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厚生労働科学研究費補助金（創薬基盤推進研究事業）
創薬バイオマーカー探索研究事業

精神・神経疾患関連バイオマーカー探索による創薬基盤研究

（H20-バイオ-一般-010）

平成24年度　総括研究報告書

研究代表者　　後　藤　雄　一

国立精神・神経医療研究センター

平成 25（2013）年 5 月

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Ⅰ．総括研究報告書

厚生労働科学研究費補助金（創薬基盤推進研究事業）
総括研究報告書

精神・神経疾患関連バイオマーカー探索による創薬基盤研究

研究代表者
後藤　雄一（国立精神・神経センター医療研究センター神経研究所　疾病研究第二部長）

研究要旨　本研究計画は（A）髄液等の患者試料と情報の収集、(B) プロテオーム解析、(C) 疾患特異的バイオマーカー同定に向けた研究、(D) 臨床応用と創薬研究、という研究内容の区分がある。平成24年度は、(A) に関しては、病院検査部及び各診療科との連携で、髄液試料確保のシステムを整備し、正常対照者、統合失調症等の精神疾患患者を中心に髄液採取が順調に登録された（平成25年3月31日までに、710検体)。(B) (C) に関しては、統合失調症10例、健常対象者10例のプロテオーム測定を終了し、さらにセカンドコホートとして統合失調症9例、健常者10例のデータを追加検討し、バイオマーカーの候補となりうるタンパク質を23個見いだした。そこで、別の統合失調症患者40例、健常者40例でELISA法を用いたバリデーションを行い、8 個で何らかの統計的有意なものを見つけており、疾患もしくは創薬標的バイオマーカーとなる可能性が高い。今後、疾患特異性の検討、血液での検討などを行い、臨床応用を目指す。(D) を目標としてきたが、結果としてそこまで研究が進展できなかった。また、他の精神疾患やパーキンソン病のバイオマーカーの探索研究は、端緒の段階に止まった。

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A．目的

精神・神経疾患はその病因、病態の複雑さのために、治療薬開発が最も遅れている分野である。ヒトゲノムプロジェクトの成果を受けて、網羅的なゲノム解析手法で疾患関連遺伝子及びその産物が同定されてきているが、それらが病態にどう関わるかについて理解し、さらに創薬に結びつけるには、「タンパク質レベル」の動態の把握が必要なことが周知の事実となっている。平成15年度ー平成19年度に行った精神・神経疾患プロテオーム研究において、血液を用いた解析に比べ、髄液を用いた解析では、数多くの神経特異的タンパク質の同定が可能で、中枢神

経の状態を直接的に反映していることが実証された。その手法を最大限活用し、各種の精神・神経疾患患者から採取した髄液のプロテオーム解析を出発点として疾患特異的に変動するタンパク質を見いだし、診断、病勢、薬効を判定する際に有効なバイオマーカーを同定し、さらにはそのタンパク質及び関連するタンパク質の機能解析を行うことで創薬に結びつけることが本研究の目的である。

B．研究方法

全体計画

1） 髄液等の患者試料と情報の収集

国立精神・神経医療研究センター病院（もしくは共同研究病院）で、IC 取得後に試料と情報を収集、登録する。精神疾患担当（有馬、吉田、功刀）、神経疾患担当（村田、山村）、小児精神・神経疾患担当（中川）で行う。

2）プロテオーム解析

当センターにおいて cICAT 法を用いたプロテオーム解析を行う（林）。

3） 疾患特異的バイオマーカー同定

ア．バイオインフォマティクス（後藤、金子）

当センターでのデータを解析し、候補バイオマーカーを選択する。

イ．バリデーション研究（村田、有馬、沼知、中川、和田、功刀、山村、後藤）

ELISA を用いた簡易測定系を開発し、バイオマーカーとして有用かどうかを判定する。その結果を踏まえて、40 例程度のバリデーション研究を行う。

4）臨床応用と創薬研究

ア．バイオマーカーの臨床応用（村田、吉田、有馬、山村、中川）

多数例を用いて臨床的な有用性の確認を行う。

イ．創薬研究（疾患担当者、小紫、茶木）

有力なバイオマーカーに関連するタンパク質の探求やそれらの生物学的機能の理解を踏まえて、新薬開発に関する研究を行う。

本年度の研究方法

1）髄液等の患者試料と情報の収集

(1) 髄液等の検体採取と受け入れシステム化

髄液採取コーディネーターチームを作り、研究への参加意思の確認から、髄液採取の実施もしくは援助、得られた検体の運搬・処置、匿名化、臨床情報の取得などを行う。

(2) 患者試料の登録

収集した試料をプロテオーム解析まで小分けしてディープフリーザー（-80℃）に凍結保存する。

2）プロテオーム解析

(1) 髄液 2mL からのプロテオーム解析

確定した解析手法で前処理を行い、QSTAR による質量分析を行う。

(2) 統合失調症症例のプロテオーム解析

統合失調症の男性患者 10 名のプロテオーム解析を行い、健常者 10 名での結果を比較検討した（1st コホート）。その際、cICAT 法のスタンダード（L 鎖標識）として、購入髄液を用いた。統合失調症群と健常対照群の年齢を合わせるとともに、すべて男性症例で検討した。通常の統計的比較(Student’s t-test もしくは Mann-Whitney U-test）に加えて、分散が大きいものを（統合失調症の一群で高値・低値を示すもの）捉える分散比および F-test を実施した。

さらに、別の統合失調症群 9 名と健常者 10 名のプロテオーム解析を行い（2nd コホート）、1st コホートで有意差のみられたタンパク質を絞り込んだ。その際、cICAT 法のスタンダード（L 鎖標識）として、正常圧水頭症患者のプール髄液を用いた。

その上で、40 例の別の患者群と 40 例の健常者について、多検体を用いて ELISA 法で検討した。

（倫理面への配慮）

研究者の所属する施設の倫理委員会に本研究に関する倫理申請を行い、承認を得て行った。診療上、髄液を採取する必要のある疾患患者に研究参加を依頼することを基本に研究計画を作成した。すでに、認知症及び神経疾患全般に関しては、余剰髄液を用いて行う研究が動いていたので、それに加えるプロ

トコールで研究計画を構築した。さらに平成21年度後半には、ボランティアにて髄液採取に協力していただける疾患患者、健常者からの髄液を研究利用するプロトコールも倫理委員会の承認を得た。

C．研究結果と考案

1）髄液等の患者試料と情報の収集

(1) 髄液等の検体採取及び受け入れのシステム化

患者への説明、同意取得、検体採取等の一連の流れと必要書類、必要物品をパッケージとして病棟に配布し、それらを用いた「検体採取のプロトコール」を作成し使用している。今年度は、2名の臨床検査技師、3名の臨床心理士を含む髄液コーディネーターチームを組織し、検体採取の流れを促進させた（図1）。

