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難病・がん等の疾患分野の医療の実用化研究事業
(精神疾患関係分野)

# 双極性障害の神経病理学に基づく診断法の開発に関する研究

(課題番号　H25－精神－実用化（精神）－一般－004)

平成２５年度　総括研究報告書

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# 双極性障害の神経病理学に基づく診断法の開発
平成25年度　総括研究報告書

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研究要旨：

福島県立医科大学では、ブレインバンク事業において、双極性障害患者の生前登録を進め、2014年5月までに、23名の双極性障害患者が登録された。登録者の分布は、北海道から九州まで全国に及んでいた。一方、実際の死後脳の集積に関しては、2014年5月現在で、5名の双極性障害患者の脳が集積された。国立精神・神経医療研究センターでは、リサーチリソースネットワークを基盤とした精神神経疾患患者の脳集積を続けており、リサーチリソースネットワークと東京都健康長寿医療センター・高齢者ブレインバンクを合わせて、13例の双極性障害症例の脳を集積している。これらの脳について、高齢者ブレインバンクにおける対照群との比較を行い、双極性障害における嗜銀顆粒の蓄積について、定量的な観点より確認すると共に、その分布の特徴について、詳細な検討を行った。また、東京都健康長寿医療センター・高齢者ブレインバンクでは、移転に伴う剖検数の一時的低下がある中でも、1例の新規双極性障害例の剖検を行い、脳の集積を進めた。理化学研究所脳科学総合研究センターでは、モデルマウスを用いて、ミトコンドリア機能障害を有する細胞を検出する方法を確立し、ヒト死後脳に応用した。その結果、ミトコンドリアDNA(mtDNA)欠失に伴うシトクロムc酸化酵素（COX）蛋白サブユニット減少を免疫組織化学的に検出できることを確認した。また、マウスでCOX陰性細胞が見られる候補部位について、ヒト脳で検討するため、抗カルレチニン抗体および抗アセチルコリンエステラーゼ抗体を用いた免疫染色を行った。これらの方法を用いて、今後双極性障害患者死後脳において検討を進める予定である。しかしながら、ホルマリン固定の期間によって、染色性の低下が予想されるため、慎重に方法論的検討を進めていく必要がある。

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國井泰人　福島県立医科大学医学部　講師

## A 研究目的

双極性障害は、躁状態、うつ状態を繰り返し、社会生活の障害を引き起こす重大な精神疾患である。他の精神疾患に比べて、比較的均一な一群であると考えられたことから、ゲノム研究、脳画像研究が進められてきたが、未だ確実な所見は乏しく、その原因については不明な点が多い。そのため、確実な生物学的診断法がなく、診断は面接によって行われるため、初発のうつ状態は大うつ病と診断せざるを得ない。そのため、潜在的な双極性障害患者において、抗うつ薬治療によって躁転・急速交代化などの問題が引き起こされる可能性があり、これが経過の悪化につながっている。初発時に双極性障害と診断できるような診断法の開発が急務である。

我々は、双極性障害患者における磁気共鳴スペクトロスコピー研究、死後脳研究、遺伝学研究、及び動物モデルの研究などから、双極性障害にミトコンドリア機能障害が関与することを示した。

さらに、平成21～23年度の厚生労働科学研究費（気分障害の神経病理学に基づく分類を目指した脳病態の解明）により、脳内の情動関連部位へのミトコンドリア機能障害細胞の蓄積が、気分障害の病態に関与する可能性を示した。一方、双極性障害患者死後脳と気分障害モデル動物の遺伝子発現解析により共通点を探索した結果、Sfpq/PSFが共通に変化していることを突き止めた(Kubota et al, 2010)。最近、SFPQがタウ遺伝子のスプライシングに関わることが見いだされ(Ray et al, J Mol Neurosci 2011)。リチウムがタウリン酸化酵素であるGSK-3βの阻害作用を持つことと併せ、タウと双極性障害の関連が注目された。

村山らは、同研究費により気分障害患者の脳を蓄積し、最近、齊藤らと共に、タウ蓄積による嗜銀顆粒(Saito et al, JNEN 2004)が双極性障害患者死後脳で脳幹部などに多い可能性を示した。

死後脳において、特定の脳部位のこれらの変化が双極性障害に特異的な変化であることが確認されれば、双極性障害の脳画像診断が可能になると期待される。

本研究の目的は、双極性障害における局在性ミトコンドリア機能障害およびタウ蓄積の意義について、患者死後脳を用い、動物モデルと比較しながら明らかにすることである。

## B. 研究方法

本研究では、病態としては、ミトコンドリア機能障害とタウを中心として解析し、研究の手法としては、死後脳の収集と、その免疫組織化学的な解析に焦点を絞る。

加藤は、死後脳および動物モデルにおける定量的かつハイスループットな免疫組織化学的解析法を開発すると共に、モデルマウスを用いて、病変候補脳部位を同定する。一方、國井と共に、双極性障害患者の献脳登録の推進に向けて、啓発活動を行う。啓発活動は、これまでに進めてきた福島精神疾患ブレインバンクを基盤として、生物学的精神医学会のブレインバンク委員会と連携して進める。

村山、齊藤、國井は、各々が関わるブレインバンク（高齢者ブレインバンク、リサーチリソースネットワーク、福島精神疾患ブレインバンク）において、生前登録した患者の剖検を推進し、双極性障害患者の死後脳の蓄積を進めると共に、既に得られた試料を用いた神経病理学的な解析を行う。

（倫理面への配慮）

本研究については、参加施設の倫理委員会の承認を受けている。これらの研究においては、ヒトゲノム指針、死体解剖保存法など、関連の法規および指針を遵守して行う。

動物実験においては、施設内の動物実験委員会の承認を得て研究を進めると共に、3Rの原則に基づき、使用する動物を最小にすること、可能な限り代替法を利用すること、苦痛を軽減することに努める。

生前登録者には、献脳について説明の上、書面にてインフォームドコンセントを得ると共に、家族の同意を得る。死亡後は家族のインフォームドコンセントに基づいて剖検を行う。剖検は、基本的に、東京都健康長寿医療センター、国立精神神経医療研究センター、福島県立医科大学の3カ所で行う。

C.研究結果

福島県立医科大学では、ブレインバンク事業において、双極性障害患者の生前登録を進め、2014年5月までに、福島県内からの5名に加え、福島県外からも18名の登録を受け、合計23名の双極性障害患者が登録された。これは、生前登録者160名の14.3%を占め、2007年まで（77名中1名、1.2%)に比べ、大きく増加している。登録に至った内訳は、加藤忠史の著書（47%）、福島ブレインバンクのホームページ（17%）、ウォールポケットを活用したパンフレット配布(17%)の他、ネット検索、講演会などであった。登録者の分布は、北海道から九州まで全国に及んでいた。一方、実際の死後脳の集積に関しては、2014年5月現在で、5名の双極性障害患者の脳が集積された。これは、同ブレインバンクに脳が集積された48例の10.4%を占めている。5例のうち1名は悪性腫瘍に罹患したことを機に生前登録し、剖検に至ったケースであった。現在のところ、これらの症例の脳については、肉眼所見を得ているのみであり、今後、神経病理学的な検討を進め、診断確定を行う必要がある。

国立精神・神経医療研究センターでは、同センター主導によるリサーチリソースネットワークを基盤として、精神神経疾患患者の脳集積を進めている。これまでに、リサーチリソースネットワークおよび東京都健康長寿医療センター・高齢者ブレインバンクで集積された双極性障害患者13例について、診療録を元に臨床情報を後方視的に検索し、神経病理学的検索との対応付けを行うと共に、高齢者ブレインバンクにおける1241例の剖検例の所見との比較を行った。BBARプロトコールに基づいて、嗜銀顆粒に関する評価（ステージング）を行ったところ、双極性障害ではステージIIIが5名、IIが3名、Iが3名、0が0名であり、対照群（IIIが6.9%、IIが8.9%、Iが16.7%、0が68.0%）に比して、嗜銀顆粒のステージが高く、双極性障害では、対照群に比して、若年より、高いステージの嗜銀顆粒を示すことが確認された。また、若年の双極性障害患者群における嗜銀顆粒の分布は、扁桃体、縫線核、青斑核等に多い傾向が見られた。

