回，本人の面接で聞き取りを調査する。この調査の客観性と公平さを確保するために，全国一律の調査票と調査マニュアルが使われている。調査項目の中で痴呆症関連の質問項目が用意されている。調査項目1つひとつについて聞き方，判定の仕方のマニュアルが調査員に手渡されている。調査員はそれに従って忠実に職務を執行しているものと思われる。点数は加点法で，1が障害がなく，2あるいは3が障害があると入力される仕組みだ。調査票とそれに基づく電算機処理の仕組みは，おそらく細密に計算し尽くされたものであろう。しかし，日頃目にする痴呆性高齢者の認定結果を見ると，やはり介護者の介護負担や主治医の印象とかけ離れている場合が多い。この点について，現行の認定調査内容とその評価の仕方そのものに問題があることをすでに他の論文で指摘したが，なお改善にはほど遠い。一部手直しが行われた2003年以降について点検しても問題が未解決だ。

そのいくつかの問題を調査票の「理解度の把握の仕方」で見てみよう。理解度や記憶を調べる項目が6つある。そのうち，「生年月日や年齢を答えることが」「1.できる」，「2.できない」のいずれかにカウントする項目を見てみる。調査員マニュアルは「生年月日が答えられない場合は年齢のみでも，いずれか一方を答えることができる場合は「1.できる」とすることになっている。痴呆症を少しでも診療したことのある臨床医なら，今の年齢が曖昧で答えられなくても生年月日は答えられるケースが圧倒的に多いことに気づいているはずだ。マニュアルのように生年月日がわからなくて年齢が正解という珍しい記憶障害は特異例だ。それはさておいても，年齢を答えることと生年月日を答えることは等価ではない。重度の知能低下でも生年月日は答えられるのが一般的だ。今の年齢は今年が何年かの見当がついていないと答えられない。自己年齢が正しく言えることと，年齢はわからないが生年月日が言えることとの間は大きい。なのに，いずれか一方といえば，つまりは生年月日が陳述できれば「1」の「できる」にカウントされる。これでは，重度の痴呆まで理解度がよいほうに組み入れられてしまうはずだ。

このことを立証するために，筆者らが，痴呆症の程度と生年月日や年齢を答えることのできる知的レベルを在宅の高齢者，特別養護老人ホーム入所者614人を対象に面接調査した。その結果は，生年月日は答えられるが年齢はわからないケースまで拾うと，MMSEでは9点から10点レベルまで入り込む(図)。すなわち，高度の痴呆症まで「できる」に組み込まれる。こうした実証されたデータに基づけば，調査の評価が実態にあうかどうかが検証されるはずである。

日常生活において問題となる行動があるかどうかを評価する項目として，19項目が設定されている。これらの行動異常も頻度で3段階評価する。一見妥当に見える項目も，マニュアル通りにカウントすると問題があちこちに生じる。たとえば，「1人で外に出たがり目が離せない」では実際に徘徊外出傾向があっても「環境上の工夫などで外に出ることがなかったりした場合」は「1.ない」にカウントされる。毎晩の徘徊に困り果て玄関に鍵をかけて戦々恐々として休んでいる介護家族にとって納得のいかない判断だろう。

同じ思想が「火の始末や火元の管理ができないこと」が「ない」，「ある」のカウントでも貫徹されている。「環境上の工夫などで，火元に近づくことがなかったり，周囲の人々によって火元が完全に管理されている場合」は「ない」になる。火の始末ができなくなる痴呆症でも，周りがガスの元栓などを止めれば火の管理ができていることと等しく扱われてしまうのだ。背景にあったはずの痴呆の障害が無視されてしまう。異食行為でさえも，その行動がみられても，「異食しそうなものを周囲に置かない場合で防げていれば」「異食行為なし」なのである。痴呆症としての障害を客観的にまっすぐに評価せずに，予防的対策や介護対応の結果として，もともとある障害が一見しては目立たないと，その分を評価から差し引いてしまうというやり方をとっている。

なぜ，こうも痴呆症の認定が軽いのかは，調査

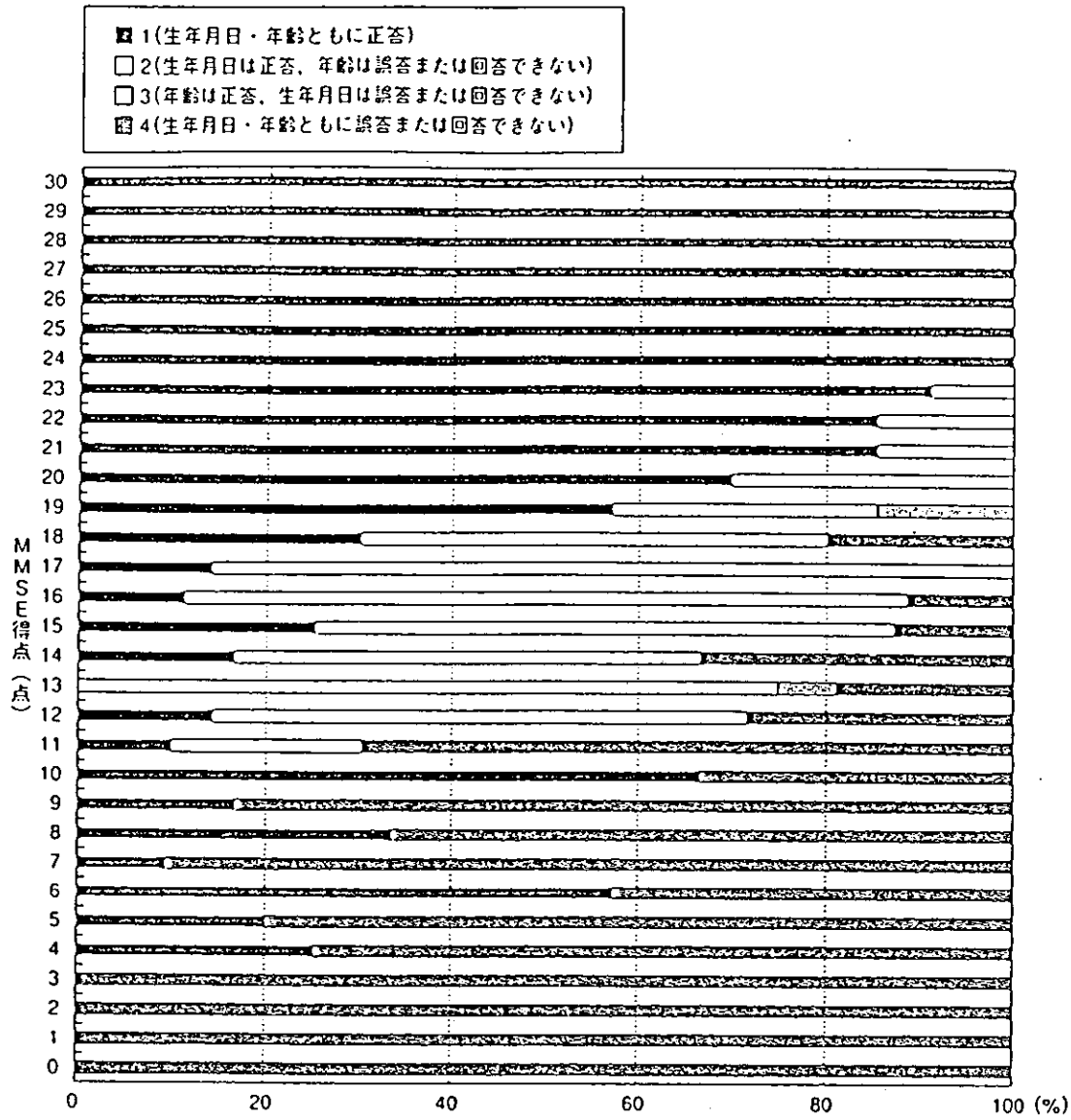

図　MMSE得点と生年月日・年齢に関する回答の関係

項目と面接調査の仕方，判断基準を見て納得がいく。マニュアルの内容を検討し，質問項目を再検討してみるとどうみてもこの調査項目に臨床現場からの目や老年精神科医の目が通ったとは考えられないのである。この質問の構成と判断基準では痴呆症の大部分は軽く判定される。これではいくらコンピュータソフトを見直し，審査委員会が頑張っても介護度は軽くなる。介護保険の公平さを維持するためにも，痴呆症の障害の程度を客観的に正確にとらえる方法と障害に対する介護の重さとをもう一度再検討し直すことが求められている。一部の手直しでは済まない問題である。

精神障害者への在宅サービス援助は現行の介護保険制度になじむか

介護保険の苦情の中で訪問介護に対する苦情は875件で，サービス内容に関する苦情の中では約32％を占め，もっとも多い。苦情内容は多岐にわたるが，「態度が悪い」「家庭内のことをよそでしゃべる」「ヘルパーにお金を貸したら返してくれない」「ヘルパーが入信している宗教を勧誘する」「ヘルパーが自分の健康のためという理由で自転車を利用せずに片道15分かけて買い物に行っている。時間がかかると料理を作ってくれない

時もある」などヘルパーの資質に関する苦情や「訪問介護を引き受けながら医療行為が伴うということで断られた」「しょっちゅうヘルパーを替え，仕事の仕方が変わる」といった事業所への不満が多い。それぞれにその都度対応がとられているが，こうした苦情，不満はヘルパー派遣件数が増えるにつれて増加する傾向にある。

見ず知らずの他人が生活の中に入り込んで利用者のできないところを援助する，というシステムだから，利用者の援助に対する理解と利用の仕方が鍵となる。1人暮らしの痴呆性高齢者の場合，「ヘルパーさん帰ってください」といった拒否で援助に入れなかったり，「ヘルパーがお金を盗んでいく」と訴えられ，処遇困難となるケースはどの事業所でも抱えている。

