値と比べ、小さいものであった。民族差による遺伝的背景の差異が大きく関与することが示唆されるが、それを超えて「リスクアレル」の統計的に有意な含有は、日本人と英国人に共通する統合失調症のリスクの存在を示唆するものであり、人類遺伝学的にも興味深いものである。

E. 結論

日本人統合失調症患者でも、polygenic componentが白人と同様に、発症の原因となっている可能性が示唆された。すなわち、リスクとしては非常に小さいものが集合することで、発症を誘発するpolygeneモデルを支持する。しかし、現状では、この解析を用いて産出された寄与率は極めて低く、診断に応用することは出来ない。今後、「リスクアレル」の精度を上昇させることや、その他の要因（例えば環境要因など）を加えることで、分離能を上昇させていくことが重要である。

F. 健康危険情報

特記すべきことなし。

G. 研究発表

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H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

該当なし。

2. 実用新案登録

該当なし。

3. その他

該当なし。

厚生労働科学研究費補助金（障害者対策総合研究事業（精神障害分野））
分担研究報告書

神経発達障害関連分子に着目したバイオマーカー・治療薬の開発

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研究要旨

上皮成長因子（EGF）は統合失調症関連遺伝子として著名なニューレグリン１と同属の分子で、共にErbB受容体を介して相互作用する成長因子である。当該遺伝子変異も、統合失調症の遺伝関連解析や患者家系において見つけられているが、その病理学的原因は明らかになっていない。これまで我々が統合失調症の神経発達障害仮説に基づき樹立、研究してきた上皮成長因子の新生児ラット投与モデルを用い、その神経回路発達の変化、認知行動変化との対応、抗精神病薬の作用機序など、その病態機序の解明を試みた。結果、本モデルは生後一旦、黒質・線条体路のドーパミン神経発達の亢進に引き続き、淡蒼球への永続的な過剰支配を呈した。薬理学的手法による淡蒼球でのドーパミン遮断は、動物の認知行動障害を改善した。これらの事実は、淡蒼球への永続的なドーパミン過剰支配が統合失調症の関連認知行動変化に関与している可能性を示唆するものである。

A. 研究目的

統合失調症のリスク因子として、周産期障害が取りざたされている。上皮成長因子(EGF)は、ヒト胎児の羊水中に数十から数百マイクログラム・L の高濃度で含まれる周産期障害関連因子であるが、中枢ではドーパミン神経にも作用しうる神経栄養因子でもある。EGF 遺伝子の変異家系の中には統合失調症発症者が見られ、SNP 遺伝解析でも、疾患との関連が報告されている遺伝子である。デコード社により発見された統合失調症感受性遺伝子「ニューレグリン１」と生体内で相互作用する因子でもあり、統合失調症との関連が疑われているが、その実態は明らかではない。統合失調症の患者死後脳において、EGF 含有量、その受容体（ErbB1）発現レベルに異常を我々が見出してから、十年以上にわたって研究を続けてきた。

最近、我々は中脳ドーパミン神経がErbB1 を発現していることを報告した。また、EGF の新生児ラットへの投与がプレパルスインヒビションや社会行動に代表される認知行動変化を成熟後に引き起こすことから、統合失調症の動物モデルとして活用できることを提唱してきた。これらの知見から本研究では、更に本 EGF モデルに焦点を当て、そのドーパミン神経回路・機能を中心に、過剰 EGF シグナルが引き起こす神経発達障害の実態を解明することを試みた。加えて、そのドーパミン神経の病理から、統合失調症関連行動障害との因果関係を検討した。

B. 研究方法

①新生児 EGF 投与モデルの作製

動物は，SD ラット（オス；日本 SLC）生後２日齢を使用した．EGF 蛋白は，大腸菌内で組み替え蛋白として精製されたヒゲタ醤油社のものを使用した。この EGF 蛋白を生理食塩水に溶解させ、生後２日目より毎日計９回（生後 10 日目まで），頚部にラット体重 1g 当たり 1.0 マイクログラム皮下投与した。生後 56－80 日齢まで成長させ、以下の実験に用いた。

②モノアミン測定

脳組織は解剖後、すぐに 0.1M の過塩素酸溶液中でホモジナイズとソニケーションにより、総モノアミンを抽出した。もしくはマイクロダイアリシスプローブ（A-I-8-02, Eicom)を淡蒼球に挿入手術し、人工脳脊髄液を 42 μL・時で透析することで局所ドーパミン放出量を推定した。祖抽出液、透析液は、電気化学検出器（EICOM、モデル３００）を装着した HPLC（島津製作所）によりＯＤＳカラム（4.6ｘ150ｍｍｍ）で分離、検出した。

③脳内薬物投与

麻酔したラット（生後５６－７０日）の頭蓋のブレグマ 0.9㎜前、3㎜横、4.5㎜下にガイドカニューレ先端を挿入・固定し、10 日間飼育して、手術からの回復を待った。ガイドカニューレを用いてハミルトンシリンジから 0.5μL のドーパミン関連薬物を淡蒼球に注入した。5－15 分後に以下の行動実験を荷した。

