1. 薬物血中濃度

抗精神病薬の臨床効果・副作用に影響を及ぼす因子の一つに薬物血中濃度がある。血中濃度以前に、抗精神病薬の効果を予測する指標としてまず注目されたのは薬物の投与量（用量）であったが、同じ用量でも、個体間に血中濃度の大きなばらつきが認められることから、問題のあることがわかった。同じ用量であっても、ハロペリドール（HAL）では3～4倍、ブロムペリドール（BRP）では約8倍の個体差があることがわかっており、用量から血中濃度を予測することは非常に困難である。そこで直接血中濃度そのものを用いて、臨床効果・副作用を予測しようとする試みがなされ、臨床応用が可能となった。従来、精神科領域では抗てんかん薬や炭酸リチウムなどの限られた薬物に対して血中濃度測定が行われてきたが、定型抗精神病薬についても、1990年にHALの血中濃度モニタリング（治療的濃度監視; TDM）が保険適応を受け、さらに1994年BRPのTDMも保険診療で行えるようになった。したがってこれらの二つの薬剤については、日常臨床で手軽にTDMを利用できるようになっている。一方、非定型抗精神病薬による薬物療法においては、TDMの意義そのものが確立されておらず、現在臨床応用が可能な客観的指標は存在しないといえる。

2. 薬物血中濃度に影響をおよぼす因子

薬物血中濃度には、抗精神病薬の代謝、併用薬との薬物相互作用など、さまざまな薬物動態学因子が影響を与えることがわかっており、定型抗精神病薬についてはある程度まとまった知見が示されている。しかし非定型抗精神病薬については、これまでにいくつかの薬物動態に関する報告はあるものの、現時点では一致した見解が得られていない。以下に薬物血中濃度に影響を及ぼす因子の代表的なものについて簡単に述べるとともに、それぞれの非定型抗精神病薬について、CYP分子種と臨床効果・副作用との関連などについて、現在までの報告をまとめる。

A. チトクローム P450

薬物代謝における個体差が、血中濃度に影響を及ぼすことが報告されており、特に肝の代謝酵素であるチトクローム P450（CYP）と抗精神病薬の血中濃度との関連が注目されている。CYP にはさまざまな分子種があり、CYP1A2、CYP2C9、CYP2C19、CYP2D6、CYP3A4 などが比較的よく知られているが、これらの分子種の遺伝子型には人種や個体間で差がある。たとえば CYP2D6 には酵素活性を減弱させる *CYP2D6*3*、*CYP2D6*4*、*CYP2D6*10*、酵素欠損を生じる *CYP2D6*5* などの対立遺伝子（アレル）があるが、*CYP2D6*3*、**4* は比較的コーカサス人（白人）で多く、*CYP2D6*5*、**10* は白人と比較してアジア人種に多いといわれている。これらのアレルをもつ個体では抗精神病薬の血中濃度が上昇する可能性があり、同じ用量の抗精神病薬で治療を受けていても、これらのアレルをもつかもたないかによって個人個人の臨床効果・副作用には違いがみられることが示唆される。

B. 薬物相互作用

近年、薬物相互作用が注目され、精神科領域に留まらず、さまざまな薬物の組合せで相互作用が起こることがわかっている。一般に、2種類以上の薬物を併用した場合、互いの薬物動態に何らかの影響を及ぼし合うのは当然であり、その意味では薬物相互作用は無数にあるともいえる。臨床薬理学領域では相互作用の機序を解明し、事前に予測可能となるように研究が進められているが十分とはいえず、実際に評価するには血中濃度測定が重要となる。たとえば抗てんかん薬であるカルバマゼピン（CBZ）の併用によりブロムペリドール（BRP）の血中濃度が低下することが知られている。BRP の代謝にもっとも強く関与しているのは CYP3A4 であるが、CBZ には CYP3A4 の活性を上昇させる作用があり、この結果相互作用が生じると考えられている。また CYP3A4 を阻害するイトラコナゾール併用により BRP 血中濃度が上昇することが知られており、BRP の血中濃度を検討する場合、CYP3A4 の酵素活性を低下させる（阻害する）、あるいは上昇させる（誘導する）薬物の併用の有無を考慮すべきであるが、その影響は個体によって大きく異なり、予測できないものである。したがってそ

の評価には個体ごとの血中濃度モニタリングが必要であるといえる。

C. その他の問題

嗜好品の中には血中濃度に影響を与える可能性をもつものが少なくない。たとえば喫煙はHALの血中濃度を低下させ、グレープフルーツジュースはその中に含まれるフラボノイド類が小腸粘膜のCYP3A4を阻害することから、CYP3A4で代謝されるBRPなどの抗精神病薬の血中濃度がグレープフルーツジュース飲用によって上昇するといわれている。また、肝機能障害や腎機能障害も血中濃度に影響を及ぼすものである。多くの抗精神病薬は脂溶性で肝代謝・排泄されるため肝機能異常がある場合には注意が必要であるが、一方、ベンザミド系のスルピリド、スルトプリドやリスペリドンは腎で排泄されるため、これらの薬物では腎機能を考慮すべきである。

D. 非定型抗精神病薬における薬物動態学的研究

①リスペリドン

リスペリドン（以下RIS）の肝における代謝には主としてCYP2D6が関与しており、特にRISの主要な代謝産物である9-水酸化リスペリドン（9-OH RIS）への代謝経路にCYP2D6の関与が大きいといわれている。CYP2D6の遺伝子型とRISおよび9-OH RISの血中濃度、臨床効果との間の関連については、コーカサス人（白人）種、日本人を含むアジア人種ともにさまざまな見解がある（Scordo et al 1999、Roh et al 2001）が、統一されたものはない。

一方でCYP3A4がRIS代謝に及ぼす影響についても報告されているが、RIS代謝においてCYP3A4が果たす役割は現時点では十分明らかにされていない。

② Clozapine

Clozapine（以下CLZ）は現在、日本では未発売の薬剤であるが、欧米では新規抗精神病薬の中でもっとも多くの薬理学的研究がおこなわれている薬剤である。CLZの肝における代謝には、主としてCYP1A2が関与していることが知られている。そのため一般にCYP1A2の酵素の働きを活性化させる（誘導する）物質（CBZ、喫煙など）はCLZ血中濃度を低下させ、CYP1A2を阻害する働きをもつ物質（カフェイン、抗生剤エリスロマイシンなど）はCLZ血中濃度を

上昇させるという。またCYP2D6を阻害させる薬剤もCLZの血中濃度を上昇させるといわれているが、その機序は明らかにされていない。

CLZの臨床効果と血中濃度に関する報告は多数あるが、いまだに一致した見解は得られていない。CLZ血中濃度と統合失調症の症状評価尺度であるBPRS (Brief Psychiatric.Rating Scale) の改善度との間に相関がみられるという報告 (Perry et al 1991、Kronig et al 1995) もあるが、その一方でCLZ血中濃度とCLZの治療反応性の間には関連がみられないともいわれている。

CLZの使用により起こりうるもっとも重篤な血液学的異常は無顆粒球症であり、易感染性をきたし、最悪の場合には死の転帰をとる場合がある。その頻度は0.8～1％であるといわれているが、発症機序については不明であり、CLZおよびその代謝産物の血中濃度と無顆粒球症発症との間に相関は認められていない。またCYP2D6遺伝子多型と無顆粒球症との関連についての研究報告もあるが、2D6の関与は否定されている。一方、CLZ誘発性の顆粒球減少症および無顆粒球症の発症予測は困難であるものの、定期的な血液モニタリングが発症率と死亡率を減少させるのに効果的であると考えられている。