これまでのヘルパー派遣事業の実績から見て，在宅の統合失調症を中心とした在宅の精神障害者への援助が可能かどうかはきわめて心もとない。高齢者への自立生活援助は，決められた時間内にその人の生活の中に入り込んで生活介護をする建前だ。そのシステムをそのまま在宅の統合失調症患者の援助に適応することを危惧する精神科医は少なくないだろう。統合失調症の生活障害の質が高齢者の生活障害の質と本質的に異なるからである。在宅の統合失調症患者の場合，基本的には身体障害はないか，軽度だ。他人との交流は避ける傾向にあり，引きこもっている。自発性に欠け，無為な生活の中で安定を保っている場合もある。身辺は不潔で，身なりも無頓着だ。生活障害は主に慢性的な精神症状によってもたらされている場合が多い。このような状況下でヘルプが必要だと思うのは本人より親族や周りの関係者だ。親族が介護保険を申請し，援助が始まったとしても，家に入り込んだヘルパーは生活障害をどう援助するか戸惑うに違いない。

幻覚や妄想を抱えて生活している精神障害者の生活援助ではなおさらのことである。利用者が「水道には毒が入っているので水は煮沸してほしい」「見張られているので買い物は電話で注文するように」「外に声が漏れないように窓に目張りをして」といった指示にヘルパーはどう動いたらよいのかなど難しい対応を迫られる。精神障害者の看護に慣れた看護師や介護者ならその場にあわせて適切な対応ができるかもしれない。それもマニュアルではなくその場で相手の緊張度や内面を推し量りながら対応するという経験がものをいう。

精神障害者への生活援助は自立支援という意味合いの他，治療的な意味も大きい。したがって統合失調症に伴う生活障害には単純な生活支援，すなわちホームヘルプではなく，精神障害によってもたらされた生活障害を立て直す生活指導の側面が期待されるはずだ。とすれば，ホームヘルプは医療的なかかわりと医療チームの中で行われていることが望ましい。ヘルパーには精神障害者への対応に習熟した資質が要求される。この条件がそろわないまま現行の制度とサービス体系に組み込まれると現場は混乱するだろう。介護保険に対する信頼性を損ないかねない。

医療に介護保険が導入された結果

医療の現場でも介護保険制度が構造改革に近いインパクトを与えた。病院に介護療養型医療施設ができたことである。入院患者の中で高齢者を多く抱える病院は，これまでも事実上，医療よりは介護目的で長期に入院しているケースが多かった。医療者側では「社会的入院」と呼んでいた一群である。慢性疾患を抱えてはいるが，医療はそこそこ，ほとんどが食事と排尿排便の世話か，せいぜい現状を維持するリハビリテーションのための入院だった。高齢者人口が増えるにしたがって，こうした「社会的入院」患者の増加が医療保険財政の圧迫要因になっていた経緯がある。

介護保険制度の発足によって，介護サービスの中に病院入院を組み入れることが可能になった。老人を多く抱える病院は，医療保険費給付の低減する傾向にある「社会的入院」患者を医療保険で診ていくか，介護保険下に組み入れられるかの選択を迫られた。多くの病院は経営上のメリットを秤にかけた。介護療養型医療施設は地域限定で数

が決められるという政策の中で，全病床を介護療養型医療施設に切り替えるところ，一般病棟と介護療養型医療施設の2つを並存するところ，介護療養型医療施設は作らないところの3つに分かれた。それぞれが，病院の質と役割，病院経営上のメリット，デメリットを計算して決断したはずだ。

筆者の勤める定床300の老人病院である浴風会病院でも150ベッドが介護療養型医療施設になった。その結果，確かに入院とは名ばかりの介護目的の「社会的入院」は市民権を得た。しかし，実際に運用してみると，介護療養型医療施設は一般病棟の急性期治療が終わっても自宅や特別養護老人ホームに復帰できない介護度の重い高齢者で占められるようになった。外来からの介護療養型医療施設への入院は，病院経営上から介護度の重い利用者を選ぶ傾向にある。介護療養型医療施設の利用者は要介護4か5で占められる。身体障害がもともと重い集団だ。その結果は，療養中に肺炎，脱水，慢性疾患の悪化が繰り返される。その都度医療措置が必要になり，一般病棟に移送し，また元に戻すというやりとりを行うことになる。一般病棟が減った結果，外来や特別養護老人ホーム，ショートステイなどで急変した患者を受け入れる病棟が少なくなり，いざという時の医療が手薄になった。高齢者の医療への依存度が高まり，医療水準への要求も高まっているのに応えられない現状がある。

介護療養型医療施設は介護保険金の上限が介護度で決められている。病院経営を健全にするためには介護療養型医療施設での医療は絶えず，控えるということを意識しないと破綻する仕組みになった。これまで徘徊や迷子で困ったケースや幻覚，妄想で興奮した痴呆性高齢者を受け入れていた痴呆病棟も，介護保険下に組み入れられたために介護度の重い車椅子レベル以上の痴呆性高齢者ばかりになった。異常行動が激しく対応困難な痴呆性高齢者でも，介護度1や2では入院させにくくなった。問題点は解決するどころかますます深刻化している。こうした事態は都市部にある老人病院に限らずどこでも一般的にみられることであろう。介護療養型医療施設のあり方を単なる介護サービスという位置づけからまず外して見直していかないと，高齢者医療の一端が崩れていくのではないかと危惧する。

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Apolipoprotein E receptor 2 is involved in neuritic plaque formation in APP sw mice

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Abstract

Apolipoprotein E receptor 2 (apoER2) is a receptor for apolipoprotein E containing lipoprotein and also for Reelin. Apolipoprotein E-associated risk of developing Alzheimer's disease (AD) may be related to its binding to and clearance by cell surface receptors, including members of the low-density lipoprotein receptor family. Otherwise there is circumstantial evidence that the Reelin signaling pathway may contribute to neurodegeneration in AD. To investigate the role of apoER2 on amyloid deposition and neurodegeneration in vivo, we examined the presence of apoER2 in the brains of APP sw transgenic mice (Tg2576) using three apoER2 monoclonal antibodies. Our immunohistochemical study revealed that apoER2 was localized in fine granular structure and reactive astrocytes surrounding amyloid plaques. The double labeling immunohistochemistry revealed that this granular structure overlaps synaptophysin-positive dystrophic neurites. These findings indicate that neuronal apoER2 may play a role for amyloid deposition and neuronal degeneration in AD.

Keywords: Alzheimer's disease; Reelin; apoER2; apoE; Transgenic mice; Aβ; Neurodegeneration; Neuritic plaque

Apolipoprotein E (apoE) occurs in three major isoforms, apoE2, apoE3 and apoE4, with apoE3 being the most common isoform. In 1993 Schmechel et al. [15] reported that the apoE4 isoform is genetically associated with late onset Alzheimer's disease (AD). The underlying pathological mechanism by which apoE4 predisposes its carriers to AD is not precisely known.

In the brain, apoE is primarily produced by glial cells, whereas its receptors are most abundantly expressed on neurons [16]. Several cellular processes or pathways in which neuronal apoE receptors may affect the pathogenesis of AD have been proposed. They include interactions with APP [18], modulation of amyloid-β clearance from the extracellular space [2] and transmission of signals to neurons [20]. Any of these functions may be modulated by binding of apoE to the extracellular domains of the different members of the low-density lipoprotein (LDL) receptor family member.

ApoE receptor 2 is a member of the LDL receptor family which is predominantly expressed in the brain [9]. ApoER2 is also a receptor for Reelin, a signaling protein that regulates neuronal migration during brain development [5]. Recently, there is a hypothesis the Reelin signaling pathway may contribute to the neurodegeneration [7]. Studies of the Reelin pathway showed that the cytoplasmic tail of apoER2 bound to Disabled 1 (Dab1) and its subsequent tyrosine phosphorylation is critical for the transmission. Mutation in the Reelin signaling pathway was shown to cause hyperphosphorylation of the microtubule-stabilizing protein tau [6].

Recently Ma et al. [11] reported that apoER2 gene polymorphisms were associated with AD. We previously reported that immunoreactivity of apoER2 was localized in neurons in

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AD and control human brains [13]. However, we did not detect any immunoreactivity which was associated with senile plaques. Therefore, we used the brains of APP sw transgenic mice (Tg2576) brain tissue, which would not be affected by post mortem intervals and analyzed the relationship between apoER2 localization and various proteins composing senile plaques using a confocal microscopy.

We performed apoER2 immunostaining using three monoclonal antibodies (CYB-5G7, CYA-6D6, and LB3-8G7). In addition to CYB-5G7 which was described previously [13], we raised CYA-6D6 and LB3-8G7. Synthetic peptides corresponding to the 17 amino acids (PAEKLSCGPTSHKCVPA) in the cytoplasmic domain was used for CYA-6D6 and a synthetic peptide corresponding to the 17 amino acids (DELHIGRTAQIGHVYPA) in the ligand-binding repeat 3 was used for LB3-8G7.

In order to confirm the specificity of the monoclonal antibodies, we performed immunoblotting against membrane fraction from Chinese hamster ovary (CHO) cell lines lacking hamster LDL receptor expressing human LDL receptor, human VLDL receptor or human apoER2 as previously described [13]. CYA-6D6 and LB3-8G7 recognized a 160 kDa band in the membrane fraction of cells expressing human apoER2 whereas it did not recognize the proteins of cells expressing human LDL receptor or VLDL receptor (Fig. 1). We reported the specificity of CYB-5G7 previously [13].