東京都健康長寿医療センター・高齢者ブレインバンクにおいては、これまでの東京都健康長寿医療センター病院症例の剖検に加え、リサーチリソースネットワークの生前登録拠点としての機能も担うこととなり、生前登録も進めつつある。新施設への移転に伴い、2013年度の剖検例は39例と、前年度の63例よりやや減少したため、今後再び剖検を促進していく必要がある。また,福島医科大学ブレインバンク検体における神経病理学的診断も担っている。双極性障害患者の剖検に関しては、2013年度には新たに1名の剖検を行った。38歳にて躁状態で発症し、その後躁状態、うつ状態を反復し、41歳にて双極性障害と診断された症例である。本症例は、リチウムにて治療されていたが、71歳時、仮面様顔貌、筋強剛などを認め、パーキンソニズムが疑われた。しかし、剖検では神経病理学的な異常所見は認められなかった。

理化学研究所脳科学総合研究センターでは、気分障害モデルマウスにおいて、ミトコンドリアDNA (mtDNA)欠失蓄積に伴い、mtDNAにコードされたシトクロムc酸化酵素（COX）蛋白サブユニットが減少している細胞（COX陰性細胞）を免疫組織化学的に検出する方法を開発すると共に、視床室傍部にmtDNA欠失が蓄積していることを見いだした。これらの所見を元に、COX陰性細胞をヒト死後脳で検出する方法の確立を進めた。ミトコンドリア病であるKearns Sayer症候群において、COX陰性細胞が多く蓄積していることを観察すると共に、年齢のほぼ一致した対照群では、COX陰性細胞ははるかに少ないことを確認した。一方、ミトコンドリア病でも、mtDNA欠失蓄積が見られないMELASにおいては、こうした所見は見られなかった。ヒト視床室傍部の解剖学的特徴を明らかにするため、マウスでこの部位の同定に用いられている、抗カルレチニン抗体および抗アセチルコリンエステラーゼ抗体を用いた免疫染色を行って、その解剖学的な特徴付けを進めた。

今後、これらの所見を元に、今後、福島医科大学ブレインバンク、リサーチリソースネットワーク、高齢者ブレインバンクの検体を用いて、COX陰性細胞の分布の検索を進め、嗜銀顆粒の所見との比較を行う予定である。しかし、ホルマリン固定期間により、染色性の低下が予想されるため、どの程度の固定期間であれば免疫染色が行えるか、方法論的な点

を確認しながら進めていく予定である。

D.考察

啓発活動により、双極性障害患者の生前登録は確実に増加しており、実際に生前登録を経て剖検に至ったケースもあった。

双極性障害患者死後脳において、嗜銀顆粒が多く見られることが多数例の対照群との比較で明らかとなった。

双極性障害の原因脳部位として疑われている部位である視床室傍部については、これまでマウスでは特徴づけられている一方、人における解剖学的な特徴付けが十分なされていなかったが、本研究により、人での検討が可能になると期待される。

なお、各施設で集積された双極性障害患者の死後脳については、本研究班における研究に限らず、幅広く研究者に提供される予定である。また、本研究で開発された免疫組織化学的手法は、今後ヒト死後脳の神経病理学的検索に用いることができる。

更に、ヒト視床室傍部の解剖学的な検証により、これまで神経病理学的な所見の記載が困難であった視床室傍部についてより詳細な所見を記載できるようになると期待される。

今後、集積した双極性障害患者死後脳を用いて、候補部位である視床室傍部を含め、タウの蓄積およびミトコンドリア機能障害に関する神経病理学的な検討を進めていく予定である。

E.結論

双極性障害患者死後脳の蓄積は困難であるが、進んでいる。本年度の研究により、今後、双極性障害の神経病理学的所見として、候補脳部位におけるタウ蓄積およびミトコンドリア機能障害について、検討を進めていくための基礎的知見を得ることができた。

F.健康危険情報

G.研究発表

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H.知的財産権の出願・登録状況
1. 特許取得
なし

2. 実用新案登録
なし

3. その他

# 研究成果の刊行一覧表（平成 25 年度）

## 加藤　忠史

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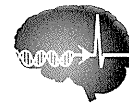

Molecular Brain

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# A role of *ADAR2* and RNA editing of glutamate receptors in mood disorders and schizophrenia

Mie Kubota-Sakashita[1†], Kazuya Iwamoto[1,2†], Miki Bundo[1,2] and Tadafumi Kato[1*]

## Abstract

**Background:** Pre-mRNAs of 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)-propanoic acid (AMPA)/kainate glutamate receptors undergo post-transcriptional modification known as RNA editing that is mediated by adenosine deaminase acting on RNA type 2 (*ADAR2*). This modification alters the amino acid sequence and function of the receptor. Glutamatergic signaling has been suggested to have a role in mood disorders and schizophrenia, but it is unknown whether altered RNA editing of AMPA/kainate receptors has pathophysiological significance in these mental disorders. In this study, we found that *ADAR2* expression tended to be decreased in the postmortem brains of patients with schizophrenia and bipolar disorder.

**Results:** Decreased *ADAR2* expression was significantly correlated with decreased editing of the R/G sites of AMPA receptors. In heterozygous *Adar2* knockout mice (*Adar2*$^{+/-}$ mice), editing of the R/G sites of AMPA receptors was decreased. *Adar2*$^{+/-}$ mice showed a tendency of increased activity in the open-field test and a tendency of resistance to immobility in the forced swimming test. They also showed enhanced amphetamine-induced hyperactivity. There was no significant difference in amphetamine-induced hyperactivity between *Adar2*$^{+/-}$ and wild type mice after the treatment with an AMPA/kainate receptor antagonist, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline.

**Conclusions:** These findings collectively suggest that altered RNA editing efficiency of AMPA receptors due to down-regulation of *ADAR2* has a possible role in the pathophysiology of mental disorders.

**Keywords:** RNA editing, Adenosine deaminase acting on RNA type 2, AMPA/kainate receptors, Serotonin 2C receptor

## Background

Mood disorders and schizophrenia are major psychiatric diseases that cause severe psychosocial impairment. Because many antidepressant and antipsychotic drugs act on the serotonin transporter and serotonin receptors, the serotonin has been implicated as having a role in these diseases [1]. However, among the drugs acting on glutamate receptors, phencyclidine causes schizophrenia-like psychosis [2] and ketamine has rapid antidepressive efficacy [3]. These findings suggest a possible role of altered glutamatergic neurotransmission in mood disorders and schizophrenia [1].

To date, numerous studies using genome analysis, positron emission tomography, and postmortem brain analysis have revealed possible roles of serotonergic and glutamatergic pathways in schizophrenia and mood disorders [1]. However, the detailed molecular mechanisms of these diseases have yet to be totally elucidated. Among the receptors in these pathways, pre-mRNAs of the serotonin 2C receptor (*HTR2C*) and two classes of ionotropic glutamate receptors, 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)-propanoic acid (AMPA) and kainate receptors, undergo RNA editing [4,5].

RNA editing is a post-transcriptional modification of pre-mRNA, which is mediated by adenosine deaminases acting on RNA (ADAR) enzymes. Research has especially focused on adenosine-to-inosine (A-to-I) editing of *HTR2C* and AMPA/kainate receptors by ADAR2 [6]. *HTR2C* undergoes editing at five sites (from A to E), which results in amino acid changes and causes functional alteration. Among the editing sites of AMPA/kainate receptors, two sites (Q/R and R/G) that result in amino acid changes and have functional significance, have been relatively well studied. The Q/R site is almost 100% edited in *GRIA2*, and loss of editing at this site causes enhanced

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$Ca^{2+}$ permeability, resulting in cellular dysfunction [6]. The R/G site is not fully edited, which changes the kinetics of desensitization [7].

Numerous studies have investigated altered RNA editing of *HTR2C* in postmortem brains of patients with mental disorders, but the findings have not been consistent. While several studies did not show any specific alteration of RNA editing in mental disorders [8-11], other studies showed disease-specific alteration, such as increased D site editing in depression and increased A site editing in suicide completers [12], decreased B site editing in schizophrenia [13], and increased E site and decreased D site editing in depression [14]. Among these studies, increased A site editing in suicide completers has been shown to be relatively consistent [9,10,12,15]. A recent study using a next-generation sequencer [11] showed no robust alteration of RNA editing of *HTR2C* in schizophrenia and depression, except that a trend of decreased editing at the C, D, and E sites in nonsuicidal depression was found. However, a trend of increased A site editing in depressive patients that died by suicide is compatible with previous studies.