③オランザピン

オランザピン（以下OLZ）の肝における代謝にはCYP1A2が関与しているといわれている。CYP1A2の阻害薬であるフルボキサミンがOLZ血中濃度を上昇させ、OLZと1A2の阻害薬であるシプロフロキサシンの併用も、OLZ血中濃度を上昇させるといわれている。またCBZはCYP1A2とCYP3A4を誘導するため、OLZとCBZの併用によりOLZ血中濃度は減少するという。喫煙もまた1A2を誘導するため、OLZ血中濃度を低下させる。

OLZ血中濃度と臨床効果との関連についてはいくつか報告があり、統合失調症患者におけるOLZ血中濃度と治療反応性、副作用との間には関連がみられるとの報告がある（Perry et al 2001、Skogh et al 2002)。

④クエチアピン

クエチアピン（以下QTP）の肝代謝には主にCYP3A4が関与しているが、CYP1A2、2C9、2C19、2D6および3A4らに対する阻害作用は示さないといわれている。したがって3A4の誘導剤はQTP血中濃度を低下させ、3A4の阻害剤はQTP血中濃度を上昇させる作用を示す。たとえば3A4の誘導剤であるフ

ェニトインはQTP血中濃度を低下させ、QTPとCBZとの併用もQTP血中濃度を減少させるといわれる。またQTP血中濃度と臨床反応との間には現時点で関連は見い出されていない。

⑤ペロスピロン

ペロスピロン（以下PER）は、わが国で開発された薬剤であり、2001年に市販された。PERは多くの代謝物を産生するが、主要代謝物はID-15036と呼ばれる1,2シクロヘキサンカルボキシリミド水酸化物である。この代謝は主にCYP3A4を介して行われ、ID-15036は神経伝達物質セロトニンに対する拮抗作用をもち、PERの経口投与後のID-15036血中濃度はPERよりもはるかに高値であることが報告されている（石橋ら 1997）。このことからPER内服患者における臨床効果・副作用の発現にはこの代謝物の関与が大きいことが示唆される。また3A4が代謝経路に関与していることから、PERとCBZなどの3A4の誘導剤を併用した場合にPERの臨床効果・副作用が影響を受ける可能性が考えられる。

3. 血中濃度を検討する際に留意すべき他の因子

以上述べたように、非定型抗精神病薬における薬物動態学的研究の結果をみると、各薬剤の血中濃度と臨床効果・副作用との間の関連が見い出されたものもある一方、関連が見い出せなかったものもあり、一致した見解が得られていない。以前から抗精神病薬の血中濃度と臨床効果・副作用との関連を評価する際に検討すべき問題はいくつか存在していたが、そうした問題が新規抗精神病薬の薬物動態を評価する場合にも十分に考慮されているとはいえない。以下にこれらの問題点についてまとめる。

A. 有効濃度域と治療的飽和

前に述べたように、定型抗精神病薬であるHALやBRPにおいてはすでにTDMが保険適応となっており、臨床の場で広く用いられている。しかし実際

には、TDMは服薬遵守の確認にしか用いられない場合も多いうえ、血中濃度を測定しても、その値と臨床効果を検討してどのように治療計画に反映させればよいのか十分に理解されておらず、TDMの臨床的意義が十分に生かされているとはいえない。臨床の場では血中濃度と臨床効果の間にはっきりとした関係性を認めにくいと考えられているようだが、その原因のひとつとして有効濃度域（therapeutic window）の問題がある。血中濃度と臨床効果を検討した研究から、有効濃度域が存在し、HALの場合は3～17 ng/ml、BRPでは4～20 ng/mlとされ、これが基準値となっている。われわれの研究ではHAL、BRPの血中濃度にはそれ以上増加させてもさらなる臨床効果が期待できない「十分濃度」が存在し、この関係を治療的飽和（therapeutic plateau）と呼んでいる（広兼ら 1997、染矢ら 1996）。つまり、「臨床効果は血中濃度上昇につれて増加するが、ある一定の濃度を超えるとそれ以上の効果は期待できない」ということである。したがって、有効血中濃度を幅で捉えるという有効濃度域の考え方では、「その範囲内に血中濃度があれば良い」と考えて薬物を十分量まで増量せず、過小な投与量のまま薬物に反応しない者として判断してしまう危険性があり、このことが血中濃度と臨床効果との関係に対する誤解を生じさせているのではないかと考えられる。

B．薬剤に対する反応性

血中濃度と臨床効果との間に関係を見い出すことを困難にしている要因として、さらに、もともとその薬物に対して反応しない者（非反応者）と反応が良好であった者とをいっしょに扱ってしまっているという問題がある。血中濃度モニタリングを用いて臨床効果を評価する際にもっとも重要なのは、いくら血中濃度を上げても臨床効果が得られないという、本当に薬物に対して反応しない者と、血中濃度が十分でないために臨床効果が得られず、見かけ上薬物に対して反応しない者となっている者を区別することであるといえる。

まとめ

以上述べたように、薬物血中濃度は、抗精神病薬の臨床効果・副作用に影響を及ぼし、これらの予測に有用であると考えられている因子である。しかし非

定型抗精神病薬における薬物動態学的研究は十分に行われてはおらず、薬物血中濃度と臨床効果・副作用との関連についての一致した見解は得られていない。また、TDMに関しても、一部の研究結果では有用であると報告されているものの、現時点では臨床応用のめどは立っていない。しかし治療濃度と副作用が出現する濃度が近接している薬剤や、重篤な副作用をもつ薬剤を投与する場合にTDMは非常に重要である。現在、抗精神病薬治療全般においてTDMは服薬遵守の確認など、限られた目的のみで用いられているが、今後の薬物動態学的研究によって、新規抗精神病薬の臨床効果・副作用予測の指標となりうる生物学的マーカーとして、TDMの有用性が確立されることを期待したい。

(澤村一司、染矢俊幸)

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ORIGINAL ARTICLE

The effects of a 5-hydroxytryptamine 1A receptor gene polymorphism on the clinical response to fluvoxamine in depressed patients

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ABSTRACT
We investigated the effects of a 5-hydroxytryptamine (5-HT) 1A receptor gene polymorphism on the clinical response to fluvoxamine (FLV) in 65 depressed outpatients who gave written consent to participate in the study. Patients visited every 2 weeks after the first examination until the week 12 end point and were evaluated by the 17-item Hamilton Rating Scale for Depression (HAM-D-17) at each visit. FLV dose was changed in response to their clinical symptoms. The Gly272Asp polymorphism of the 5-HT1A receptor gene was identified by a PCR method. The subjects with the Asp allele had a significantly higher % reduction in the HAM-D-17 score than those with the Gly/Gly genotype at week 2 ($P=0.009$), week 6 ($P=0.036$), and week 12 ($P=0.031$). There was a significant difference in the genotype distribution between the responders and nonresponders. These results suggest that the Gly272Asp polymorphism of the 5-HT1A receptor gene may predict the response to FLV.
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INTRODUCTION

