労災疾病臨床研究事業費補助金

「うつ病患者の復職成功の鍵は何か」に関する研究

（課題番号：１４０１０１０１－０１）

平成２６年度　研究報告書

研究代表者　吉村　玲児

平成２７（２０１５）年　５月

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Ｈ２６年度労災疾病臨床研究事業補助金

主任研究報告書

「うつ病患者の復職成功の鍵は何か」に関する研究
うつ病患者におけるカテコールアミンと脳由来神経栄養因子

研究代表者　吉村　玲児　産業医科大学精神医学・准教授

研究要旨

大うつ病性障害(MDD)患者のカテコールアミン主要代謝産物である 3-methoxy-4-hydroxyphenylglycol (MHPG)と homovanillc acid (HVA)の血中濃度とうつ状態の重症度の指標となる brain-derived neurotrophic factor (BDNF)の血中濃度を検討した。その結果、MHPG や HVA の血中濃度が平均値より偏倚している症例では BDNF 濃度が低値であるという結果を得た。うつ状態の時のカテコールアミンの変動は防御反応あるいは回復力（レジリアンス）と関連しているかも知れない。

研究分担者

中村　純	産業医科大学	精神医学　・教授
興梠　征典	産業医科大学	放射線科学・教授
掛田　伸吾	産業医科大学	放射線科学・講師
堀　輝	産業医科大学	精神医学　・助教

A．研究目的

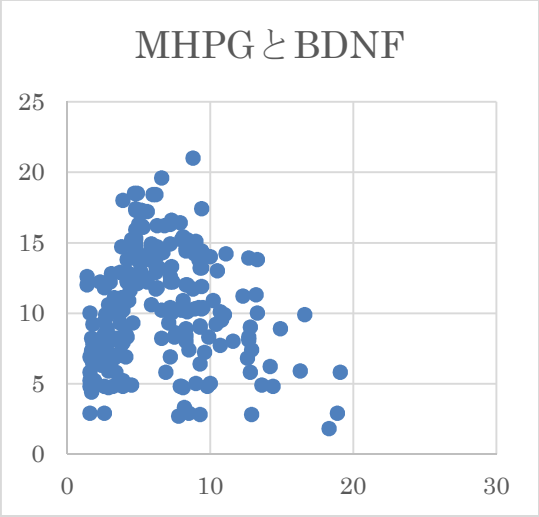
日本人の約 10%が生涯の内うつ病を発症する。うつ病を一旦発症した場合、その後 10 年間の内 25%の期間はうつ状態あるいは閾値下うつ状態にあることを我々は報告した。また、うつ病の経済的損失も大きく年間約 15 兆円である。今年度からストレスチェック制度が導入されるが、その項目には全くバイオマーカーが組み込まれておらず、患者の主観性のみに大きく依存している。我々は、うつ病の発症初期や回復状態の指標として、MHPG, HVA, BDNF の血中濃度が有用であるとの仮説を検討する事が本研究の目的である。

B．研究方法

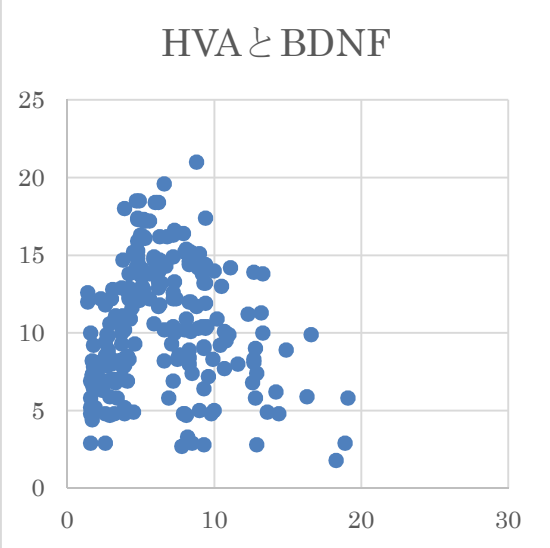
219 例の大うつ病性障害(DSM-IV or DSAM-IV-TR)の診断基準を満たす患者（M/F; 81/138, Age: 42±19 yr）を対象にして、採血を行い電気検出器付高速液体クロマトグラフィーでMHPG, HVA 濃度を測定し、ELISA 法で BDNF 濃度を測定した。また、採血時の薬物服用の有無は問わなかった。本研究は産業医科大学倫理員会の承認を得ており、患者からは文書による同意を得た。

C．研究結果

MHPG, HVA 濃度は平均値から偏倚するほど BDNF 濃度は低値を示していた。(図 1) (図 2)。



縦軸：MHPG 濃度(ng/ml),
横軸：BDNF(ng/ml)



縦軸:HVA 濃度(ng/ml), 横軸:BDNF(ng/ml)

D．考察

我々は大うつ病性障害を MHPG, HVA が高い不安焦燥型うつ病と MHPG, HVA が低い精神運動制止うつ病の分類が出来る事を提示した。今回の結果は、両タイプのうつ病でも共に BDNF は低下しており、さらにうつ状態の程度との有意な関連を示していた（つまり、うつ状態は強い程、BDNF は低値を示す)。

E．結論

MHPG, HVA, BDNF の血中濃度測定は精神的ストレスやうつ状態の指標となる。

F．健康危機情報　　なし

G．研究発表

1．論文発表

Atake K, **Yoshimura R**, Hori H, Katsuki A, Nakamura J. Catechol-O-methyltransferase Val158Met genotype and the clinical responses to duloxetine treatment or plasma levels of 3-methoxy-4-hydroxyphenylglycol and homovanillic acid in Japanese patients with major depressive disorder. Neuropsychiatry Dis Treat 2015 Apr 3;11:967-74. doi: 10.2147/NDT.S80953. eCollection 2015.