The number of studies regarding the RNA editing of AMPA/kainate receptors is relatively small. While several studies showed no alteration in schizophrenia [16,17] and bipolar disorder [17], a recent study revealed altered editing of the I/V site of *GRIK2* in bipolar disorder [6]. The role of RNA editing of glutamate receptors has drawn attention because the glutamatergic hypothesis of mood disorders has recently been established [18].

As already mentioned, there is no consensus on what kind of alteration of RNA editing is characteristic of schizophrenia and mood disorders. Possible reasons for such discordance include the effect of confounding factors in postmortem brain studies, such as medication and premortem or postmortem changes, and complex interactions between the cause of death (such as suicide) and mental disorders. Recently, Lyddon and colleagues argued that there are two factors involved in the altered editing of *HTR2C* in mood disorders; one is decreased *ADAR2* expression associated with decreased RNA editing of *HTR2C*, and the other is increased A site editing in suicide [15].

Collectively, it is difficult to elucidate how RNA editing is related to mental disorders by postmortem brain analysis alone. To understand the pathophysiological significance of altered *ADAR2* expression and RNA editing in mental disorders, integration of human postmortem brain analysis and animal model studies is crucial. In this study, we investigated the possible roles of altered *ADAR2* expression and RNA editing of AMPA/kainate glutamate receptors through an analysis of these factors in human postmortem brains. Molecular, behavioral, and pharmacological analyses of heterozygous *Adar2* knockout mice ($Adar2^{+/-}$ mice) were also conducted to elucidate the roles of RNA editing in schizophrenia and mood disorders.

## Results

### Overview

We analyzed the gene expression levels of the *ADAR2* and RNA editing status of AMPA/kainite glutamate receptors in two sets of postmortem human brain samples donated by the Stanley Medical Research Institute, Array Collection and Neuropathology Consortium (Table 1). To elucidate the molecular neurobehavioral consequence of reduced *ADAR2* expression, we analyzed the RNA editing status of *Htr2c* and AMPA/kainate glutamate receptors in $Adar2^{+/-}$ mice; the behavior of the mice was analyzed by a comprehensive battery of behavioral tests. To further understand the molecular basis of the hyperactivity of the mice, pharmacological experiments using amphetamine and the selective AMPA receptor antagonist, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline (NBQX) were performed.

### *ADAR2* expression in human postmortem brains

In the Array Collection samples, there was a significant difference in the *CFL1* (*cofilin 1*)-normalized *ADAR2* expression level between diagnoses [one-way analysis of variance

**Table 1 Subjects for RNA editing and expression analysis of ADARs**

| | Bipolar disorder | Schizophrenia | Control | Depression |
|---|---|---|---|---|
| **Array Collection (all samples)** | | | | |
| n | 32 | 35 | 34 | NA |
| Sex (F:M) | 17:15 | 9:26 | 9:25 | |
| Age[a)] | 45.6 ± 11.0 | 42.6 ± 8.5 | 44.1 ± 7.7 | |
| PMI[b)] | 36.3 ± 17.9 | 31.4 ± 15.5 | 29.6 ± 13.0 | |
| Brain pH[c)] | 6.43 ± 0.30[g)] | 6.47 ± 0.24[h)] | 6.60 ± 0.27 | |
| **Array Collection (pH-adjusted)** | | | | |
| n | 19 | 24 | 29 | NA |
| Sex (F:M) | 11:8 | 9:15 | 6:23 | |
| Age[d)] | 46.1 ± 9.9 | 42.6 ± 8.5 | 44.6 ± 7.7 | |
| PMI[e)] | 39.9 ± 20.2 | 35.0 ± 14.8 | 30.2 ± 12.5 | |
| Brain pH[f)] | 6.63 ± 0.15 | 6.61 ± 0.14 | 6.69 ± 0.17 | |
| **Neuropathology Consortium Samples[i)]** | | | | |
| n | 11 | 13 | 14 | 11 |
| Sex (F:M) | 3:8 | 5:8 | 5:9 | 5:6 |
| Age | 39.4 ± 12.4 | 43.5 ± 13.6 | 49.0 ± 10.4 | 46.3 ± 10.5 |
| PMI | 31.5 ± 15.5 | 33.0 ± 14.9 | 22.6 ± 9.2 | 27.0 ± 11.9 |
| Brain pH | 6.25 ± 0.20 | 6.15 ± 0.25 | 6.30 ± 0.21 | 6.18 ± 0.24 |

NA, not available.
[a)]One way ANOVA, $F = 0.94$, $P = 0.39$, [b)]$F = 1.61$, $P = 0.20$, [c)]$F = 3.5$, $P = 0.031$.
[d)]One-way ANOVA, $F = 0.89$, $P = 0.41$, [e)]One-way ANOVA, $F = 2.2$, $P = 0.11$.
[f)]One-way ANOVA, $F = 1.7$, $P = 0.18$, [g)]$t = -2.4$, $P = 0.017$ to control.
[h)]$t = 2.0$, $P = 0.042$ to control.
[i)]No significant difference among 4 groups were found for age, PMI, brain pH by One-way ANOVA.

(ANOVA), $P < 0.05$]. Patients with schizophrenia showed significantly lower *ADAR2* expressions in both Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*)- and *CFL1*-normalized data ($P < 0.05$). Patients with bipolar disorder also showed a nonsignificant trend in the same direction, and it was close to significance ($P = 0.05$) for the *CFL1*-normalized *ADAR2* expression level (Table 2). There was a significant correlation between brain pH and *ADAR2* expression (*GAPDH*-normalized *ADAR2*: $r = 0.28$, $P = 0.001$; *CFL1*-normalized *ADAR2*: $r = 0.222$, $P < 0.05$). Because low pH in brain affected the measurement of postmortem brain gene expression [19], we selected samples with high brain pH (pH 6.4 or higher). This threshold was determined in our previous gene expression study [20]. We found that the results were similar after selection of high pH samples (Table 2). A similar nonsignificant trend for decreased *ADAR2* expression was found in the Neuropathology Consortium samples (Table 2). There was no significant difference of *ADAR1* expression between diagnoses (Table 2).

### RNA editing changes in $Adar2^{+/-}$ mice

The initial report showed that homozygous *Adar2* knockout is lethal in mice due to seizures caused by a marked decrease of the Q/R site of *Gria2*, which results in enhanced $Ca^{2+}$ permeability of the AMPA receptors [21]. $Adar2^{+/-}$ mice showed less prominent alteration of RNA editing and were viable. Thus, $Adar2^{+/-}$ might be a better model for mental disorders.

We first characterized the effect of $Adar2^{+/-}$ on RNA editing status. Examination of *Htr2c* editing revealed that the E and C sites were not altered, whereas the A, B, and D sites had significantly decreased editing efficiency, except in the cerebellum, where only the D site showed significant alteration (Figure 1a). The alteration of the A and B sites was relatively modest (10% or less), whereas the change in the D site was larger (up to 20%).

