ALF DNA sequencerで解析中である。

NT-3多型について:

PCRは、Hattoriらの報告にのっとり、5' GGCTTGTGTCTTCCCCAAAGTT3'および5' AGGGGAGGAGGTGGAGAA3'のプライマーを用い、annealing 65℃ 60sec、extension 72℃ 120sec、denaturation 94℃ 60sec、35cyclesでおこない、PCR産物をPharmacia ALF DNA sequencerで解析中である。

MPAsについて:

Waldropスケールを用いて、診断やその他の情報についてブラインドの状態で、2人の評価者が評価している。

なお、この研究は長崎大学倫理委員会の承認を受けている。

C.研究結果

既に、統合失調症患者男性80名、女

性56名の血液試料からDNA抽出を終え、BDNF多型、NT-3多型の解析およびMPAsの評価を進めている。

D.考察

統合失調症の神経発達障害仮説から神経栄養因子の遺伝子は関連研究の対象として注目されてきた。特に、NT-3については、既にNankoらが日本人統合失調症患者との関連を報告しており、候補遺伝子として有力である。

ところで、われわれの共同研究者であるFujimaruは、日本人統合失調症患者313人と健常対照者128人を対象にWaldropスケールでMPAsを調査し、変形耳介、溝状舌、小頭囲、高尖塔口蓋が患者群において健常対照者群より有意に多いことを報告した。これは、統合失調症患者に妊娠第一、第二3半期に起源する形態異常、その中でも特に頭蓋顔面部の異常が多いということを示すものであり、神経発達障害仮説を間接的に支持するものといえる。われわれは、候補遺伝子の神経発達障害仮説における役割をより明確にするために、最終的には患者群および対照群をそれぞれ高MPAs群と低MPAs群に分けて候補遺伝子との関連を調査する予定である。

E.結論

NT-3多型とBDNF多型と統合失調症との関連を調査中である。既に、統合失調症患者男性80名、女性56名の血液試料からDNA抽出を終え、解析中である。今後は、対象者を増やしながら、高MPA群、低MPA群に分けた解析も行う。

F.健康危険情報

なし

G.研究発表

1.論文発表

辻田高宏:統合失調症のエピジェネティックス(後成的遺伝子修飾機構)、キーワード精神第3版、先端医学社、印刷中

Matsumoto S., Sasaki T., Imamura A., Matsuo K., Kayashima T., Hashida A., Ono S., Tsujita T., Matsumto S., Nakane Y., Tokunaga K., Okazaki Y: HLA classI distribution in Japanese patients with schizophrenia. Am J Med Genet. 114: 42-45, 2002

Fujimaru K., Imamura A., Tsujita T., Uraguchi M., Hashida A., Mori T., Matsumoto S., Matsumoto S., Okazaki Y., Nakane Y.: Minor Physical Anomalies in Japanese Patisents with Schizophrenia. Acta Med. Nagasaki 47: 133-137, 2002

藤丸浩輔、辻田高宏：一卵性双生児のゲノムの不一致について、分子精神医学、2、260-261、2002

2.学会発表

Takahiro Tsujita: Genomic Methylation Discordance between Monozygotic Twins Discordant for Psychosis., XII World Congress of Psychiatry, 2002.8.24.-8.29., Yokohama

加藤忠史、石渡みずほ、垣内千尋、田島　治、秋山　剛、辻田高宏、岡崎祐士、久住一郎：双極性障害患者の培養リンパ芽球細胞内Ca2＋反応　～一卵性双生児不一致例における検討～、第24回日本生物学的精神医学会、2002.4.10.-4.12.、さいたま

垣内千尋、岩本和也、石渡みずほ、久住一郎、辻田高宏、岡崎祐士、加藤忠史：Gene Chipを用いた一卵性双生児双極性障害不一致例における遺伝発現の差異の検討、第24回日本生物学的精神医学会、2002.4.10.-4.12.、さいたま

辻田高宏、山下秀次、今村　明、小田利香、茅島智彦、藤丸浩輔、橋田あおい、松尾勝久、与那城竹亮、菊池妙子、小野慎治、森　貴俊、林田雅希、三好　修、加藤忠史、陣野吉広、中根允文、新川詔夫、大石道夫、岡崎祐士：精神疾患の発症に関するepigeneticsの解明　～一卵性双生児精神疾患不一致例を対象として～、平成14年度厚生労働省精神・神経疾患研究委託費精神疾患関連研究班研究報告会、2002.12.16-12.18.、東京