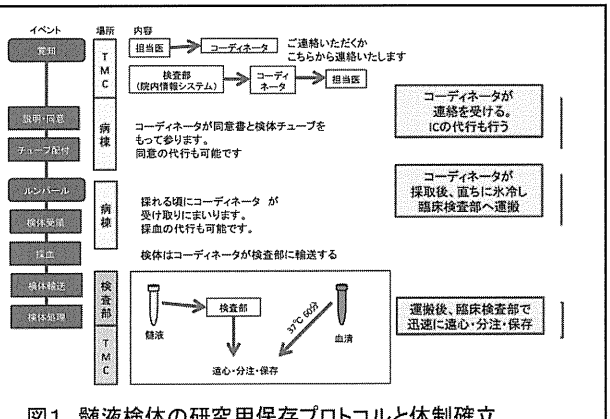

図1 髄液検体の研究用保存プロトコルと体制確立

また、臨床情報及び検体情報を登録するデータベースシステムを構築した（図2A、図2B）。

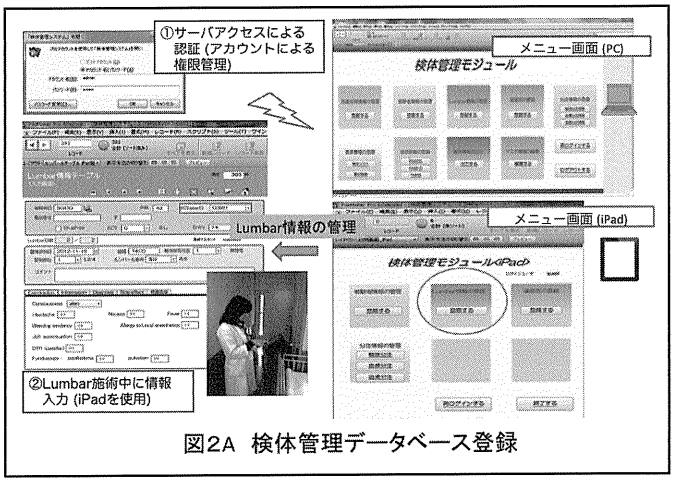

図2A 検体管理データベース登録

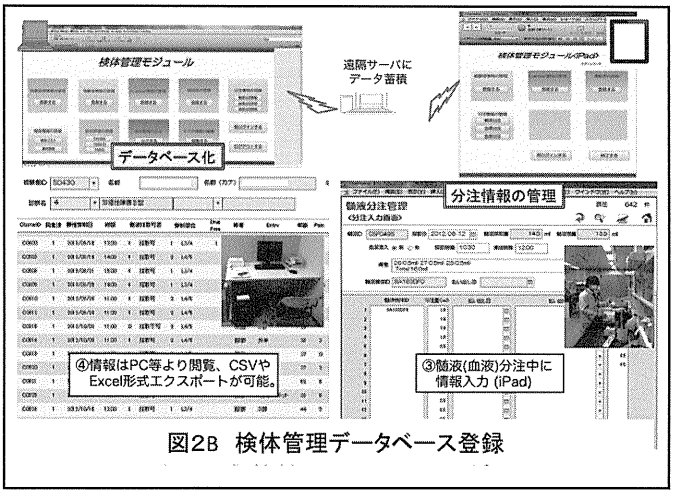

図2B 検体管理データベース登録

NCNP内サーバーに検体管理モジュールを作成し、アクセスはアカウント制限の認証を用いてセキュリティー管理を行った。また、デスクトップPCとiPadからの入力管理を行えるシステムを構築した。

具体的には、コーディネーターチームが病棟に赴いて、説明と同意を行う際や実際に腰椎穿刺に立ち会う際に病棟でiPadを用いて情報を入力できるようにした（図3）。

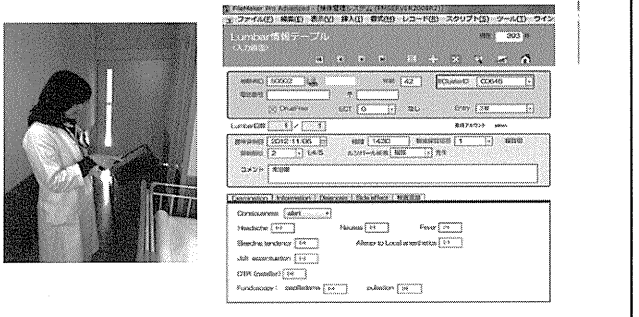

図3 携帯端末(iPad)による情報入力

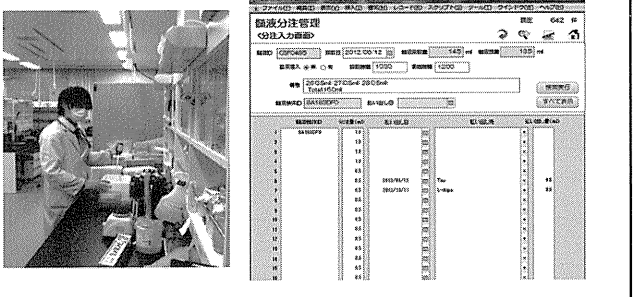

図4 検体管理

また、髄液検体をラボに搬入し、処理を行った上で、分注してフリーザーに保存する際に検体情報を iPad を用いて入力できるようにした（図４）。

入力された情報は PC 等で閲覧が可能で、CSV ファイルやエクセルファイルで出力できる（図５）。

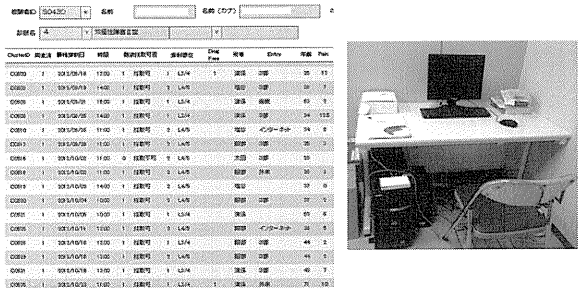

図5　データベース化

(2) 患者試料の登録

平成 25 年 3 月末までに 576 例(710 検体)の髄液採取ができた。精神疾患患者からの検体数が飛躍的に伸び、小児神経科、神経内科、脳神経外科からの登録も得た。2011 年 2 月末では総数が 150 検体、2012 年 3 月で 270 検体、2013 年 3 月で 710 検体の登録を行う事ができた。特に統合失調症の検体は、3 年間で 150 検体以上に達した。その内訳を次表に示す。

分類	内訳	検体数	症例数	測定数
精神疾患（159症例）	統合失調症	159	83	30
	大うつ病	93	72	17
	双極性障害	46	36	5
	健常対照	92	72	25
神経内科疾患（69症例）	パーキンソン病	76	76	5
	脊髄小脳変性症	7	7	2
	正常圧水頭症	31	24	5
	認知症	54	54	2
	その他	132	132	5
小児神経疾患（20症例）	てんかん	11	11	0
	精神遅滞	5	5	0
	その他	4	4	0
Total		710	576	96

表1　髄液検体の内訳　（2013年3月末現在）

２）プロテオーム解析

(1)　髄液 2mL からのプロテオーム解析

平成 23 年度までの研究で確定した初期量 2mL からのプロテオーム解析のプロトコールを実施した。

その後、QSTAR-XL を用いて質量分析を行い、データをプロテオームファクトリーで開発した質量分析データ処理ソフト（Mascot ベース）を用いて解析した。しかし、自動的に 2 つのラベル化ペプチド（H と L）の量比（H/L）が出ない場合が多く、その場合はペプチド量の生データから値を引き出してくる必要があった。この作業は研究補助者 4 人を雇い、マニュアルで行うため相当の時間がかかった。

(2)　統合失調症症例のプロテオーム解析

統合失調症の男性患者 10 名のプロテオーム解析を行い、健常者での結果を比較検討した（1st コホート）。健常対照者の場合と同様に、一回の質量分析で、270ー300 前後のタンパク質を同定できた。

さらに、男女を含む、別な統合失調症患者 9 名、健常者 10 名の結果を比較検討した(2nd コホート)。

1st コホート

	N	Sex (M/F)	Age
Schizo	10	10/0	43.0±12.2
Control	10	10/0	41.4±13.3

未治療1例を含む

2nd コホート

	N	Sex (M/F)	Age
Schizo	9	5/4	40.8±10.6
Control	10	6/4	39.7±12.2

未治療1例、Drug free 1例を含む

表2　検体の内訳

個々の質量分析で同定できているタンパク質の中で、タンパク解析ソフトで自動的に同定されるタンパク質が複数例で同定できているタンパク質は少なく、1st コホートでは、結果としてのべ 682 個のタンパク質が同定できた。

そこで、統合失調症群で上昇している、もしくは低下しているタンパク質は、$P<0.05$ のものが 37 個、$P<0.01$ のものが 19 個存在した。その中から代表的なタンパク質の例を以下に示す。

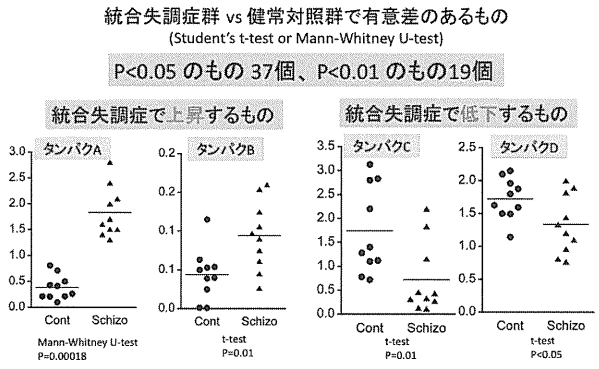

図6 統合失調症に共通のバイオマーカーの探索

また、統合失調症にはいくつかのサブグループが存在すると想定されており、それらの解析を分散比とF-testで行い、他の検体と飛び抜けて値の違うタンパク質を探索することで、サブグループ特異的なマーカーを探し出す試みをした。その中で以下の図に示すようなタンパク質が同定できた。