④音驚愕反応の測定

小動物驚愕反応測定装置（San Diego Instruments）にて驚愕反応強度およびプレパルスインヒビションを測定した．驚愕反応を誘発する感覚刺激としては，音刺激（120ｄＢ、110ｄＢ）を用い，プレパルス刺激として環境騒音レベルより 5, 10, 15 デシベル高い音圧の刺激を与えた．驚愕音刺激単独の時の驚愕反応とプレパルスを組み合わせた時の反応比の減少分をプレパルスインヒビション(PPI)とした。

⑤運動量の測定と社会行動の測定

赤外線によるラット用の自動運動量測定装置（Med Associates）にてラットの水平・垂直運動量を測定した。１時間後の新規環境になれた時点で、異なるケージ育った同性の標的真数を測定装置中に入れ、EGF 投与ラットの標的ラットに対する社会行動（匂いカギ、追尾、マウンテイング）を計測した。

（倫理面への配慮）

これらの動物実験は、新潟大学動物実験倫理委員会からその実験法についての承認を得て実施した。

C. 実験結果

①新生児ラットへの EGF 末梢投与のドーパミン神経発達への効果

新生児ラットへの EGF 蛋白の末梢連続投与により、生後 10 日齢時点ではドーパミン生合成律速酵素であるチロシン水酸化酵素（TH)の発現量が、線条体、側座核、淡蒼球において上昇した。しかし、生後 60 日齢にいたると、その TH の上昇は淡蒼球に限局し、また同時に同部位におけるドーパミン含量の上昇も観察された。

②EGF 投与モデル動物の淡蒼球でのマイクロダイアリシス

成長後の EGF 投与マウスの淡蒼球において、実際にドーパミン放出量が、合成量と含量の上昇に伴って上がっているかどうかを、マイクロダイアリシスと微量ドーパミン計測によって検討した。EGF 投与動物の淡蒼球では定常状態でドーパミン放出量が 2 倍近く亢進していた。また脱分極刺激を負荷しても、その亢進は維持されていた。各動物のドーパミン放出量を、当該動物のプレパルスインヒビションスコアと対比させたところ、強い負の相関が観察された。

③淡蒼球のドーパミン活性と認知行動異常

実際に淡蒼球でのドーパミン放出が認知行

動異常の発現に関連しているかどうか、淡蒼球にモノアミンを枯渇させるレセルピンやドーパミン受容体のアゴニスト、アンタゴニストを注入して、EGF 投与動物の行動変化を観察した。レセルピンの投与は、淡蒼球のドーパミン含量を低下させるとともに、プレパルスインヒビション障害を著しく改善した。代わって D1 受容体アンタゴニスト（SCH23390）や D2（raclopride）受容体アンタゴニスト（raclopride）を局所注入すると、raclopride の場合にのみ、用量依存的にプレパルスインヒビション障害が改善した。

この部位特異性を検証する目的で、D2 受容体アゴニスト（quinpirole）を、逆にコントロールラットの淡蒼球局所に注入するとプレパルスインヒビション障害が誘発された。

D. 考察

神経発達期の過剰 EGF 暴露は黒質・線条体路のドーパミン神経発達の異常な亢進をまねき、淡蒼球では成熟後もこの過剰神経支配が持続していた。黒質における EGF 受容体の分布は、淡蒼球支配をしうるドーパミン神経の細胞体の分布と一致しており、EGF の新生児投与により、これらの神経が刺激され、過剰発達したものと推定される。しかし、EGF の供給がなくなった時点で、黒質—淡蒼球路以外のドーパミン神経終末は正常化したと推察される。唯一、淡蒼球では、この部位からの内在性 EGF 類の発現・放出上昇を招いたことで、過剰支配が持続したものと考えられる。

実際に EGF 投与動物の淡蒼球でのドーパミン放出量は異常に亢進していて、その量はプレパルスインヒビション障害と有意な相関を示した。淡蒼球局所へのレセルピンや D2 ブロッカーの注入により、コントロールレベルにまで、プレパルスインヒビションが改善している。したがって EGF 投与モデルのプレパルス障害は、淡蒼球でのドーパミン亢進に起因する行動異常であると推察された。

このようなドーパミン神経機能の亢進は統合失調症の病態で疑われる所見である。今回注目された脳部位、淡蒼球は、間接路とよばれるドーパミン D2 受容体で制御されている神経回路に対応する。つまり定型抗精神病薬の主要な作用点でもある脳部位がドーパミンの過剰神経支配を受けていたのである。この事実は、定型抗精神病薬の作用を解説しうるものである。加えて脳画像研究では、実際の統合失調症患者において、淡蒼球のサイズ、活動、異方性についての異常報告があることから、EGF モデル動物に限らず、より一般化できうる病態かもしれない。

E. 結論

本 EGF モデル研究から統合失調症の関連病態には、間接路を成す淡蒼球のドーパミン機能亢進が関与する可能性を提唱している。

F. 健康危険情報

なし

G. 研究発表

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H. 知的財産権の出願・登録状況

なし

研究成果の刊行に関する一覧表

雑 誌

雑誌

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Expression of Ca^{2+}-dependent activator protein for secretion 2 is increased in the brains of schizophrenic patients