Hatano M, Ikeda M, Kondo K, Saito T, Shimasaki A, Esaki K, Umene-Nakano W, **Yoshimura R**, Nakamura J, Ozaki N, Iwata N. No support for replication of the genetic variants identified by a recent mega-analysis of the treatment response to antidepressants. J Hum Genet 2015 Mar 5. doi: 10.1038/jhg.2015.21. [Epub ahead of print]

H．知的財産権の出版・登録状況　　なし

Ｈ２６年度労災疾病臨床研究事業補助金

分担研究報告書

「うつ病患者の復職成功の鍵は何か」に関する研究

うつ病患者における脳由来神経栄養因子(BDNF)とその前駆体(proBDNF)

研究分担者　中村　純　　産業医科大学精神医学・教授

研究要旨

大うつ病性障害(MDD)患者の brain-derived neurotrophic factor (BDNF)の血中濃度とその前駆体である(proBDNF)を検討した。その結果、MDD 患者では HC 群と比較して血清 BDNF 濃度が低下していたが、血清 proBDNF 濃度に差はなかった。

A．研究目的

脳由来神経栄養因子は神経可塑性(BDNF)に関与する重要な分子の一つである。BDNF は血液脳関門を通過する。うつ病では血小板および脳内からの BDNF 分泌が低下しており、健常者と比較して有意に血中(血漿・血清)BDNF 濃度が低値である。BDNF はその前駆物質である proBDNF から合成されるが、proBDNF もまた生理活性を有する。すなわち、BDNF が TrkB 受容体に結合してシナプス可塑性に関与するのに対して、proBDNF は P75 受容体に結合して神経細胞死(アポトーシス)に関与する。すなわち、BDNF と proBDNF は神経に対して全く逆の作用をする(陰陽仮説)。本研究では、うつ病患者の proBDNF, BDNF の血清濃度を比較した。さらに、SSRI 単剤治療による治療の BDNF, proBDNF 影響も検討した。

B．研究方法

88 例の DSM-IV-TR で大うつ病性障害(MDD)の診断基準を満たす drug-naïve 患者(M/F: 39/49, Age<mean±s.d.: 34±17 yr)と 108 例の健常対象者(HC)(M/F: 42/66, Age<mean±s.d.: 38±19 yr)である。抑うつ状態の症状評価にはハミルトンうつ病評価尺度(17 項目)(HAMD17)を用いた。全例抗うつ薬単剤治療が行われた。投与された薬物は fuluvoxamine 32 例、paroxetine 26 例、milnacipran 10 例、sertraline 10 例、duloxetine 10 例であった。**血清 BDNF, proBDNF 測定**：ELISA 法にて行った。BDNF は ELISA kit (SK00572-06, Adipo Bioscience, Santa Clara, CA, USA)、proBDNF は ELISA kit (SK00572-01, Adipo Bioscience, Santa Clara, CA, USA)を用いた。測定は duplicate で行い平均値を求めた。**統計解析**：2 群間のパラメーター比較には student's t-test、2 群間のパラメーター相関検定には Pearson's correlation、血清 BDNF, proBDNF の時間経過による変化の検定には one-way ANOVA 法を用いた。

本研究は産業医科大学倫理委員会の承認を受けており、被験者からは文書による同意を得た。

C．研究結果

(1)血清 BDNF 濃度は両群とも全例で測定することが出来た。血清 proBDNF 濃度は MDD 群で 72/88 例(49%)、HC 群で 93/108 例(63%)で測定することが出来た。(2)血清 BDNF 濃度は MDD 群では HC 群と比較して有意に低値であった。(HC 群: 12881±5878 pg/ml, MDD 群: 8081±3886 pg/ml, t=3.287, p=0.0024, 1-β=87.3%) (2)血清 proBDNF 濃度は 2 群間で差はなかった。(MDD 群: 1876±568 pg/ml, HC 群: 1743±503 pg/ml, t=-0.979, p=0.833) (3)baseline の HAMD17 得点と血清 BDNF 濃度には負の相関傾向があった。(r=-1.837, p=0.073)。(4)baseline の HAMD17 得点と血清 proBDNF 濃度は関連がなかった。(r=0.092, p=0.421)。(5)baseline proBDNF/BDNF は MDD 群 HC 群に差はなかった。(MDD 群: 0.242 ± 0.074, HC 群: 0.2141 ± 0.1090, t=1.1934, p=0.2330) (6)MDD 群では baseline proBDNF/BDNF と HAMD17 得点には関連がなかった。(r=-0.130, p=0.190)(7)MDD 群では fluvoxamine 投与後(平均最大投与量: 103±38mg/d)により 25/51 例(51%)が 4 週間までに反応した。(HAMD17 得点で 50%以上の改善)(8)fluvoxamine 反応群と非反応群(HAMD17 得点で 50%未満の改善)で baseline proBDNF(t=0.090, p=0.336)と BDNF 濃度(t=-0.084, p=0.730)に差はなかった。(9)fluvoxamine 投与前、投与 2 週間後、投与 4 週間後の血清 BDNF 濃度(F=0.579, p=0.561)および proBDNF 濃度(F=2.580, p=0.080)に差は認められなかった。(10)4 週間後の fluvoxamine 濃度と血清 BDNF 濃度変化(r=0.117, p=0.691)および proBDNF/BDNF(r=0.211, p=1.514)に関連がなかった。