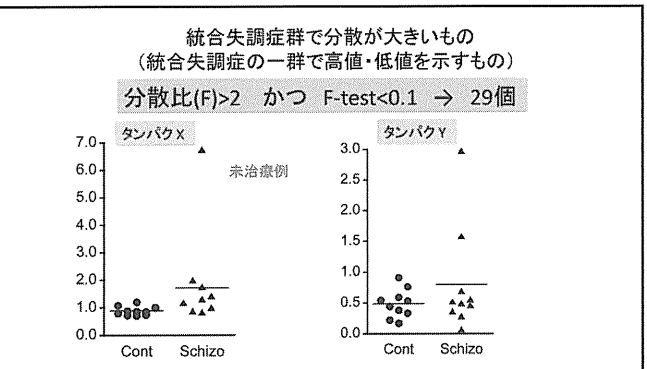

図7 統合失調症のサブタイプの探索

次いで、2nd コホートの解析結果を合わせ、以下の基準で候補となるタンパク質を選び出した。

1) ランクA：1st コホートと 2nd コホートが明らかに同じ傾向を示すもの → 7種
ランクA-：症状評価から残したもの
→ 6種
2) ランクB：1st コホートと 2nd コホートがある程度同じ傾向を示すもの → 4種
3) ランクC：上記以外のもの。ランクAやランクBで同定されたタンパク質から、病態に関わる可能性のあるパスウェイが見出された場合に関連のある分子など。 → 6種

これら24種のバイオマーカー候補に関して、さらに、患者群 40 例、健常者群 40 例を用いて、ELISA 法を用いた多検体での検証を行った。

その際、タンパク量の結果を用いた、T-test, F-test に加え、PANSS の陰性症状・陽性症状及び総合症状の評価、使用薬剤の等価計算、BACS の評価項目を用いての解析も追加した。

その結果、表4のような結果を得た。すなわち、T-テストで有意な結果を得たものが5種、BACS の症状との関連性を示したものが 4 種（1種は両方で相関あり）であった。

タンパク番号	評価	1st	2nd	多検体解析
1	A	T-test	F-test	T-test
2	A	T-test	T-test	有意差なし
3	A	T-test	T-test	BACS
4	A	T-test	T-test	T-test
5	A	F-test	T-test	T-test
6	A	T-test	T-test	抗体なし
7	A	T-test	T-test	有意差なし
8	A	T-test	T-test	有意差なし
9	A-	総合	総合	抗体なし
10	A-	陰性	陰性	有意差なし
11	A-	陰性	陰性	BACS
12	A-	総合	総合	T-test, BACS
13	A-	陽性	陽性	有意差なし
14	A-	陰性	陰性	抗体なし
15	B	陽性	陽性	抗体なし
16	B	F-test	F-test	抗体なし
17	B	T, F-tests	F-test	有意差なし
18	B	T-test	F-test	抗体なし
19	C	病態	病態	T-test
20	C	病態	病態	抗体なし
21	C	病態	病態	有意差なし
22	C	病態	病態	BACS
23	C	病態	病態	抗体なし

表4 多検体解析の結果

これら 9 種のバイオマーカー候補に対して、1）疾患特異性の評価、2）薬剤との関連性から、drug-free 例での解析、3）血液や尿などのアクセスしやすい検体での評価、など臨床応用に向けてのさらなる検討が必要であり、現在進行中である。

E．結論

平成24年度においては、(A) 髄液等の患者試料と

情報の収集、(B) プロテオーム解析、(C) 疾患特異的バイオマーカー同定に向けた研究、(D) 臨床応用と創薬研究、という研究内容の区分のうち、(A) 髄液等の患者試料と情報の収集が格段に進んだが、(B) プロテオーム解析の結果から、(C) 疾患特異的バイオマーカー同定の研究に踏み込むことができたが、バイオマーカー候補が 9 種に絞られたところまでの研究に終わり、D) の臨床応用にまで到達できなかった。また、当初の全体計画では多種類の疾患を対象としてあげていたが、大幅に進捗が遅れたことは否めない。

一方で、精神疾患を中心に、多数の高品質の髄液を収集できたことは、今後の研究に積極的に利用することで、新たなバイオマーカーを同定できると確認している。NCNP に登録させた検体を広く共同研究を行って利用促進を図る予定である。

F．健康危険情報

特になし

G．研究発表

1．論文発表

1) Sasayama D, Hattori K, Teraishi T, Hori H, Ota M, Yoshida S, Arima K, Higuchi T, Amano N, Kunugi H. Negative correlation between cerebrospinal fluid oxytocin levels and negative symptoms of male patients with schizophrenia. Schizophrenia Research 139: 201-206, 2012.

2) Hattori K, Tanaka H, Yamamoto N, Teraishi T, Hori H, Kinoshita Y, Matsuo J, Kawamoto Y, Kunugi H. Blood CADPS2 Delta Exon3 expression is associated with intelligence and memory in healthy adults. Biological Psychology 89: 117-122, 2012.

3) Fujii T, Ota M, Hori H, Sasayama D, Hattori K, Teraishi T, Yamamoto N, Hashikura M, Tatsumi M, Higuchi T, Kunugi H. Association between the functional polymorphism (C3435T) of the gene encoding P-glycoprotein (ABCB1) and major depressive disorder in the Japanese population. Journal of Psychiatric Research 46: 555-559, 2012

4) Sasayama D, Hori H, Teraishi T, Hattori K, Ota M, Matsuo J, Kawamoto Y, Kinoshita Y, Hashikura M, Amano N, Higuchi T, Kunugi H. More severe impairment of manual dexterity in bipolar disorder compared to unipolar major depression. Journal of Affective Disorders 136:1047-1052, 2012.

2．学会発表　なし

H．知的財産権の出願・登録状況

「統合失調症の判定方法」(出願準備中)

Ⅱ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Sasayama D, Hattori K, Teraishi T, Hori H, Ota M, Yoshida S, Arima K, Higuchi T, Amano N, Kunugi H	Negative correlation between cerebrospinal fluid oxytocin levels and negative symptoms of male patients with schizophrenia.	Schizophrenia Research	139	201-206	2012
Hattori K, Tanaka H, Yamamoto N, Teraishi T, Hori H, Kinoshita Y, Matsuo J, Kawamoto Y, Kunugi H	Blood CADPS2 Delta Exon3 expression is associated with intelligence and memory in healthy adults.	Biological Psychology	89	117-122	2012
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Sasayama D, Hori H, Teraishi T, Hattori K, Ota M, Matsuo J, Kawamoto Y, Kinoshita Y, Hashikura M, Amano N, Higuchi T, Kunugi H	More severe impairment of manual dexterity in bipolar disorder compared to unipolar major depression	Journal of Affective Disorders	136	1047-1052	2012

Ⅲ. 主な刊行物・別刷

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Negative correlation between cerebrospinal fluid oxytocin levels and negative symptoms of male patients with schizophrenia

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ABSTRACT

Background: Accumulating evidence indicates that oxytocin plays an important role in social interactions. Previous studies also suggest altered oxytocin function in patients with schizophrenia and depression. However, few studies have examined the central oxytocin levels in these disorders.
Methods: Cerebrospinal fluid (CSF) oxytocin levels were measured by ELISA in male participants consisting of 27 patients with schizophrenia, 17 with major depressive disorder (MDD), and 21 healthy controls.
Results: CSF oxytocin levels of patients with schizophrenia or MDD did not differ significantly with healthy controls. The antidepressant dose or the Hamilton depression rating scale score did not significantly correlate with the oxytocin levels in MDD patients. CSF oxytocin levels in schizophrenic patients significantly negatively correlated with second generation antipsychotic dose ($r=-0.49$, $P=0.010$) but not with first generation antipsychotic dose ($r=-0.13$, $P=0.50$). A significant correlation was observed between oxytocin levels and negative subscale of PANSS ($r=-0.38$, $P=0.050$). This correlation remained significant even after controlling for second generation antipsychotic dose ($r=-0.47$, $P=0.016$).
Conclusions: We obtained no evidence of altered CSF oxytocin levels in patients with schizophrenia or those with MDD. However, lower oxytocin levels may be related to higher second generation antipsychotic dose and more severe negative symptoms in schizophrenia.

1. Introduction

Oxytocin is produced in the supraoptic and paraventricular nuclei of hypothalamus and is secreted into the blood stream from the posterior pituitary. Its release is induced by a variety of stressful stimuli, including noxious stimuli, conditioned fear, and exposure to novel environments (Onaka, 2004). Accumulating evidence indicates that oxytocin plays an important role in social interactions (Lim and Young, 2006; Bartz et al., 2010). Deficits in social functioning observed in psychiatric disorders including schizophrenia (Couture et al., 2006; Sparks et al., 2010) and mood disorders (Inoue et al., 2004; Montag et al., 2010; Wolkenstein et al., 2011) imply the possible involvement of oxytocin in the pathophysiology of these disorders.