Depression is one of the most common psychiatric diseases, with an incidence of about 4% and a life-time prevalence of 15–20%.[1,2] Although a large number of antidepressants are available in the clinical situation, approximately 30% of patients fail to respond satisfactorily to the treatment administered.[3] Moreover, all antidepressant treatments require an administration of at least 2 weeks before inducing a clinically significant improvement.[4] Preclinical studies have indicated that the delayed action of serotonergic antidepressants is partly due to the existence of a negative feedback mechanism involving 5-hydroxytryptamine-1A (5-HT1A) autoreceptors. Most antidepressants, especially the selective serotonin reuptake inhibitors (SSRIs), increase the concentration of 5-HT present in the synaptic cleft.[5,6] The increased levels of 5-HT will act on postsynaptic receptors as well as presynaptic receptors including 5-HT1A somatodendritic receptors, which exert negative feedback on the axon, reducing the release of new 5-HT into the cleft, and thereby reducing the effect of SSRI antidepressant medication.[7,8] It has also been indicated that during treatment with SSRIs, synaptic concentrations of 5-HT do not increase until after the 5-HT1A receptors have been functionally desensitized.[9–12] The delay in the onset of therapeutic benefits is thought to be

caused by this process. Clinical trials based on these hypotheses have revealed that combination therapy with SSRIs and an 5-HT1A autoreceptor antagonist increased the therapeutic efficacy in depressive patients.[13,14]

On the other hand, a number of polymorphisms have been identified in the human 5-HT1A receptor gene and some of the genetic variations alter the extracellular amino-terminal domain of the 5-HT1A receptor.[15] Several 5-HT1A receptor gene variants have been detected to date in Caucasian populations, but their frequencies are very low in the Japanese population.[15–17] Recently, Kawanishi *et al*[17] detected a new structural polymorphism, Gly272Asp, in the Japanese population. The effect of this polymorphism on the function of 5-HT1A is unclear, and to the best of our knowledge, there have been no studies investigating the effect of this polymorphism on the response to SSRIs.

In this study, we investigated the effect of the Gly272Asp polymorphism of the 5-HT1A receptor gene on the clinical response to fluvoxamine (FLV).

RESULTS

Among the 65 patients, 10 patients with the Gly/Gly genotype could not complete the 12-week study due to side effects or for unknown reasons. The genotypes of three patients could not be identified. Genetic variation was in the Hardy–Weinberg equilibrium. The genotype frequencies, as well as the clinical and demographic characteristics of the sample, are shown in Table 1. Since the Asp/Asp genotype group contained only one sample, the analysis was performed between the subjects with the Gly/Gly genotype and those with the Gly/Asp or Asp/Asp genotypes. No significant differences were demonstrated for age and sex between the two genotype groups. There were also no significant differences between the two genotype groups for the baseline 17-item Hamilton Rating Scale for Depression (HAM-D-17) scores. Figure 1 shows the time courses of the HAM-D-17 scores. A two-way repeated-measures analysis of variance of the HAM-D-17 scores showed a highly significant effect of time ($F=50.319$; d.f. $=3.255$; $P<0.0001$), and a significant effect of the genotype ($F=2.700$; d.f. $=3.255$; $P=0.043$). The subjects with the Gly/Asp or Asp/Asp genotypes had a significantly higher % reduction in the HAM-D-17 score than the subjects with the Gly/Gly genotype at week 2 (53.5 ± 20.3 *vs* $26.3\pm27.0\%$, $P=0.009$), week 6 (64.5 ± 19.1 *vs* 44.9 ± 35.0, $P=0.036$), and week 12 (80.8 ± 16.4 *vs* 61.6 ± 40.9, $P=0.031$).

There was a significant difference in the genotype distribution between the responders and nonresponders, while the percentages of remitters were similar among the genotype groups (Table 1).

DISCUSSION

The frequencies of the Gly/Gly, Gly/Asp and Asp/Asp genotypes were 87, 11, and 2%, respectively. There are only two previous studies that have reported the distribution of the Gly272Asp polymorphism of the 5-HT1A receptor gene in the Japanese population. Kawanishi *et al*[17,18] reported that the genotype frequencies of Gly/Gly, Gly/Asp, and Asp/Asp were 95, 5, and 0% and Nishiguchi *et al* reported 91, 8, and 1%, respectively. The genotype frequencies observed in this study are consistent with these previous studies.

Our results show a significant effect of the Gly272Asp polymorphism of the 5-HT1A receptor gene on the clinical response to FLV, and to our knowledge, this is the first study reporting a relationship between the 5-HT1A receptor gene polymorphism and the antidepressant response. The subjects with the Gly/Asp or Asp/Asp genotypes had a significantly higher % reduction in the HAM-D-17 score at weeks 2, 6, and 12, and there was a particularly large difference in the % change in the HAM-D-17 score between the Gly/Gly genotype group and the Gly/Asp or Asp/Asp genotype group at week 2. At week 2, the Gly/Asp or Asp/Asp group had a 53.5% reduction in the HAM-D-17 score, while the other group had only a 26.3% reduction. This result suggests that the 5-HT1A receptor gene polymorphism might affect the initial response to FLV. There are several studies that have indicated that the delay in the onset of the

Table 1 Demographic data and fluvoxamine therapeutic response among the genotype groups

	5-HT1A receptor genotypes		*P*
	Gly/Gly $n=44$	*Gly/Asp or Asp/Asp* $n=8$	
Sex (M/F)	26/18	3/5	0.441
Age	41.4 (14.5)	38.0 (13.7)	0.544
Baseline HAM-D-17 score	20.0 (5.6)	23.1 (3.9)	0.136
Responders/nonresponders	27/17	8/0	0.042
Remitters/nonremitters	24/20	6/2	0.442

Data are the means (SD). Responders were defined as subjects having at least a 50% decrease in the total HAM-D-17 score after 12 weeks of treatment. Remitters were defined as subjects having a HAM-D-17 score of seven or less points after 12 weeks of treatment. HAM-D-17: 17-item Hamilton Rating Scale for Depression.

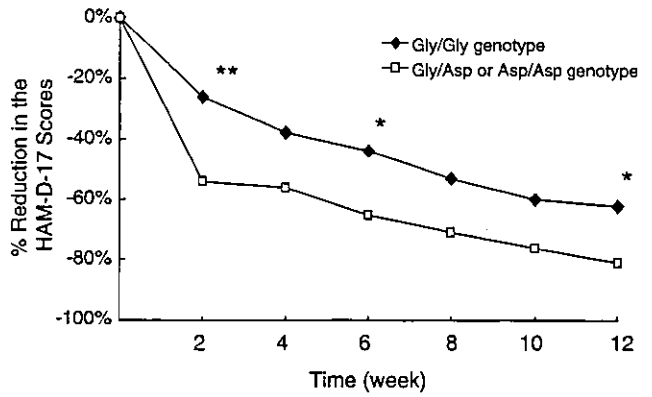

Figure 1 Change of mean HAM-D-17 score. Comparison by 5-HT1A receptor gene polymorphism. *P<0.05, **P<0.01.

antidepressant therapeutic effect is caused by 5-HT1A autoreceptors.[4,9–12] Our results appear to be consistent with these previous findings.

It is supposed that 5-HT1A receptor antagonists can cancel the delay in the onset of the therapeutic effects of antidepressants. A more rapid onset of action of SSRIs has been reported to be induced by 5-HT1A antagonists, such as pindolol, in recent studies. Tome *et al*[19] reported a significant acceleration in the onset of the effect of paroxetine observable from day 4 in a group receiving pindolol. It has been also indicated that a greater number of patients showed improvement in the pindolol plus paroxetine group than in the placebo plus paroxetine group at day 10.[13] Perez *et al*[20] reported that the number of days required to reach a sustained response was lower in the fluoxetine and pindolol group than in the fluoxetine and placebo group. These clinical findings also show that 5-HT1A receptors play an important role in the delay of the onset of the therapeutic effect.