Fifteen-month-old transgenic (Tg) 2576 mice [8] ($n = 6$, age 15 months) (Immuno-Biological Laboratories, Tokyo, Japan) were obtained. Mice were sacrificed by cervical dislocation, and the brains were removed and fixed by immersion in Bouin solution for 2 days. The brains were then cut coronally into five blocks, dehydrated in pure ethanol, and embedded in paraffin. Serial sections were cut at 6-μm thickness. Slides were incubated with formic acid for 5 min at room temperature. Endogenous peroxidase was blocked by 3% H_2O_2 (15 min at room temperature). Nonspecific antigens were blocked with 10% goat serum in PBS (30 min at room temperature). An apoER2 monoclonal antibody (1:50) was incubated overnight at 4 °C and then sections were incubated with a secondary anti-mouse biotinylated antibody for 60 min, followed by HRP-conjugated streptavidin for 60 min. For immunostaining amyloid, rabbit polyclonal anti-amyloid β 1–42 antibody 1:300 (IBL, Gunma, Japan) was used. Finally Diaminobenzidine (DAB) was used as chromogen. For triple immunofluorescence CYB-5G7 was labeled with Alexa 633 using Alexa Fluor 633 protein labeling kit (Molecular Probes, Leiden, Netherlands). For astrocyte immunostaining, a mouse anti-glial fibrillary acidic protein (GFAP) antibody 1:2000 (Nichirei, Japan) was used. For immunostaining neuritic plaque, goat polyclonal anti-apoE antibody 1:300 (CHEMICON, CA, USA), mouse monoclonal anti-synaptophysin antibody 1:50 (PROGEN, Heidelberg, Germany) and mouse monoclonal anti-phosphorylated tau antibody AT81:400 (Innogenetics, Belgium) were used. Alexa 594-conjugated goat anti-rabbit IgG (H + L) 1:500 (Molecular probes), TRITC conjugated swine anti-rabbit immunoglobulins 1:500 (DAKO, Denmark), Alexa 488-conjugated goat anti-mouse IgG (H + L) 1:100 (Molecular probes) or Alexa 488-conjugated rabbit anti-goat IgG (H + L) 1:100 (Molecular probes) were used for visualization. Fluorescent images were then visualized with a confocal laser microscopy (LSM 510 Version 2.5, Carl Zeiss, Heidelberg, Germany).

Abundant senile plaques with giant cores were labeled by anti-amyloid β 1–42 antibody (Fig. 2a). Interestingly fine granular structure surrounding amyloid cores were labeled with all three anti-apoER2 antibodies (Fig. 2a and b). CYB-5G7, CYA-1D9 and LB3-8G7 labeled approximately 34%, 44% and 25.5% of all senile plaque cores, respectively. The triple immunostaining showed that the apoER2-positive granular structures did not overlap with the amyloid β-positive cores while apoER2-positive structures overlapped with synaptophysin-positive dystrophic neurites (Fig. 3e–h). These apoER2-positive granular structures overlapped neither with apoE-positive amyloid cores (Fig. 3i–l) nor tau-positive dystrophic neurites (Fig. 3m–q).

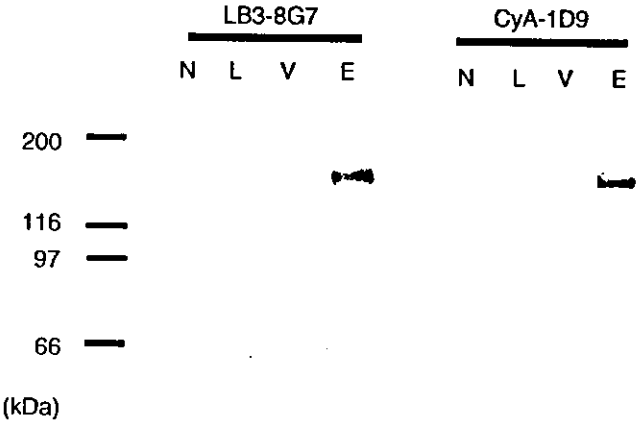

Fig. 1. Immunoblots showing the specificity of two monoclonal apoER2 antibodies. N: CHO cells lacking hamster LDL receptor, L: CHO cells lacking hamster LDL receptor expressing human VLDL receptor, V: CHO cells lacking hamster LDL receptor expressing human apoER2.

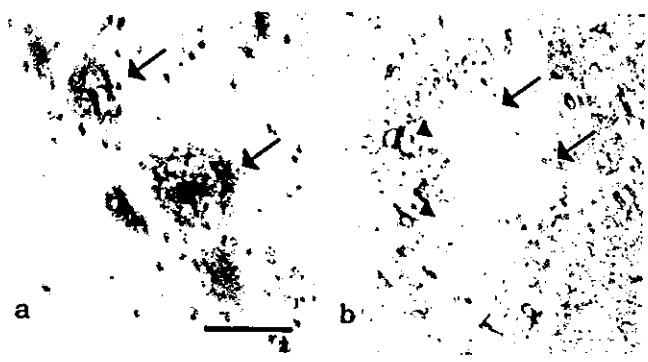

Fig. 2. Serial sections of the Tg 2576 brain with antibodies to Aβ 1–42 (a) and apoER2 (b). Anti-Aβ 1–42 antibody labeled senile plaques with cores (arrow) (a). CYB-5G7 did not stain amyloid cores (arrow). However, CYB-5G7 stained the granular structure surrounding amyloid cores and glial cells (arrow head). Bar = 20 μm.

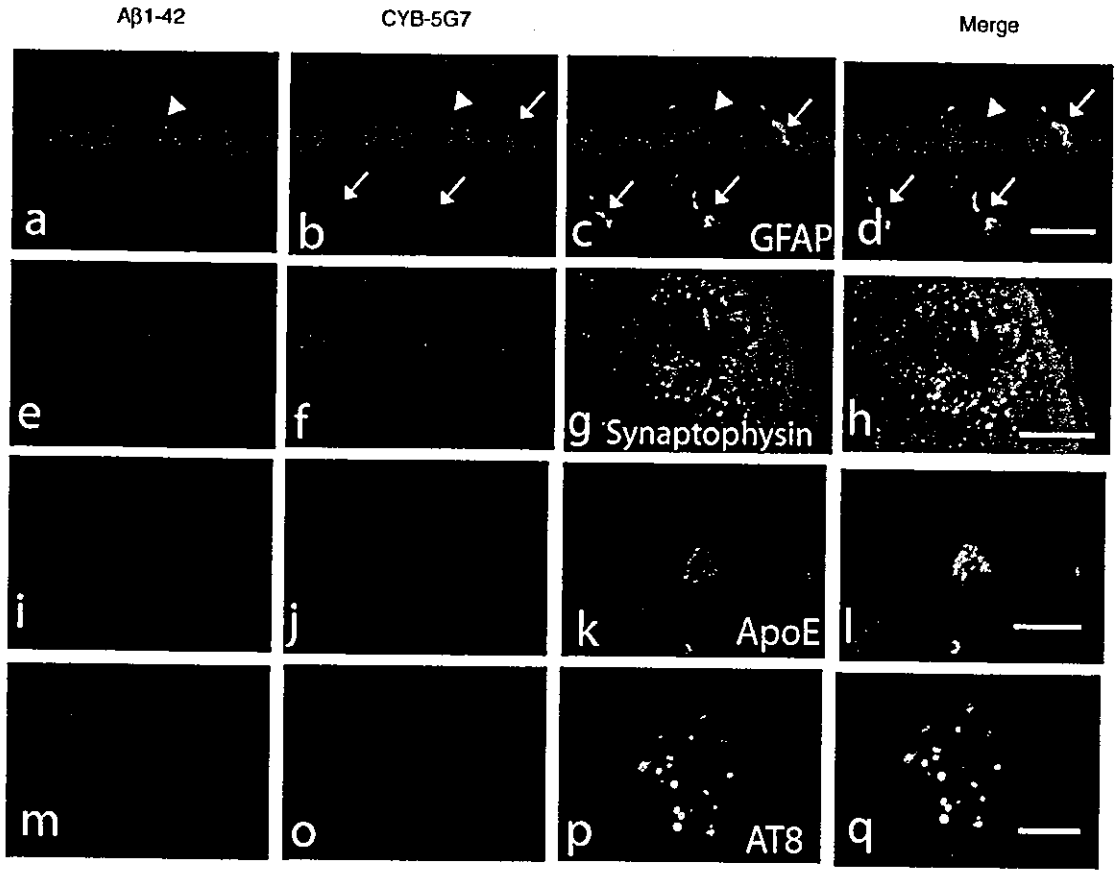

Fig. 3. Triple labeling immunohistochemistry of neuritic plaque with Aβ1–42 (a–p), CYB-5G7 (a–p), GFAP (a–d), Synaptophysin (e–h), apoE (i–l) and AT8 (phosphorylated tau) (m–p). Merged images (Aβ1–42 is labeled with Alexa 594 or TRITC [red], GFAP, synaptophysin and apoE with Alexa 488 [green], CYB-5G7 with Alexa 633 [blue] and double-labeling with light blue and yellow) are shown in d, h, l and q. (a–d) CYB-5G7 immunostained glial cells (arrow) surrounding Aβ1–42-positive amyloid core (arrow head). Merged images (d) shows these astrocytes are GFAP-positive (light blue, arrow). (e–h) CYB-5G7 stained granular structures surrounding amyloid core (arrow). Merged images show this structure overlapped with Synaptophysin-positive structure (light blue). (i–l) CYB-5G7-positive granular structure is not overlapped with apoE-positive amyloid core. (m–q) CYB-5G7-positive granular structure is not overlapped with AT8-positive dystrophic neurites. Bar = 20 μm.

Anti-synaptophysin antibody and AT8 labeled the dystrophic neurites in approximately 52% and 65% of all senile plaques, respectively. CYB-5G7, CYA-1D9 and LB3-8G7 also immunostained neuronal cell bodies in the hippocampus, the cortex and the thalamus.

In the cerebral cortex abundant glial cells were labeled with CYB-5G7 (Figs. 2b and 3a–c). Triple immunostaining revealed those glial cells were GFAP-positive astrocytes (Fig. 3j–l). In the thalamus and brain stem, CYB-5G7 labeled almost exclusively neurons but not glial cells. Since neuritic plaques were absent in the thalamus and the brain stem, these findings may indicate that CYB-5G7-positive astrocytes are reactive astrocytes. CYA-1D9 and LB3-8G7 labeled perineural oligodendroglia in the cerebral cortex. Reactive astrocytes were faintly labeled by these two antibodies (data not shown).

The presence of apoER2-positive astrocytes in the cerebral cortex may indicate that β amyloid induces apoER2 expression on astrocytes since apoER2 is present exclusively in neurons in normal brain [4,13]. In general glial cells express low-density lipoprotein receptor (LDLR) and LDLR-associated protein (LRP) in the LDLR family. By immunostaining Aβ-induced activated cultured astrocytes express both LDLR and LRP [10]. ApoE antagonist receptor-associated protein (RAP) blocks Aβ-induced activation. To date, apoER2 was not examined about the presence of cultured activated glial cells. Since RAP binds several apoE receptors including apoER2, apoER2 may mediate the effects on Aβ on astrocyte as well as LDLR and LRP.