Many studies have investigated the possible link between oxytocin and psychiatric disorders. Some previous studies reported altered oxytocin function in patients with schizophrenia (Linkowski et al., 1984; Beckmann et al., 1985; Mai et al., 1993). Higher plasma oxytocin levels in schizophrenic patients were associated with lower symptom severity (Rubin et al., 2010). A clinical study showed that administration of this hormone ameliorated symptoms of schizophrenia (Feifel et al., 2010). In a preclinical study, systemically administered oxytocin reversed prepulse inhibition deficits induced by amphetamine and the phencyclidine analog in rats (Feifel and Reza, 1999). Oxytocin dysfunction has been implicated in the pathophysiology of depression as well. Two studies have shown that peripheral oxytocin levels and depressive symptoms were significantly correlated in patients with major depressive disorder (MDD) (Scantamburlo et al., 2007; Cyranowski et al., 2008). Moreover, oxytocin knock-out mice have shown dysregulated stress responses to psychological stimuli (Mantella et al., 2005) and enhanced anxiety behaviors (Mantella et al., 2003).

Oxytocin secreted from the pituitary gland generally does not re-enter the brain through the blood-brain barrier (Ermisch et al., 1985). Therefore, the behavioral effects of oxytocin are likely to be due to the release from centrally projecting oxytocin neurons. Since

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oxytocin in the nervous system can be transported to blood (Durham et al., 1991), peripheral oxytocin levels may reflect brain levels to some extent. However, central and peripheral oxytocin is regulated independently, and the half-life of oxytocin is less than 5 minutes in the blood (Ryden and Sjoholm, 1969) while that in the brain is 19.1 - minutes (Durham et al., 1991). Therefore, measurement in the CSF is necessary for the direct assessment of central oxytocin levels.

To our knowledge, two studies have previously examined the cerebrospinal fluid (CSF) levels of oxytocin in patients with schizophrenia. One reported elevated oxytocin levels in schizophrenia compared with controls (Beckmann et al., 1985), while the other did not obtain such a finding (Glovinsky et al., 1994). Only one study has examined the CSF levels of oxytocin in patients with depression, in which no difference was found compared with controls (Pitts et al., 1995). No study to date has examined the association of CSF oxytocin levels with symptom severity of these disorders. Since symptom severity forms a continuous spectrum ranging from mild to severe state, an association with the severity of the disease would suggest that oxytocin levels reflect the state of the disease.

In the present study, the oxytocin levels in the CSF of patients with schizophrenia and those with depression were measured and compared to that of healthy controls. Furthermore, we investigated the correlation between CSF oxytocin levels and symptom severity of these disorders. From the findings of previous studies examining peripheral oxytocin levels (Scantamburlo et al., 2007; Rubin et al., 2010), we hypothesized that CSF oxytocin levels would be lower in patient groups compared to healthy controls and that symptom severity would be negatively correlated with the oxytocin levels.

2. Materials and methods

2.1. Subjects

Participants were 27 patients with schizophrenia (mean age (standard deviation): 42.6 (8.5) years), 17 patients with major depressive disorder (MDD) (age: 39.5 (8.0) years), and 21 healthy controls (age: 38.3 (15.3) years). Demographic and clinical characteristics of the subjects are summarized in Table 1. All subjects were males to avoid gender effects and were biologically unrelated Japanese recruited from the outpatient clinic of the National Center of Neurology and Psychiatry Hospital, Tokyo, Japan or through advertisements in free local information magazines and by our website announcement. None of the healthy controls were on psychotropic medication, while 70.6% of the patients with MDD were treated with antidepressant medication at the time of the study. Most of the schizophrenic patients were prescribed antipsychotic medication, and all of those prescribed antipsychotics were on the medication for more than 3 years. Consensus diagnosis by at least 2 psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition criteria (American Psychiatric Association, 1994), on the basis of unstructured interviews and information from medical records. The controls were healthy volunteers with no current or past history of psychiatric treatment and were screened using the Japanese version of the Mini International Neuropsychiatric Interview (Sheehan et al., 1998; Otsubo et al., 2005) by a research psychiatrist to eliminate the possibility of any axis I psychiatric disorders. Participants were excluded if they had prior medical histories of central nervous system diseases or severe head injury or if they met the criteria for substance abuse or dependence or mental retardation. The study protocol was approved by the ethics committee at the National Center of Neurology and Psychiatry, Japan. After describing the study, written informed consent was obtained from every subject.

2.2. Clinical measures

Schizophrenic symptoms and depressive symptoms were assessed immediately after the lumbar puncture by an experienced research psychiatrist using the Japanese version of the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987; Yamada et al., 1991) and the Japanese version of the GRID Hamilton Depression Rating Scale, 17-item version (HAMD-17) (Hamilton, 1967), which have both been demonstrated to show good inter-rater reliability (Igarashi et al., 1998; Tabuse et al., 2007). Medication status at the time of lumbar puncture was recorded. Daily doses of antipsychotics in patients with schizophrenia and antidepressants in patients with MDD were

Table 1
Demographic and clinical characteristics.

	Controls ($N=21$)	Schizophrenia ($N=27$)	Depression ($N=17$)	Analysis
Age (years)	38.3 (15.3)	42.6 (8.5)	39.5 (8.0)	ANOVA: $F=0.97$, n.s.
BMI	23.9 (4.1)	26.0 (6.2)	23.9 (4.5)	ANOVA: $F=1.06$, n.s.
Duration of illness (years)		16.3 (9.8)	7.7 (7.3)	t-test: $t=2.8$, $P<0.01$
Treatment duration (years)		15.5 (9.1)	5.8 (6.9)	t-test: $t=3.4$, $P<0.01$
Medication status				
on antipsychotic medication				
first generation (%)	0	59.3	11.8	
second generation (%)	0	66.7	23.5	
first and/or second generation (%)	0	96.3	35.3	
on antidepressant medication (%)	0	25.9	70.6	
on benzodiazepine medication (%)	0	81.5	76.5	
on mood stabilizer medication (%)	0	14.8	5.9	
CP equivalent dose				
first generation (mg/day)		361.8 (445.0)		
second generation (mg/day)		402.4 (498.3)		
total (mg/day)		764.2 (591.6)		
IMI equivalent dose (mg/day)			167.2 (141.5)	
PANSS				
Positive symptoms score		12.5 (3.8)		
Negative symptom score		16.0 (5.8)		
General symptom score		6.8 (1.3)		
Total score		55.6 (12.6)		
HAMD-17 score			13.4 (9.6)	

Values are shown as mean (standard deviation).
BMI: body mass index; CP: chlorpromazine; IMI: imipramine.
PANSS: Positive and Negative Syndrome Scale; HAMD-17: 17 item Hamilton Rating Scale for Depression.
ANOVA: analysis of variance; n.s.: not significant.

converted to chlorpromazine and imipramine equivalent doses, respectively, using published guidelines (Inagaki et al., 1999).

2.3. Lumbar puncture and oxytocin assay

Lumbar puncture was performed with the subject in the left decubitus position. CSF was withdrawn from the L3–L4 or L4–L5 interspace. After the removal of 2 ml of CSF, a further 6 ml of CSF was collected and immediately transferred on ice to be centrifuged at 4 °C and aliquoted for storage at −80 °C until assay. CSF oxytocin levels were analyzed using a commercial ELISA kit (Enzo Life Sciences, INC., NY). Using the results from two separate runs of standard concentrations, the inter-assay coefficient of variation (CV) was less than 10%.

2.4. Statistical analysis

Statistical differences between groups were calculated using Student's *t*-test, Welch's *t*-test, or one-way analysis of variance (ANOVA). Correlations were assessed using Pearson's correlation coefficient. Since the CSF oxytocin levels were not normally distributed, log transformation was applied prior to statistical analyses to achieve normal distribution. Because previous studies suggest that some antipsychotic and antidepressant medications increase oxytocin secretion (Uvnas-Moberg et al., 1992, 1999), chlorpromazine and imipramine equivalent doses were examined as possible confounders. Statistical analyses were performed using the Statistical Package for the Social Sciences version 11.0 (SPSS Japan, Tokyo, Japan). All statistical tests were two-tailed, and $P<0.05$ indicated statistical significance.