Although the therapeutic finding has not been consistently regulated, if the Gly/Asp or Asp/Asp genotypes accelerate the initial response to SSRIs, SSRIs-pindolol augmentation therapy should be chosen for subjects with the Gly/Gly genotype from the start of the medication.

Therefore, it may be possible to select the most suitable medication for the treatment of depression based on a genetic factor.

Furthermore, it was observed that the subjects with the Asp allele also showed a greater % reduction in the HAM-D-17 score at week 12. This result may suggest that the more rapid onset of the action of FLV is correlated with a superior outcome at week 12. In the study by Tome *et al*,[19] patients who were originally treated with pindolol showed a better outcome at week 24 than patients taking paroxetine alone. In the study by Perez *et al*,[20] the difference between pindolol and placebo administrations in the cumulative percentages of patients with a sustained response was maintained until the end of the 6-week period. Although these preceding studies have shown a significant effect of the more rapid onset of action of SSRIs on a prolonged response, the function of the 5-HT1A receptor gene polymorphism remains unclear and needs to be discussed.

In this study, the only patient with the Asp/Asp genotype showed a 75% reduction in the HAM-D-17 score at week 2, and the HAM-D-17 score changed from 28 points at week 0 to 7 points at week 2. None of the subjects with the Gly/Asp genotype showed a greater % reduction in the HAM-D-17 score than the subject with the Asp/Asp genotype. Although it is impossible to clarify the differences in the response to FLV between the Gly/Asp and Asp/Asp genotypes because there was only one patient with the Asp/Asp genotype in this study, this finding suggests the possibility that subjects with the Asp/Asp genotype may have a better response to FLV than subjects with the Gly/Asp or Gly/Gly genotypes.

The Gly272Asp mutation was recently identified in the Japanese population. Although Kawanishi *et al*[17] expected that this substitution might alter the signal transduction function through G-protein coupling, the function of the amino-acid change is still unclear. Several other mutations of the 5-HT1A receptor gene have been detected in previous studies, but their functions also remain unclear. Therefore, the effects of these other mutations on the clinical response to FLV cannot be excluded. Furthermore, although the subjects who are diagnosed with major depressive disorder should be enrolled in this study, the sample is not homogeneous as for diagnosis. It is supposed that this heterogeneity of diagnosis affects the response to FLV. However, since all subjects with Asp allele are diagnosed with major depressive disorder, the effect of different diagnosis on our result may be not so great.

There is a limitation within our results that the frequency of this mutation in Caucasian samples has not been studied to date. If the frequency is very low, our results will not be useful for Caucasian patients.

It was demonstrated that patients with the Asp allele had a more rapid onset of the action of FLV, and that their final response rate to FLV was also greater than that of patients without the Asp allele. However, this study has the limitation of a small sample size and much research remains to be done to explain the mechanism. Further studies are needed to clarify the effect of the 5-HT1A receptor gene polymorphism on the clinical response to SSRIs.

MATERIALS AND METHODS

Subjects

This study was conducted at the Niigata University Medical Hospital. The study protocol was approved by the Ethics Committee of Niigata University Medical Hospital, and each subject provided written informed consent before enrolment. The subjects were 65 Japanese depressed outpatients (34 males, 31 females), and their mean age$\pm$SD was 40.5$\pm$14.0 years. In all, 58 subjects had DSM-IV diagnoses of major depressive disorder, three had adjustment disorder with depressed mood, and four had a depressive disorder not otherwise specified. The exclusion criteria were additional diagnoses on Axis I or Axis II of the DSM-IV. All the patients were free from psychotropic drugs for at least 14 days before their entry into the present study. Demographic data, medical histories, and laboratory data, including hematology, serology, electrolytes, and urine analysis, were collected for each patient. Patients with obvious physical illnesses were excluded from the present study. All patients were orally treated with FLV for their psychiatric illnesses.

Study Design

On the first examination (week 0), after the patients had given their informed consent, their symptoms were evaluated by HAM-D-17 and they were treated with FLV at a starting dose of 25 mg/day for the first week. The patients visited at weeks 1, 2, 4, 6, 8, 10, and 12 after the first examination. The HAM-D-17 score and side effects were assessed at each visit. If the improvement rate in the HAM-D-17 score was less than 40% in comparison to the score on the last visit, the FLV dose was increased from 25 mg/day to 50, 100, 150, and 200 mg/day after that. When the patients

achieved remission (the HAM-D-17 score reached less than eight points), the FLV dose was not subsequently changed.

Data Collection

Blood sampling was performed using a Venoject® tube containing EDTA-Na (Terumo Japan, Tokyo, Japan) at week 1. In all, 7 ml of venous blood was collected, and genomic DNA was extracted from the samples within 2 h of collection. The target mutation of the 5-HT1A receptor gene was characterized by a single base-pair substitution (815G→A) at the position of codon 272 that resulted in an amino-acid exchange (Gly272 to Asp). To detect this mutation, polymerase chain reaction (PCR) was performed with the following primers: 5′ GGAGCTTTCTACATCCCGCTG 3′–5′ CTGGCGGCAGAACTTACACTT 3′. The amplification was carried out in a 10 μl volume containing 50 ng genomic DNA, 2 pmol of each primer, 1 μl 10 × Ex Taq buffer, 0.8 μl dNTP mixture, and 0.25 U of Ex Taq (Takara Biomedicals, Tokyo, Japan). After an initial 4 min denaturation at 94°C, 40 cycles of denaturing at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min were conducted, followed by a final extension step at 72°C for 4 min, using a thermal cycler. The PCR products were refined and the target mutation of the 5-HT1A receptor gene was identified using a MegaBASE Single Nucleotide Primer Extension (SNuPe) Genotyping kit (Amersham Biosciences, USA).

Statistical Analysis

The clinical and demographic characteristics were compared between groups with unpaired *t*-tests or Fisher's exact tests. Comparisons of the time courses of the HAM-D-17 scores were performed using a two-way repeated-measures analysis of variance, with time (week) as a within-subjects factor, and genotype as a between-subjects factor. The genotype distributions were analyzed using Fisher's exact test. The level of significance was set at less than 0.05.

DUALITY OF INTEREST

None declared.

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Regular Article

Transmission disequilibrium test and haplotype analysis of the *NOTCH4* gene in Japanese patients with schizophrenia

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Abstract A recent study reported that the *NOTCH4* gene was highly associated with schizophrenia in the British population. To confirm this association for another population, a case–control study was conducted and a transmission disequilibrium test (TDT) analysis was performed on a group of Japanese subjects (235 pairs of schizophrenia patients and controls, and 78 trios consisting of probands and their parents) using two single nucleotide polymorphisms and three microsatellite markers for the *NOTCH4* gene. Haplotype analysis was also studied in case–control and family based data sets. In all markers except for (CTG)n ($P = 0.012$, before correction for multiple testing), no differences were found in the case–control study. The TDT analysis also revealed only a weak transmission disequilibrium in (TTAT)n (genotype-wise $P = 0.012$). The finding of the present study could not support the original findings that the *NOTCH4* gene itself is associated with susceptibility to schizophrenia.