H.知的財産権の出願・登録状況

なし

Brain derived neurotrophic factor gene and schizophrenia: polymorphism screening and association analysis

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Abstract

In view of both the neurodevelopmental hypothesis and the dopamine theory of schizophrenia, brain derived neurotrophic factor (BDNF) is a strong candidate gene for the illness. We searched for polymorphisms in the coding region of the BDNF gene with polymerase chain reaction and single strand conformational polymorphism (PCR-SSCP) analysis. Furthermore, we performed an association study between the BDNF gene and schizophrenia in a Japanese sample of 178 patients with schizophrenia (DSM-IV) and 332 control subjects. A single nucleotide substitution (A758G) in the coding region which leads to an amino acid change (Val/Met) was detected. Concerning this polymorphism, there was no significant difference in the genotype or allele distribution between patients and controls. With respect to the C270T polymorphism in the 5' noncoding region, however, a significantly increased frequency of carrying the 270T allele was observed in the patients than in the controls ($p<0.05$, odds ratio 2.2, 95% CI: 1.1 ~ 4.6). These results suggest that the missense polymorphism (A758G) has no major role in the pathogenesis of schizophrenia. However, the C270T polymorphism in the noncoding region of the BDNF gene may give susceptibility to the schizophrenia.

1. Introduction

Brain-derived neurotrophic factor (BDNF) belongs to the neurotrophic factor family and promotes the development, regeneration, survival and maintenance of function of neurons (Maisonpierre et al., 1990). A recent study has suggested that BDNF elicits long-term neuronal adaptations by controlling the responsiveness of its target neurons to the important neurotransmitter, dopamine (Guillin et al., 2001). BDNF from dopamine neurons was shown to be responsible for inducing normal expression of the dopamine D3 receptor in nucleus accumbens both during development and in adulthood. In view of both the neurodevelopmental hypothesis (Weinberger,1987; Jones and Murray, 1991; Murray, 1994) and the dopamine theory (Kahn and Davis, 1995) of schizophrenia, the BDNF gene is therefore a strong candidate gene for the illness. Indeed, an elevated BDNF level was observed in the postmortem brains of schizophrenic patients specifically in the anterior cingulate cortex and hippocampus (Takahashi et al., 2000). Altered expression of BDNF mRNA in response to stress was observed in mice with neonatal lesion of the ventral hippocampus, a possible animal model of schizophrenia (Molteni et al., 2001).

The BDNF gene maps to chromosome 11p13 (Maisonpierre et al., 1991). A microsatellite (GT repeat) located 1 kb upstream from the transcription site of the BDNF gene has been extensively examined for allelic association with schizophrenia, although effects of this polymorphism on gene function are unclear. The majority of the studies failed to find evidence that the microsatellite marker is associated with the development of schizophrenia (Sasaki T et al., 1997; Hawi et al., 1998; Wassink et al., 1999; Krebs et al., 2000; Virgos et al., 2001).

Recently we have performed polymorphism screening in the 5' upstream and noncoding regions of the BDNF gene. We found a single nucleotide substitution (C270T) in the 5' noncoding region and detected a significant association with late-onset al.zheimer's disease (Kunugi et al., 2001). To our knowledge, this polymorphism has not yet been examined

for the possible association with schizophrenia. Furthermore, there is no study that performed systematic polymorphism screening on the coding region of the BDNF gene in any patient group.

The aim of the present study is to search for polymorphisms in the coding region of the BDNF gene. Then we examined polymorphisms of the BDNF gene for allelic association with schizophrenia.

2. Materials and methods

2.1. Subjects

To search for polymorphisms, we screened genomic DNA from 20 patients with schizophrenia and 20 with Alzheimer's disease. Consensus diagnosis of schizophrenia was made for each patient by at least two psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV; American Psychiatric Association, 1994), based on unstructured interviews and medical records. Diagnosis of Alzheimer's disease was made according to the NINCDS-ADRDA criteria for "probable AD" (McKhann et al., 1984).

Association analyses between schizophrenia and the BDNF polymorphisms were performed in a sample of 178 patients with schizophrenia (90 men and 88 women; mean age 39.7 years [SD 12.7]) and two control groups. The patients were recruited from the psychiatric clinic at the Teikyo University Hospital.