We suggest that the labeling of synaptophysin-positive neurites indicates the accumulation of apoER2 associated with synaptic vesicle membranes during neurodegeneration. In an adult brain, mice lacking ApoER2 show deficiencies in long-term potentiation [20]. Furthermore physiological studies of mice deficient in Reelin reveal an attenuation of rod-driven retinal responses which is associated with a decrease in an abnormal distribution of processes which implies the Reelin pathway contributes to the formation of retinal synaptic circuitry [14].

Masliah et al. [12] reported that ultrastructual observations of transgenic mice harboring human APP showed that abnormal, dilated neurites in plaques contained neurofilament, accumulations of multivesicular bodies, multilamellar

bodies, degraded mitochondria and synaptic vesicles. Most of these share several critical subcellular alterations with AD. They suggested that plaque formation was initiated with local synaptic alterations induced by possible abnormalities in APP processing and Aβ formation. Furthermore Brion suggested that synaptic vesicle accumulation in dystrophic neurites might witness for axoplasmic flow disturbances in these neurites since synaptic vesicles would move along microtubules [3]. The c-Jun N-terminal kinase (JNK)-interacting protein-1b/islet-brain-1 (JIP-1) is also an adaptor protein which interacts with ApoER2 in the Reelin signaling pathway [19]. JIPs and APP also bind to the anterograde molecular motor kinesin [19]. Thus, apoER2 may be involved in the disturbance of the axonal flow leading to neurodegeneration.

ApoER2 antibody did not immunostain the tau-positive dystrophic neurites. One hypothesis has been suggested that Reelin/apoE-receptor/Dab1 complex may initiate a signal transduction cascade that controls tau phosphorylation [6]. Our results did not support this hypothesis. Our results indicate that apoER2 may play a role in disturbing axonal flow rather than paired helical filament formation.

Increasing evidence suggests that apoER2 may function as a signal transduction rather than receptor-mediated endocytosis. The first the binding affinity of RAP (the receptor-associated protein, a substrate for receptor-mediated endocytosis) to apoER2 was 25-fold lower than the binding affinity of Reelin [1]. The second ApoER2 contains one distinct structural feature in its cytoplasmic domain, encoded by a single exon, containing three PXXP motifs. PXXP motifs bind src homology domains, most of which are found in proteins involved in signal transduction pathways. In addition chimeras comprising the ectodomain and transmembrane domain of the LDL receptor fused to the cytoplasmic domain of apoER2 lacking the PXXP motifs are able to mediate clathrin-dependent endocytosis of LDL but not if the PXXP motifs is present in the protein [17]. Although the role of the Reelin pathway in the adult brain is not precisely known, there is one hypothesis that the binding of Reelin to apoER2 is inhibited by recombinant apoE [5]. Further studies will be needed to elucidate the underlying mechanism by which apoE4 predisposes its carriers to AD.

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ORIGINAL RESEARCH ARTICLE

Molecular characterization of bipolar disorder by comparing gene expression profiles of postmortem brains of major mental disorders

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We performed the oligonucleotide microarray analysis in bipolar disorder, major depression, schizophrenia, and control subjects using postmortem prefrontal cortices provided by the Stanley Foundation Brain Collection. By comparing the gene expression profiles of similar but distinctive mental disorders, we explored the uniqueness of bipolar disorder and its similarity to other mental disorders at the molecular level. Notably, most of the altered gene expressions in each disease were not shared by one another, suggesting the molecular distinctiveness of these mental disorders. We found a tendency of downregulation of the genes encoding receptor, channels or transporters, and upregulation of the genes encoding stress response proteins or molecular chaperons in bipolar disorder. Altered expressions in bipolar disorder shared by other mental disorders mainly consisted of upregulation of the genes encoding proteins for transcription or translation. The genes identified in this study would be useful for the understanding of the pathophysiology of bipolar disorder, as well as the common pathophysiological background in major mental disorders at the molecular level. In addition, we found the altered expression of *LIM* and *HSPF1* both in the brains and lymphoblastoid cells in bipolar disorder. These genes may have pathophysiological importance and would be novel candidate genes for bipolar disorder.
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Keywords: microarray; schizophrenia; depression; lymphoblastoid

Introduction

Bipolar disorder is a severe mental disorder characterized by recurrent manic and depressive episodes. Twin, adoption, family and linkage studies suggested that bipolar disorder is a complex disease caused by multiple genetic and environmental risk factors.[1] Pharmacological evidence suggests the involvement of monoaminergic systems and intracellular second messenger systems in bipolar disorder.[2,3] However, the etiology of bipolar disorder has not been established. The uncertainty of phenotype definition and complex mode of inheritance impede the understanding of bipolar disorder at the molecular level by conventional strategies.

Genome-wide gene expression analysis using DNA microarray, by which expression of thousands of genes can be monitored, has a great advantage to identify the genes or specific molecular cascades involved in the complex diseases, especially mental disorders.[4] Pioneering studies, which examined the postmortem brains of patients with schizophrenia by DNA microarray, revealed the downregulation of the genes encoding proteins functioning at the presynapses,[5] genes expressed in the oligodendrocytes,[6] neuropeptide Y,[7] or the upregulation of apolipoprotein genes in schizophrenia.[8] On the other hand, there is one DNA microarray study of bipolar disorder, which revealed the altered expression of the genes encoding the signal transduction proteins.[9]

Bipolar disorder shares signs and symptoms of depressive episodes with major depression, and also shares clinical features with schizophrenia, such as their chronic and relapsing course, and psychotic symptoms.[10] The molecular basis of the uniqueness of bipolar disorder and its similarity to the other two disorders remains to be studied. Here, we carried out the microarray analysis in bipolar disorder, major depression, schizophrenia, and control subjects using postmortem frontal cortices provided by the Stanley Foundation Brain Collection. The differentially expressed genes in bipolar disorder were compared with those in other mental disorders.

Among the genes whose differential expressions in the brains were confirmed by RT-PCR, the expressions of *LIM* and *HSPF1* were also altered in lymphoblastoid cells of patients with bipolar disorder. These genes may have some pathophysiological importance such as the genetic abnormality in bipolar disorder.

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Materials and methods

Brain samples

Samples of postmortem prefrontal cortex (Broadmann's Area 10) were donated by the Stanley Foundation Brain Collection. They were derived from patients with bipolar disorder, major depression, schizophrenia, and control subjects. Each group consisted of 15 subjects. Diagnoses had been made according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.[11] A summary of the demographic information of subjects used in this study is shown in Table 1. Detailed information of the original set of subjects was described elsewhere.[12]

Microarray procedure

Total RNA was extracted from 0.1 g of frozen tissues using Trizol (Invitrogen, Groningen, The Netherlands). After cleaning up using an RNeasy column (Qiagen, Hilden, Germany), the purity and integrity of total RNA was evaluated by OD measurements and denaturing agarose gel electrophoresis, respectively. Microarray analysis was performed according to the manufacturer's protocol (Affymetrix, Santa Clara, CA, USA). Briefly, 8–10 μg of total RNA was used to synthesize cDNA. This was used to generate biotinylated cRNA. cRNA was fragmented and first applied to the Test2Chip (Affymetrix) to assess the sample

Table 1 Summary of the demographic variables of subjects used in this study

	N	*Age (years)*	*Gender*	*PMI (h)*	*Medication*	*Cause of death*
			Male : female		*Medicated : nonmedicated*	*Suicide :nonsuicide*
Postmortem brains						
Control subject	15	48±11	9M:6F	24±10	0M:15NM	0S:15NS
Bipolar disorder	11	39±12	8M:3F	32±16	9M:2NM	8S:3NS
Major depression	11	46±10	6M:5F	27±12	9M:2NM	4S:7NS
Schizophrenia	13	44±14	8M:5F	33±15	10M:3NM	4S:9NS
Lymphoblastoid cells						
Control subject	11	51±10	8M:3F	—	—	—
Bipolar disorder	14	53±11	9M:5F	—	—	—

Table 2 Primer sequences used in this study

	Size (bp)	*Forward primer (5′ to 3′)*	*Reverse primer (5′ to 3′)*
GAPDH	107	ATCATCAGCAATGCCTCCTGC	ATGGCATGGACTGTGGTCATG
Beta actin	152	ATTGCCGACAGGATGCAGAA	TTGCTGATCCACATCTGCTGG
Cofilin	73	CGCCCCTTAAGAGCAAAATG	TGCAATTCATGCTTGATCCC
HSP27	113	GTCCCTGGATGTCAACCACTTC	AGATGTAGCCATGCTCGTCCTG
IFITM3	90	TCGTCTGGTCCCTGTTCAACA	TCCTGTCCCTAGACTTCACGGA
CEBPD	101	CATCGACTTCAGCGCCTACAT	CCTTGTGATTGCTGTTGAAGAGGT
APM2	96	TGACGACTCCACAGATACCCC	GCTGACACGGCTTCCTGG
AQP4	144	TGTCTTCTACATCGCAGCCCA	TCAACCAGGAGACCATGACCA
HEPH	237	AGGAAAATGTGGCAACCCATG	TCTCGCCATTCCGATAGAGGA
LIM	111	TCCTTGGAGAAGTCATCAATGC	CACCATCCTCCAAGTGAAAAAC
KIAA0133	137	AGTATGGAAGCGTCTTCCCGA	CCCTTCCCGCATAACTGAAAA
CACNA1A	90	ACCTCAGTACCATCTCAGACACCAG	CCAGCGAGTAATCGTCCAGG
GRM1	99	TCTGTGAAGGCATGACAGTGCG	TGTCTGCCCATCCATCACTTC
GRIK1	86	GGCGGTTACAGATGGATCAACA	TGCTGCTCATGAAAGCCCA
DTNA	134	TGTCTTCCATCCGGTTGAGTG	TATGAGAACCACCGGCATGTC
HSP40	139	TGATGTCATTTATCCTGCCAGG	AGGAACTTTTCGCCGCATG
DKFZp564H203	84	GCAGACAGAAAACCTCAACCCC	AACAAGCACTTCCTGTGCCCAG
COL16A1	89	GACATTGGTATTGGCATTGCAG	GTTGCACCCATCTTGCCAT
SPRAC	113	AATGACAAGTACATCGCCCTGG	GAGAATCCGGTACTGTGGAAGG
BTN3A3	144	TACGCTGCAACAGAGCAAGAA	CACATCCGCAGGTTTGAAGA
TAF6L	81	GGACTTGCAGACGAACTCCAA	TGGCTTACAGATTTCACCCCA
GOLGA4	128	GGACACCTTACAAAGGTGGCAA	TGCCATGGTCTTAGTCTCACGA
KIAA0645	154	GACCCTGGATGTTGACGTGAA	CAACCTTGGACCATCTCGAAG
KDR	94	AGAGCCGGCCTGTGAGTGTAAA	CCACTGTCCGTCTGGTTGTCAT

quality, and then applied to the HU95A chip (Affymetrix), which contains probes for about 12 000 genes. The hybridization signal on the chip was scanned using a scanner (HP GeneArray scanner, Hewlett-Packard, Palo Alto, CA, USA), and was processed by GeneSuite software (Affymetrix).