Key words case–control study, linkage, microsatellite marker, single nucleotide polymorphism, transmission disequilibrium test.

INTRODUCTION

Schizophrenia (MIM 181500) is a severe and chronic mental disorder expressed through a wide variety of symptoms, including psychotic symptoms such as delusions and hallucinations, and abnormalities in emotional expression and social interaction. It affects approximately 1% of the population in several countries.[1] Genetic epidemiological studies, such as family, twin, and adoption studies have shown that genetic factors play an important role in the pathogenesis of schizophrenia.[2–4] As for other complex diseases, the inheritance of schizophrenia is not Mendelian form, and several genes contribute to its development. Despite an enormous number of molecular genetic studies, no major gene has been identified as the cause of schizophrenia, and results have not been consistent.

Linkage studies have repeatedly shown that the chromosome 6p is a possible susceptibility region for schizophrenia.[5–13] The possibility for linkage at 6p21.3 was found in several studies.[7,12–14] Recently, the *NOTCH4* gene, which is located at chromosome 6p21.3, was demonstrated to be highly associated with schizophrenia in British subjects.[15] Interestingly, Notch signaling plays an important role in the neural system, including neuronal growth.[16,17] However, follow-up molecular genetic studies with subjects from several ethnic backgrounds failed to confirm original findings.[18–24] Very recently, Skol *et al.* reported a possible linkage and association between schizophrenia and the *NOTCH4* gene.[25] To determine whether this gene is linked with schizophrenia, we analyzed *NOTCH4* gene polymorphisms in Japanese subjects by means of a case–control study and the transmission disequilibrium test (TDT).

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METHODS

Subjects

The subjects in the case–control study consisted of 235 Japanese schizophrenic patients (122 male and 113 female patients). Control subjects were matched on dimensions such as sex and ethnicity and were healthy volunteers with no history of psychiatric disorders. Each control was selected to pair with each case patient of the nearest age. As a result, cases and controls were well matched in younger (<40 years old) subjects (n = 80; mean age: cases, 30.7 ± 6.4 years; controls, 30.5 ± 7.0 years), whereas the mean age of cases was higher than those of controls in older (≥40 years old, which is higher than the peak age of onset of schizophrenia) subjects (n = 155; cases, 54.0 ± 7.5 years; controls, 49.2 ± 6.1 years). The overall mean age of the cases and controls was 46.0 ± 13.3 years and 42.8 ± 10.8 years, respectively.

The TDT analysis and family based haplotype analysis included 78 trios, made up of patients and both their parents. The patients consisted of 44 men and 34 women, with a mean age of 30.0 ± 9.4 years. All patients were diagnosed as having schizophrenia by trained psychiatrists according to the *Diagnostic and Statistical Manual of Mental Disorders* (4th edn; DSM-IV) criteria.[26] Written informed consent was obtained from all patients and their parents prior to participation in the present study. None of the patients or controls, including those participating in the TDT analysis, were related. All subjects were residents of the Niigata area, in northern Japan. The present study was approved by the ethics committee on genetics of the Niigata University School of Medicine.

Genotyping

Genomic DNA was extracted from peripheral blood by the standard phenol/chloroform method. Two single nucleotide polymorphisms (SNP) and three short tandem repeat polymorphisms as described by Wei and Hemmings[15] were genotyped. SNP1 (dbSNP rs387071) is an A to G substitution and SNP2 (rs367398) is a T to C substitution in the 5′ flanking region of the *NOTCH4* gene. The (TAA)n repeat polymorphism is present in the 5′ flanking region of the *NOTCH4*, the (CTG)n repeat in exon 1 and the (TTAT)n repeat in intron 17. Their order and physical location are shown in Fig. 1. Each region containing SNP or short tandem repeat polymorphism was amplified by means of a polymerase chain reaction (PCR) using the following primer sets: (TAA)n: 5′-TCATGACCAGCAACATAGGG-3′, 5′-TACACACTACCATTCCTGGG-3′; SNP1: 5′-TGCT GGCTCACGGGCTTCC-3′, 5′-TGGATTGCAGTG GCACGACC-3′; SNP2: 5′-AAACAGCAGGGCTGG GACTG-3′, 5′-ACCTCTGGGTCTGACCACTG-3′; (CTG)n: 5′-AATGCAGCCCCCTTCACTG-3′, 5′-TC CTCCATCCAGCATCCCT-3′; and (TTAT)n: 5′-TGA ATACACCCTTCCTCCTC-3′, 5′-ACAGACTGGGA CTCCATCTC-3′.

Each forward primer of repeat polymorphisms was labeled with fluorescence. The PCR products of SNP1 and SNP2 were genotyped on 3% agarose gel after digestion using Msp I. Those of the repeat polymorphisms were genotyped by means of an ABI 377 genetic analyzer (Applied Biosystems, USA) using GeneScan program ver. 2.1 (Applied Biosystems). Genotyping was performed under blind-to-diagnosis conditions. The number of repeats was confirmed by sequencing PCR products of homozygotes for each repeat as templates, using ABI 377 and Sequencing Analysis program ver. 3.0 (Applied Biosystems). Each non-fluorescent forward and reverse primer listed here was used as a sequencing primer and a DNA Sequencing Kit was also used (Big Dye Terminator Cycle Sequencing, v2.0 Ready Reaction; Applied Biosystems). Genotypes from family samples were checked by examination of Mendelian inheritance using the PEDCHECK program (supplied by J. R. O'Connell).

Statistical analysis

Statistical analysis was performed using the χ^2 method for SNP1 and SNP2. The CLUMP program ver-

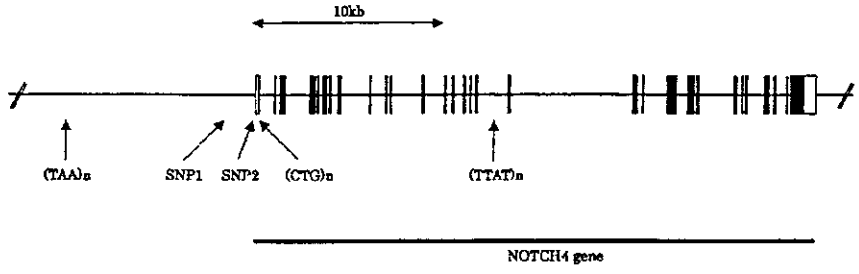

Figure 1. Locations of the markers used in the present study. Rectangles indicate exons and black rectangles indicate coding regions. The (TAA)n repeat is 8.3 kb away from single nucleotide polymorphism 1 (SNP1); SNP1 is present at base −1745 of the translation initiation site of *NOTCH4* and SNP2 at base −25; and the (CTG)n repeat is 40 bp away from SNP2 and 12.3 kb away from the (TTAT)n repeat.

sion 2.2 (supplied by P. C. Sham and D. Curtis), which tests contingency tables with small cell counts using the Monte Carlo method,[27] was used for microsatellite markers in the case–control study because of their sparse distribution. The significance is assessed by performing repeated simulations to count the number of times that a simulated table yields a χ^2 value greater than or equal to that obtained from the real table. It produces four statistics, T1 to T4, which differ in the way of clumping columns. The number of simulations was 10 000 in each test and we adopted the T1 statistic, which uses a raw, not clumped table, because of the authors' recommendation in the document with the program. Haplotype frequencies were analyzed using the FASTEHPLUS program (supplied by J. Zhao), which estimates haplotype frequencies based on genotype data from a group of unrelated individuals, and performs permutation tests.