D．考察

今回の研究結果は MDD 患者では、HC と比較して血中（血清・血漿）BDNF 濃度が低下しているという我々の先行研究やメタ解析結果を再確認した。MDD では、血中（血清・血漿）BDNF 濃度が低下していることは、うつ病の biological marker (state marker)として確実であるが一方、血中 BDNF はその特異度の低さに問題がある。血清 proBDNF 濃度は両群間に差がなかった。しかし、血清 proBDNF は ELISA kit 使用による測定方法では、約 50%しか測定できない（半分は検出限界以下となる）。現在我々は MAGPIX を用いて proBDNF, BDNF を測定中であり良好な結果を得ている。最近、難治性うつ病に対する即効性が期待されている ketamine も BDNF への作用が強く血漿 BDNF 濃度を迅速(6 時間)に増加させることが報告されている 3。今回の検討から、血清 proBDNF 濃度が MDD 患者で増加するという知見は得られなかった。さらに、fluvoxamine は血清 proBDNF に影響を与えなかった。今回の結果の解釈として、(1)測定方法(ELIZA 法)の問題、(2)血清 BDNF ほど proBDNF はうつ状態を鋭敏に反映しない可能性などが考えられる。

E．結論

(1) MDD 患者では HC 群と比較して血清 BDNF 濃度が低下していた。
(2) MDD 患者と HC 群に血清 proBDNF 濃度に差はなかった。

F．健康危機情報　なし

G．研究発表

1．論文発表

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H. 知的財産権の出版・登録状況
１．特許取得　　なし
２．実用新案登録　なし
３．その他　　なし

平成２６年度労災疾病臨床研究事業費補助金
分担研究報告書

「うつ病患者の復職成功の鍵は何か」に関する研究

大うつ病性障害における脳皮質厚と血中コルチゾール値の関係：初回エピソード未治療群での検討

研究分担者　氏　名　　興梠征典　産業医科大学 放射線科学・教授

研究要旨

うつ病患者 30 例と正常被験者 48 例に対して、Voxel-based morphometry(VBM)を用いて血中コルチゾール値と脳形態の変化との関係を調べた。うつ病患者は正常被験者と比較して、右眼窩前頭皮質の皮質厚が有意に減少しており、かつ同部位の皮質厚は血中コルチゾール値と有意な負の相関関係を示した。今回の結果は、血中コルチゾール値が、うつ病患者の脳形態に関連する可能性を示唆する。

A. 研究目的

うつ病の発症に関わる環境因子の一つにストレスがある。うつ病患者ではストレスに対してコルチゾールの過剰分泌が生じることが知られており、過剰なコルチゾールによる神経障害がうつ病の病態に関係しているとの推測もある。今回、我々は Voxel-based morphometry(VBM)を用いてうつ病患者における血中コルチゾール値と脳形態の変化との関係を調べた。

B. 研究方法

DSM-IV-TRで大うつ病性障害(Major Depressive Disorder)の診断基準を満たした未治療のうつ病患者30例と年齢と性別を患者とマッチングした正常被験者48例を対象とした。全例に3T MRIで高分解能三次元FSPGR (fast spoiled gradient recalled acquisition)を撮像し、朝（午前9-10時）に採取した血液サンプルから血中コルチゾール値の測定を行なった。VBM解析のソフトにはFreeSurferを使用し、得られた脳画像から皮質厚を自動計測した。画像統計解析により、うつ病群と正常被験者群の皮質の厚さ(皮質厚)の比較、うつ病群の皮質厚と血中コルチゾール値の相関関係を検討した。

（倫理面への配慮）

本研究は産業医科大学倫理委員会の承認を受けており、対象者からは文書による同意を得た。

C. 研究結果

うつ病群の血中コルチゾール値は正常被験者群と比較し、有意に高かった（平均±標準偏差: 12.3±5.4 vs 9.5±3.4 nmol/l）。うつ病患者は正常被験者と比較して、右眼

窩前頭皮質の皮質厚が有意に減少しており（図1）、かつ同部位の皮質厚は血中コルチゾール値と有意な負の相関関係を示した（図2）。また、うつ病患者では、左前部帯状回、右舌状回、左下側頭回でも皮質厚と血中コルチゾール値に有意な負の相関を認めた（図2）。

D. 考察

本研究において、うつ病患者は、正常被験者と比較して、眼窩前頭皮質厚が有意に減少し、同部位は血中コルチゾール値と有意な負の相関を示した。コルチゾールは神経毒性を有し、過剰な血中コルチゾールは細胞障害を引き起こすことが報告されている。また、グルココルチコイド受容体は、今回検出された前頭前皮質（眼窩前頭皮質など）や海馬に多く分布しており、コルチゾールの主な作用部位である。今回の結果は、うつ病において血中コルチゾール値の上昇が眼窩前頭皮質の形態異常と密接に関与することを示している。また眼窩前頭皮質の機能異常は、うつ病における感受性の低下や興味や喜びの喪失に関連することが知られており、今回の結果は、これらの症状への過剰な血中コルチゾールの関与を示唆する。今回用いた VBM による脳統計解析は、脳容積の群間比較だけでなく、血中コルチゾールなど臨床データを因子とした解析も可能であり、今後のうつ病の病態解明に有用な手法と考えられる。

E. 結論

今回の結果は、血中コルチゾール値が、未治療うつ病患者の脳形態に関連する可能性を示唆する。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