3. Results

Fig. 1 shows the CSF oxytocin levels in each diagnostic group. A one-way ANOVA using the transformed oxytocin levels as the dependent variable indicated no significant difference between diagnostic groups ($F=1.08$, $P=0.35$). The transformed oxytocin levels showed no significant correlation with age or body weight. Figs. 2 and 3 show the relation of CSF oxytocin levels with symptom severity and psychotropic dose, respectively. The antidepressant dose or the HAMD-17 score did not significantly correlate with the transformed oxytocin levels in patients with MDD (antidepressant dose: $r=-0.15$, $P=0.57$; HAMD-17: $r=-0.19$, $P=0.46$). The transformed oxytocin levels were significantly negatively correlated with negative subscale of PANSS ($r=-0.38$, $P=0.050$). Correlations between transformed oxytocin levels and other subscales of PANSS were not statistically significant. The transformed oxytocin levels in schizophrenic patients were significantly negatively correlated with chlorpromazine equivalents of total antipsychotic dose ($r=-0.51$, $P=0.0064$) and second generation antipsychotic (SGA) dose ($r=-0.49$, $P=0.010$) but not with chlorpromazine equivalents of first generation antipsychotic (FGA) dose ($r=-0.13$, $P=0.50$). Those prescribed SGA had significantly lower CSF oxytocin levels compared to those not prescribed SGA (Welch's *t* test: $t=2.6$, $df=10.4$, $P=0.024$). Comparison between patients prescribed and not prescribed FGA did not yield significant difference (Student's t test: $t=1.1$, $df=25$, $P=0.27$). Although none of the subscales of PANSS were correlated with FGA, SGA, or total chlorpromazine equivalent dose in the present study (all $P>0.1$), a previous study (Sim et al., 2009) reported an association between antipsychotic dose and the severity of positive as well as negative symptoms of schizophrenia. Therefore, we considered antipsychotic dose as a confounding factor for the association between oxytocin levels and symptom severity. Thus, we also examined the correlation between the oxytocin levels and PANSS scores controlling for prescribed antipsychotic dose. Partial correlation between transformed oxytocin levels and negative subscale of PANSS, removing the linear effects of total antipsychotic dose, was statistically significant ($r=-0.39$, $P=0.047$). Removing the linear effects of SGA dose instead of total antipsychotic dose also resulted in significant correlation of transformed CSF oxytocin levels with negative subscale ($r=-0.47$, $P=0.016$) as well as with total PANSS score ($r=-0.47$, $P=0.016$). SGA dose-controlled partial correlations between transformed oxytocin levels and other subscales of PANSS were not statistically significant (positive subscale: $r=-0.24$, $P=0.23$; general subscale: $r=-0.33$, $P=0.099$).

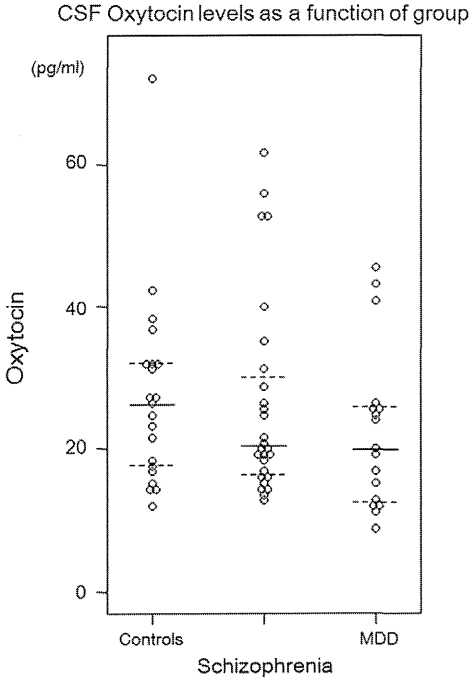

Fig. 1. Cerebrospinal fluid oxytocin levels as a function of group. The cerebrospinal fluid oxytocin levels in healthy controls and patients with schizophrenia and major depressive disorder are shown. Solid bars indicate median values and the dotted lines indicate interquartile range. No significant difference was observed between the diagnostic groups.

4. Discussion

Consistent with some previous studies (Glovinsky et al., 1994; Pitts et al., 1995), CSF oxytocin levels did not significantly differ between healthy controls and patients with schizophrenia and MDD. However, the present results showed that higher levels of CSF oxytocin may be associated with less severe symptoms of schizophrenia.

The observed negative correlation between antipsychotic dose and CSF oxytocin levels points to the possibility that antipsychotic medication lowers oxytocin levels. A recent study suggests that an inhibitory feedback loop may exist between prolactin-secreting lactrophs and oxytocinergic paraventricular neurons (Sirzen-Zelenskaya et al., 2011). Therefore, the disinhibition of prolactin secretion due to the D_2 receptor blockade by antipsychotics may have resulted in the suppression of oxytocin secretion. This, however, does not explain the stronger correlation of SGA dose compared to FGA dose. Kiss et al (2010) showed that SGAs have a more potent influence than haloperidol on the activity of oxytocin magnocellular neurons. This also seems contradictory to the present finding that SGA is negatively correlated with oxytocin levels. An alternative explanation for this negative correlation is that patients with low oxytocin levels may respond poorly to antipsychotic medication, and thus, higher dose was prescribed to such patients. Nevertheless, despite the relatively strong correlation with the antipsychotic dose, the cross-sectional design of the present study hinders any causal inferences. One previous study (Glovinsky et al., 1994) demonstrated that CSF oxytocin levels were unchanged by antipsychotic medication. Thus, further investigation is necessary to elucidate the effects of antipsychotic medication on oxytocin levels.

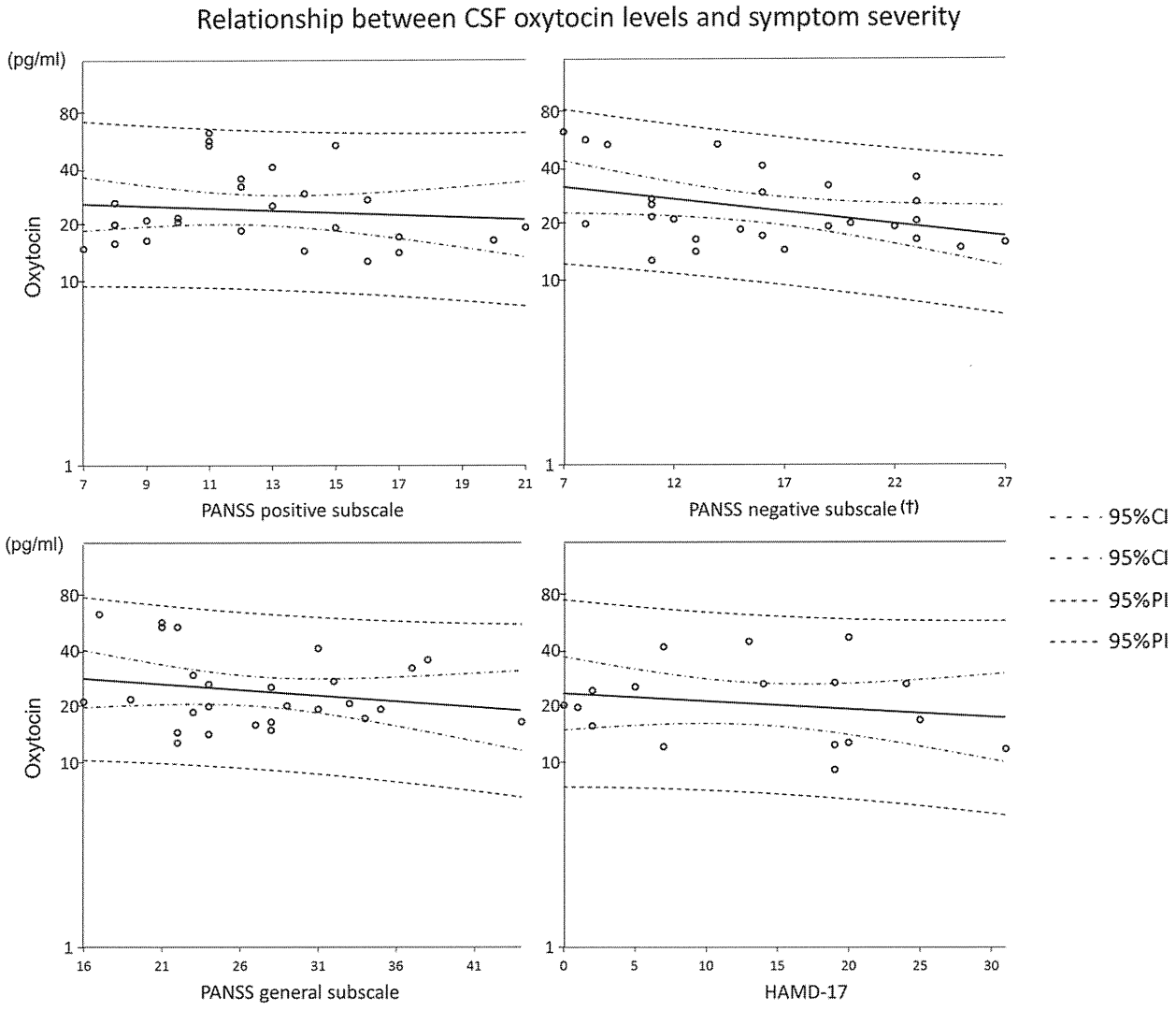

Fig. 2. Relationship between cerebrospinal fluid oxytocin levels and symptom severity. The association between cerebrospinal oxytocin levels and symptom severity is shown. Oxytocin levels are shown in logarithmic scale. Solid lines indicate fitted regression lines, unevenly dashed lines indicate 95% confidence intervals, and evenly dashed lines indicate 95% prediction intervals. (†): Correlation at significance level of $P<0.05$. PANSS: Positive and Negative Syndrome Scale, HAMD-17: Hamilton Depression Rating Scale, 17-item version, 95%CI: 95% confidence interval, 95%PI: 95% prediction interval.