With regard to the AMPA/kainate receptors, the Q/R site of *Gria2* was almost fully edited (Figure 1b). Editing of the Q/R sites of *Grik1* and *Grik2* was slightly decreased. There are two alternative spliced isoforms of flip and flop types that have different desensitization kinetics. Because the R/G site is located just before this alternative spliced exon [7], the R/G sites were separately examined for two alternative spliced isoforms. We found that RNA editing of the R/G site was generally decreased in both the flip and flop isoforms for all receptors investigated (*Gria2*,

**Table 2 Expression levels of ADARs in postmortem brains of patients with mental disorders**

| **Array collection samples** | | | | |
|---|---|---|---|---|
| | All samples (normalized by *GAPDH*) | | All samples (normalized by *CFL1*) | |
| Diagnosis | *ADAR1* | *ADAR2* | *ADAR1* | *ADAR2* |
| Bipolar disorder | 0.0219 ± 0.0090 | 0.0025 ± 0.0011 | 0.0397 ± 0.0163 | 0.0046 ± 0.0018[b)] |
| Schizophrenia | 0.0216 ± 0.0086 | 0.0024 ± 0.0008[a)] | 0.0393 ± 0.0192 | 0.0043 ± 0.0015[c)] |
| Control | 0.0232 ± 0.0063 | 0.0029 ± 0.0009 | 0.0477 ± 0.0380 | 0.0054 ± 0.0015 |
| One-way ANOVA | NS | $F = 2.11$, $P = 0.120$ | NS | $F = 3.86$, $P = 0.024$ |
| | pH-adjusted (normalized by *GAPDH*) | | pH-adjusted (normalized by *CFL1*) | |
| Diagnosis | *ADAR1* | *ADAR2* | *ADAR1* | *ADAR2* |
| Bipolar disorder | 0.0028 ± 0.0010 | 0.0244 ± 0.0096 | 0.0050 ± 0.0016 | 0.0442 ± 0.0180 |
| Schizophrenia | 0.0025 ± 0.0008[d)] | 0.0231 ± 0.0088 | 0.0043 ± 0.0013[e)] | 0.0401 ± 0.0149 |
| Control | 0.0031 ± 0.0008 | 0.0238 ± 0.0054 | 0.0054 ± 0.0014 | 0.0420 ± 0.0080 |
| One-way ANOVA | $F = 3.09$, $P = 0.052$ | NS | $F = 4.37$, $P = 0.016$ | NS |
| **Neuropathology Consortium Samples** | | | | |
| | Normalized by *GAPDH* | | Normalized by *CFL1* | |
| Diagnosis | *ADAR1* | *ADAR2* | *ADAR1* | *ADAR2* |
| Bipolar disorder | 0.0025 ± 0.0016[f)] | 0.0110 ± 0.0071 | 0.0122 ± 0.0097 | 0.0026 ± 0.0017[g)] |
| Depression | 0.0030 ± 0.0012 | 0.0107 ± 0.0036 | 0.0043 ± 0.0013[e)] | 0.0039 ± 0.0012 |
| Schizophrenia | 0.0029 ± 0.0006 | 0.0107 ± 0.0045 | 0.0151 ± 0.0051 | 0.0039 ± 0.0010 |
| Control | 0.0033 ± 0.0008 | 0.0119 ± 0.0054 | 0.0133 ± 0.0062 | 0.0037 ± 0.0010 |
| One-way ANOVA | NS | NS | NS | $F = 5.6$, $P = 0.008$ |

Values are given in ± mean SD.
[a)] $t = 2.22$, $P = 0.029$ to control, [b)] $t = -1.92$, $P = 0.05$ to control.
[c)] $t = 2.90$, $P = 0.005$ to control, [d)] $t = -2.65$, $P = 0.01$, [e)] $t = -3.12$, $P = 0.003$.
[f)] $t = -1.70$, $P = 0.09$ to control, [g)] $t = -2.0$, $P = 0.06$ to control.
NS: not significant.
For one of 13 subjects with schizophrenia in Neuropathology Consortium, data was missing.

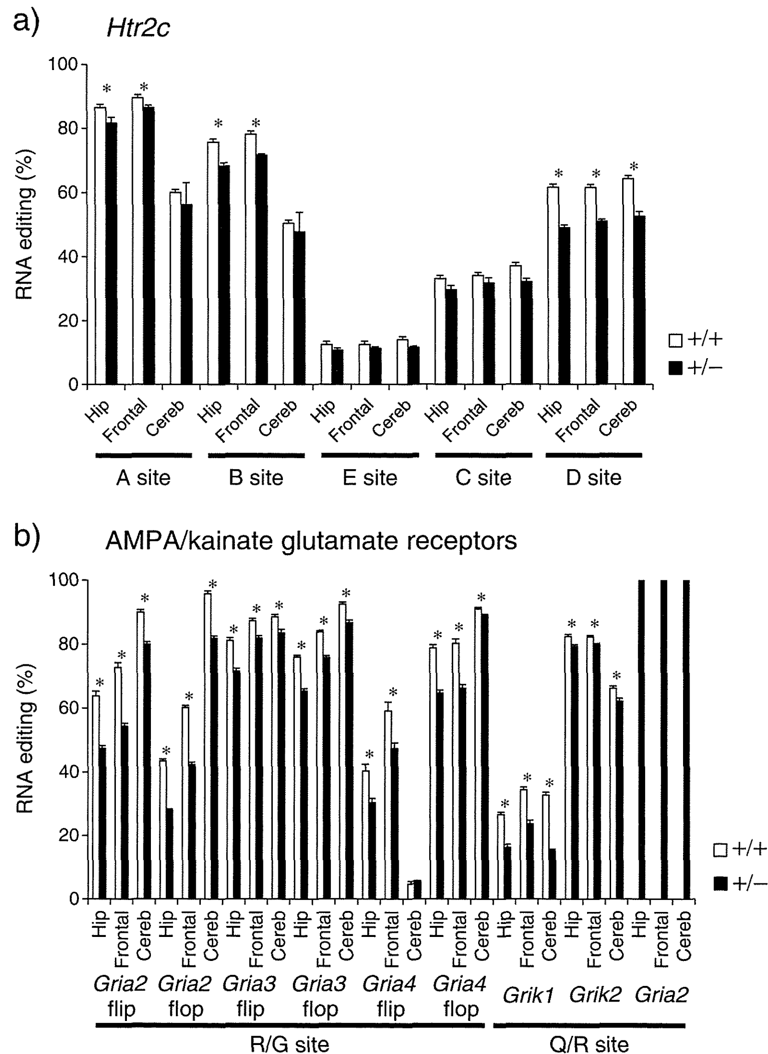


**Figure 1 RNA editing of *Htr2c* and AMPA/kainate glutamate receptors in *Adar2*$^{+/-}$ mice. a)** *Htr2c*. **b)** AMPA/kainate glutamate receptors. $P < 0.05$ by Student *t*-test. Hip denotes hippocampus, Frontal denotes frontal cortex, and Cereb denotes cerebellum. White bars indicate the WT mice, black bars indicate the *Adar2*$^{+/-}$ mice. Data represent mean and error bars indicated by standard errors (n = 10 for each genotype).

*Gria3*, and *Gria4*). The R/G site showed a 10–36% decrease except for *Gria4* in the cerebellum, where minimal or no alteration in editing efficiency was observed.

## RNA editing in human postmortem brains

We previously reported RNA editing efficiency of *HTR2C* in the Consortium samples, which showed an increase at the D site in depression and an increase at the A site in suicide completers [12]. In this study, we measured the editing efficiency of AMPA/kainate receptors in the same sample set. As expected, the Q/R sites were almost fully edited in all groups. There was no significant alteration in RNA editing of R/G sites, but there was a tendency toward decreased editing efficiency in mood disorders (Table 3). The editing efficiency at the R/G site was significantly correlated with the *ADAR2* expression level for all transcripts investigated ($r = 0.30$–$0.64$, $P < 0.05$ for *GAPDH*-normalized and $r = 0.25$–$0.64$, $P < 0.05$ for *CFL1*-normalized data) (Table 3); however, the Q/R site of *GRIA2* was not ($P > 0.05$). As shown in Figure 2, some of the patients showed a prominent decrease in both editing efficiency of R/G sites and *ADAR2* expression levels.

## Behavioral analysis of *Adar2*$^{+/-}$ mice

The present findings suggest that *ADAR2* is downregulated in schizophrenia and bipolar disorder, which correlates with decreased R/G site editing of AMPA receptors. Thus, decreased *ADAR2* levels and the resultant alteration of editing efficiency at the R/G sites of AMPA receptors might have some pathophysiological significance in mood disorders and schizophrenia. To test this,

**Table 3 RNA editing of AMPA receptors in the postmortem brains of patients with mental disorders**

| | R/G sites | | | | | | Q/R sites |
|---|---|---|---|---|---|---|---|
| | *GRIA2* flip | *GRIA2* flop | *GRIA3* flip | *GRIA3* flop | *GRIA4* flip | *GRIA4* flop | *GRIA2* |
| Bipolar disorder | 64.4 ± 16.4 | 54.4 ± 12.5 | 66.3 ± 9.5 | 79.4 ± 10.6 | 56.0 ± 17.1 | 64.8 ± 15.8 | 96.6 ± 3.4 |
| Depression | 62.5 ± 13.8 | 54.9 ± 5.3 | 61.9 ± 9.6 | 82.6 ± 7.7 | 55.0 ± 15.8 | 69.9 ± 10.7 | 97.6 ± 3.0 |
| Schizophrenia | 66.0 ± 10.8 | 53.7 ± 8.0 | 66.0 ± 5.9 | 83.5 ± 9.4 | 60.5 ± 9.7 | 71.5 ± 6.1 | 99.0 ± 1.9 |
| Control | 70.1 ± 4.8 | 58.1 ± 4.4 | 65.0 ± 12.1 | 85.5 ± 3.5 | 63.2 ± 9.5 | 71.2 ± 7.5 | 98.8 ± 2.3 |
| $r^*$ (*GAPDH*) | 0.508 | 0.476 | 0.300 | 0.644 | 0.527 | 0.565 | ns |
| $r^*$ (*CFL1*) | 0.477 | 0.430 | 0.251 | 0.642 | 0.509 | 0.600 | ns |