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3.その他

H. 知的所有権の出願・登録状況

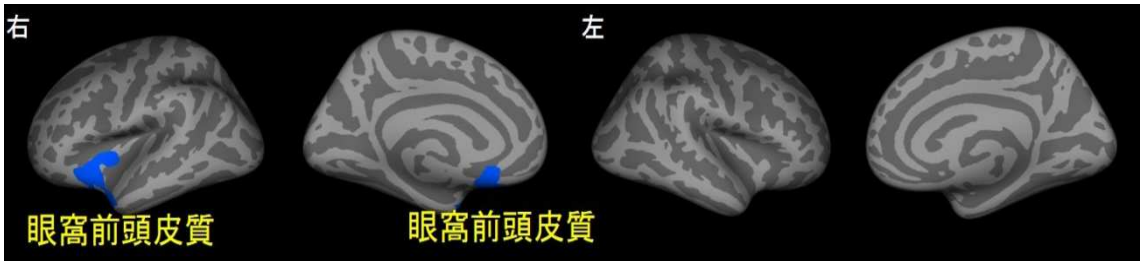
1. 特許取得

なし

2. 実用新案登録

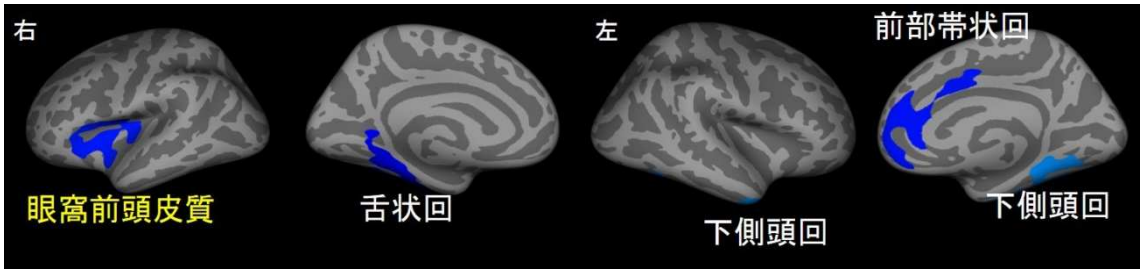
なし

図1　うつ病患者と健常者の皮質厚の2標本t検定



うつ病患者では、健常者と比較して、右眼窩前頭皮質の皮質厚が有意に減少している（青で示す領域）。

図2　うつ病患者における皮質厚と血中コルチゾール値の相関解析



右眼窩前頭皮質、左前部帯状回、右舌状回、左下側頭回の皮質厚と血中コルチゾール値に有意な負の相関関係を認める（青および水色で示す領域）。

平成２６年度労災疾病臨床研究事業費補助金
分担研究報告書

「うつ病患者の復職成功の鍵は何か」に関する研究
うつ病患者における灰白質体積の変化：BDNF遺伝子 Val166Met多型との関連性

研究分担者　氏　名　　掛田伸吾 産業医科大学 放射線科学・講師

研究要旨
うつ病患者 30 例と正常被験者 30 例に対して、脳由来神経栄養因子(brain-derived neurotrophic factor :BDNF)遺伝子 Val166Met 多型を調べ、4群(うつ病患者 Met-carrier 群、うつ病患者 Val/Val-carrier 群、正常被験者 Met-carrier 群、正常被験者 Val/Val-carrier 群)に分類し、BDNF 遺伝子 Val166Met 多型と脳灰白質体積の関連性を検討した。Voxel-based morphometry(VBM)解析を行った結果、うつ病患者 Met-carrier 群では、Val/Val-carrier 群に比べ、前頭前皮質に有意な容積減少を認めた。今回の結果は、うつ病患者において、BDNF 遺伝子多型が前頭前皮質の萎縮に関与している可能性を示唆する。

A. 研究目的
脳由来神経栄養因子(brain-derived neurotrophic factor :BDNF)は、中枢神経における発達、分化、生存、可塑性などに重要な役割を果たしており、最近、BDNF 遺伝子 Val66Met 多型とうつ病発症との関連が示唆されている。今回、我々は Voxel-based morphometry(VBM)を用いてうつ病患者および正常被験者における BDNF 遺伝子 Val166Met 多型と灰白質体積の関連性を検討した。

B. 研究方法
DSM-IV-TRで大うつ病性障害(Major Depressive Disorder)の診断基準を満たしたうつ病患者30例、患者と年齢と性別をマッチングした正常被験者30例を対象として、BDNF遺伝子Val166Met多型について調べ、4群(うつ病患者Met-carrier群:25例、うつ病患者Val/Val-carrier群:5例、正常被験者Met-carrier群:20例、正常被験者Val/Val-carrier群:10例)に分類した。全例に3T MRIで高分解能三次元FSPGR(fast spoiled gradient recalled acquisition)を撮像し、画得られた脳画像データについて、VBMを用いて局

所的な灰白質容積を算出した。また統計処理では、SPM8を用いてうつ病の有無および遺伝子型を因子として2要因分散分析を行った。

（倫理面への配慮）

本研究は産業医科大学倫理委員会の承認を受けており、対象者からは文書による同意を得た。

C. 研究結果

うつ病群において、Met-carrierはVal/Val-carrierと比較し、左中前頭回（前頭前皮質）の灰白質体積が有意に小さかった(P < 0.01)。また、Met-carrierとVal/Val-carrierでの左中前頭回の体積差は、正常被験者群と比較してうつ病群で有意に大きかった（P < 0.01)。 脳統計解析の結果を図に示す。

D. 考察

過去のうつ病患者におけるVBM研究では、前頭前皮質、前帯状皮質、海馬の容積減少が報告されている。本研究の結果は、前頭前皮質の容積減少が、うつ病のBDNF遺伝子多型(Met-carrier）に関連があることを示している。また、前頭前皮質はうつ病におけるワーキングメモリの障害に関連する脳領域であることが知られており、 今回の結果は、うつ病のワーキングメモリ障害におけるBDNF遺伝子多型の関与を示唆している。今回用いたVBMによる脳統計解析は、脳容積の群間の比較だけでなく、血液所見など臨床データを因子とした解析も可能であり、遺伝子解析など今後のうつ病における病態解明に有用な手法と考えられる。