Microarray data analysis

The gene expression data generated by microarray analysis were imported into GeneSpring 5.0. software (SiliconGenetics, Redwood, CA, USA). Data of each array were then normalized by dividing the median of its gene expression value. Data were then filtered based on the following criteria: (i) genes marked as present (detected) in at least half of the samples in each diagnostic group, (ii) genes that showed their expression changes were not associated with aging, postmortem interval (Pearson's correlation coefficient, $P \geq 0.05$), and gender (Student's *t*-test, $P \geq 0.05$). Of approximately 12 000 genes, 5138 genes passed these filtering procedures. Differentially expressed genes compared with the control group were defined based on the following criteria: (i) 1.3-fold or greater change in the mean expression level, (ii) $P < 0.05$ in the two-tailed Student's *t*-test. Statistical analysis was performed using SPSS 10.0J software (SPSS Co. Ltd, Tokyo, Japan).

Consideration of the effects of medication status, alcohol abuse, and drug abuse on the gene expressions in bipolar disorder

The possible effect of each class of medication, as a confounding factor for genes differentially in bipolar disorder, was considered by dividing all subjects regardless of their diagnoses ($N = 50$; see the Results and discussion section) into medicated and nonmedicated groups. Two-tailed Student's *t*-test was employed in the statistical analysis ($P < 0.05$ was considered to be significant). The medication status considered was as follows: lithium-treated ($N = 6$) and nontreated groups ($N = 44$), anticonvulsants treated ($N = 4$) and nontreated groups ($N = 46$), antidepressants treated ($N = 13$) and nontreated groups ($N = 37$), antipsychotics treated ($N = 16$) and nontreated groups ($N = 34$), benzodiazepine treated ($N = 6$) and nontreated groups ($N = 44$). In case of the consideration of the effects of alcohol abuse and drug abuse, all subjects were divided into two groups according to the qualitative ratings (on a 1–5 scale) of severity of abuse: moderate to severe use (ratings 3–5) of alcohol ($N = 10$) and none or light use (ratings 1–2) groups ($N = 34$), moderate to severe use of drugs ($N = 12$) and none or light use ($N = 38$) groups. Some subjects whose status of alcohol use was not available were excluded from the analysis.

a

accession	gene	R	P	N
AF060538	vesicle-associated membrane protein 1 (synaptobrevin 1)	0.356	0.011	50
M63138	cathepsin D	0.332	0.019	50
J04177	collagen, type XI, alpha 1	-0.324	0.022	50
AF038660	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 2	-0.309	0.029	50

b

accession	gene	symbol	locus	male	N	female	N	M/F ratio	P
L25270	Smcx homolog, X chromosome	SMCX	Xp11	1.08	31	1.47	19	0.74	<0.001
AF000994	ubiquitously transcribed tetratricopeptide repeat gene, Y chromosome	UTY	Yq11	0.42	31	0.03	16	13.19	<0.001
U79247	protocadherin 11 X-linked	PCDH11X	Xq21	0.57	31	0.18	19	3.21	<0.001
AA669799	acetylserotonin O-methyltransferase-like	ASMTL	Xp22	1.54	31	1.10	19	1.40	<0.001
U52191	Smcy homolog, Y chromosome	SMCY	Yq11	1.01	31	0.11	18	9.48	<0.001
M86934	DNA segment, numerous copies, expressed probes (GS1 gene)	DXF68S1E	Xp22	0.41	31	0.64	19	0.63	<0.001
AF000984	DEAD/H box polypeptide, Y chromosome	DBY	Yq11	1.20	31	0.03	16	34.26	<0.001
AB018328	Ac-like transposable element	ALTE	Xp22	3.03	31	2.57	19	1.18	<0.001
AF000982	DEAD/H box polypeptide 3	DDX3	Xp11	1.31	31	1.76	19	0.75	<0.001
Y14391	Pseudoautosomal GTP-binding protein-like	PGPL	Xp22	2.39	31	1.73	19	1.38	<0.001
M18279	antigen identified by monoclonal antibodies 12E7, F21 and O13	MIC2	Xp22	1.55	31	0.73	19	2.13	<0.001
M58459	ribosomal protein S4, Y-linked	RPS4Y	Yp11	3.70	31	0.05	16	77.40	<0.001
Y15801	protein kinase, Y-linked	PRKY	Yp11	0.32	31	0.22	18	1.45	0.001
M58458	ribosomal protein S4, X-linked	RPS4X	Xq13	15.94	31	17.73	19	0.90	0.002
Y15521	acetylserotonin O-methyltransferase-like	ASMTL	Xp22	1.30	31	0.85	19	1.53	0.003
U78575	phosphatidylinositol-4-phosphate 5-kinase, type I, alpha	PIP5K1A	1q22-q24	0.89	31	1.10	19	0.82	0.004
X89887	HIR histone cell cycle regulation defective homolog A	HIRA	22q11	0.44	31	0.59	19	0.73	0.004
AB002354	KIAA0356 gene product	KIAA0356	17q21	0.51	31	0.67	19	0.77	0.004
AF051160	protein tyrosine phosphatase type IVA, member 1	PTP4A1	6q12	2.67	31	3.26	19	0.82	0.005
M58525	catechol-O-methyltransferase	COMT	22q11	1.20	31	1.46	19	0.82	0.005

Figure 1 Genes whose expressions were influenced by aging or gender. (a) Representative examples of the aging-influenced gene expressions, which were previously identified by microarray analysis of aging.[14] *R*, Pearson's correlation coefficient; *P*, probability value; *N*, number of the samples used for calculation. (b) List of the top 20 genes whose expressions were influenced by gender. The pairs of NRY gene and its X homologue are colored. The mean expression levels in males and females are shown. The values less than 1 in the M/F ratio means downregulation in males. The complete lists are available on request (kato@brain.riken.go.jp).

Lymphoblastoid cells

Lymphoblastoid cell lines of control subjects and patients with bipolar disorder were established by standard protocols.[13] A summary of demographic information of subjects is shown in Table 1. Briefly, lymphocytes were separated from peripheral blood using Ficoll-Paque (Pharmacia-Upjohn, Peapack, NJ, USA), and cultured with RPMI 1640 medium containing 20% fetal bovine serum (FBS), penicillin, streptomycin, cyclosporin A, and filtered supernatant of the B95-8 cell culture infected by Epstein–Barr Virus. The cells were subcultured every week until the cell line was established. Thereafter, the cells were subcultured three times a week using a similar medium, except for the addition of 10% FBS and no cyclosporin A. The cells were kept frozen until the experiment. After reculturing, total RNA of the cells was extracted using Trizol reagent and then was treated with DNase I. This study was approved by the Ethical Committee of the Brain Science Institute, RIKEN, and written informed consent was obtained from each subject.

Real-time quantitative PCR

A measure of 1–5 μg of total RNA was used for cDNA synthesis by oligo(dT) and SuperScript II reverse transcriptase (Invitrogen). Real-time quantitative PCR using SYBR/GREEN I dye (Applied Biosystems, Foster City, CA, USA) was performed with ABI PRISM 7700 or 7900HT (Applied Biosystems). The comparative Ct method was employed for quantification of transcripts according to the manufacturer's protocol (User Bulletin #2, Applied Biosystems). In addition to the two housekeeping genes conventionally used for normalization (beta actin and GAPDH), cofilin 1, which encodes actin-binding protein, was selected as a control gene by identifying a gene showing the constant expression levels by DNA microarray analysis across the samples (data not shown). Measurement of delta Ct was performed at least in triplicate. Amplification of the single product in RT-PCR was confirmed by monitoring the dissociation curve and by agarose gel electrophoresis. One-tailed and two-tailed Student's *t*-tests were employed in the statistical analysis of the results of postmortem brains and lymphoblastoid cells, respectively ($P<0.05$ was considered to be significant). The genes examined by RT-PCR were as follows: *HSP27, IFITM3, CEBPD, APM2, AQP4, HEPH, LIM, KIAA0133, CACNA1A, GRM1, GRIK1, DTNA, HSPF1, DKFZp564H203, COL16A1, SPARC, BTN3A3, TAF6L, GOLGA4, KIAA0645,* and *KDR*. They were chosen for the quantitative PCR analysis, since they were also identified as the differentially expressed genes by the preliminary data analysis based on different normalization procedures (data not shown). Primer sequences used for RT-PCR are listed in Table 2.