In the TDT analysis, the McNemar test was conduced to test the transmission distortion for SNP markers. A program utilizing the Monte Carlo approach, MCETDT,[28,29] which performs TDT even if the marker is highly polymorphic, was used for microsatellite markers to obtain empiric *P* values. The number of simulations was 10 000 in each test. The haplotype transmission was analyzed by the TRANSMIT program ver.2.5.2 (supplied by D. Clayton). Rare haplotypes with frequencies <1% were aggregated in this analysis.

The level of significance was 0.05.

RESULTS

Tables 1,2 and Tables 3,4 show the allele and genotype frequencies for each marker in the case–control study. The genotype frequencies of SNP were not significantly different from the values expected from Hardy–Weinberg equilibrium (χ^2 test for goodness of fit). A slight difference was found in the allele frequencies of the (CTG)n repeat polymorphism between patients and controls ($P = 0.012$). However, after correction for multiple testing (i.e. the level of significance/number of tests performed; 0.05/5), the *P* value for the difference did not exceed the level of significance. Significant differences in allele frequencies between patients and controls were not found in any other markers. There was also no difference in the overall genotype frequencies of markers. In haplotype analysis of case–control samples, modest significance (empiric $P = 0.028$) was found in the SNP2-(CTG)n haplotype (Table 5). We tested the marker–marker association using the FASTEHPLUS program and found strong marker–marker associations between each marker ($P < 1 \times 10^{-4}$).

Case–control studies have potential problems of population stratification. In contrast, family based analyses such as TDT are effective in eliminating population stratification therefore we performed TDT analysis in 78 trios for each polymorphism. The TDT results are shown in Table 6 for each SNP and Table 7 for the microsatellite markers. A trend for transmission distortion in the (TATT)n repeat polymorphism was observed (allele-wise, $P = 0.024$; genotype-wise, $P = 0.012$, before correction for multiple testing). The allele-wise analysis attempts to establish a pattern of preferential transmission of certain alleles across genotypes (i.e. it determines whether certain alleles are preferentially transmitted over other alleles across genotypes having those alleles). The genotype-wise analysis considers every heterozygous parental genotype separately, and examines whether each allele of a genotype is transmitted to affected offspring on 50% of occasions. In our TDT analysis, all the *P* values for the difference after the correction did not exceed the required level of significance. We analyzed transmission of multilocus haplotypes and did not find significant excess transmission in any haplotypes (Table 8).

DISCUSSION

Wei and Hemmings conducted a study on linkage disequilibrium mapping for the chromosome 6p MHC locus, a candidate region associated with the onset of schizophrenia.[15] They reported that there was a strong association between the *NOTCH4* gene and the development of schizophrenia. Proteins of the Notch family are transmembrane receptors associated with the destination of apoptosis and regulation of differentiation.[16] They are also associated with the differentiation suppression and survival of nerve stem cells in the nervous system.[17] Hence, it was believed that there was a possibility that *NOTCH4* was a vulnerability gene for neurogenetic diseases. However, more recent studies, including those on Japanese subjects, have yielded negative results for such an association.[18–21] It should be noted, however, that these reports, with some exceptions, dealt with only the (CTG)n repeat polymorphism, which showed the highest association with schizophrenia in the study by Wei and Hemmings. In the present study, we analyzed two microsatellite markers and two SNP for the *NOTCH4* gene, as well as the (CTG)n repeat polymorphism and conduced haplotype analysis of these markers.

The results of our case–control study revealed a slightly different distribution of (CTG)n repeat allele frequency ($P = 0.012$). However, the *P* value that we obtained did not exceed the required level of significance after correction for multiple testing. In the other

Table 1. Allele frequencies of SNP1 and SNP2 in schizophrenia patients and controls

	SNP1		SNP2	
Allele	G	A	C	T
Schizophrenia (Freq.)	280 (0.60)	190 (0.40)	248 (0.53)	220 (0.47)
Control (Freq.)	302 (0.64)	168 (0.36)	235 (0.50)	233 (0.50)
	$P = 0.139$		$P = 0.395$	

Table 2. Genotype frequencies of SNP1 and SNP2 in schizophrenia patients and controls

	SNP1			SNP2		
Genotype	G/G	G/A	A/A	C/C	C/T	T/T
Schizophrenia (Freq.)	82 (0.35)	116 (0.49)	37 (0.16)	66 (0.28)	116 (0.50)	52 (0.22)
Control (Freq.)	92 (0.39)	118 (0.50)	25 (0.11)	56 (0.24)	123 (0.53)	55 (0.24)
		$P = 0.233$			$P = 0.574$	

Table 3. Allele frequencies of microsatellite markers in case and control subjects

	(TAA)n			(CTG)n			(TTAT)n	
Repeat	Case (%)	Control	Repeat	Case	Control	Repeat	Case	Control
3	8 (1.7)	8 (1.7)	5	0 (0.0)	1 (0.2)	8	1 (0.2)	1 (0.2)
7	0 (0.0)	1 (0.2)	6	92 (19.6)	69 (14.7)	10	6 (1.3)	7 (1.5)
8	80 (17.0)	77 (16.4)	7	0 (0.0)	4 (0.9)	11	271 (57.9)	255 (54.5)
9	230 (48.9)	239 (50.9)	8	0 (0.0)	5 (1.1)	12	165 (35.3)	183 (39.1)
10	12 (2.6)	10 (2.1)	9	146 (31.1)	168 (35.7)	13	23 (4.9)	14 (3.0)
11	29 (6.2)	22 (4.7)	10	190 (40.4)	192 (40.9)	14	2 (0.4)	8 (1.7)
12	2 (0.4)	1 (0.2)	11	40 (8.5)	30 (6.4)			
13	52 (11.1)	50 (10.6)	12	1 (0.2)	0 (0.0)			
14	46 (9.8)	49 (10.4)	13	1 (0.2)	1 (0.2)			
15	9 (1.9)	13 (2.8)						
16	2 (0.4)	0 (0.0)						
	χ^2 (T1) = 5.567			χ^2 (T1) = 17.266			χ^2 (T1) = 7.284	
	d.f. = 10			d.f. = 8			d.f. = 5	
	$P = 0.892$			$P = 0.012$			$P = 0.183$	

Table 4. Genotype frequencies (%) of microsatellite markers in case and control subjects

	(TAA)n													
	3/9	8/8	8/9	8/11	8/13	8/14	9/9	9/10	9/11	9/13	9/14	9/15	13/14	Others
Case	0.9	2.6	14.9	3.8	4.3	3.8	24.3	2.6	6.0	12.8	9.8	1.7	1.3	12.8
Control	3.0	3.0	17.0	1.3	2.1	4.7	23.4	1.7	4.7	13.6	11.5	2.6	2.6	8.9
	χ^2(T1) = 28.702 d.f. = 35 $P = 0.849$													

Genotypes with frequencies <2% are aggregated.

markers, all were in strong linkage disequilibrium, no significant differences between patients and controls were found. Ujike *et al.* examined three markers, SNP1, SNP2 and (CTG)n,[18] and Imai *et al.* studied only a (CTG)n marker in a Japanese group.[21] They failed to find an association between the *NOTCH4* gene and schizophrenia and their allelic distribution in the controls did not differ from those of the present study. The negative results of SNP1 and SNP2 analysis were similar to those of the present study. From these findings, it was not possible to conclude that there is an association with schizophrenia. Almost of the studies with subjects from several ethnic backgrounds such as Caucasian, African-American and Chinese have shown no association between schizophrenia and the *NOTCH4* gene. Recently, Fan *et al.* conducted a case-control