To ensure any conclusion, we employed two control groups. One group (control A; 87 men and 83 women; mean age 31.6 years [SD 12.7]) was recruited from hospital staffs who were not assessed for psychiatric symptoms, although they showed good social functioning. The other control group (control B; 64 men and 98 women; mean age 57.0 [SD 7.9]) consisted of medical patients. When the two control groups were combined, there were 332 controls (151 men and 181 women) with mean age of 44.0 years (SD 16.6). All the patients and controls were Japanese and biologically unrelated to each other. Written informed consent for the participation of the study was obtained from all the subjects. The study protocol was approved by the institutional ethical

committees.

2.2 Polymorphism screening and genotyping

Venous blood was drawn and genomic DNA was extracted according to standard procedures. We searched for polymorphisms for the coding region of the BDNF gene referring to DNA sequence (GenBank accession M61181) reported by Maisonpierre et al. (1991). Polymerase chain reaction (PCR) amplifications and single strand conformational polymorphism (SSCP) analyses were performed for approximately 1kb DNA sequence which encompasses the coding region of the BDNF gene by using 6 sets of oligonucleotide primers (Table 1). Gel electrophoresis in the SSCP analysis was performed on at least two differential conditions for each target sequence: 10% polyacrylamide SSCP gel with 5% glycerin for 3 hours on 200V at room temperature and 20% gel with 5% glycerin overnight on 200V at 4°C. Band patters were visualized with silver staining. Differential band patterns were subject to direct sequencing with an autosequencer (ABI prism 310 Genetic Analyzer, Perkin Elmer, Chiba, Japan).

Genotyping for the C270T polymorphism in the 5'-noncoding region was performed according to the method that we described previously (Kunugi et al., 2001). Genotyping for the A758G polymorphism was done by PCR with primers of BDSS2F and BDSS2R (Table 1) and digestion by the restriction enzyme *PmaC*I, followed by 5% polyacrylamide gel electrophoresis with ethidium bromide staining. The thermal cycling for this PCR was initial one cycle of 95 °C for 5 min, 30 cycles of three stages of 95 °C for 30 sec, 60°C for 30 sec and 72 °C for 60 sec, and the final extension at 72 °C for 10 min.

2.3 Statistical analysis

The presence of Hardy-Weinberg equilibrium for the genotype distributions in the patients and controls was examined by using the χ^2 test for goodness of fit. The differences in the genotype and allele distributions between patients and controls were examined by using the χ^2 test for independence or the Fischer's exact test. All p-values reported are two-tailed and the critical p-value was set at 0.05.

3. Results

3.1 Polymorphism screening

We detected differential band patterns in one DNA fragment. Subsequent direct sequencing revealed a single nucleotide substitution (A758G) which results in an amino acid change (Val/Met) of the BDNF precursor protein. This polymorphism had already been described by Maisonpierre et al. (1991) (GenBank accession M61176). This polymorphism was detectable by PCR amplification with primers BDSS2F and BDSS2R (Table 1) and digestion by a restriction enzyme of *BbrP*I, *Eco72*I, *PmaC*I, *or Hsp92*II. Although we performed the PCR-SSCP analyses on two differential conditions, we found no other polymorphism in the coding region of the BDNF gene.

3.2 Association analyses

Then we examined the two polymorphisms (the A758G missense polymorphism and C270T polymorphism in the 5'-noncoding region) for an association with schizophrenia. The genotype and allele distributions of the A758G polymorphism in the patients and controls are shown in Table 2. The genotype distributions in the patients and control groups were not significantly deviated from the Hardy-Weinberg equilibrium (for the patients: χ^2= 0.2603, df=1, p=0.61; for the control A: χ^2=0.1, df=1, p=0.77; for the control B: χ^2=1.6, df=1, p=0.20). The genotype and allele distributions for the patients were quite similar to those for the two control groups. There was no statistically significant difference in genotype or allele distribution between the patients and control groups (for statistics, see Table 2).

The genotype and allele distributions with respect to the C270T polymorphism in the patients and controls are shown in Table 3. The genotype distributions in the patients and control groups were not significantly deviated from the Hardy-Weinberg equilibrium (for the patients: χ^2= 0.45, df=1, p=0.50; for the control A: χ^2=0.1, df=1, p=0.75; for the control B: χ^2=0.1, df=1, p=0.78). There was no individual who was homozygous for the 270T allele. The frequency of heterozygous individuals in the patients

(9.6%) was approximately twice as high as those observed in the control A (4.7%) and control B (4.3%). There was a trend ($p<0.10$) towards an increased frequency of carrying the mutated type (270T) in the patients compared with each control group. When the two control groups were combined, there was a significant difference in the frequency of carrying the 270T allele between the patients and the total control subjects ($p=0.034$, odds ratio 2.2, 95% CI: 1.1 ~ 4.6). An allelewise comparison also yielded a significant difference between the patients and the total controls ($p=0.037$).