Results and discussion

Filtering procedures

Of the 60 samples initially analyzed, 10 were not suitable for DNA microarray analysis estimated by denaturing agarose gel electrophoresis or Test2Chip analysis. Thus, we could obtain gene expression profiles from 50 samples. These included 11 patients with bipolar disorder, 11 with major depression, 13 with schizophrenia, and 15 control subjects. Although there were no significant differences in the demographic variables among four groups, we removed the genes whose expressions were associated with aging, gender or postmortem interval (PMI). The genes previously reported to be altered with aging were confirmed, such as aging-dependent upregulation of cathepsin D and downregulation of collagen, type XI (Figure 1a).[14] The genes influenced by gender mainly consisted of the genes on the sex chromosomes (Figure 1b), including three nonrecombining region of the Y chromosome (NRY) class I genes. X homologues of NRY class I genes were also highly expressed in females, which is compatible with the finding that they are not subject to X

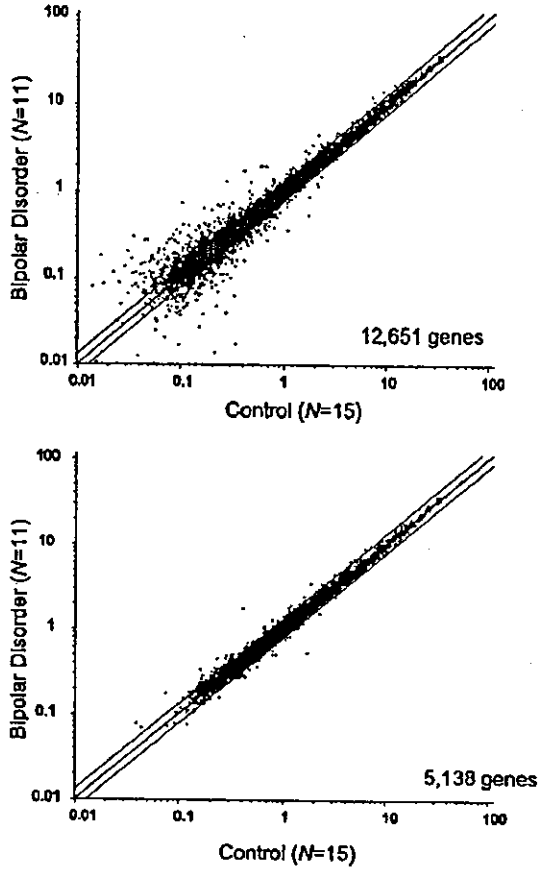
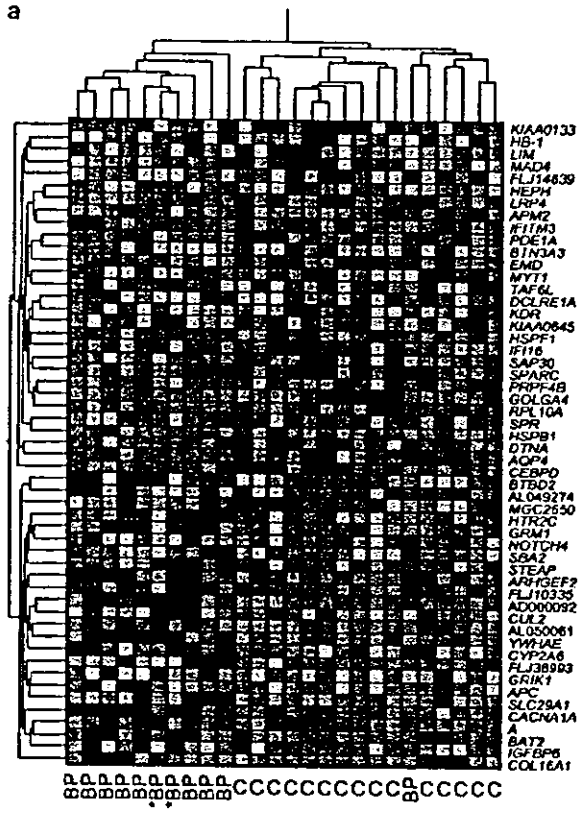


Figure 2 Scatterplots of the mean expression levels of bipolar disorder *vs* control groups. The plots were drawn based on all genes on the microarray (*left*) or the genes after the filtering procedures (*right*). The middle line in each plot indicates that the mean expression levels of the genes on this line are not different between the two groups. The upper and lower lines indicate the 1.3-fold up- and downregulation, compared with the control group, respectively.

inactivation in females.[15] These results imply the reliability of our microarray analysis. Although these genes might offer some insight into the significance of the pathophysiology, they were eliminated from the analysis since the sample size was not large enough to be subject to subgroup analysis.

Among the previously reported DNA microarray studies of mental disorders, Hakak *et al* reported the differentially expressed genes in schizophrenia, using a microarray system comparable with ours.[6] Comparison of the microarray results by the clustering analysis revealed that schizophrenics could be rela-



c

Acc no.	Symbol	Gene product	Fold change		
Channel/Receptor/Transporter			BP	MD	SZ
U49516	*HTR2C*	serotonin receptor 2C	-1.55	-	-1.46
U79666	*CACNA1A*	calcium channel, P/Q type, alpha 1A	-1.47	-	-
L76627	*GRM1*	glutamate receptor, metabotropic 1	-1.44	-	-
L19068	*GRIK1*	glutamate receptor, ionotropic, kainate 1	-1.43	-	-
U47924	*A*	protein "A"	-1.41	-	-
M62402	*IGFBP6*	insulin-like growth factor binding protein 6	-1.39	-	-
AB014551	*ARHGEF2*	rho/rac guanine nucleo. exchange factor 2	-1.37	-	-
U61375	*SLC29A1*	solute carrier family 29, member 1	-1.34	-	-
U95299	*NOTCH4*	Notch homolog 4	-1.34[b]	-	-
AC005053	*STEAP*	six transm. epithelial antigen of the prostate	-1.33	-	-
U90548	*BTN3A3*	butyrophilin, subfamily 3, member A3	1.32	-	-
AF035121	*KDR*	kinase insert domain receptor	1.35[b]	-	-
AB011540	*LRP4*	LDL receptor-related protein 4	1.35	1.36	-
U34646	*AQP4*	aquaporin 4	1.52[b]	1.45	-
Stress response/Chaperon			BP	MD	SZ
Z23090	*HSP27*	heat shock 27kDa protein 1	1.32	-	1.30
M63838	*IFI16*	interferon, gamma-inducible protein 16	1.35	1.52	-
D86429	*HSPF1*	heat shock protein 40	1.36[c]		-
X57352	*IFITM3*	interferon induced transm. protein 3	1.39	-	-
Transcription/Translation			BP	MD	SZ
AL022721	*RPL10A*	ribosomal protein L10a	1.31	-	-
AF089735	*TAF6L*	PCAF-associated factor, 65kDa	1.34	-	1.43
A3029973	*MYT1*	myelin transcription factor 1	1.38	-	1.34
AB011108	*PRPF4B*	PRP4 homolog B	1.38	1.36	1.31
AF055993	*SAP30*	sin3-associated polypeptide 30kDa	1.42[a]	1.38	-
D42045	*DCLRE1A*	DNA cross-link repair 1A	1.42	1.83	-
AF040963	*MAD4*	MAX dimerization protein 4	1.44	-	-
M63667	*CEBPD*	C/EBP, delta	1.56		1.74
Signal Transduction			BP	MD	SZ
M73548	*APC*	adenomatosis polyposis coli	-1.35	-	-
AA923149	*SBA2*	SS box-containing WD protein	-1.32	-	-
U54778	*YWHAE*	14-3-3 Protein, Epsilon	-1.30	-	-
U40370	*PDE1A*	PDE1A, calmodulin-dependent	1.40[a]	-	-
AF081258	*LIM*	LIM protein	1.56	1.43	1.48
U26742	*DTNA*	dystrobrevin, alpha	1.71	-	-
Others/Unknown			BP	MD	SZ
AC005306	*BTBD2*	BTB domain containing 2	-1.73	-	-
AL049274		cDNA DKFZp564H203	-1.60	-	-
AI885381	*MGC2650*	hypothetical protein MGC2650	-1.56	-	-
AF070524	*FLJ38993*	hypothetical protein FLJ38993	-1.45	-	-
AI962638	*COL16A1*	collagen, type XVI, alpha 1	-1.44	-	-1.58
AL043470	*FLJ10335*	hypothetical protein FLJ10335	-1.37	-	-
AD000092		EST	-1.35	-	-
M33318	*CYP2A6*	CYP2A, polypeptide 6	-1.35	-	-
AL050061		cDNA DKFZp566J123	-1.33	-	-
U83410	*CUL2*	cullin 2	-1.32	-	-
M33509	*BAT2*	HLA-B associated transcript 2	-1.30	-	-
X82834	*GOLGA4*	golgi autoantigen, golgin subfamily a, 4	1.31[b]	-	-
M76231	*SPR*	sepiapterin reductase	1.34	-	1.48
J03040	*SPARC*	osteonectin	1.34[a]	-	-
AB014545	*KIAA0645*	KIAA0645 gene product	1.34	-	-
X86810	*EMD*	emerin	1.37	1.36	-
AI361790	*APM2*	adipose specific 2	1.39		-
AA152202	*FLJ14639*	hypothetical protein FLJ14639	1.47	-	-
AB014596	*HEPH*	hephaestin	1.56	-	-
AF103864	*HB-1*	minor histocompatibility antigen HB-1	1.92	-	-
D50923	*KIAA0133*	KIAA0133 gene product	2.05[b]	-	-

Figure 3 Differentially expressed genes in bipolar disorder. (a) Two-way hierarchical clustering analysis based on the expression profiles of the differentially expressed genes in patients with bipolar disorder. Each column represents the gene expression levels in individual samples and each row represents the individual gene. The expression value of the individual gene is normalized by dividing its median value. Blue and red colors indicate that the values are less than and greater than the mean, respectively. Gene symbols are given in the right. BP, patients with bipolar disorder; *, medication-free patients; C, control subjects. (b) Venn diagram drawn based on the differentially expressed genes in bipolar disorder, major depression, and schizophrenia, compared with controls. (c) List of the differentially expressed genes. The genes were functionally classified using the NetAffix database (http://www.affymetrix.com/index.affx, Affymetrix). The fold change was determined by comparing the mean expression level of each disease and control subject. Negative value in the fold change column means fold decrease. Genes showing upregulations compared with controls are highlighted in red. BP, bipolar disorder; MD, major depression; SZ, schizophrenia. The dash indicates statistically not significant. [a,b] and [c] indicate that significant effects of anticonvulsants, antipsychotics, and drug abuse, respectively, were found.

tively well separated from control subjects, despite independent samples being used (Figure 4). In addition, the reduced expression of neuropeptide Y in schizophrenia[7] was also observed in this study (data not shown). These would suggest the consistency and reproducibility of the microarray analyses using postmortem samples performed by independent studies.