E. 結論

うつ病患者において、BDNF遺伝子多型は前頭前皮質の萎縮に関与している可能性がある。

F. 健康危険情報

なし

G. 研究発表

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COMT Val158Met, but not BDNF Val66Met, is associated with white matter abnormalities of the temporal lobe in patients with first-episode, treatment-naïve major depressive disorder: a diffusi

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H. 知的所有権の出願・登録状況

1. 特許取得

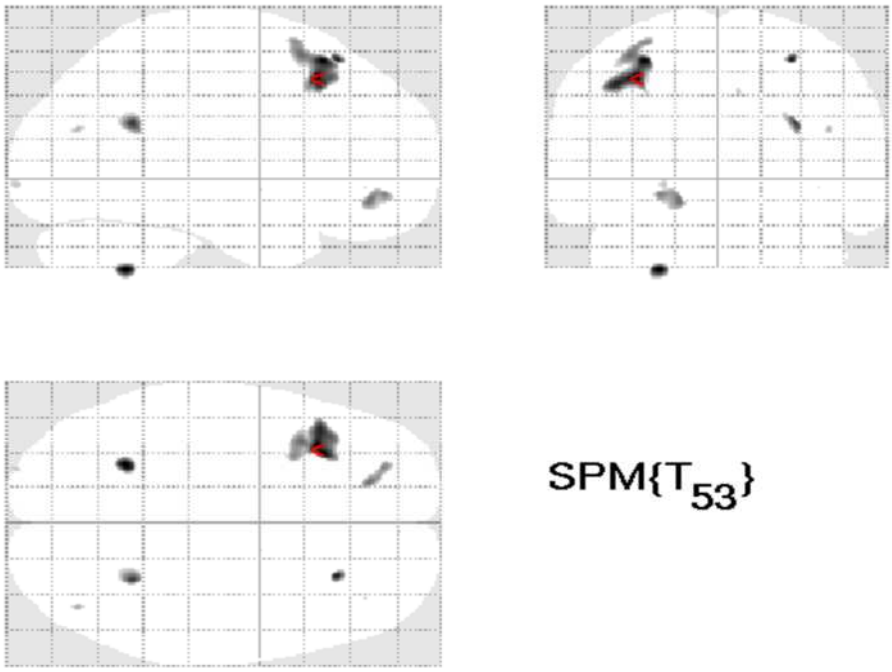
なし

2. 実用新案登録

なし

3.その他

図　脳容積の解析結果：Met-carrierとVal/Val-carrierの比較
Val/Val-carrierと比較し、Met-carrierで容積減少を認めた左中前頭回（前頭前皮質）が赤矢頭で示されている。



Ｈ２６年度労災疾病臨床研究事業補助金

分担研究報告書

「うつ病患者の復職成功の鍵は何か」に関する研究
～復職時の運動療法の負荷療法の復職継続率に対する影響～

研究分担者　堀　輝　　産業医科大学精神医学教室・助教
研究協力者　香月あすか　産業医科大学精神医学教室・助教
　　柴田　裕香　産業医科大学精神医学教室・修練医

研究要旨

職域のうつ病対策が重要視されている。その対策の一つとして、うつ病勤労者の復職後の再休職率をいかに減らすことかが重要である。我々は以前にうつ病勤労者の中で復職失敗者は復職成功者と比較して活動性が低いことを報告した。

今回の研究では就労予測因子の一つとして活動性の低さがハザード比 3.28 であることが分かった。さらに休職中のうつ病勤労者に対して無作為割り付け試験を行った。評価指標としては復職成功率、仕事のパフォーマンスの指標である認知機能、睡眠覚醒リズムに対する影響について検討したところ、運動介入群は就労継続率が高い傾向であり、睡眠効率が有意に改善し認知機能も改善していた。本研究からうつ病勤労者に対する運動療法の付加治療は有効であることが示唆された。

A．研究目的

職域におけるうつ病勤労者が増加している。職場における長期休職者の大半がうつによるものである。勤労者がうつ病を発症すると、病状が改善し、復職に至ったとしても、多くの患者が早期に再休職に至ることを我々は報告（堀ら, 2013）した。さらに、復職を決めた際に、戸外での活動性が低いうつ病勤労者の方が再休職に至りやすいことが明らかとなり（Morita et al. in submission）、復職時には活動性を維持することが重要であることが示唆された。

また、うつ病勤労者のコスト面からの検討においては、presenteeism による損失が大きいことが示唆されており、復職時のパフォーマンスの低さが疑われる。うつ病は寛解期においても認知機能障害が残存していることが報告されている。認知機能障害は手順の学習や効率的な処理過程、問題点の抽出と問題解決など、多くの職業的能力に基礎的に関わっており、社会生活機能を含めた全体的機能転帰に関連している可能性がある（北川ら, 2011）。

今回我々は、休職中のうつ病勤労者を対象として運動療法を付加することによる影響について検討を行った。

B．研究方法

対象者は現在休職中で、DSM-IV-TR の診断基準によって、大うつ病性障害（うつ病）と診断された患者で当院外来通院中の患者である。対象者は 17 項目ハミルトンうつ評価尺度（HAM-D）で 13 点以下までうつ状態は改善しており、労作性狭心症などの心疾患や