The present results showed that the negative symptoms of schizophrenia were negatively correlated with CSF oxytocin levels. The correlation coefficient between CSF oxytocin levels and total PANSS score was also significant, controlling for SGA dose. Rubin et al. (2010) reported that higher peripheral oxytocin levels were associated with more prosocial behaviors in female patients with schizophrenia. Furthermore, previous studies have demonstrated improvement of social behaviors with administration of intranasal oxytocin (Macdonald and Macdonald, 2010; Pedersen et al., 2011). Since strong relationships between negative symptoms and social difficulties have been demonstrated in schizophrenia (Weinberg et al., 2009), the present finding associating higher CSF oxytocin levels with lower negative subscale is in accord with what has previously been described for peripheral oxytocin. Whether the peripheral oxytocin levels reflect the CSF oxytocin levels, or whether a different mechanisms of action in the brain and the peripheral result in a similar effect, remains to be explored.

Previous studies examining CSF oxytocin levels in patients with schizophrenia (Beckmann et al., 1985; Glovinsky et al., 1994) and depression (Pitts et al., 1995) showed mean oxytocin levels of less than 10 pg/ml, which is lower than that in the present study (>20 pg/ml). Such outcome may have resulted from some of the methodological differences between previous studies and the present one. Previous three studies measured oxytocin levels using radioimmunoassay (RIA), while the present study used a commercially available ELISA kit. A recent study that used the same ELISA kit to measure CSF oxytocin levels (Heim et al., 2009) also demonstrated higher levels of oxytocin (mean oxytocin levels of 17 pg/ml in women without a history of emotional abuse) compared to the previous studies using RIA. Thus, the different measurement techniques may have influenced the values.

A number of other methodological differences exist between the present study and previous ones examining CSF oxytocin levels (Beckmann et al., 1985; Glovinsky et al., 1994; Pitts et al., 1995). One of the major differences was that the present study did not require fasting prior to lumbar puncture, while Beckmann et al (Beckmann et al., 1985) collected CSF in patients with schizophrenia after 12 hours fasting. Although a previous study (Challinor et al., 1994) reported that peripheral oxytocin levels were not affected by 20 hours of fasting, the influence of fasting on CSF levels is unknown. Furthermore, Beckmann et al used Research Diagnostic Criteria to select a patient group consisting entirely of paranoid schizophrenia. Such difference in composition of participants may have affected the outcome of the study by Beckmann et al (1985), which showed significantly higher CSF oxytocin levels in schizophrenic patients compared to healthy controls. The findings by Glovinsky et al (1994) and Pitts et al (1995) were consistent with the present study in that no significant difference in CSF oxytocin levels was found between patients and controls. However,

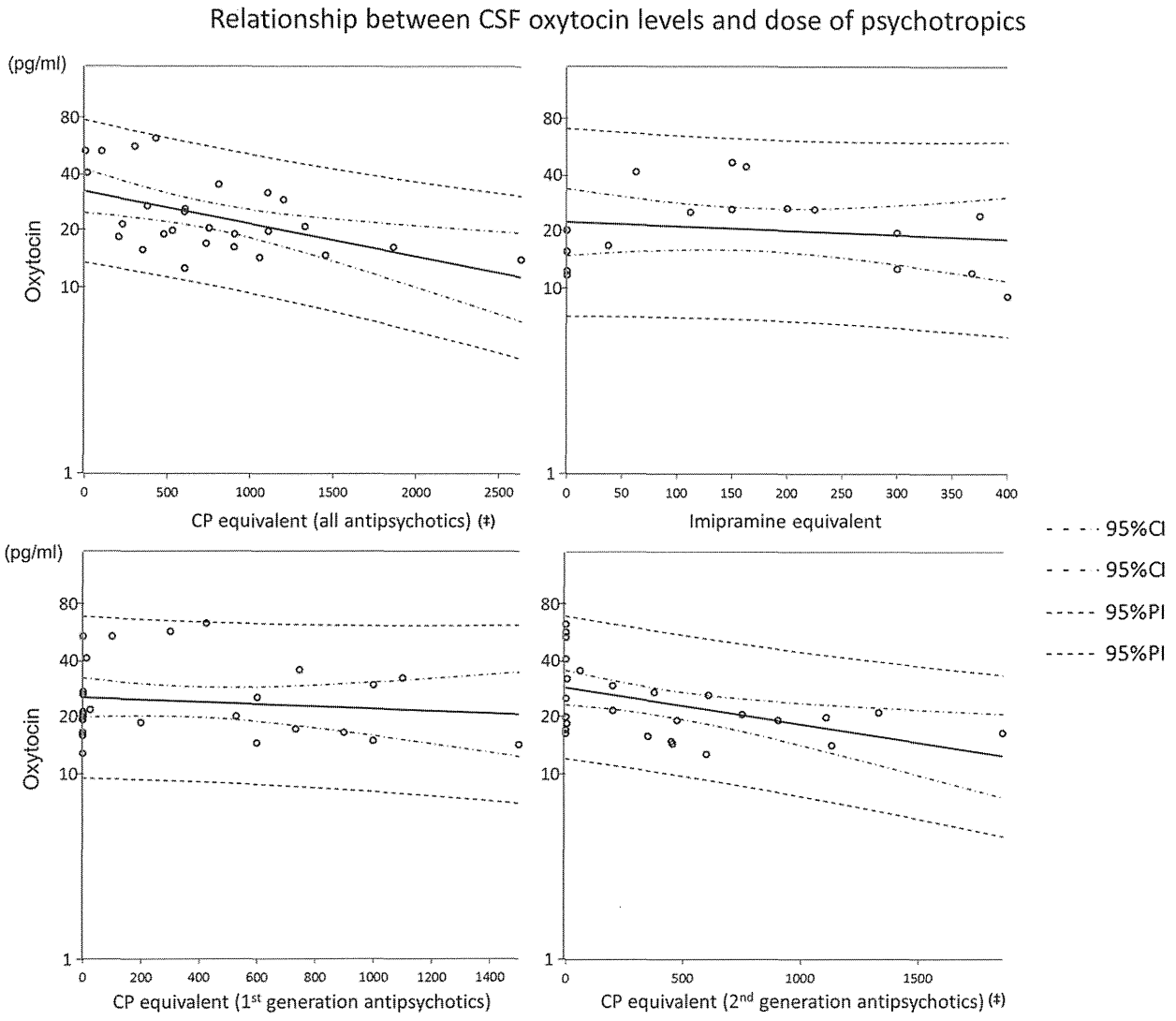

Fig. 3. Relationship between cerebrospinal fluid oxytocin levels and dose of psychotropics. The association between cerebrospinal oxytocin levels and dose of psychotropics is shown. Oxytocin levels are shown in logarithmic scale. Solid lines indicate fitted regression lines, unevenly dashed lines indicate 95% confidence intervals, and evenly dashed lines indicate 95% prediction intervals. (‡): Correlation at significance level of $P<0.01$. CP equivalent: chlorpromazine equivalent, 95%CI: 95% confidence interval, 95%PI: 95% prediction interval.

participants in these studies also differed from that of the present study in that both genders were included. Furthermore, MDD patients in the study by Pitts et al (1995) all scored 18 or above on the HAMD-17, while the MDD patients in the present study included those in a remitted state. These differences in composition of study samples should be carefully considered when comparing findings across studies.