*$r$ = Correlation coefficient between editing efficiency of AMPA receptors and *ADAR2* expression level.

we applied a battery of conventional behavioral tests to $Adar2^{+/-}$ mice. The results are summarized in Figure 3. In open-field test, RMANOVA with the intrasubject factor of time (1–20 min, $df$ = 19) and the intersubject factor of genotype showed a trend-level effect of genotype ($F$ = 4.0, $P$ = 0.054) for locomotor activity. The $Adar2^{+/-}$ mice tended to be more hyperactive than the wild-type (WT) mice (Figure 3a, $t$ = 2.01, $P$ = 0.05). No significant difference was found in the rearing scores (Figure 3b). There was no alteration in prepulse inhibition (Figure 3c), which does not support the hypothesis that $Adar2^{+/-}$ mice show schizophrenia-like sensorimotor gating abnormality. Among the factors affecting the results of the open-field test, a possible effect of anxiety was not supported because there was no significant alteration by the elevated plus maze (Figure 3d, 3e). The Morris water maze test did not show any difference, indicating that there was no marked impairment in spatial memory (Figure 3f, 3g). No significant alteration in passive avoidance test (Figure 3h) is in accordance with lack of significant alteration in elevated plus maze test. In the active avoidance test, there was no significant time × genotype interaction in the avoidance latency or number of avoidances by repeated measures (RM) ANOVA (RMANOVA) (Figure 3i, 3j). In the forced swimming test (Figure 3k, 3l), the ratio of the immobility time on the second day to that of the first day, which is an indicator for behavioral despair, tended to be smaller in the $Adar2^{+/-}$ mice ($t$ = 1.9, $P$ = 0.06). This suggests that $Adar2^{+/-}$ mice showed a tendency of resistance to behavioral despair.

### Pharmacological experiments

To further elucidate the mechanism of increased locomotor activity in $Adar2^{+/-}$ mice, we examined the effect of amphetamine administration in the mice. Amphetamine is known to evoke a delayed overflow of glutamate in the brain in addition to having an impact on the dopaminergic system. It causes acute hyperactivity and behavioral sensitization [22,23]. Less editing in the GluR2 receptors would result in high $Ca^{2+}$ permeability in AMPA receptors, which may enhance glutamate

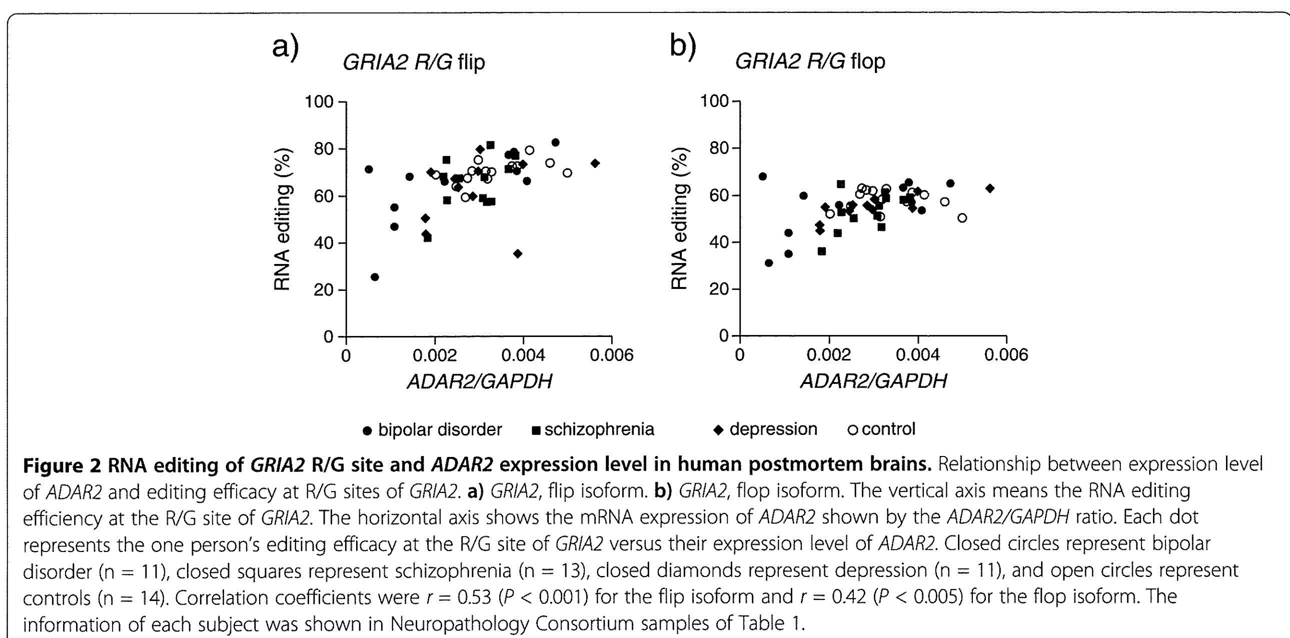


**Figure 2 RNA editing of *GRIA2* R/G site and *ADAR2* expression level in human postmortem brains.** Relationship between expression level of *ADAR2* and editing efficacy at R/G sites of *GRIA2*. **a)** *GRIA2*, flip isoform. **b)** *GRIA2*, flop isoform. The vertical axis means the RNA editing efficiency at the R/G site of *GRIA2*. The horizontal axis shows the mRNA expression of *ADAR2* shown by the *ADAR2/GAPDH* ratio. Each dot represents the one person's editing efficacy at the R/G site of *GRIA2* versus their expression level of *ADAR2*. Closed circles represent bipolar disorder (n = 11), closed squares represent schizophrenia (n = 13), closed diamonds represent depression (n = 11), and open circles represent controls (n = 14). Correlation coefficients were $r$ = 0.53 ($P$ < 0.001) for the flip isoform and $r$ = 0.42 ($P$ < 0.005) for the flop isoform. The information of each subject was shown in Neuropathology Consortium samples of Table 1.