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ABSTRACT

Ca^{2+}-dependent activator protein for secretion 2 (CADPS2), a secretory granule associate protein, mediates monoamine transmission and the release of neurotrophins including brain-derived neurotrophic factor (BDNF) which have been implicated in psychiatric disorders. Furthermore, the expression of CADPS2deltaExon3, a defective splice variant of CADPS2, has been reported to be associated with autism. Based on these observations, we examined whether expression levels of CADPS2 and CADPS2deltaExon3 are altered in psychiatric disorders. Quantitative polymerase chain reaction analysis was performed for postmortem frontal cortex tissues (BA6) from 15 individuals with schizophrenia, 15 with bipolar disorder, 15 with major depression, and 15 controls (Stanley neuropathology consortium). The mean CADPS2 expression levels normalized to human glyceraldehyde-3phosphate dehydrogenase (GAPDH) or TATA-box binding protein levels was found to be significantly increased in the brains of the schizophrenia group, compared to the control group. On the other hand, the ratio of CADPS2deltaExon3 to total CADPS2 was similar in the 4 diagnostic groups. We then analyzed CADPS2 expression in blood samples from 121 patients with schizophrenia and 318 healthy controls; however, there was no significant difference between the two groups. Chronic risperidone treatment did not alter the expression of CADPS2 in frontal cortex of mice. The observed increase in the expression of CADPS2 may be related to the impaired synaptic function in schizophrenia.

1. Introduction

Ca^{2+}-dependent activator protein for secretion (CADPS) family, which consists of two members, CADPS1 and CADPS2, is a secretory granule-associated proteins involved in Ca^{2+}-dependent exocytosis of large dense-core vesicles containing diverse array of modulators including neurotrophines, monoamines and neuropeptides (Liu et al., 2008; Sadakata et al., 2004). CADPS2 mediates the release of neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3. Mouse CADPS2 protein is associated with BDNF-containing secretory vesicles and promotes activity-dependent release of BDNF (Sadakata et al., 2004). BDNF release is significantly reduced in cultured neurons prepared from the brain of CADPS2 deficient mice (Sadakata et al., 2007a,b).

A number of findings suggest that BDNF action is impaired in psychiatric disorders including schizophrenia, bipolar disorder and depression. Several studies have shown decreased levels of BDNF or its receptor, TrkB, in the postmortem brains of patients with schizophrenia (Hashimoto et al., 2005; Iritani et al., 2003; Weickert et al., 2003), although there are contradictive reports (Chen et al., 2001; Dunham et al., 2009; Durany et al., 2001; Takahashi et al., 2000). The contribution of BDNF in depression has been suggested from animal studies that demonstrated stressful environments decrease, and antidepressive treatments increase BDNF levels in the brain (Duman and Monteggia, 2006; Martinowich et al., 2007). Also, centrally administered BDNF has an antidepressant-like effect in rat models (Siuciak et al., 1997). Thus, the molecules that contribute to the trafficking and release of BDNF may be a culprit of these disorders.

CADPS family also mediate monoamine transmission. Both CADPS1 and CADPS2 mediate the refilling of catecholamine to the releasable vesicles, and catecholamine secretion is significantly suppressed in the CADPS1/2 double deficient cells. (Liu et al., 2008). Another study supports that CADPS family are involved in monoamine storage as antibodies against CADPS1 or 2 inhibit monoamine

Abbreviations: ANCOVA, Analysis of covariance; BDNF, Brain-derived neurotrophic factor; CADPS2, Ca^{2+}-dependent activator protein for secretion 2; CCK, Cholecystokinin; DSM—IV, Diagnostic and Statistical Manual of Mental Disorders, 4th edition; FST, Freezer storage time; M.I.N.I., Mini-International Neuropsychiatric Interview; NT, Neurotensin; PCR, Polymerase chain reaction; PMI, Postmortem interval; SD, Standard deviation; TBP, TATA-box binding protein.

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sequestration by synaptic vesicles from mice brain (Brunk et al., 2009).

Dysregulation of monoamine neurotransmission has been hypothesized to play a central role in the etiology of psychiatric disorders including schizophrenia and mood disorders. In schizophrenia, not only classical evidence that dopamine agonists induce and dopamine D2 receptor antagonists ameliorate psychoses but also brain imaging studies on drug naïve patients have suggested that dopamine transmission is affected in this disorder (Lyon et al., 2009). In major depression, reduced monoamine transmission hypothesis was derived from the finding that most anti-depressants increase monoamine levels in the synaptic cleft and that reserpine, a monoamine-depleting drug, worsen depressive symptoms in a subset of patients with mood disorder (Krishnan and Nestler, 2008), although imaging, postmortem, or cerebrospinal fluid studies have yet to find the definitive evidence for altered monoamine neurotransmission in this disorder (Belmaker and Agam, 2008; Nikolaus et al., 2009).

While, to our knowledge, CADPS2 expression in schizophrenia or mood disorders have not yet been examined, aberrant splicing of CADPS2 mRNA was reported in autism (Sadakata et al., 2007b). In this study, an exon-3 skipped isoform, CADPS2ΔExon3, was detected in the bloods of several autistic patients but not in those of healthy controls. They also showed that CADPS2ΔExon3 was deficient in proper axonal transport, which results in the loss of local synaptic BDNF release. Though the CADPS2ΔExon3 expression in the brains of patients with autism is unclear, the aberrant splicing of CADPS2 could contribute to susceptibility to autism by affecting neurotrophin release.