整形疾患を有さない者とした。本研究は産業医科大学倫理委員会で承認を得ており、対象者からは書面で同意を得た。

エントリー時に、運動介入群と通常治療群の2群に封筒法を用いて無作為に割り付けた。ここでの通常治療とは主治医による薬物療法と精神療法のことである。運動介入群には、通常治療に加え、運動療法を上乗せする。通常治療群には、通常治療のみを行った。運動療法の内容は最大下多段階ステップテストを自覚的運動強度から中強度相当で行うこととした。ステップ台を貸し出し、自宅において1日合計60分（4回までの分割可）以上、週に3回､1か月以上の計13回以上の介入を行った。運動は復職が決定するまで継続するよう指示し、トレーニングを確認するために生活記録日誌の記載を行い、2 週間に一回の外来通院時に確認し運動促進を定期的に行った。2週間の平均で75%以下の達成時間の患者は運動介入群を脱落とすることとした。

両群ともにエントリー時と復職時にアクチグラフによる活動量の測定と睡眠効率の測定、精神症状の評価、認知機能検査を行った。認知機能検査の内容は、N-back 課題によるワーキングメモリの評価と CPT（Continuous Performance Test）課題による注意の評価、ロンドン塔（Tower of London）課題による遂行機能の評価を行った。復職後に再休職に至るまでの復職継続日数を365日までのフォローアップを行った。解析は通常治療群と運動介入群間の比較には正規分布している場合には t 検定を用い、正規分布していない場合にはMann-Whitney U 検定を用いた。また同一群内での変化の解析ではpaired-t検定を行った。復職の継続日数についての解析は Kaplan-Meier 法による生存分析を行った。

C．研究結果

（1）患者背景

休職中のうつ病勤労者 25 名がエントリーし、封筒法による無作為割り付けによって12名が通常治療群に、13名が運動介入群に割り付けられた。年齢は45.8±7.9(SD)歳､性別は男女比17:4と男性が多かった。教育年数14.9±2.2（SD）年、休職中の会社への勤労年数 19.7±10.5（SD）年、これまでの転職回数1.2±1.4（SD）回、過去の休職回数 1.6±0.5（SD）回であった。HAM-D は9.4±3.8（SD）点であった。

（2）活動量

通常治療群は894.7±625.9 (SD) kcal、運動介入群は 1651.3±873.9（SD）kcal で有意に活動量が多い傾向（.064）であった。

（3）睡眠効率

アクチグラフを用いて睡眠効率を評価した。エントリー時の睡眠効率には通常治療群と運動介入群間に有意差は見られなかった。運動介入群では睡眠効率は有意に改善していた（p=.008）が、通常治療群ではエントリー時も復職時も同等で有意な変化は見られなかった。

（4）認知機能

各認知機能検査のエントリー時の両群間で差はなく、障害の程度は同等であった。通常治療群に比べ運動介入群の方が 2-back 課題（p=.006）と 3-back 課題（p=.024）で正答率は有意に良く、つまり、ワーキングメモリの障害が有意に少なかった。

（5）復職の継続率

メインアウトカムである運動介入が復職を成功させるのかについて検討を行った。復職は復職することがゴールではなく、継続し、職場で十分な生産性を発揮することが重要である。今回、フォローアップ中の

患者をその時点までの日数の打ち切りとして扱い、Kaplan-Meier 法による生存分析を行った。運動介入群の方が通常治療群よりも就労の継続日数が有意に継続できている傾向（p=.054）を示した。

D．考察

今回の結果から就労成功予測因子としては一番大きい因子は復職決定時の活動性の高さであることが推察された。復職決定時にうつ状態が改善していたとしてもある程度の活動性が維持できていないとその後の復職継続が困難になる可能性が考えられた。

本研究ではそれらの結果をもとに、復職決定前に運動療法を付加することによる復職決定時の活動性を高めることの有効性について検討したものであるが、運動療法の付加によって睡眠リズムの確立、認知機能の改善作用を介した復職準備性を高める可能性があると推察された。

しかしながらまだまだ症例数が少ないために十分な結論には至っていない。

E．結論

うつ病勤労者に対して運動療法を付加することは睡眠改善作用、認知機能改善作用を有し結果的に就労継続率を高める可能性がある。

F．健康危機情報　　なし

G．研究発表

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H．知的財産権の出版・登録状況

1．特許取得　　なし

2．実用新案登録　なし

3．その他　　なし

研究成果の刊行に関する一覧表
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ANNALS OF GENERAL PSYCHIATRY

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Serum proBDNF/BDNF and response to fluvoxamine in drug-naïve first-episode major depressive disorder patients

Reiji Yoshimura[1*], Taro Kishi[2], Hikaru Hori[1], Kiyokazu Atake[1], Asuka Katsuki[1], Wakako Nakano-Umene[1], Atsuko Ikenouchi-Sugita[1], Nakao Iwata[2] and Jun Nakamura[1]

Abstract

Background: We investigated the association between serum proBDNF, a precursor of brain-derived neurotrophic factor (BDNF), and response to fluvoxamine in patients with major depressive disorder (MDD) using the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR): physically healthy and free of current alcohol or drug abuse, comorbid anxiety, or personality disorders.

Methods: Fifty-one patients with MDD (M/F, 19:32; age, 38 ± 19 years) and 51 healthy controls (M/F, 22:29; age, 34 ± 17 years) were studied using DSM-IV-TR: physically healthy and free of current alcohol or drug abuse, comorbid anxiety, or personality disorders. Serum levels of proBDNF and MDNF were measured by sandwich enzyme-linked immunosorbent assay (ELISA).

Results: Serum mature BDNF levels in the MDD patients were significantly lower than those in the healthy controls ($t = 3.046$, $p = 0.0018$). On the other hand, no difference was found in serum proBDNF between the MDD patients and the healthy controls ($t = -0.979$, $p = 0.833$). A trend of negative correlation was found between baseline serum BDNF and baseline scores of the 17 items of the Hamilton Rating Scale for Depression (HAMD17) ($r = -0.183$, $p = 0.071$). No correlation was however found between HAMD17 scores and proBDNF at baseline ($r = 0.092$, $p = 0.421$). Furthermore, no correlation was observed between baseline HAMD17 scores and baseline proBDNF/BDNF ($r = -0.130$, $p = 0.190$). No changes were observed in serum levels of proBDNF and BDNF during the treatment periods.