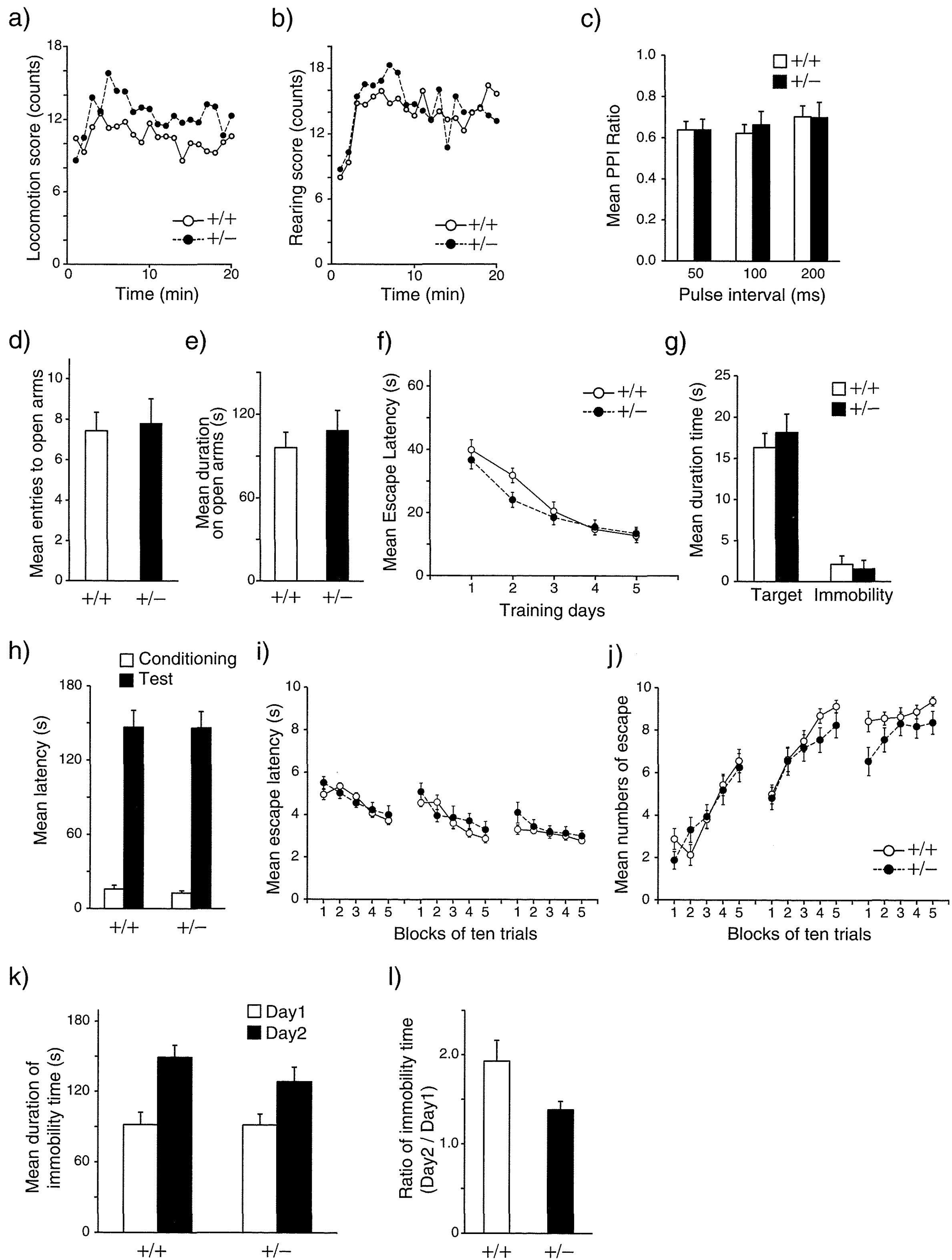


**Figure 3** (See legend on next page.)

(See figure on previous page.)
**Figure 3 Behavioral battery in *Adar2*$^{+/-}$ mice. a,b)** Open-field test. The vertical axis is the counts measured by the interruption of infrared beams. **a)** Locomotor activity. **b)** Rearing. Closed circles indicate the *Adar2*$^{+/-}$ mice (+/−). Open circles indicate the WT mice (+/+). No significant genotype × time interaction was found for locomotor activity. Locomotor count tended to be higher in the *Adar2*$^{+/-}$ mice than that in the WT mice ($t$ = 2.0, $P$ = 0.05). RMANOVA with the intrasubject factor of time (1–20 min, df = 19) and the intersubject factor of genotype showed no significant effect of genotype or genotype × time interaction in rearing count. **c)** PPI test. There was no significant difference between genotypes. **d,e)** Elevated plus maze test. **d)** Number of entries to open arms. **e)** Time spent on open arms. There was no significant difference in the number of entries and time spent on open arms between genotypes. **f,g)** Morris water maze test. **f)** Escape latency. No significant effect of genotype was found for the escape latency by RMANOVA. **g)** Probe test. There was no significant difference between genotypes. **h)** Passive avoidance test. There was no significant effect of genotypes by two-way ANOVA. **i,j)** Active avoidance test. There was no significant interaction between time ($df$ = 14) and genotype ($df$ = 1) in the avoidance latency **(i)** or number of avoidances **(j)** by RMANOVA ($P$ = 0.05). **k,l)** Forced swimming test. **k)** Duration of immobility. **l)** The ratio of immobility time on the second day to the first day. Data represent mean and error bars indicated by standard errors (n = 16 for each genotype).

transmission in *Adar2*$^{+/-}$ mice. Previously, it was shown that the AMPA receptor antagonist NBQX by itself had no effect on locomotor activity but prevented hyperactivity after treatment with amphetamine [24,25]. In accordance with previous studies, the locomotor activity gradually declined in two groups with treatment of saline or NBQX in both the WT and the *Adar2*$^{+/-}$ mice in our experiment. There was no significant difference of activity level between saline injection and NBQX injection both in the WT mice and the *Adar2*$^{+/-}$ mice [see Additional file 1: Figure S1]. Thus, we examined this behavioral trait in relation to amphetamine treatment of *Adar2*$^{+/-}$ mice. The amphetamine treatment enhanced the activity level in both the WT and the *Adar2*$^{+/-}$ mice (Figure 4a, 4b). RMANOVA showed significant effects of genotype ($df$ = 1, $F$ = 17.8, $P$ <0.005) and drug ($df$ = 1, $F$ = 70.9, $P$ < 0.001), as well as a significant interaction of genotype × drug ($df$ = 1, $F$ = 7.1, $P$ < 0.05). The enhancement was significantly larger in the *Adar2*$^{+/-}$ mice than in the WT mice (Figure 4c, $t$ = 3.07, $P$ = 0.015). Amphetamine treatment after the NBQX treatment also enhanced the locomotor activity in the *Adar2*$^{+/-}$ mice (9.03 ± 7.16) and the WT mice (7.51 ± 5.19) (Figure 4d, 4e). RMANOVA showed significant effects of drug ($df$ = 1, $F$ = 58.1, $P$ < 0.001) but no significant effect of genotype ($df$ = 1, $F$ = 0.37, $P$=0.54) and no significant interaction of genotype × drug ($df$ = 1, $F$ = 0.29, $P$ = 0.59). There was no significant difference in the enhancement between genotypes (Figure 4f, $t$ = 0.146, $P$ = 0.56).

## Discussion

In this study, we performed an integrated analysis of *ADAR2* and RNA editing of AMPA/kainate glutamate receptors in human postmortem brains and model mice. Recent expression studies using postmortem brains showed no significant alteration of *ADAR2* [6,26] and significant down-regulation of *ADAR2* in major depressive disorder [15]. In our study, although the results were not consistent between two independent sample sets, we observed general down-regulation of *ADAR2* in the brains of patients with schizophrenia or mood disorders. The discrepancy in the results across studies may be partly due to the complexity of *ADAR2* transcripts [27]. In this study, we examined the gene expression level of *ADAR2* transcripts containing exons 11 and 12. Another notable feature was that some patients, but not all patients, showed drastic down-regulation of *ADAR2*, which was accompanied by decreased RNA editing at the R/G sites.

To model the down-regulation of *ADAR2* in patients with mental disorders, we analyzed *Adar2*$^{+/-}$ mice. Altered RNA editing of *Htr2c* was characterized by a relatively large decrease at the D site with smaller changes at the A and B sites. A decrease of D site editing in depression has been reported in some [11,14], but not all [9,10,12,15] studies. The altered RNA editing of AMPA/kainate receptors in *Adar2*$^{+/-}$ mice was characterized by preserved Q/R site editing of *Gria2* and decreased editing of R/G sites. This is consistent with a previous study using semiquantitative analysis of sequence chromatograms [21]. Although it is a limitation of the study that we did not measure the protein levels of Adar2, the alteration of RNA editing status might suggests that haploinsufficiency of *Adar2* would cause a decrease of Adar2 protein level and subsequently reduced Adar2 activity in *Adar2*$^{+/-}$ mice [21]. Because there was some residual Q/R site editing of *Gria2* in homozygous *Adar2* knockout mice, *Adar1* might also play some role in the editing of this site as well as RNA editing of the R/G site of AMPA receptors and *Htr2c*. However, *Adar1* was not upregulated in homozygous *Adar2* knockout mice, suggesting that compensatory upregulation of *Adar1* do not play a major role in RNA editing changes in *Adar2*$^{+/-}$ mice.