Based on above findings, the present study was aimed to examine whether the expression of CADPS2 transcripts is altered in the frontal cortex of patients with psychiatric disorders including schizophrenia, major depression and bipolar disorder. The CADPS2 expression levels in the blood of schizophrenia were also examined.

2. Materials and methods

2.1. Brain samples

Frozen postmortem samples of frontal cortex (BA6) were obtained from the Stanley Foundation Neuropathology Consortium (Torrey et al., 2000). The collection consists of 60 subjects: 15 with schizophrenia, 15 bipolar disorder, 15 major depression and 15 unaffected controls. All groups were matched for age, sex, race, pH and hemispheric side (Table 1), although postmortem interval (PMI) and freezer storage time differed across the groups. The brain tissues obtained were coded. Once our blind study was complete, we sent the data to the Stanley Foundation who then returned the codes, demographic and clinical data. In a cold-room, each frozen brain tissue was broken into powder in the plastic bag using dry-ice block and dry-ice-cold hammer. The powder was then transferred and kept in dry-ice-cold tubes. Temperature of the tubes and instruments that directly contacted to the samples was frequently measured by infrared–thermometer (AD-5613A, A&D Company, Japan) and kept under −20 °C. Then, 30 to 40 mg of brain powder was used for cDNA synthesis. RNA was extracted using RNAqueous (Applied biosystems, Foster City, CA) according to manufacturer's instructions with a slight modification, i.e., after homogenization, samples were washed twice with 500 μl of chloroform, and then applied to the spin-column. Extracted RNA was quantified by optical density reading at 260 nm using NanoDrop ND-1000 (Thermo Scientific, Rockford, IL). Then, the obtained RNA (14 μl) was used for cDNA synthesis using SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA).

Table 1
Demographic information on brain specimens of Stanley Neuropathology Consortium.

	Control	Schizophrenia	Bipolar disorder	Major depression
Age (years)	48.1 (29–68)	44.2 (25–62)	42.3 (25–61)	46.4 (30–65)
Gender (M/F)	9/6	9/6	9/6	9/6
Race	14 C, 1 AA	13 C, 2 A	14 C, 1 AA	15 C
PMI (hours)	23.7 (8–42)	33.7 (12–61)	32.5 (13–62)	27.5 (7–47)
pH	6.3 (5.8–6.6)	6.1 (5.8–6.6)	6.2 (5.8–6.5)	6.2 (5.6–6.5)
Side of brain frozen (R/L)	7/8	6/9	8/7	6/9
Freezer storage time (months)	11.3 (1–26)	20.7 (2–31)	20.7 (7–28)	14.5 (3–31)

AA, African American; A, Asian; C, Caucasian; F, female; M, male; and PMI, postmortem interval.

2.2. Blood samples

Subjects were 121 patients with schizophrenia (84 males and 37 females; age 44.1 ± 13.7 (mean ± SD) years) and 318 controls (90 males and 228 females; age 43.1 ± 15.3 years). All subjects were biologically unrelated Japanese and recruited from the same geographical area (Western part of Tokyo Metropolitan). Consensus diagnosis by at least two psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM—IV) criteria (American Psychiatric Association, 1994) on the basis of unstructured interviews and information from medical records. The controls were healthy volunteers recruited from the community, through advertisements in free local magazines and our website announcement. Control individuals were interviewed by the Japanese version of the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Otsubo et al., 2005; Sheehan et al., 1998) and those who had a current or past history of psychiatric treatment were not enrolled in the study. After the nature of the study procedures had been fully explained, written informed consent was obtained from all subjects. The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

Blood collection and RNA isolation were performed using the PAXgene blood RNA system (Qiagen, Valencia, CA). Blood samples were collected around 11 A.M. Extracted RNA was quantified as described above. Samples that contained more than 40 ng/μl of total RNA were used for analysis; 8 μl from each sample was reverse transcribed using SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA).

2.3. Chronic risperidone treatment to mice

C57BL/6J male mice aged 10 weeks were purchased from Crea Japan. Chronic oral risperidone treatment was performed according to Belforte et al., (Belforte et al., 2010). In brief, 2.5 mg/kg/day of risperidone (Rispadal liquid, Janssen Pharmaceutical, Tokyo, Japan) in drinking water freshly made every 72 h had been administered continuously for 3 weeks. Control mice received solvent (1.4 mM tartaric acid neutralized to pH 6–7). All experimental procedures were in accordance with the guidelines of the United State's National Institutes of Health (1996) and were approved by the Animal Care Committee of the National Institute of Neuroscience, NCNP.