Some limitations must be considered when interpreting the results of this study. First, the effects of medication could not be fully controlled due to the variability in types and doses. Future studies should examine oxytocin levels in untreated patients to elucidate the role of oxytocin in the pathophysiology of schizophrenia and depression. Treatment duration may also affect oxytocin levels. However, since all of the schizophrenic patients that were prescribed antipsychotics were on chronic treatment with the medication, treatment duration is unlikely to have confounded the main findings of the present study. Secondly, as mentioned above, the cross-sectional design did not allow for any definitive conclusions regarding the causal relationship between the CSF oxytocin levels, psychotropic medication, and symptom severity. Thirdly, only male participants were included in the present study. Previous studies suggest that effects of peripheral and intranasal oxytocin may differ between men and women (Domes et al., 2010; Rubin et al., 2010, 2011). Therefore, the present findings cannot be generalized to women. Finally, the risk of type II error was high due to the small sample size. The sample size in the present study was comparable to those of the previous studies that examined CSF oxytocin levels in patients with schizophrenia and depression (Beckmann et al., 1985; Glovinsky et al., 1994; Pitts et al., 1995). However, the power to detect a moderate difference (effect size of 0.50) in CSF oxytocin levels between patients and controls was relatively low (schizophrenia: 39%; MDD: 32%; calculated by G*Power 3.1.3 (Faul et al., 2007)). A larger sample may be necessary to detect small to moderate change in CSF oxytocin levels in psychiatric disorders.

In conclusion, we obtained no evidence of altered CSF oxytocin levels in patients with schizophrenia or those with MDD. However, lower CSF oxytocin levels may be related to higher SGA dose and more severe negative symptoms in schizophrenia, which is in line with the possibility that central oxytocin may ameliorate the severity of some symptoms of schizophrenia by improving social functioning.

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no role in the study design; the collection, analysis and interpretation of data, in the writing of the report; and in the decision to submit the paper for publication.

Contributors

Daimei Sasayama and Kotaro Hattori designed the study. Daimei Sasayama, Kotaro Hattori, and Toshiya Teraishi performed the lumbar punctures. Daimei Sasayama, Kotaro Hattori, Toshiya Teraishi, Hiroaki Hori, Miho Ota, Sumiko Yoshida, Kunimasa Arima, and Hiroshi Kunugi screened and diagnosed the study participants. Daimei Sasayama wrote the draft of the manuscript. Hiroshi Kunugi supervised the writing of the paper. Teruhiko Higuchi and Naoji Amano gave critical comments on the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest statement

The authors declare no conflicts of interest.

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References

American Psychiatric Association, 1994. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th edition. American Psychiatric Press, Washington D.C.

Bartz, J.A., Zaki, J., Ochsner, K.N., Bolger, N., Kolevzon, A., Ludwig, N., Lydon, J.E., 2010. Effects of oxytocin on recollections of maternal care and closeness. Proc. Natl. Acad. Sci. U. S. A. 107 (50), 21371–21375.

Beckmann, H., Lang, R.E., Gattaz, W.F., 1985. Vasopressin–oxytocin in cerebrospinal fluid of schizophrenic patients and normal controls. Psychoneuroendocrinology 10 (2), 187–191.

Challinor, S.M., Winters, S.J., Amico, J.A., 1994. Pattern of oxytocin concentrations in the peripheral blood of healthy women and men: effect of the menstrual cycle and short-term fasting. Endocr. Res. 20 (2), 117–125.

Couture, S.M., Penn, D.L., Roberts, D.L., 2006. The functional significance of social cognition in schizophrenia: a review. Schizophr. Bull. 32 (Suppl. 1), S44–S63.

Cyranowski, J.M., Hofkens, T.L., Frank, E., Seltman, H., Cai, H.M., Amico, J.A., 2008. Evidence of dysregulated peripheral oxytocin release among depressed women. Psychosom. Med. 70 (9), 967–975.

Domes, G., Lischke, A., Berger, C., Grossmann, A., Hauenstein, K., Heinrichs, M., Herpertz, S.C., 2010. Effects of intranasal oxytocin on emotional face processing in women. Psychoneuroendocrinology 35 (1), 83–93.

Durham, D.A., Banks, W.A., Kastin, A.J., 1991. Carrier-mediated transport of labeled oxytocin from brain to blood. Neuroendocrinology 53 (5), 447–452.

Ermisch, A., Barth, T., Ruhle, H.J., Skopkova, J., Hrbas, P., Landgraf, R., 1985. On the blood–brain barrier to peptides: accumulation of labelled vasopressin, DesGlyNH2-vasopressin and oxytocin by brain regions. Endocrinol. Exp. 19 (1), 29–37.

Faul, F., Erdfelder, E., Lang, A.G., Buchner, A., 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods 39 (2), 175–191.

Feifel, D., Reza, T., 1999. Oxytocin modulates psychotomimetic-induced deficits in sensorimotor gating. Psychopharmacology (Berl.) 141 (1), 93–98.

Feifel, D., Macdonald, K., Nguyen, A., Cobb, P., Warlan, H., Galangue, B., Minassian, A., Becker, O., Cooper, J., Perry, W., Lefebvre, M., Gonzales, J., Hadley, A., 2010. Adjunctive intranasal oxytocin reduces symptoms in schizophrenia patients. Biol. Psychiatry 68 (7), 678–680.

Glovinsky, D., Kalogeras, K.T., Kirch, D.G., Suddath, R., Wyatt, R.J., 1994. Cerebrospinal fluid oxytocin concentration in schizophrenic patients does not differ from control subjects and is not changed by neuroleptic medication. Schizophr. Res. 11 (3), 273–276.

Hamilton, M., 1967. Development of a rating scale for primary depressive illness. Br. J. Soc. Clin. Psychol. 6 (4), 278–296.

Heim, C., Young, L.J., Newport, D.J., Mletzko, T., Miller, A.H., Nemeroff, C.B., 2009. Lower CSF oxytocin concentrations in women with a history of childhood abuse. Mol. Psychiatry 14 (10), 954–958.

Igarashi, Y., Hayashi, N., Yamashina, M., Otsuka, N., Kuroki, N., Anzai, N., Kazamatsuri, H., 1998. Interrater reliability of the Japanese version of the Positive and Negative Syndrome Scale and the appraisal of its training effect. Psychiatry Clin. Neurosci. 52 (5), 467–470.

Inagaki, A., Inada, T., Fujii, Y., Yagi, G., 1999. Equivalent Dose of Psychotropics. Seiwa Shoten, Tokyo.

Inoue, Y., Tonooka, Y., Yamada, K., Kanba, S., 2004. Deficiency of theory of mind in patients with remitted mood disorder. J. Affect. Disord. 82 (3), 403–409.

Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The Positive and Negative Syndrome Scale (PANSS) for schizophrenia. Schizophr. Bull. 13 (2), 261–276.

Kiss, A., Bundzikova, J., Pirnik, Z., Mikkelsen, J.D., 2010. Different antipsychotics elicit different effects on magnocellular oxytocinergic and vasopressinergic neurons as revealed by Fos immunohistochemistry. J. Neurosci. Res. 88 (3), 677–685.

Lim, M.M., Young, L.J., 2006. Neuropeptidergic regulation of affiliative behavior and social bonding in animals. Horm. Behav. 50 (4), 506–517.

Linkowski, P., Geenen, V., Kerkhofs, M., Mendlewicz, J., Legros, J.J., 1984. Cerebrospinal fluid neurophysins in affective illness and in schizophrenia. Eur. Arch. Psychiatry Neurol. Sci. 234 (3), 162–165.

Macdonald, K., Macdonald, T.M., 2010. The peptide that binds: a systematic review of oxytocin and its prosocial effects in humans. Harv. Rev. Psychiatry 18 (1), 1–21.

Mai, J.K., Berger, K., Sofroniew, M.V., 1993. Morphometric evaluation of neurophysin-immunoreactivity in the human brain: pronounced inter-individual variability and evidence for altered staining patterns in schizophrenia. J. Hirnforsch. 34 (2), 133–154.

Mantella, R.C., Vollmer, R.R., Li, X., Amico, J.A., 2003. Female oxytocin-deficient mice display enhanced anxiety-related behavior. Endocrinology 144 (6), 2291–2296.

Mantella, R.C., Vollmer, R.R., Amico, J.A., 2005. Corticosterone release is heightened in food or water deprived oxytocin deficient male mice. Brain Res. 1058 (1–2), 56–61.

Montag, C., Ehrlich, A., Neuhaus, K., Dziobek, I., Heekeren, H.R., Heinz, A., Gallinat, J., 2010. Theory of mind impairments in euthymic bipolar patients. J. Affect. Disord. 123 (1–3), 264–269.

Onaka, T., 2004. Neural pathways controlling central and peripheral oxytocin release during stress. J. Neuroendocrinol. 16 (4), 308–312.