Table 5. Haplotype frequency analysis between schizophrenia and control for markers in *NOTCH4* region

Haplotype	Empirical *P*
(TAA)n-SNP1	0.801
SNP1-SNP2	0.161
SNP2-(CTG)n	0.028
(CTG)n-(TTAT)n	0.084
(TAA)n-SNP1-SNP2	0.909
SNP1-SNP2-(CTG)n	0.117
SNP2-(CTG)n-(TTAT)n	0.135

Table 6. TDT results for SNP1 and SPN2

	SNP1		SNP2	
Allele	G	A	C	T
Transmitted	40	33	45	42
Not Transmitted	33	40	42	45
	$P = 0.200$		$P = 0.374$	

Table 7. TDT results for microsatellite markers

Repeat	(TAA)n Transmitted	Not trans	Repeat	(CTG)n Trans	Not trans	Repeat	(TTAT)n Trans	Not trans
3	5	4	5	1	0	10	0	3
7	0	1	6	19	25	11	38	24
8	24	21	9	33	34	12	21	35
9	39	36	10	34	37	13	5	1
10	3	1	11	15	6	14	0	1
11	8	13	13	1	1			
13	16	15						
14	12	16						
15	1	0						
16	0	1						
Allele-wise								
	$\chi^2 = 7.598$ $P = 0.776$			$\chi^2 = 5.852$ $P = 0.431$			$\chi^2 = 11.371$ $P = 0.024$	
Genotype-wise								
	$\chi^2 = 15.872$ $P = 0.990$			$\chi^2 = 11.272$ $P = 0.369$			$\chi^2 = 14.762$ $P = 0.012$	

(CTG)n											(TTAT)n				
6/6	6/9	6/10	6/11	8/10	9/9	9/10	9/11	10/10	10/11	Others	11/11	11/12	11/13	12/12	Others
3.0	13.2	17.0	3.0	0.0	8.1	25.5	6.4	15.7	6.8	1.3	41.0	26.9	4.7	20.9	6.4
1.3	13.2	12.8	0.9	2.1	12.8	26.0	5.1	17.0	6.0	3.0	35.9	31.2	3.8	22.6	6.4
$\chi^2(T1) = 21.868$ d.f. = 15 $P = 0.078$											$\chi^2(T1) = 11.892$ d.f. = 12 $P = 0.472$				

Table 8. Haplotype transmission analysis in trio samples for markers in *NOTCH4* region

	χ^2	d.f.	*P*
(TAA)n-SNP1	8.987	12	0.704
SNP1-SNP2	0.802	3	0.849
SNP2-(CTG)n	6.403	8	0.602
(CTG)n-(TTAT)n	21.305	15	0.127
(TAA)n-SNP1-SNP2	23.910	20	0.246
SNP1-SNP2-(CTG)n	8.866	12	0.714
SNP2-(CTG)n-(TTAT)n	16.028	14	0.312

study and TDT using the same markers that we used[22] and reported that there were no significant differences. In haplotype analysis of case–control samples, we found different distribution of SNP2-(CTG)n haplotype frequency. However, the significance is very modest and no differences were observed in all other haplotypes.

We conducted a TDT analysis because the TDT is a powerful test that largely eliminates the influence of population stratification, which leads to erroneous conclusions in case–control studies.[27,30,31] Further, family based studies facilitate detection of typing errors by examination of Mendelian inheritance. The (TTAT)n repeat polymorphism tended to show transmission disequilibrium as determined by the test ($P = 0.024$, allele-wise; $P = 0.012$, genotype-wise) but the *P* values that we obtained did not exceed the required level of significance after correction for multiple testing. In the original report (TTAT)n transmission was not shown to be distorted. In addition, there was no difference in the polymorphism in our case–control study. Further, we did not observe transmission distortion of any other markers to schizophrenic offspring. Very recently, Skol *et al.* reported excess transmission of $(TAA)_8$ and $(TAA)_{13}$ alleles to African–American schizophrenic subjects, but the significance was marginal (before correction for multiple testing, $P = 0.06$ and $P = 0.04$, respectively).[25] The preceding studies did not show consistent significant results in TDT. In haplotype analysis of parents–offspring trios there was no significant difference of transmission of any haplotypes. Considering the results from case–control and family based analysis together, we could not discard a possibility of an association between the *NOTCH4* gene and schizophrenia, but it may be implausible because the results were conflicting between our case–control and TDT analysis.

In the present study we failed to confirm an association between the *NOTCH4* gene and schizophrenia but the possibility still requires clarification. It is still likely that the relevant locus is a causative region for the disease, and further investigation will be necessary.

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Adult-onset leukoencephalopathy with vanishing white matter with a missense mutation in *EIF2B5*

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Abstract—We report of a woman aged 52 years born to consanguineous parents and seeking treatment for progressive dementia and delusion. Neurologic examination revealed dementia and emotional instability, indifference, and confabulation. There was also mild spasticity of the bilateral lower limbs. MRI revealed diffuse white matter hyperintensity on T2-weighted images accompanied by hypointense areas on fluid-attenuated inversion recovery images. A homozygous missense mutation was identified in *EIF2B5*.

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Leukoencephalopathy with vanishing white matter (VWM; MIM #603896) is a rare autosomal recessive disease.[1] On MRI, increasing areas of the abnormal white matter that show signal intensities close to those of CSF on all pulse sequences. Magnetic resonance spectroscopy (MRS) of the abnormal white matter areas shows a decrease in the levels of all the compounds detected in normal white matter. The clinical presentations of this disease include chronic progressive cerebellar ataxia and spasticity, often accompanied by episodes of rapid deterioration of clinical symptoms after minor head trauma or infection.[1] Point mutations in the five eIF2B subunit genes have also been demonstrated in patients with VWM.[2-6] Most patients with VWM exhibit neurologic deterioration by age 6 years.[1] We report one patient with adult-onset VWM with dementia and carrying a missense mutation in *EIF2B5*.

Patients and methods. *Case report.* A Japanese woman aged 52 years born to parents who were first cousins. None of her relatives had neurologic symptoms. She worked as an office clerk until age 38 years. Two years later, she fractured her right femur in a traffic accident. After this accident, her family noticed that she became increasingly disorganized. By age 45 years, she had become markedly forgetful. Although she had never committed any illegal acts, she began shoplifting despite repeated arrests. She spent 19 months in jail at age 51 years. She was referred to a psychiatrist (T.S.) for delusional behavior and was admitted to our hospital. Neurologic examination revealed spastic gait and exaggerated tendon reflexes. She did not exhibit cerebellar ataxia, extrapyramidal signs, or epileptic episodes. Dementia was determined by a Mini-Mental State Examination score of 15. She achieved a verbal IQ of 58, a performance IQ of 52, and a full-scale IQ of 51. She had prominent declines in recent memory and orientation. She was euphoric and emotionally unstable. She was not delusional during hospitalization. She was sometimes indifferent. In addition to these symptoms, she exhibited defective planning of her activities and a lack of initiative and flexibility. During hospitalization, she confabulated and often caused serious troubles with other inpatients. However, she was not antisocial.