2.4. Quantitative real-time polymerase chain reaction

Polymerase chain reaction (PCR) amplifications were performed in triplicate (5 μl volume) on 384-well plates using ABI prism 7900HT (Applied Biosystems, Foster City, CA). Each reaction contained 0.28 μl of cDNA sample, qPCR QuickGoldStar Mastermix Plus (Eurogentec, Seraing, Belgium) and a primer of the target, i.e. human CADPS2 (Hs01095968_m1 at Exon 4–5 on NM_017954.9), mouse CADPS2 (Mm00462577_m1), human CADPS2ΔExon3 (Forward primer: GTAGCTGACGAAGCATTTTGCA,

Reverse Primer: TGATCTGGGCTGCTTGTTCAT, Reporter: CTGCGTTATC-CAGCTCAT) and a primer of the housekeeping gene human glyceraldehyde-3phosphate dehydrogenase (GAPDH, 4326317E), mouse GAPDH (4352339E) and human TATA-box binding protein (TBP, Hs99999910_ml) all purchased from Applied Biosystems (Foster City, CA). Negative control reactions were carried out with "no RNA" samples. The real time PCR reactions ran at 50 °C for 2 min, at 95 °C for 10 min and in 40 or 45 cycles changing between 95 °C for 15 s and 60 °C for 1 min. A standard amplification curve was made by serial dilution of a "standard" pooled cDNA sample in each plate. The mean value of triplicate of each sample was normalized to the standard curve. Then, the values of CADPS2 and CADPS2ΔExon3 from each sample were normalized to those of GAPDH.

2.5. Statistical analyses

Data analyses were performed with SPSS software (Version 11, SPSS Japan, Tokyo, Japan). Effect of age, brain pH, postmortem interval (PMI), and freezer storage time on each brain analysis was assessed by Pearson's correlations (Table 2). Variables showing significant correlations were included as covariates in the main analysis. Levene's test was used to assess the equality of variances across diagnostic group. Analysis of covariance (ANCOVA) was used to identify overall effects of diagnosis and significant main effects of diagnosis were investigated by planned post hoc contrasts. In the blood sample analyses, CADPS2 expression levels were converted to 10-log scale before statistical analysis in order to obtain a normal distribution (Castensson et al., 2005). The effect of diagnosis on blood CADPS2 expression was assessed by ANCOVA with sex and age as covariates after Levene's test. The effect of diagnosis on blood CADPS2ΔExon3 expression was assessed by logistic regression, controlling for sex and age as covariates. The effect of risperidone on CADPS2 expression in mice brain was assessed by student's *t*-test after F-test.

3. Results

3.1. CADPS2 expression levels in the postmortem brain (BA6)

We first analyzed the effects of age, brain pH, postmortem interval (PMI), and freezer storage time (FST) on each expression analysis (Table 2). Brain pH was significantly correlated with GAPDH expression levels or raw CADPS2 expression levels. PMI also tended to be correlated with GAPDH expression levels or raw CADPS2 expression levels. If the effects were analyzed separately within each diagnostic group, no significant correlation was detected.

CADPS2 expression levels normalized to GAPDH expression levels (CADPS2/GAPDH) in each sample are shown in Fig. 1A. ANCOVA with brain pH as covariates detected a significant effect of diagnosis on CADPS2/GAPDH levels ($F=3.4$, $df=3$, $p=0.025$) and post hoc test detected a significant difference between schizophrenia and control groups ($p=0.03$). Even if PMI was added as another covariate, the difference was significant ($p=0.002$). There was no significant difference between bipolar disorder and controls or between depression and controls. There was no significant correlation between CADPS2/GAPDH levels and lifetime dose of antipsychotic drugs (data not shown). There was a significant effect of diagnosis on GAPDH expression levels ($F=3.4$, $df=3$, $p=0.023$, Fig. 1B). GAPDH levels in the control group was significantly higher than that of schizophrenia ($p=0.012$), bipolar disorder ($p=0.009$) or major depression group ($p=0.013$). Raw CADPS2 levels did not differ among the diagnostic groups ($F=1.0$, $df=3$, $p=0.38$, Fig. 1C). There was a significant correlation between GAPDH expression levels and raw CADPS2 expression levels (Pearson's correlation 0.69, $p<0.001$, Fig. 1D).

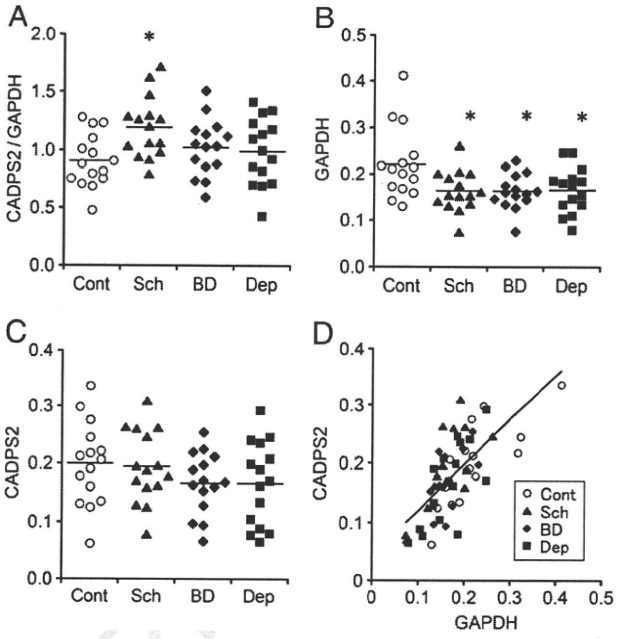

Fig. 1. CADPS2 expression levels in the postmortem brains of psychiatric disorder. (A) CADPS2 expression levels normalized by GAPDH levels. Scatter plots display the variability and differences in the CADPS2 mRNA expression levels normalized by each GAPDH expression levels. A crossbar on each scatter plot represents mean expression levels for each group. (B) GAPDH expression levels (C) Raw CADPS2 expression levels. (D) Correlation between GAPDH levels and raw CADPS2 levels. Cont, control; Sch, schizophrenia; BD, Bipolar Disorder; and Dep, Depression. *, statistically significant difference ($p<0.05$).