3.3 Linkage disequilibrium

When the possible linkage disequilibrium was examined between the A758G and C270T polymorphisms (Table 4), these polymorphisms were significantly linked ($\chi^2=11.7$, df=2, $p=0.003$). There was no individual who carried the 270T allele among those who were homozygous for the 758A allele, suggesting that the 270T allele in the 5'-noncoding region is tightly linked to the 758G allele.

4. Discussion

In our polymorphism screening, we confirmed the A758G polymorphism in our Japanese subjects which was described previously in a Caucasian population (Maisonpierre et al., 1991). However, we did not find any other polymorphism in the coding region. A potential limitation is that the SSCP analysis cannot detect all polymorphisms even in the examined DNA sequences, although we performed the SSCP analysis on two different conditions for each target sequence. Furthermore, we examined genomic DNA from 20 patients with schizophrenia and 20 with Alzheimer's disease; it is possible that we have missed rare mutations.

The A758G polymorphism results in an amino acid change of Val/Met at position -63 of the BDNF precursor protein which may affect processing from precursor protein to mature peptide. Thus we examined this polymorphism for allelic association with schizophrenia; however, we found no significant difference in the genotype or allele distribution between the patients and controls, suggesting that the A758G polymorphism has no major role in

giving susceptibility to the illness.

With respect to the C270T polymorphism in the 5' noncoding region, on the other hand, there were significant differences in the genotype and allele distributions between the patients and the total controls. The frequency of individuals who carried the mutated type (270T) was significantly elevated in the patients compared with the total controls, suggesting that the 270T allele may confer susceptibility to schizophrenia. Since the 270T allele was tightly linked to the 758G allele, the haplotype 758G/270T may play a role. However, the obtained significant level of the association was weak ($p<0.05$) and further studies in other samples are required to draw any conclusion.

We previously examined the neurotrophin-3 (NT-3) gene for the possible association with schizophrenia. We found that a dinucleotide repeat polymorphism of the NT-3 gene was associated with the development of schizophrenia (Nanko et al., 1994). Subsequently some studies supported the association between the NT-3 gene and schizophrenia (Dawson et al., 1995; Jonsson et al., 1997; Virgos et al., 2001), although others did not (Nimgaonkar et al., 1995; Arinami et al., 1996; Gill et al., 1996). This polymorphism was also suggested to be related to smaller hippocampal volume in patients with schizophrenia (Kunugi et al., 1999). These previous findings and present results warrant further studies to examine the possible role of neurotrophins (BDNF and NT-3) in the development and brain abnormalities of schizophrenia.

References

American Psychiatric Association, 1994. Diagnostic and Statistical Manual of Mental Disorders, 4th edn. American Psychiatric Association, Washington, DC.

Arinami, T., Takekoshi, K., Itokawa, M., Hamaguchi, H., Toru, M., 1996. Failure to find associations of the CA repeat polymorphism in the first intron and the Gly-63/Glu-63 polymorphism of the neurotrophin-3 gene with schizophrenia. Psychiatr. Genet. 6, 13-15.

Dawson, E., Powell, J.F., Sham, P.C., Nothen, M., Crocq, M.A., Propping, P., Korner, J., Rietschel, M., van Os, J., Wright, P., Murray, R.M., Gill, M., 1995. An association study of a neurotrophin-3 (NT-3) gene polymorphism with schizophrenia. Acta Psychiatr. Scand. 92, 425-428.

Gill, M., Hawi, Z., O'Neill, F.A., Walsh, D., Straub, R.E., Kendler, K.S., 1996. Neurotrophin-3 gene polymorphisms and schizophrenia: no evidence for linkage or association. Psychiatr. Genet. 6, 183-186.

Guillin, O., Diaz, J., Carroll, P., Griffon, N., Schwartz, J.-C., Sokoloff, P. 2001. BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization. Nature 411, 86-89.

Hawi, Z., Straub, R.E., O'Neill, A., Kendler, K.S., Walsh, D., Gill, M., 1998. No linkage or linkage disequilibrium between brain-derived neurotrophic factor (BDNF) dinucleotide repeat polymorphism and schizophrenia in Irish families. Psychiatry Res. 81, 111-116.

Jones, P., Murray, R.M., 1991. The genetics of schizophrenia is the genetics of neurodevelopment. Br. J. Psychiatry 158, 615-623.