Gene expression changes in bipolar disorder

The scatterplot drawn based on the genes after the filtering procedures showed that the gene expressions of most of the genes were similar between bipolar disorder and control groups from high to low expression levels (Figure 2). We defined the differentially expressed genes based on the fold change (1.3-fold or greater) and the *P*-value ($P<0.05$) of the mean expression values. The number of genes that met our criteria was 53. As expected, the hierarchical clustering analysis based on the expression level of these genes could separate patients well from control subjects (Figure 3a).

Importantly, some of the genes encoding channels, receptors, or transporters were downregulated, suggesting the impairment of the diverse neuronal functions within the prefrontal cortex in patients with bipolar disorder, similar to the results of microarray studies of schizophrenia.[5,6] On the other hand, genes encoding the stress response proteins or molecular chaperones, whose expressions were usually induced by environmental insult, were upregulated in bipolar disorder (Figure 3c). Several lines of evidence suggest their neuroprotective roles by preventing the formation of protein aggregations[16] or the neuronal cell death induced by injury.[17] Their altered expressions might be involved in part of the pathophysiology of bipolar disorder.

Many lines of evidence suggest that the dysregulation of calcium-dependent signal transductions are involved in bipolar disorder.[18] The downregulation of channels or receptors, *CACN1A*, *HTR2C*, *GRM1*, *GRIK1*, and the upregulation of *LIM* would directly affect the intracellular calcium ion concentration. The glutamatergic system is the main excitatory neurotransmitter system responsible for fundamental brain functions, and hypofunction of this system has been postulated in schizophrenia[19] and bipolar disorder.[20] The downregulation of two glutamate receptors, *GRIK1* and *GRM1*, would be involved in the hypofunction of this system in bipolar disorder. In addition to *GRIK1*, *HTR2C* and *NOTCH4* have been the candidate genes for association studies of schizophrenia and bipolar disorder, although consistent results have not been obtained.[21–28] It may be interesting to note the upregulation of dystrobrevin alpha gene, *DTNA*, in bipolar disorder, since genetic variation of *DTNBP1* (dystrobrevin-binding protein 1) was recently reported to be associated with schizophrenia.[29,30] DTNA and DTNBP1 are the components of dystrophin-associated protein complex (DPC),[31] and the altered function of DPC may cause the dysregulation of maturation and maintenance of the synapses.[32]

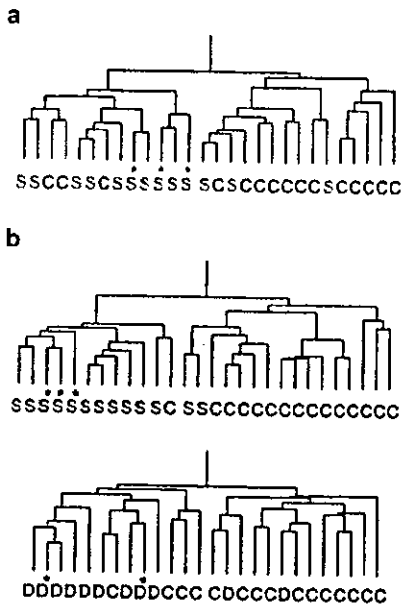


Figure 4 Clustering analysis. (a) Hierarchical clustering analysis based on the expression profiles of the previously reported 89 genes by microarray analysis in schizophrenia.[6] (b) Hierarchical clustering analysis based on the expression profiles of the differentially expressed genes in patients with schizophrenia (*upper*; 117 genes) or major depression (*lower*; 67 genes). S, patients with schizophrenia; D, patients with major depression; *, medication-free patients; C, control subjects.

Table 3 Correlation between microarray and RT-PCR data using the three control genes

	N	*Mean of R*	*Dev.*
Beta actin	21 (10)	0.360 (0.465)	0.213 (0.246)
GAPDH	21 (10)	0.300 (0.437)	0.222 (0.231)
Cofilin	21 (10)	0.340 (0.417)	0.199 (0.231)

Pearson's correlation coefficients were calculated in each of the 21 genes tested. Correlation coefficients of each gene were derived from 26 (bipolar disorder and control), 37 (bipolar disorder and major depression and control), 39 (bipolar disorder, schizophrenia and control), or 50 comparisons (all). The mean of the correlation coefficients of the 21 genes and its standard deviations are given. Results of the 10 genes, whose differential gene expressions in microarray were confirmed by RT-PCR using all the three control genes for normalization, are shown in brackets. *N*, number of the genes; *R*, correlation coefficient.

Uniqueness of bipolar disorder and its similarity to major depression and schizophrenia at the molecular level

We compared the differentially expressed genes in bipolar disorder with those in major depression and

schizophrenia (Figure 3b). The hierarchical clustering analysis revealed that the differentially expressed genes identified in major depression and schizophrenia were useful for the separation of patients from control subjects (Figure 4b), suggesting the appropriateness of data analysis in major depression and schizophrenia. Interestingly, most of the differentially expressed genes in bipolar disorder, as mentioned above, were not shared with these two diseases, suggesting that they are distinctive diseases at the molecular level. Unexpectedly, despite bipolar disorder and major depression sharing symptomatic similarities and genetic background, these were clearly different with regard to gene expression patterns. Most of the genes showing altered expressions in two or more disorders turned out to encode the proteins responsible for transcription or translation (Figure 3c). This finding may imply that the common pathophysiological background underlies the different mental disorders to some extent.

Among the not many commonly altered gene expressions in bipolar disorder and the other two mental disorders, altered expression of *AQP4*, *HTR2C*, and *LIM* would be of particular interest. AQP4 is associated with DPC by interacting syntrophin, a component of DPC,[33] and is involved in the water permeability across the blood–brain barrier and cerebrospinal fluid–brain interface.[34] Impaired water homeostasis due to the upregulation of *AQP4* could be involved in the pathophysiology common to bipolar disorder and depression such as white matter hyperintensity observed by magnetic resonance imaging,[35] although the effects of medication could not be excluded.

Considering the wide-ranging physiological roles of serotonin including regulation of mood, appetite, and sexual behavior, and the mechanisms of drugs such as the antipsychotic properties of serotonin–dopamine antagonists, and the hallucinogenic effects of serotonin agonists,[36] downregulation of *HTR2C* would contribute to the pathophysiology of common clinical features of bipolar disorder and schizophrenia such as psychotic symptoms.

LIM protein, initially identified as a protein kinase C (PKC)-beta-1-binding protein,[37] was recently found to regulate the N-type calcium channel activity by

Table 4 Summary of the results of the quantitative RT-PCR analysis

	Control gene					
	Beta actin		*GAPDH*		*Cofilin*	
	Fold change[a]	*P-value*[b]	*Fold change*	*P-value*	*Fold change*	*P-value*
Postmortem brains[c]						
GRIK1	−1.39	0.0344	−1.43	0.0209	−1.47	0.0307
HSP27	1.65	0.0238	1.63	0.0116	1.63	0.0146
APM2	1.60	0.0298	1.73	0.0173	1.49	0.0199
HSPF1	1.91	0.0035	1.77	0.0041	1.79	0.0164
KIAA0133	1.99	0.0002	1.93	0.0015	2.12	0.0012
IFITM3	1.86	0.0091	1.96	0.0047	1.80	0.0080
LIM	2.10	0.0102	2.05	0.0070	2.06	0.0089
AQP4	2.46	0.0011	2.34	0.0019	2.47	0.0060
HEPH	2.88	0.0021	2.78	0.0021	2.64	0.0035
CEBPD	3.04	0.0002	3.21	0.0002	2.81	0.0001
Lymphoblastoid cells						
GRIK1	nd		nd		nd	
HSP27	1.10	0.5934	1.04	0.6513	1.09	0.6513
APM2	1.04	0.9142	−1.09	0.9927	1.00	0.9927
HSPF1	1.85	**0.0016**	1.74	**0.0010**	1.84	**0.0010**
KIAA0133	−1.09	0.4850	−1.18	0.3304	−1.13	0.3304
IFITM3	1.10	0.4621	1.00	0.5384	1.08	0.5384
LIM	−2.81	**0.0003**	−3.11	**0.0004**	−3.04	**0.0004**
AQP4	nd		nd		nd	
HEPH	nd		nd		nd	
CEBPD	1.54	0.1772	1.40	0.2415	1.45	0.2415

[a]Fold change was determined by comparing the mean expression level of bipolar disorder and control groups. Negative value means fold decrease.

[b]The *P*-values are derived from one-tailed (postmortem brains) or two-tailed (lymphoblastoid cells) Student's *t*-test. $P<0.05$ in the lymphoblastoid cells are denoted in bold.

[c]Among the 21 genes tested, genes whose differential expressions were confirmed by three different control genes are shown.
nd, RT-PCR product was not detected in the lymphoblastoid cells.