We compared relative CADPS2 expression levels among diagnostic groups using another endogenous control, TATA-box binding protein (TBP), and obtained similar result (Fig. S1, this experiment was done after uncode the sample). ANCOVA with brain pH as covariates detected a significant effect of diagnosis on CADPS2/TBPlevels ($F=3.3$, $df=3$, $p=0.027$) and post hoc test detected a significant

Table 2
The effect of age, pH, postmortem interval, and freezer storage time on each brain expression analysis.

		GAPDH	CADPS2	ΔExon3	CADPS2/GAPDH	ΔExon3/G APDH	ΔExon3/C ADPS2
Age	Pearson's	0.013	−0.13	0.19	−0.18	0.088	0.27
	P	0.92	0.34	0.37	0.16	0.51	0.041
pH	Pearson's	0.36	0.26	0.25	0.031	0.12	0.090
	p	0.005	0.048	0.058	0.81	0.38	0.50
Post mortem Pearson's interval (hours)	−0.23	−0.22	−0.13	−0.040	0.039	0.15	
	P	0.076	0.098	0.30	0.76	0.77	0.25
Freezer storage time (months)	Pearson's	−0.22	−0.034	−0.041	0.21	0.12	0.052
	P	0.092	0.80	0.75	0.11	0.36	0.69

ΔExon3, CADPS2ΔExon3; and Pearson's, Pearson's correlation.

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difference between schizophrenia and control groups ($p=0.019$). Even if PMI was added as another covariate, the difference was significant ($p=0.012$).

With respect to CADPS2ΔExon3/GAPDH level (Fig. 2A), the effect of age was detected in the control group (Pearson's correlation 0.58, $p=0.023$) and the effect of pH was detected in the bipolar disorder group (Pearson's correlation 0.60, $p=0.018$). ANCOVA with age and brain pH as covariates detected the marginal effect of diagnosis ($F=2.8$, $df=3$, $p=0.050$) and the mean expression level was significantly increased in the schizophrenia group, compared to the control group ($p=0.030$). When the ratio of CADPS2ΔExon3 to raw (total) CADPS2 expression levels was compared, the ratio was similar in the 4 diagnostic groups ($F=1.1$, $df=3$, $p=0.36$, Fig. 2B). Neither the effect of diagnosis on raw CADPS2ΔExon3 levels was observed ($F=1.9$, $df=3$, $p=0.15$, Fig. 2C). There was a significant correlation between GAPDH expression levels and raw CADPS2ΔExon3 expression levels (Pearson's correlation 0.66, $p<0.001$, Fig. 2D).

3.2. Cortical CADPS2 expression after chronic antipsychotic treatment in mice

To see whether antipsychotics alter the mRNA expression of CADPS2, we measured the CADPS2 levels in the frontal cortex of mice, following chronic treatment with an antipsychotic risperidone. Oral administration of risperidone (2.5 mg/kg, $n=15$ for the controls and 16 for the risperidone group) for 3 weeks did not alter CADPS2 expression ($F=1.5$, $df=29$, $p=0.61$).

3.3. CADPS2 expression in blood sample

Since we observed increased expression of CADPS2 in postmortem brains of schizophrenia patients, we then examined whether such an alteration exists in peripheral blood samples. The CADPS2/GAPDH expression levels were converted to 10-logarism before statistical analyses to obtain normal distribution. The mean (Standard deviation) CADPS2 expression level was 0.17 (1.29) in the control group and 0.32 (1.46) in the schizophrenia group. ANCOVA controlling for age and sex did not detect the significant effect of diagnosis on CADPS2/GAPDH level ($F=1.67$, $df=1$, $p=0.20$). We also measured CADPS2ΔExon3 levels in the blood samples. Compared to brain samples, the expression levels were quite low and could not detect in the majority of samples. Thus, we defined "expressed" when at least 2 tubes in triplet analyses of each sample were detected until 45 cycles. CADPS2ΔExon3 expression was detected in 36 of 318 control samples (ratio = 0.11), and 21 of 121 schizophrenia samples (ratio = 0.17). There was no significant effect of diagnosis on CADPS2ΔExon3 expression by the logistic regression analysis controlling for age and sex (odds ratio 1.51, [95% CI 0.80–2.86], $p=0.21$). Even when men and women were examined separately, there was no significant difference between the patients and controls for each sex (data not shown).