In the postmortem brains of patients with schizophrenia and mood disorders, statistically significant alteration of RNA editing of AMPA/kainate receptors was not found. The lack of prominent alteration of the Q/R site in schizophrenia is consistent with the pioneering work of Akbarian and colleagues [16]. However, there was a significant correlation between *ADAR2* expression and R/G site editing. This suggests that decreased *ADAR2*

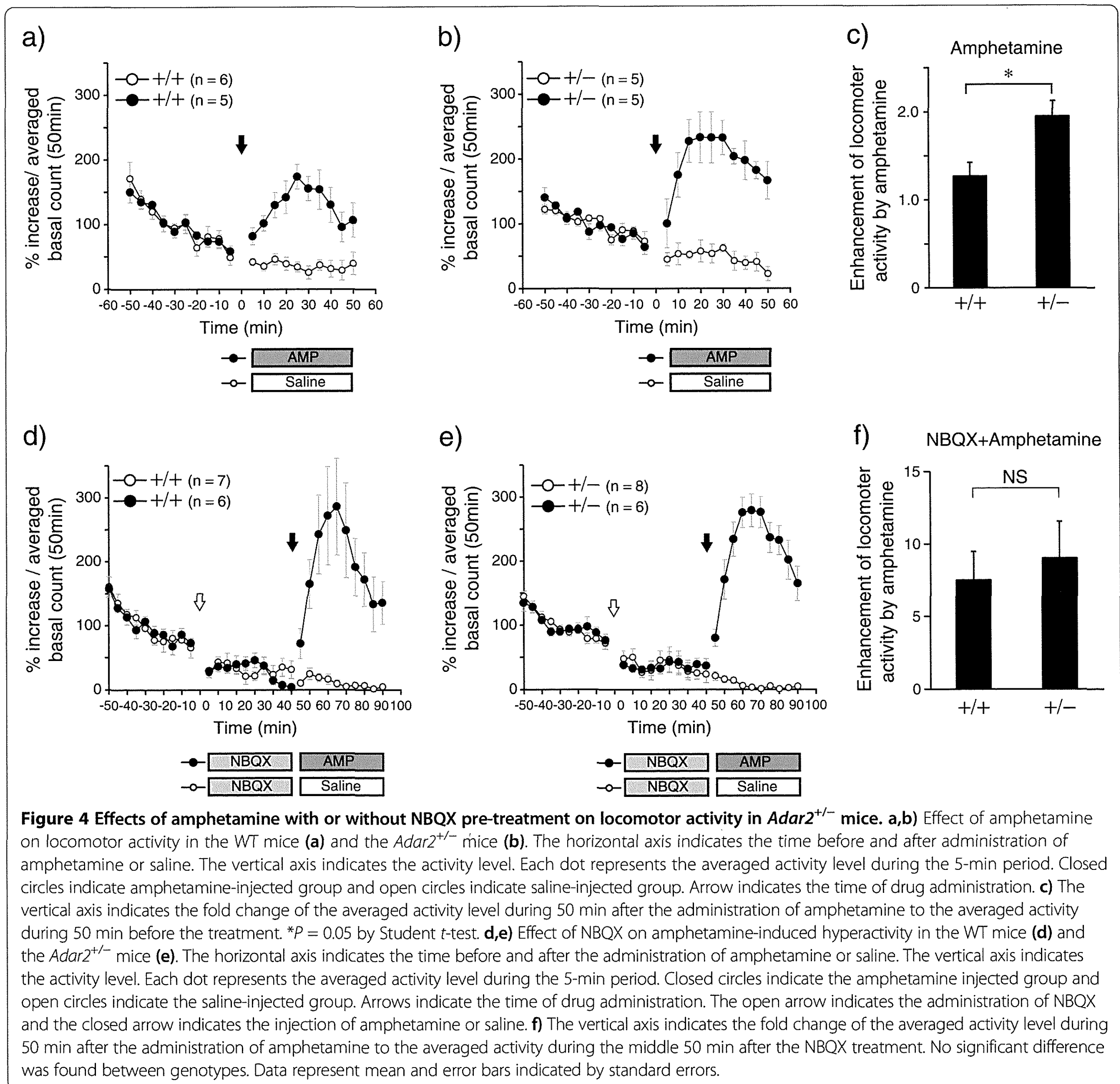


**Figure 4 Effects of amphetamine with or without NBQX pre-treatment on locomotor activity in *Adar2*$^{+/-}$ mice. a,b)** Effect of amphetamine on locomotor activity in the WT mice **(a)** and the *Adar2*$^{+/-}$ mice **(b)**. The horizontal axis indicates the time before and after administration of amphetamine or saline. The vertical axis indicates the activity level. Each dot represents the averaged activity level during the 5-min period. Closed circles indicate amphetamine-injected group and open circles indicate saline-injected group. Arrow indicates the time of drug administration. **c)** The vertical axis indicates the fold change of the averaged activity level during 50 min after the administration of amphetamine to the averaged activity during 50 min before the treatment. $^*P = 0.05$ by Student *t*-test. **d,e)** Effect of NBQX on amphetamine-induced hyperactivity in the WT mice **(d)** and the *Adar2*$^{+/-}$ mice **(e)**. The horizontal axis indicates the time before and after the administration of amphetamine or saline. The vertical axis indicates the activity level. Each dot represents the averaged activity level during the 5-min period. Closed circles indicate the amphetamine injected group and open circles indicate the saline-injected group. Arrows indicate the time of drug administration. The open arrow indicates the administration of NBQX and the closed arrow indicates the injection of amphetamine or saline. **f)** The vertical axis indicates the fold change of the averaged activity level during 50 min after the administration of amphetamine to the averaged activity during the middle 50 min after the NBQX treatment. No significant difference was found between genotypes. Data represent mean and error bars indicated by standard errors.

expression in patients with mental disorders has some functional impact on RNA editing, similar to that observed in *Adar2*$^{+/-}$ mice. Indeed, the editing of R/G sites tended to be decreased in mood disorders as a whole (Table 3), and some patients showed markedly decreased editing efficiency (Figure 2). Thus, down-regulation of *ADAR2* and the resultant decreased editing of R/G sites might have some pathophysiological significance, at least in a subgroup of patients. The causes of downregulation of *ADAR2* in these disorders are unknown. A recent study searched for proteins regulating *ADAR2* and found three RNA binding proteins, RPS14, SFRS9 and DDX15 [28]. We previously performed gene ontology analysis of differentially expressed genes in the postmortem brains of patients with bipolar disorder and found that genes related to RNA binding and RNA splicing were significantly enriched [29]. Indeed, *SFRS9*, a splicing factor, was downregulated in the postmortem brain of patients with schizophrenia and bipolar disorder (unpublished finding). Thus, RNA machinery might be somehow dysregulated in bipolar disorder and schizophrenia.

Lyddon and colleagues examined the RNA editing in AMPA/kainate receptors in the same samples (i.e., the Neuropathology Consortium samples) [17]. Their finding that the flop isoform of *GRIA3* and *GRIA4* showed higher R/G site editing compared with the flip isoform

was replicated in this study. They also reported that the diagnosis did not affect the RNA editing efficiency, which is compatible with the present finding that there is no significant difference between diagnoses.

In this study, *Adar2*$^{+/-}$ mice showed slight tendencies of altered behavior by comprehensive behavioral battery. The *Adar2*$^{+/-}$ mice showed hyperactivity in the open-field test, but they did not show altered prepulse inhibition, a candidate endophenotype in schizophrenia.

Amphetamine-induced hyperactivity was significantly enhanced in the *Adar2*$^{+/-}$ mice. However, the difference in this enhancement was no longer significant after the pretreatment with NBQX, an inhibitor of the AMPA/kainate receptor. This suggests that the enhanced response to amphetamine seen in *Adar2*$^{+/-}$ mice might be mediated by the enhanced glutamatergic signaling caused by altered function of AMPA receptors due to the decreased RNA editing of these receptors. However, a possibility that NBQX enhanced amphetamine-induced hyperactivity in WT mice but not in *Adar2*$^{+/-}$ mice cannot be totally ruled out, because we did not set a control group to examine the effect of amphetamine after saline injection. Moreover, editing of *Htr2c* was also affected by haploinsufficiency of *Adar2*, and its contribution to the enhanced response cannot be ruled out.

In addition to the *HTR2C* and AMPA/kainate receptors, recent studies using next-generation sequencers showed numerous previously unidentified editing sites [30-34], although some of the initial findings could contain false-positive sites [35,36]. In spite of the controversy surrounding the bioinformatics analysis of RNA–DNA differences, many other A-to-I editing sites such as *Cyfip2*, *Kcna1*, *Blcap*, *Gabra3*, *Flna*, *Flnb*, and *Cadps*, have been experimentally validated as target editing sites of *Adar2* [37]. Thus, alteration of RNA editing of transcripts other than those for glutamate receptors and *HTR2C* can also contribute to the phenotypes observed in this study.