The results of routine laboratory tests, including those of CSF, are available on the Neurology Web site (see table E-1 on the Neurology Web site). EEG exhibited diffuse δ- and θ-wave abnormalities.

MRI and MRS studies. A 1.5-T MR system (Magnetom Vision, Siemens, Erlangen, Germany) was used to perform MRI studies. A 3-T MR system (Signa, GE Medical Systems, Waukesha, WI) was used to perform proton MRS (^{1}H-MRS). The studies were performed according to the human research guidelines of the Institutional Review Board of Niigata University. Informed consent for the 3-T MRS study was obtained from the patient and her family.

Single-voxel ^{1}H-MRS was performed using a point-resolved spectroscopy (PRESS) sequence combined with a chemical shift-selective excitation (CHESS) sequence to suppress the water signal (repetition time, 2,000 ms; echo time, 80 ms/144 ms). Referring to the axial MRIs acquired using T2-weighted fast spin-

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See also pages 1464, 1503, 1509, and 1598

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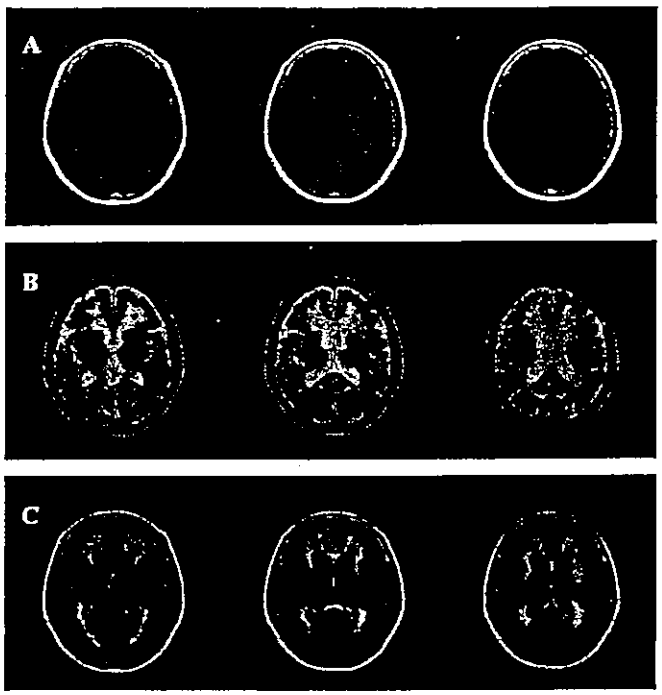

Figure 1. Axial T1-weighted (A), T2-weighted (B), and fluid-attenuated inversion recovery (FLAIR; C) MRIs. T2-weighted and FLAIR MRIs revealed hyperintense lesions in the white matter, predominantly in the frontal lobe (B, C). The affected white matter adjacent to the anterior and posterior horns of lateral ventricles had a signal intensity identical to that of CSF (A, C).

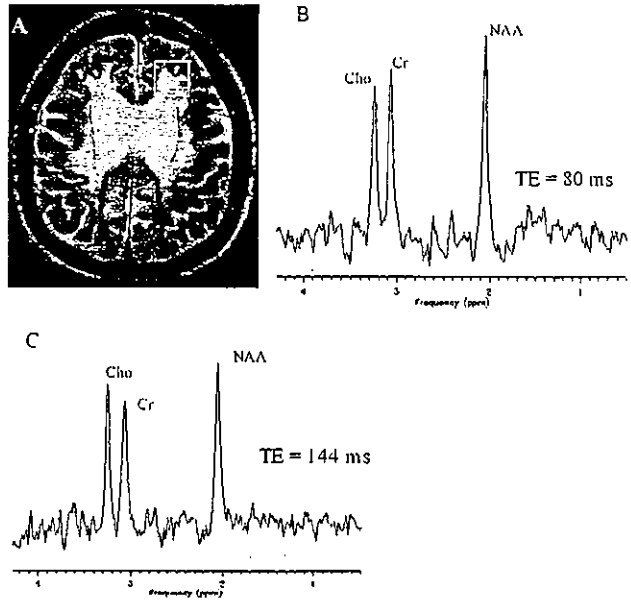

Figure 2. Proton magnetic resonance spectra obtained from the voxel of the left frontal white matter (A) at echo times (TE) of 80 ms (B) and 144 ms (C). N-acetyl-aspartate (NAA) level substantially decreased relative to creatine and phosphocreatine (Cr) level. The level of choline-containing compounds (Cho) relative to that of Cr also slightly decreased.

echo sequences, the volumes of interest (VOIs) for ^{1}H-MRS were selected as follows: 1) the left frontal white matter (20 × 20 × 20 mm; 64 acquisitions); 2) the right parietal white matter (20 × 20 × 20 mm; 64 acquisitions); 3) the left basal ganglia (15 × 27 × 15 mm; 128 acquisitions); and 4) the left dentate nucleus (15 × 15 × 15 mm; 96 acquisitions). The ^{1}H-spectra were quantified by peak area measurements with a GE spectral analysis program (SAGE). The concentrations of metabolites were calculated relative to creatine and phosphocreatine (Cr).

DNA analysis. After informed consent from the patient was obtained, high molecular weight genomic DNA was extracted from the peripheral leukocytes of the patient according to standard protocols. The entire coding regions of EIF2B2 and *EIF2B5* were amplified using previously described primer pairs.[7] The PCR products were subjected to direct nucleotide sequence analysis using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Results. T2-weighted MRIs showed diffuse hyperintense lesions in the cerebral white matter, which were more prominent in the frontal lobe than in the occipital lobe (figure 1). Parts of the white matter lesion adjacent to the anterior horn of the lateral ventricles and the posterior horn of the right lateral ventricles had signal intensities identical to those of CSF on fluid-attenuated inversion recovery (FLAIR) images, suggesting focal rarefaction and cystic degeneration. The atrophy of gyri and dilatation of ventricles were marked. ^{1}H-MR spectra revealed a markedly decreased N-acetyl-aspartate level relative to the level of Cr in the frontal white matter (figure 2) and dentate nucleus. The levels of choline-containing compounds compared with those of Cr were also slightly decreased in the frontal and parietal white matters and dentate nucleus. These findings suggest that the concentrations of all the compounds detected in normal white matters were decreased in the white matter lesions.

Mutational analysis revealed that this patient had a homozygous C545T transition in exon 4 of *EIF2B5* (see figure E-1 on the *Neurology* Web site). This mutation results in the substitution of methionine for threonine at codon 182 (T182M). This mutation has not been described previously and was not present in 45 unrelated Japanese control subjects.[2-6]

Discussion. Most patients with mutations in the eIF2B subunit had onset of VWM before age 6 years.[1] The neurologic signs of the disease include cerebellar ataxia and spasticity. Five adults with VWM have been reported.[2,6,8,9] Their MRI findings were compatible with the diagnosis of VWM. Two of the five patients were described as presymptomatic with molecularly confirmed VWM.[2] One of the two patients was noted to be clumsy in his motor activities at age 22 years and had learning difficulties.[9] However, the deterioration of his mental or motor symptoms was not evident. Two of the five adult patients with mutations in the eIF2B subunit gene had ovarian failure and secondary amenorrhea at ages 27 and 31 years.[6] Although both of them completed high school without difficulty, they presented with spastic gait in their 30s. In addition to these patients, another patient with clinically diagnosed adult-onset VWM has been reported.[8] He exhibited deteriorating cognitive abilities by age 38 years. Two