Jonsson, E., Brene, S., Zhang, X.R., Nimgaonkar, V.L., Tylec, A., Schalling, M., Sedvall, G., 1997. Schizophrenia and neurotrophin-3 alleles. Acta Psychiatr. Scand. 95, 414-419.

Kahn, R.S., Davis, K.L., 1995. New developments in dopamine and schizophrenia. In: Bloom, F.E., Kupfer, D.J. (Eds.), Psychopharmacology: The Fourth

Generation of Progress. Raven Press, New York, pp. 1193-1203.

Krebs, M.O., Guillin, O., Bourdell, M.C., Schwartz, J.C., Olie, J.P., Poirier, M.F., Sokoloff, P., 2000. Brain derived neurotrophic factor (BDNF) gene variants association with age at onset and therapeutic response in schizophrenia. Mol. Psychiatry 5, 558-562.

Kunugi, H., Hattori, M., Fujii, K., Kato, T., Nanko, S., 1999. Dinucleotide repeat polymorphism in the neurotrophin-3 gene and hippocampal volume in psychoses. Schizophr. Res. 37, 271-273.

Kunugi, H., Ueki, A., Otsuka, M., Isse, K., Hirasawa, H., Kato, K., Nabika, T., Kobayashi, S., Nanko, S., 2001. A novel polymorphism of the brain-derived neurotrophic factor (BDNF) gene associated with late-onset al.zheimer's disease. Mol. Psychiatry 6, 83-86.

Maisonpierre, P.C., Belluscio, L., Friedman, B., Alderson, R.F., Wiegand, S.J., Furth, M.E., Lindsay, R.M., Yancopoulos, G.D., 1990. NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression. Neuron 5, 501-509.

Maisonpierre, P.C., Le Beau, M.M., Espinosa, R., Ip, N.Y., Belluscio, L., de la Monte, S.M., Squinto, S., Furth, M.E., Yancopoulos, G.D., 1991. Human and rat brain-derived neurotrophic factor and neurotrophin-3: gene structures, distributions, and chromosomal localizations. Genomics 10, 558-568.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., Stadlan, E.M., 1984. Clinical diagnosis of Alzheimer's disease. Neurology 34, 939-944.

Molteni, R., Lipska, B.K., Weinberger, D.R., Racagni, G., Riva, M.A., 2001. Developmental and stress-related changes of neurotrophic factor gene expression in an animal model of schizophrenia. Mol. Psychiatry 6, 285-292.

Murray, R.M., 1994. Neurodevelopmental schizophrenia: the rediscovery of dementia praecox. Br. J. Psychiatry (suppl.), 6-12.

Nanko, S., Hattori, M., Kuwata, S., Sasaki, T., Fukuda, R., Dai, X.Y., Yamaguchi, K., Shibata, Y., Kazamatsuri, H., 1994. Neurotrophin-3 gene polymorphism associated with schizophrenia. Acta Psychiatr. Scand. 89, 390-392.

Nimgaonkar, V.L., Zhang, X.R., Brar, J.S., DeLeo, M., Ganguli, R., 1995. Lack of association of schizophrenia with the neurotrophin-3 gene locus. Acta Psychiatr. Scand. 92, 464-466.

Sasaki, T., Dai, X.Y., Kuwata, S., Fukuda, R., Kunugi, H., Hattori, M., Nanko, S., 1997. Bain-derived neurotrophic factor gene and schizophrenia in Japanese subjects. Am. J. Med. Genet. 74, 443-444.

Takahashi, M., Shirakawa, O., Toyooka, K., Kitamura, N., Hashimoto, T., Maeda, K., Koizumi, S., Wakabayashi, K., Takahashi, H., Someya, T., Nawa, H., 2000. Abnormal expression of brain-derived neurotrophic factor and its receptor in thecorticolimbic system of schizophrenic patients. Mol. Psychiatry 5, 293-300.

Virgos, C., Martorell, L., Valero, J., Figuera, L., Civeira, F., Joven, J., Labad, A., Vilella, E., 2001. Association study of schizophrenia with polymorphisms at six candidate genes. Schizophr. Res. 49, 65-71.

Wassink, T.H., Nelson, J.J., Crowe, R.R., Andreasen, N.C., 1999. Heritability of BDNF alleles and their effect on brain morphology in schizophrenia. Am. J. Med. Genet. 88, 724-728.

Weinberger, D.R., 1987. Implications of normal brain development for the pathogenesis of schizophrenia. Arch. Gen. Psychiatry 44, 660-669.