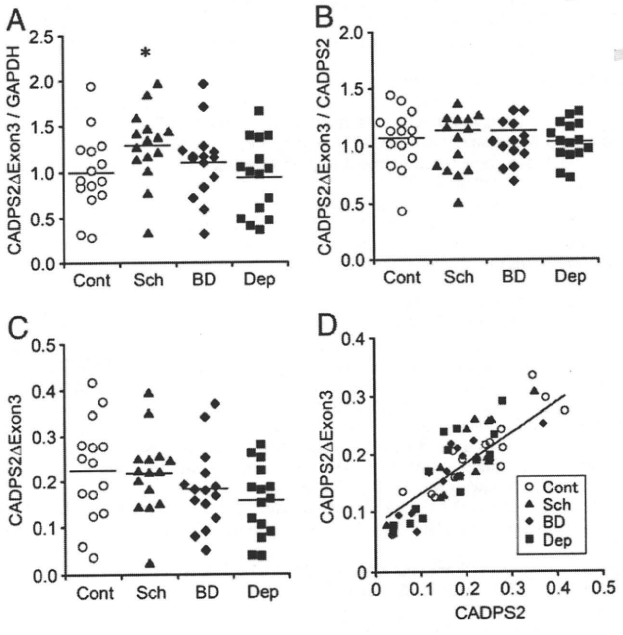

Fig. 2. CADPS2ΔExon3 expression levels in the postmortem brains of psychiatric disorder. (A) CADPS2ΔExon3 expression levels normalized by GAPDH levels. Scatter plots display the variability and differences in the CADPS2ΔExon3 mRNA expression levels normalized by each GAPDH expression levels. A crossbar on each scatter plot represents mean expression levels for each group. (B) CADPS2ΔExon3 levels normalized to each total CADPS2 expression levels. (C) Raw CADPS2ΔExon3 expression levels. (D) Correlation between GAPDH expression levels and raw CADPS2ΔExon3 expression levels. Cont, control; Sch, schizophrenia; BD, Bipolar Disorder; and Dep, Depression. *, statistically significant difference ($p<0.05$).

4. Discussion

4.1. Main findings

In the present study, we analyzed the expression of CADPS2 mRNA in the postmortem brains (BA6) of psychiatric patients (schizophrenia, major depression and bipolar disorder) and controls. A significant increase in the CADPS2 expression was detected in the brains of the schizophrenia group, compared to the control group. No change was detected in other disease groups. While a CADPS2 splice variant, CADPS2ΔExon3 showed a non-significant increase in the schizophrenia group, its ratio to the total CADPS2 levels was not different from the control group. Chronic risperidone treatment did not alter the CADPS2 levels in mice brain. We also analyzed CADPS2 or CADPS2-ΔExon3 expression levels in the blood samples of schizophrenia and control subjects; however, the levels were not significantly different between the two groups.

4.2. Brain analysis

4.2.1. Drug effect

A large number of gene expressions in the brain are affected by antipsychotic treatments (Girgenti et al.,; Mehler-Wex et al., 2006; Thomas, 2006). Therefore, the observed increase in CADPS2 mRNA in the schizophrenia group could be the result of antipsychotic treatment. However, our results did not support this assumption because the CADPS2 levels did not correlate to life-time antipsychotic dose and chronic risperidone treatment in mice did not alter CADPS2 expression on their cortexes, although caution is required for the interpretation of those results because we don't have data for the latest dose before death and other drugs such as chlorpromazine, haloperidol and clozapine might be used in the patients. Q1

4.2.2. Possible relevance to BDNF secretion, dopamine transmission, and neuropeptide release

Considering that defective BDNF signaling has been suggested in schizophrenia and mood disorders (Angelucci et al., 2005) and that CADPS2 mediates BDNF release in neurons (Sadakata et al., 2004), we initially expected that CADPS2 levels would be decreased in frontal cortex in patients with these psychiatric disorders. However, in our results, CADPS2 levels were not altered in mood disorders but increased in schizophrenia. In addition, the relative levels of defective CADPS2 isoform, CADPS2ΔExon3 were not altered in those disorders. Thus, it is unlikely that altered CADPS2 expression might be a cause of BDNF deficits in schizophrenia. It may be rather a compensatory consequence of reduced BDNF signaling.

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CADPS2 also promotes monoamine storage in neurons (Brunk et al., 2009; Liu et al., 2008). CADPS2 is highly expressed in the dopamine-rich brain areas such as ventral tegmental area and substantia nigra of mice brain (Sadakata et al., 2006) and it is reported to interact with dopamine D2 receptor (Binda et al., 2005). Growing evidence has demonstrated increased presynaptic dopamine levels in the striatum of schizophrenia patients (Lyon et al., 2009). If the observed increase in the expression of CADPS2 occurs in the subcortical regions including striatum and midbrain as well as frontal cortex, it might be the cause of hyper-dopamine transmission that reflects psychotic state (Howes et al., 2009).

Furthermore, large dense-core vesicles contain not only neurotrophins and monoamines but also neuropeptides (Salio et al., 2006). Neuropeptides such as endorphins, cholecystokinin (CCK), neurotensin (NT), somatostatin, Neuropeptide Y and neuregulin 1 have been implicated in schizophrenia (Caceda et al., 2007). Especially reduced levels of CCK and NT have been repeatedly reported in the disorder (Caceda et al., 2007), which may have caused compensatory increase in the CADPS2 expression in schizophrenia.