Horsch and colleagues performed behavioral analysis of homozygous *Adar2* knockout mice under the background of homozygous knock-in alleles of an edited version of *Gria2* to rescue severe phenotypes due to loss of Q/R site editing of *Gria2* [37]. These mice had dramatically decreased R/G site editing of *Gria2* (15%) and *Gria4* (10%) as well as profound alterations in RNA editing of *Htr2c* [21], and showed increased passive rotation in a rotarod test, impaired hearing ability, increased raring in open field test and impaired prepulse inhibition. Some of these findings might be attributable to decreased editing of *Htr2c*. *Adar2*$^{+/-}$ mice did not show impairment in hearing ability and prepulse inhibition or altered response to open field test, possibly because of milder impairment in RNA editing of *Htr2c* and/or R/G site of AMPA receptors. However, regarding the open-field test, they showed increased rearing during the first 5 min but did not show any alteration in locomotor activity [37]. This difference may be due to different methodologies; however, less extensive editing abnormality in heterozygous knockout might cause a different phenotype.

Another group generated transgenic mice expressing *Adar2* [38] and found that these mice showed increased immobility time and decreased activity in the open-field test. Pairing this information with our findings that *Adar2*$^{+/-}$ mice showed a tendency of increased activity in the open-field test and a tendency of resistance to immobility in the forced swimming test, we can postulate that the levels of *Adar2* and the resultant RNA editing changes might be related to the activity level or liability to the behavioral despair exhibited in the forced swimming test. In the *Adar2* transgenic mice, editing of the A, C, D, and E sites of *Htr2c* was also increased [39]. Together with the decreased editing of A, B, and D sites in *Adar2*$^{+/-}$ mice, it is possible that altered A and D site editing of *Htr2c* might also contribute to the behavioral phenotypes of *Adar2*$^{+/-}$ mice.

At this stage, we should be cautious about directly connecting the behavioral features of mice such as activity in open-field tests, forced swimming tests, and enhanced amphetamine response to mental disorders such as schizophrenia, depression, or mania. To extend this preliminary finding, other animal models and effects of psychotropic medications should also be examined. In spite of its limitations, the present study suggests that an altered expression level of *Adar2* due to haploinsufficiency affects the behavior of mice at least partly through the altered RNA editing efficiency of AMPA/kainate receptors.

## Conclusion

In conclusion, *ADAR2* expression is decreased in the postmortem brains of patients with schizophrenia and bipolar disorder, and decreased *ADAR2* expression is correlated with decreased RNA editing of the R/G site in AMPA glutamate receptors. *Adar2*$^{+/-}$ mice showed decreased RNA editing of the R/G site of AMPA receptors. These mice showed slight behavioral changes such as hyperlocomotion in the open-field test, attenuated immobility response to the forced swimming test, and enhanced response to amphetamine. The difference of amphetamine response was not seen after the treatment with the AMPA/kainate receptor antagonist, NBQX. These findings collectively suggest a possible role of altered RNA editing efficiency of AMPA receptors due to down-regulation of *ADAR2* in the pathophysiology of mental disorders.

## Methods

### Postmortem brains

Two sets of postmortem brain samples donated by the Stanley Medical Research Institute were used for this

study (https://www.stanleygenomics.org) [40]. One set was the Array Collection, consisting of 104 RNA samples extracted from the prefrontal cortex (Brodmann area 46) (34 bipolar disorder, 35 schizophrenia and 35 controls). The other set comprised frozen brain tissue samples (Brodmann area 10) from the Neuropathology Consortium. They were derived from patients with bipolar disorder (n = 15), major depression (n = 15), and schizophrenia (n = 15) and 15 controls. Diagnoses had been made according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition [41]. Because of the RNA quality obtained, 101 and 49 samples from the Array Collection and Neuropathology Consortium, respectively, could be used for this study (Table 1). Subjects' demographic information is shown in Table 1. This study was approved by the Ethics Committee of RIKEN.

### Animals

*Adar2* knockout mice were developed by Higuchi and colleagues as described [21]. In brief, a targeting vector to replace exon 4 of *Adar2* with a PGK–neo gene was used for generation of a targeted embryonic stem cell line. Chimeric mice were generated by injection of this clone into C57BL/6-derived blastocysts, and homozygous *Adar2* knockout mice were bred thereafter. Genotyping of the mice was performed as described [21].

The mice were maintained in a 12-h light/12-h dark cycle. All animal experiments were approved by the local animal experiment committees of RIKEN and the Behavioral and Medical Sciences Research Consortium (BMSRC) (Akashi, Japan). Animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering.

### Real-time quantitative reverse transcription polymerase chain reaction analysis in human brain samples

Three to five micrograms of total RNA was used for cDNA synthesis by oligo (dT) and SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA). Reverse transcription polymerase chain reaction (RT-PCR) using SYBER/GREEN I (Applied Biosystems, Foster City, CA) was performed with an ABI PRISM 7900HT (Applied Biosystems). The comparative Ct method was used for quantification according to the manufacture's protocol (Applied Biosystems). Measurement of delta Ct was done at least in triplicate. Amplification of the single product was confirmed by monitoring the dissociation curve and by gel electrophoresis. We used two control genes (*GAPDH* and *CFL1*) for normalization. The validity of the use of *CFL1* as an internal control gene in postmortem brain samples has been shown previously [20]. Primer sequences used for the measurement of *ADAR2* are shown in Additional file 2: Table S1.

### RNA editing analysis

RNA editing levels of *GRIA2, GRIA3,* and *GRIA4* were determined in human samples. In the case of mice, those of *Gria2, Gria3, Gria4, Grik1,* and *Grik2* as well as *Htr2c* were determined. The primers used for this assay are listed in Additional file 2: Table S1. Because of the limited amount of RNA samples, RNA editing analysis in human brain was performed only on the Neuropathology Consortium samples by the primer extension combined with denaturing high-performance liquid chromatography (PE-DHPLC) method, according to a previous report [42]. In brief, after RT-PCR, extension of the primer was performed before the editing site, and it was terminated by incorporation of ddNTPs. The reaction mixture was separated and quantified by denaturing HPLC using a WAVE DNA fragment analysis system with the DNASep column (Transgenomic, Hillington, United Kingdom). The gradient was prepared by mixing buffer A [0.1 M triethylammonium acetate buffer (TEAA), pH 7.0] and buffer B [25% acetonitrile in 0.1 M TEAA]. Extension products were typically eluted using a linear gradient from 18% B to 38% B. RNA editing efficiency was calculated by comparing the area of the peak corresponding to the edited and nonedited extension products.

For the mouse study, heterozygous *Adar2* knockout mice ($Adar2^{+/-}$ (n = 10) and the WT littermates (n = 10) were used for the RNA editing analysis by pyrosequencing [43]. Each group included five males and five females. Three brain areas (cerebral cortex, hippocampus, and cerebellum) were dissected from the brain of each mouse. RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA). In brief, after RT-PCR with a biotinylated primer, streptavidin-sepharose beads (GE Healthcare Life Sciences, Uppsala, Sweden) and the binding buffer (10 mM Tris–HCl, 1 mM EDTA, 2 M NaCl, 0.1% Tween 20 at pH 7.6) were mixed with the RT-PCR product. The reaction mixture was placed onto a MultiScreen-HV clear plate (Millipore, Billerica, MA). After applying the vacuum, the beads were treated with a denaturation solution (0.2 N NaOH). The beads were then suspended in annealing buffer (20 mM Tris-acetate, 2 mM Mg-acetate at pH 7.6) containing a sequencing primer. The template-sequencing primer mixture was transferred onto a PSQ96 Plate (Qiagen, Venlo, Netherlands). Sequencing reactions were performed with a PSQ96 SNP Reagent Kit (Qiagen) using PSQ96MA (Qiagen) according to the manufacturer's instructions.

### Behavioral analyses

Behavioral analyses were performed at BMSRC (Akashi, Japan) with $Adar2^{+/-}$ (n = 16) and the WT littermates (n = 16). All were males, aged 8–10 weeks at the initiation of the behavioral analyses. The analyses were performed in the following order: open-field test, prepulse inhibition