Otsubo, T., Tanaka, K., Koda, R., Shinoda, J., Sano, N., Tanaka, S., Aoyama, H., Mimura, M., Kamijima, K., 2005. Reliability and validity of Japanese version of the Mini-International Neuropsychiatric Interview. Psychiatry Clin. Neurosci. 59 (5), 517–526.

Pedersen, C.A., Gibson, C.M., Rau, S.W., Salimi, K., Smedley, K.L., Casey, R.L., Leserman, J., Jarskog, L.F., Penn, D.L., 2011. Intranasal oxytocin reduces psychotic symptoms and improves Theory of Mind and social perception in schizophrenia. Schizophr. Res. 132 (1), 50–53.

Pitts, A.F., Samuelson, S.D., Meller, W.H., Bissette, G., Nemeroff, C.B., Kathol, R.G., 1995. Cerebrospinal fluid corticotropin-releasing hormone, vasopressin, and oxytocin concentrations in treated patients with major depression and controls. Biol. Psychiatry 38 (5), 330–335.

Rubin, L.H., Carter, C.S., Drogos, L., Pournajafi-Nazarloo, H., Sweeney, J.A., Maki, P.M., 2010. Peripheral oxytocin is associated with reduced symptom severity in schizophrenia. Schizophr. Res. 124 (1–3), 13–21.

Rubin, L.H., Carter, C.S., Drogos, L., Jamadar, R., Pournajafi-Nazarloo, H., Sweeney, J.A., Maki, P.M., 2011. Sex-specific associations between peripheral oxytocin and emotion perception in schizophrenia. Schizophr. Res. 130 (1–3), 266–270.

Ryden, G., Sjoholm, I., 1969. Half-life of oxytocin in blood of pregnant and non-pregnant women. Acta Endocrinol. (Copenh.) 61 (3), 425–431.

Scantamburlo, G., Hansenne, M., Fuchs, S., Pitchot, W., Marechal, P., Pequeux, C., Ansseau, M., Legros, J.J., 2007. Plasma oxytocin levels and anxiety in patients with major depression. Psychoneuroendocrinology 32 (4), 407–410.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 59 (Suppl. 20), 22–33 quiz 34-57.

Sim, K., Su, H.C., Fujii, S., Yang, S.Y., Chong, M.Y., Ungvari, G., Si, T., He, Y.L., Chung, E.K., Chan, Y.H., Shinfuku, N., Kua, E.H., Tan, C.H., Sartorius, N., 2009. High-dose antipsychotic use in schizophrenia: a comparison between the 2001 and 2004 Research on East Asia Psychotropic Prescription (REAP) studies. Br. J. Clin. Pharmacol. 67 (1), 110–117.

Sirzen-Zelenskaya, A., Gonzalez-Iglesias, A.E., Boutet de Monvel, J., Bertram, R., Freeman, M.E., Gerber, U., Egli, M., 2011. Prolactin induces a hyperpolarising current in rat paraventricular oxytocinergic neurones. J. Neuroendocrinol. 23 (10), 883–893.

Sparks, A., McDonald, S., Lino, B., O'Donnell, M., Green, M.J., 2010. Social cognition, empathy and functional outcome in schizophrenia. Schizophr. Res. 122 (1–3), 172–178.

Tabuse, H., Kalali, A., Azuma, H., Ozaki, N., Iwata, N., Naitoh, H., Higuchi, T., Kanba, S., Shioe, K., Akechi, T., Furukawa, T.A., 2007. The new GRID Hamilton Rating Scale for Depression demonstrates excellent inter-rater reliability for inexperienced and experienced raters before and after training. Psychiatry Res. 153 (1), 61–67.

Uvnas-Moberg, K., Alster, P., Svensson, T.H., 1992. Amperozide and clozapine but not haloperidol or raclopride increase the secretion of oxytocin in rats. Psychopharmacology (Berl.) 109 (4), 473–476.

Uvnas-Moberg, K., Bjokstrand, E., Hillegaart, V., Ahlenius, S., 1999. Oxytocin as a possible mediator of SSRI-induced antidepressant effects. Psychopharmacology (Berl.) 142 (1), 95–101.

Weinberg, D., Shahar, G., Davidson, L., McGlashan, T.H., Fennig, S., 2009. Longitudinal associations between negative symptoms and social functioning in schizophrenia: the moderating role of employment status and setting. Psychiatry 72 (4), 370–381.

Wolkenstein, L., Schonenberg, M., Schirm, E., Hautzinger, M., 2011. I can see what you feel, but I can't deal with it: impaired theory of mind in depression. J. Affect. Disord. 132 (1–2), 104–111.

Yamada, H., Masui, K., Kikuimoto, K., 1991. The Japanese version of The Positive and Negative Syndrome Scale (PANSS) Rating Manual. Seiwa, Tokyo.

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Blood *CADPS2ΔExon3* expression is associated with intelligence and memory in healthy adults

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ABSTRACT

Ca^{2+}-dependent activator protein for secretion 2 (CADPS2), a secretory granule associate protein, mediates monoamine transmission and neurotrophin release. Both monoamines and neurotrophins play a crucial role in cognition, learning and memory. An aberrant splice variant of *CADPS2*, *CADPS2ΔExon3*, was reported to be associated with autism. Therefore, we examined the possible association between the expression of *CADPS2/CADPS2ΔExon3* in peripheral blood and brain functions such as intelligence and memory. Quantitative polymerase chain reaction analysis was performed in 271 healthy adults (age range 20–74 years, mean ± SD 43.3 ± 15.3). Data on intelligence quotient (IQ) and memory were obtained by using full versions of the Wechsler Adult Intelligence Scale-Revised (WAIS-R), and the Wechsler Memory Scale-Revised (WMS-R), respectively. *CADPS2* expression levels were not significantly associated with any scores/sub-scores of these scales. However, *CADPS2ΔExon3* expression was significantly associated with lower IQ ($p = 0.022$; effect size: $\eta_p^2 = 0.031$), particularly verbal IQ of WAIS-R ($p = 0.019$; $\eta_p^2 = 0.032$), lower verbal memory ($p = 0.026$; $\eta_p^2 = 0.026$) and delayed recall ($p = 0.042$; $\eta_p^2 = 0.021$) of WMS-R. Our results suggest that CADPS2ΔExon3 affects intelligence and memory in the non-clinical population.

1. Introduction

Ca^{2+}-dependent activator protein for secretion 2 (CADPS2) is a secretory granule-associated protein involved in the release of neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3). Mouse CADPS2 protein is associated with BDNF-containing secretory vesicles and promotes activity-dependent release of BDNF (Sadakata et al., 2004). Accordingly, BDNF release is significantly reduced in the cultured neurons prepared from the cerebellum, neocortex and hippocampus of CADPS2 deficient mice (Sadakata et al., 2007a, 2007b).

BDNF plays a crucial role in the development and maintenance of brain function, including formation of synapses and neural circuits. Reduced long-term potentiation and impaired spatial memory have been reported in conditional BDNF deficient mice or mice after infusion of antisense BDNF (Mizuno et al., 2000; Monteggia et al., 2004). A polymorphism in *BDNF*, Val66Met was reported to affect human memory and hippocampal activity (Egan et al., 2003). That polymorphism may also affect intelligence (Tsai et al., 2004), and susceptibility to psychiatric disorders including depression, schizophrenia (Rybakowski, 2008) and Alzheimer's disease (Fukumoto et al., 2010), although there are also negative reports; i.e. cognition (Houlihan et al., 2009), memory (Strauss et al., 2004), psychiatric disorders (Naoe et al., 2007; Zhang et al., 2006).

CADPS2 also mediates monoamine transmission. CADPS2, together with its family protein, CADPS1, mediates 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 CADPS2 is involved in monoamine storage as antibodies against CADPS2 inhibit monoamine sequestration by synaptic vesicles (Brunk et al., 2009). Monoamine-containing neurons project to diverse brain regions including the hippocampus, neocortex, amygdala and neocortex, and regulate the mode of their function (Robbins and Arnsten, 2009). Dopamine neurotransmission is critical for basic reinforcement learning, noradrenalin modulate attention/concentration, while serotonin mediates cognitive flexibility (Kehagia et al., 2010). CADPS2's roles in synaptic functions suggest that CADPS2 may also mediate human brain functions, especially in learning, memory and cognition.

The regulation of learning/memory by CADPS2 could also be developmental. A comprehensive voxelwise genome-wide association study (GWAS) study found that a single nucleotide polymorphism (SNP) in *CADPS2* was associated with brain structure (Stein et al., 2010). In that study, the association between whole voxels from brain images of the 740 elderly subjects and SNPs were

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