Table 1 Oligonucleotide primer sequences for the PCR-SSCP analysis of the coding region of the BDNF gene

	cDNA No[$]	Name	Sequence	Bp
1	487 – 509	BDSS1F	CGGTGAAAGAAAGCCCTAACCAG	232
	728 – 709	BDSS1R	AGCCTCTTGAACCTGCCTTG	
2	689 – 708	BDSS2F	CTGGAGAGCGTGAATGGGCC	206
	894 – 871	BDSS2R	TCCAGCAGAAAGAGAAGAGGAGG C	
3	844 – 864	BDSS3F	GATGCTCAGTAGTCAAGTGCC	184
	1027 – 1006	BDSS3R	AGTCTTTTTGTCTGCCGCCGTT	
4	984 – 1004	BDSS4F	GTGACAGTATTAGTGAGTGGG	187
	1170 – 1149	BDSS4R	TGCCTTTTGTCTATGCCCCTGC	
5	1126 – 1147	BDSS5F	CATGGGTTACACAAAAGAAGGC	135
	1260 – 1239	BDSS5R	ATCCTTATGAATCGCCAGCCAA	
6	1152 - 1171	BDSS6F	GGGGCATAGACAAAAGGCAT	342
	1493 – 1474	BDSS6R	TGTTTCCCTTCTGGTCATGG	

[$] DNA numbering is according to Maisonpierre et al. (1991)(GenBank accession M61181). The BDNF precursor protein is encoded by nucleotides between 663 and 1406, and mature peptide between 1047 and 1403.

Table 2 Genotype distributions and allele frequencies for the A758G polymorphism of the BDNF gene in the patients with schizophrenia and controls

	Genotype distributions (%)				Allele frequencies (%)		
	n	A/A	A/G	G/G	n	A	G
Patients	178	32	83	63	356	147	209
		(18.0%)	(46.6%)	(35.4%)		(41.3%)	(58.7%)
Controls							
Total	332	55	172	105	664	282	382
		(16.6%)	(51.8%)	(31.6%)		(42.5%)	(57.5%)
Control A	170	30	85	55	340	145	195
		(17.6%)	(50.0%)	(32.4%)		(42.6%)	(57.4%)
Control B	162	25	87	50	324	137	187
		(15.4%)	(53.7%)	(30.9%)		(42.3%)	(57.7%)

Genotypewise comparisons: patients v. total controls: χ^2=1.3, df=2, p=0.53; patients v. control A: χ^2=0.4, df=2, p=0.80; patients v. control B: χ^2=1.7, df=2, p=0.43
Allelewise comparisons: patients v. total controls: χ^2=0.1, df=1, p=0.72; patients v. control A: χ^2=0.1, df=1, p=0.72; patients v. control B: χ^2=0.1, df=1, p=0.79

Table 3 Genotype distributions and allele frequencies for the C270T polymorphism in the 5'-noncoding region of the BDNF gene in the patients with schizophrenia and controls

	Genotype distributions (%)				Allele frequencies (%)		
	n	C/C	C/T	T/T	n	C	T
Patients	178	161 (90.4%)	17 (9.6%)	0 (0.0%)	356	339 (95.2%)	17 (4.8%)
Controls							
Total	332	317 (95.5%)	15 (4.5%)	0 (0.0%)	664	649 (97.7%)	15 (2.3%)
Control A	170	162 (95.3%)	8 (4.7%)	0 (0.0%)	340	332 (97.6%)	8 (2.4%)
Control B	162	155 (95.7%)	7 (4.3%)	0 (0.0%)	324	317 (97.8%)	7 (2.2%)

Genotypewise comparisons in the frequency of heterozygotes (C/T): patients v. total controls: p=0.034 (Fisher's exact probability); patiens v. control A: p=0.097; patients v. control B: p=0.088

Allelewise comparisons: patients v. total controls: p=0.037 (Fisher's exact probability); patients v. control A: p=0.10; patients v. control B: p=0.094

Table 4 Linkage disequilibrium between the A758G polymorphism in the coding region and the C270T in the 5'-noncoding region of the BDNF gene among the total 510 subjects

	A758G (%)			
C270T	A/A	A/G	G/G	Total
C/C	87	241	150	478
	(18.2%)	(50.4%)	(31.4%)	(100%)
C/T	0	14	118	32
	(0.0%)	(43.8%)	(56.2%)	(100%)
Total	87	255	168	510
	(17.1%)	(50.0%)	(32.9%)	(100%)