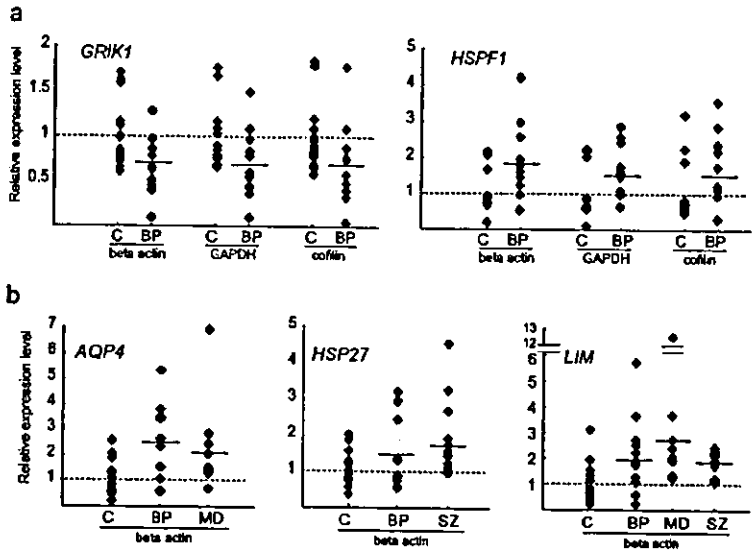


Figure 5 Differentially expressed genes examined by quantitative RT-PCR in postmortem brains. Representative examples of the altered gene expressions in bipolar disorder (a) and the commonly altered gene expressions between and among the mental disorders (b) are shown. Expression value is normalized by dividing the mean of the value of control subjects (C, $N=15$), which is indicated by the dotted line in each plot. Bars indicate the mean of the values of patients (BP, bipolar disorder, $N=11$; MD, major depression, $N=11$; SZ, schizophrenia, $N=13$). In each analysis, expression values were determined by using three different control genes. For simplicity, only the data normalized by beta actin are shown in (b). The differences shown here are significant (one-tailed Student's *t*-test, $P<0.05$). See Table 4 for the *P*-values with respect to bipolar disorder. The *P*-values with respect to major depression are $P=0.0156$ (*AQP4*) and $P=0.0150$ (*LIM*), and those with respect to schizophrenia are $P=0.0034$ (*HSP27*) and $P=0.018$ (*LIM*). The *P*-value after excluding a subject with major depression showing extremely high-level expression of *LIM* (12.63) was still significant ($P=0.0122$).

interacting with both calcium channel and PKC.[38] Considering that PKC activity is altered in bipolar disorder, and antibipolar drugs such as lithium and valproate regulate the PKC signaling cascade,[3,18] upregulation of *LIM* may be involved in the pathophysiology of bipolar disorder, and may also be related to the common pathophysiology of major mental disorders.

Effects of medication status, alcohol abuse, and drug abuse on the gene expressions in bipolar disorder

Among the 53 genes differentially expressed in bipolar disorder patients, the expressions of nine genes were significantly affected by anticonvulsants, antipsychotics, or drug abuse (Figure 3c). No significant effects of lithium, antidepressants, and alcohol use were found. Since patients were usually treated with multiple drugs and had complex histories of medication status, alcohol, and drug abuse, the effects of these confounding factors on gene expressions are difficult to define. However, it would be interesting to note that two drug-free patients were clustered within one patient branch (Figure 3a). This suggests that the altered gene expressions identified in this study largely reflect the pathophysiological conditions of bipolar disorder, rather than the effects of medication. More detailed studies will be needed to examine the effects of these factors as well as other confounding factors such as duration of illness and terminal condition of subjects.

Quantitative RT-PCR analysis

To consider the general consistency of microarray analysis, we compared the mRNA expression levels of each of the representative 21 genes measured by microarray and quantitative RT-PCR (see Materials and methods section). Although the correlations between the results by the two methods were highly variable and dependent on the gene tested, many of them were significantly correlated with each other (Table 3). We then examined whether the differential expressions identified by microarray analysis were confirmed by RT-PCR. Among the 21 genes tested, the altered expressions in bipolar disorder were confirmed in 10 genes using three control genes for normalization (Table 4; Figure 5). This relatively low success rate of confirmation may be caused by multiple reasons such as strict criteria of quantitative RT-PCR analysis using three control genes, relatively small level of fold change in microarray analysis, and the type of genes we examined. In general, the downregulated genes encoding receptors or channels were difficult to obtain the consistency by quantitative RT-PCR analysis. This may be partly attributable to the complexity of their transcripts in the brain.[39]

We also confirmed the gene expression changes observed in bipolar disorder and in other disorders; *AQP4* in bipolar disorder and major depression, *HSP27* in bipolar disorder and schizophrenia, *LIM* in three mental disorders (Figure 5b).

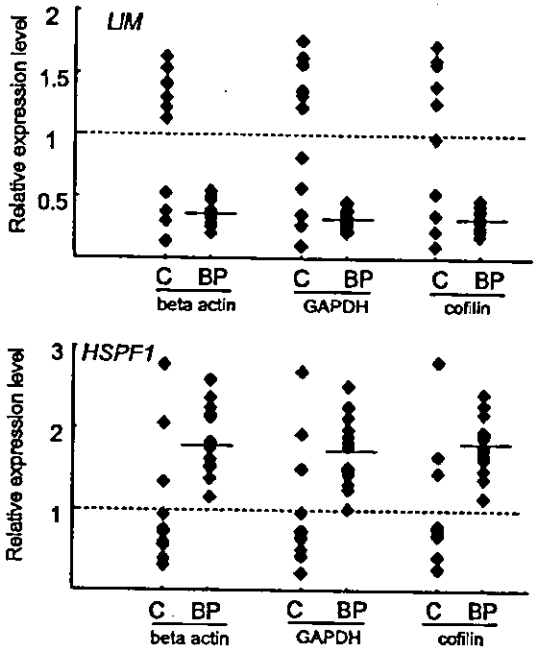


Figure 6 Differentially expressed genes examined by quantitative RT-PCR in the lymphoblastoid cells. Expression value is normalized by dividing the mean of the value of control subjects (C, $N=11$), which is indicated by the dotted line in each plot. Bars indicate the mean of the values of patients (BP, bipolar disorder, $N=14$). In each analysis, expression values were determined by using three different control genes. All the differences shown here are significant (two-tailed Student's *t*-test, $P<0.05$). See Table 4 for the *P*-values.

Altered gene expressions in lymphoblastoid cells established from patients with bipolar disorder

Lymphoblastoid cell lines are the only available tissue that can be cultured semipermanently from patients. Although lymphoblastoid cells have clear limitations in that they are unrelated to neuronal cells, they have advantages in that they are free from the effects of medication, and their altered gene expression may have some pathophysiological importance such as the genetic abnormality. We examined the mRNA expression levels of the 10 genes, whose differential expressions were confirmed by RT-PCR, in the lymphoblastoid cells (Table 4). We could not detect the transcripts of the three genes, *GRIK1*, *AQP4*, and *HEPH*. Although no significant differences in the expression levels were found between bipolar disorder and control groups for most genes, *HSPF1* and *LIM* showed the differential expressions in the lymphoblastoid cells (Table 4; Figure 6).

In contrast to the upregulation in the brain, the expression of *LIM* was downregulated in the lymphoblastoid cells of patients with bipolar disorder (Figures 5b and 6). The cause of these changes in the opposite directions remains unknown, but may be explained by the difference of tissue type.

HSPF1 (HSP40) modulates the activity of HSP70 and direct unfolded proteins to HSP70, which leads to the translocation of proteins into the mitochondria and endoplasmic reticulum (ER).[40] Several recent studies delineated the altered function of mitochondria and ER in bipolar disorder. The altered levels of calcium ions, which were intracellularly stored in the mitochondria and ER, were reported in the lymphoblastoid cells in patients with bipolar disorder.[41,42] In addition, the altered functions of ER were suggested by the increased levels of molecular chaperones in ER by valproate, and by the altered expression of molecular chaperons in the lymphoblastoid cells of patients with bipolar disorder (CK *et al*, in preparation). Considering the above findings, the altered expression of *HSPF1* (Figures 5a and 6) could be involved in the aberration of protein translocation systems into the mitochondria and/or ER, which in turn affects the functions of these organelles.

Conclusion

In summary, we have performed microarray analysis of bipolar disorder using postmortem brains. By profiling the gene expression patterns of a large number of samples, we were able to remove possible effects of the confounding factors including aging, gender, and postmortem interval. By comparing the gene expression patterns of similar but different mental disorders, we identified the unique expression changes in bipolar disorder, and common expression changes between and among mental disorders. The genes identified in this study would be useful for the understanding of the pathophysiology of mental disorders, as well as the molecular genetic studies and drug development.

Since bipolar disorder as well as other psychiatric disorders are considered to be heterogeneous diseases, more detailed studies using a larger number of independent postmortem samples will be clearly needed to explore the gene expression differences among the possible subgroups of bipolar disorder. It is also not clear whether these gene expression changes in bipolar disorder are attributable to anatomical changes in brain such as glial loss or selective neuronal death. Histochemical analyses such as *in situ* hybridization, or single-cell transcript analysis [43] may be needed to address such question.

We successfully found the altered expression of two genes, *LIM* and *HSPF1*, in the lymphoblastoid cells established from patients with bipolar disorder. These findings will be needed to explore a larger number of samples and in the different ethnic groups. Even though it remains unclear whether the altered expressions of *LIM* and *HSPF1* are caused by the genetic variations within these genes in patients, or by the secondary effects induced by other unidentified causes, these two genes would be novel candidate genes for bipolar disorder.

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Synaptic plasma membrane-bound acetylcholinesterase activity is not affected by docosahexaenoic acid-induced decrease in membrane order

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Abstract

We investigated the effect of administration of docosahexaenoic acid (C22:6, n-3; 300 mg/kg·day, for 12 weeks) on the degree of membrane order and membrane-bound acetylcholinesterase activity of the cerebral cortex synaptic plasma membrane in male Wistar rats. Docosahexaenoic acid levels in the synaptic plasma membrane increased significantly by 16% over levels in control rats concomitant with an increase in the molar ratio of docosahexaenoic acid to arachidonic acid. Synaptic plasma membrane order, assessed by 1,6-diphenyl-1,3,5-hexatriene, which measures order of the bulk internal hydrophobic lipid core, decreased significantly in the docosahexaenoic acid-fed rats. Lateral mobility of both global and annular lipids measured by pyrene also increased. Acetylcholinesterase activity of the synaptic plasma membrane was unaffected, and synaptic plasma membrane phospholipid contents increased in the docosahexaenoic acid-fed rats, with a concomitant decrease in the cholesterol/phospholipid molar ratio. Lipid peroxide and reactive oxygen species, indicators of tissue oxidative stress, decreased in both the cerebral cortex synaptosome and homogenate of the docosahexaenoic acid-fed rats. Arrhenius plot showed a break point in acetylcholinesterase activity at 22 °C and 24 °C in plasma membranes from docosahexaenoic acid-fed and control rats, respectively. The present experiment indicates that chronic administration of docosahexaenoic acid does not affect synaptic

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