Conclusions: These results suggest that there is no association between serum proBDNF/BDNF and fluvoxamine response in MDD patients at least within 4 weeks of the treatment.

Keywords: BDNF, proBDNF, Major depressive disorder, Serum, Fluvoxamine

Background

Major depressive disorder (MDD) is a common and major psychiatric disorder that affects as many as about 20% of individuals within their lifetime [1-3]. A wide variety of pharmaceuticals are available for treating depression, including tricyclic antidepressants, monoamine oxidase inhibitors, and selective serotonin reuptake inhibitors (SSRIs). Fluvoxamine is an SSRI that is widely used for treatment of depression and other psychiatric disorders and has been suggested to have early effects when used as an antidepressant drug [4,5]. In addition, the results of a meta-analysis have shown that significant improvements in Hamilton Rating Scale for Depression (HAMD) scores achieved in the first few weeks were maintained after 6 weeks of treatment [6]. Results of a recent meta-analysis also suggest that treatment with fluvoxamine leads to symptomatic improvements in patients with MDD by the end of the first week of use [6].

Mature brain-derived neurotrophic factor (BDNF) is initially synthesized as a precursor protein. ProBDNF is converted to BDNF by extracellular proteases such as matrix metalloproteinase-9 (MMP-9). BDNF is biologically active. In contrast, proBDNF, which localizes intracellularly, serves as an inactive precursor. In short, new evidence shows that

* Correspondence: yoshi621@med.uoeh-u.ac.jp
[1]Department of Psychiatry, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan
Full list of author information is available at the end of the article

Table 1 Demographics of participants

	Values
Age (years)	38 (19)
Female (%)	62
Daily dose at week 4 (max) (mg)	103 (38)
DUP (months)	2.1 (0.9)
HAMD17 (baseline)	19.3 (2.8)

proBDNF and BDNF elicit opposing effects via the neurotrophin receptor p75 (p75NTR) and tropomyosin-related kinase B (TrkB) receptors, respectively, and that both proBDNF and mature BDNF play important roles in several physiological functions for neurons, which might be related to the pathology of psychiatric disorders such as mood disorders and schizophrenia [7-9]. Sen et al. [10] first performed a meta-analysis and demonstrated that serum BDNF levels are abnormally low in patients suffering from major depressive disorder and that the BDNF levels are elevated following a course of antidepressant treatment. Although the relationship of our findings to the pathophysiology of depression and the mechanism of drug action remains to be determined, the measure may have potential use as a biomarker for psychiatric disorders or as a predictor of antidepressant efficacy [10,11]. Recently, Yoshida et al. [12] reported that it was initially thought that only secreted mature BDNF was biologically active and that proBDNF, which localizes intracellularly, served as an inactive precursor. However, new evidence shows that proBDNF and BDNF elicit opposing effects via the p75NTR and TrkB receptors, respectively, and that both proBDNF and BDNF play important roles in several physiological functions [8,12]. In recent decades, the role of BDNF in first-episode major depressive disorder MDD patients has received intensive attention. However, the relationship between proBDNF and MDD has not been clearly elucidated. We hypothesized that (1) serum levels of BDNF, proBDNF, and proBDNF/BDNF are different between MDD patients and healthy controls, (2) fluvoxamine decreases serum proBDNF level and proBDNF/BDNF ratio and increases serum BDNF level, and (3) the plasma level of fluvoxamine is related to serum levels of BDNF and the HAMD17 scores.

This study aimed to determine whether serum levels of proBDNF/BDNF were different between MDD patients and the healthy controls. We also examined serum levels of proBDNF/BDNF between the responders and the nonresponders to fluvoxamine in patients with first-episode MDD. In addition, we also investigated longitudinal changes in proBDNF and BDNF in MDD patients treated with fluvoxamine before and after the treatment.

To the best of our knowledge, this is the first study investigating serum proBDNF/BDNF and response to fluvoxamine.

Materials and methods

Participants

Fifty-one drug-naïve and first-episode patients with MDD were studied. In the MDD group, 19 were males and 32 were females, ranging in age from 29 to 71 (mean ± standard deviation (SD), 38 ± 19) years. In 51 cases of the healthy control (HC) group, 22 males and 29 females, ranging in age from 24 to 68 (mean ± SD, 35 ± 16) years, were enrolled in the present study. Prior to the commencement of the study, all subjects provided written informed consent, after receiving a full explanation of the study as well as any potential risks and benefits of study participation. The study was approved by the Ethics Committee of the University of Occupational and Environmental Health and performed in accordance with the Declaration of Helsinki II. The demographics of the participants are shown in Table 1, ranging in age from 29 to 71 (mean ± SD, 38 ± 19)