4.3. CADPS2 expression in the blood

4.3.1. CADPS2 expression and diagnosis

Following the report that 4 of 16 patients with autism expressed CADPS2ΔExon3 in peripheral bloods but none in 24 normal subjects (Sadakata et al., 2007b), another group reported that they detected CADPS2ΔExon3 in some control subjects (Eran et al., 2009). Thus we assumed that the ratio of CADPS2ΔExon3 to total CADPS2 rather than whether CADPS2ΔExon3 exists or not is important and therefore we applied quantitative real-time PCR to measure their expression. The pilot experiment in the present study indicated that our quantification method using SuperScript VILO and random-hexamer, was 4 to 8 fold more sensitive than one step real-time PCR using gene specific primers and could detect 10 to 100 clones of CADPS2 or CADPS2-ΔExon3 sequence-containing vector. Compared with the brains, CADPS2 expression was 32 to 128 fold lower in the blood. Unlike in the brain, CADPS2ΔExon3 could not be detected in most blood samples. So we performed qualitative analysis for each subject. As a result, we didn't detect any significant difference in the expression of CADPS2ΔExon3 in the blood between patients with schizophrenia and controls. The CADPS2ΔExon3 was abundantly expressed in the brain and the levels were unchanged across the diagnostic groups. Thus, it is unlikely that the expression or the splicing balance should relate to diseases we analyzed.

5. Conclusion

In conclusion, we found increased mRNA expression of CADPS2 in the postmortem frontal cortex of schizophrenia patients which might have some relevance to dysregulation in the release of dopamine, neurotrophins, and/or neuropeptides in the disorder. This increase was unlikely to be attributable to antipsychotic medication. We also analyzed the CADPS2ΔExon3 in human brains and found that it is abundantly present in the frontal cortex in any diagnostic group. We obtained no evidence for the specific role of the splice variant in schizophrenia or mood disorders. Future research should include the evaluation of CADPS2 expression in other brain areas, and basic studies on the cause and consequence of increased CADPS2 expression.

Supplementary materials related to this article can be found online at doi:10.1016/j.pnpbp.2011.05.004.

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Poor sleep is associated with exaggerated cortisol response to the combined dexamethasone/CRH test in a non-clinical population

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ABSTRACT

Although sleep disturbance has been shown to be associated with psychological distress and the hypothalamic-pituitary-adrenal (HPA) axis function, the simultaneous relationship between sleep, distress and HPA axis function is less clear. Here we examined the relationship between sleep quality as assessed with the Pittsburgh Sleep Quality Index, psychological distress as assessed with the Hopkins Symptom Checklist, and cortisol responses to the dexamethasone (DEX)/corticotropin-releasing hormone (CRH) test in 139 non-clinical volunteers. Poor sleep was significantly correlated with greater cortisol response to the combined DEX/CRH challenge, but not with the cortisol level just before CRH challenge. When subjects were divided into three groups based on the suppression pattern of cortisol (i.e., incomplete-, moderate-, and enhanced-suppressors), poor sleep was significantly associated with the incomplete suppression in women while no significant association was found between sleep and the enhanced suppression. The association between poor sleep and exaggerated cortisol response to the CRH challenge became more clear in the regression analysis where the confounding effect of psychological distress was taken into consideration. These results indicate that poor sleep would be associated with exaggerated cortisol reactivity. The observed association of poor sleep with reactive cortisol indices to the CRH challenge, but not with the cortisol level after DEX administration alone, might add to the well-established evidence demonstrating the role of CRH in the regulation of sleep. Our findings further suggest that the mediation model would work better than the bivariate approach in investigating the relationship between sleep, distress and HPA axis reactivity.

1. Introduction

Sleep is recognized as a physiological function necessary for optimal daytime functioning. Sleep disturbance due to various challenges imposed by modern society can represent serious threats to our health and well-being, such as cognitive impairments (Ferrara et al., 2000), impaired glucose regulation (Scheen et al., 1996) and elevation of cortisol (Leproult et al., 1997). The hypothalamic-pituitary-adrenal (HPA) axis mediates the reaction to all sorts of stressors, and plays an important role in the regulation of sleep as well. Poor sleep status such as chronic insomnia (Vgontzas et al., 2001) and short sleep duration (Kumari et al., 2009) can lead to HPA axis overactivation; likewise, hyperactive HPA axis could have many unfavorable effects on sleep, including increased light sleep and wakefulness and decreased slow wave sleep (Buckley and Schatzberg, 2005; Steiger, 2002). Accumulated evidence thus shows multiple reciprocal relationships between sleep and HPA axis function (Buckley and Schatzberg, 2005). Indeed, HPA axis is proposed as a potential mechanism by which sleep could be associated with physical and mental health (McEwen, 2006).

Previous studies investigating HPA axis function in relation to a variety of sleep parameters have employed different cortisol measures, including diurnal cortisol profiles (Lasikiewicz et al., 2008), cortisol awakening response (Stetler and Miller, 2005), and cortisol reactivity to psychosocial challenge tests (Räikkönen et al., 2010). Besides these measurements, several recent studies have

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