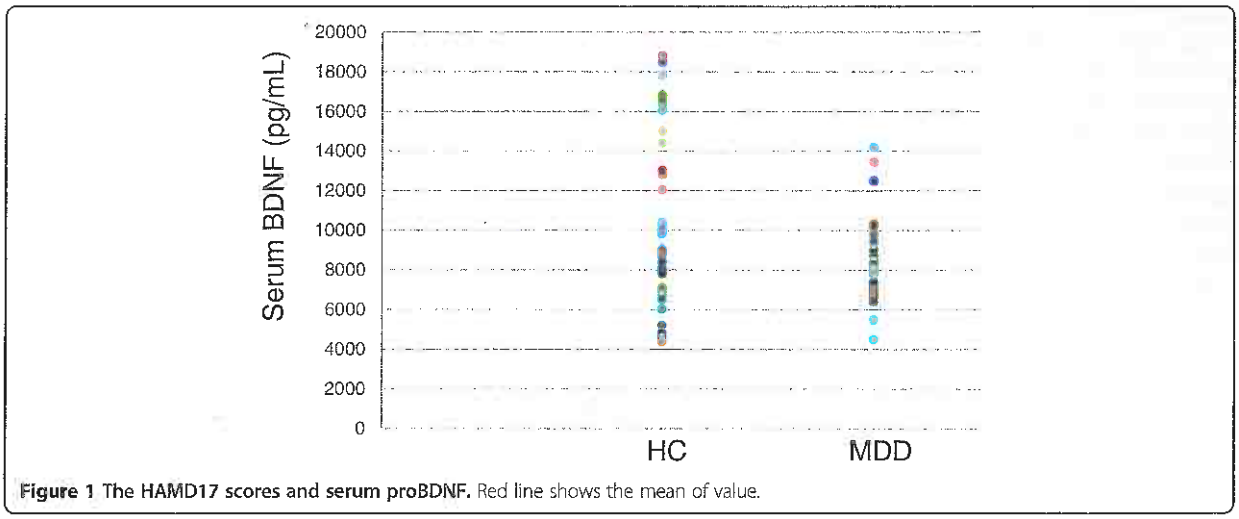

Figure 1 The HAMD17 scores and serum proBDNF. Red line shows the mean of value.

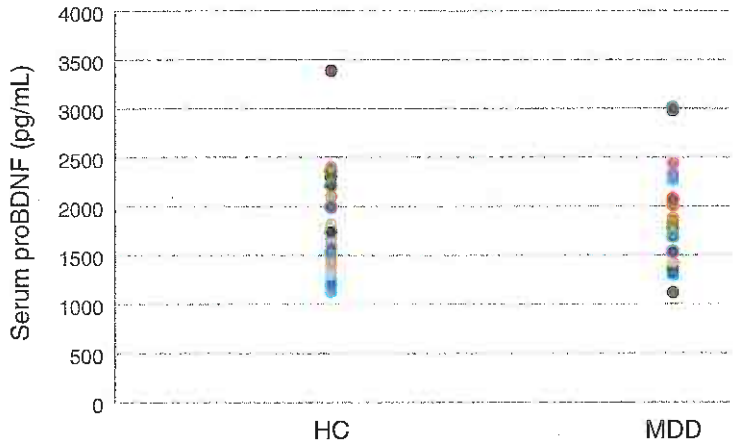

Figure 2 Serum proBDNF change during treatment with fluvoxamine. Red line shows the mean of value.

years. All patients fulfilled the MDD criteria using the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR): physically healthy and free of current alcohol or drug abuse, comorbid anxiety, or personality disorders. We defined the responders as those whose scores of the 17 items of the Hamilton Rating Scale for Depression (HAMD17) decreased 50% or more. All patients consented to participate after having been informed of the study's purpose. Benzodiazepines were the only hypnotics permitted, and their dosages were kept constant throughout the study period. The dosages of fluvoxamine varied among patients and were not fixed for ethical reasons.

Assessment of clinical variables

Depression was assessed using the 17 items of the Structured Interview Guide for the Hamilton Depression Rating Scale (SIGH-D) by an experienced psychiatrist (R.Y.).

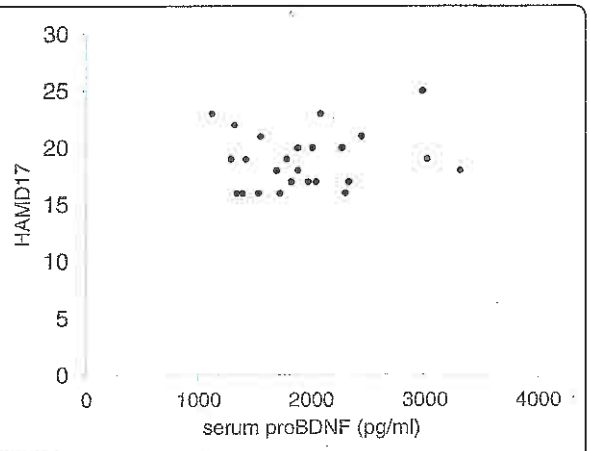

Figure 3 Serum proBDNF in the responders' and the nonresponders' treatment with fluvoxamine.

Serum proBDNF and BDNF assay

Blood was drawn at 9:00 a.m. Serum levels of BDNF and proBDNF were measured in duplicate using the human proBDNF enzyme-linked immunosorbent assay (ELISA) kit SK00572-06 (Adipo Bioscience, Santa Clara, CA, USA) and the human matureBDNF ELISA kit SK00572-01 (Adipo Bioscience, Santa Clara, CA, USA). All experiments were performed in duplicate. Protocols were performed according to the manufacturer's instructions. In short, 96-well microplates were coated with anti-BDNF monoclonal antibody and incubated at 4°C for 18 h. The plates were incubated in a blocking buffer for 1 h at room temperature. The samples were diluted 100-fold with an assay buffer, and BDNF standards were kept at room temperature with horizontal shaking for 2 h and then washed with the appropriate washing buffer. The plates were incubated with antihuman BDNF polyclonal antibody at room temperature for 2 h and washed with the washing buffer. Then, they were incubated with anti-IgY antibody conjugated to horseradish peroxidase for 1 h at room temperature and incubated again in peroxidase substrate and tetramethylbenzidine solution to induce a color reaction. The reaction was stopped with 1 mol/L hydrochloric acid. The absorbance at 450 nm was measured with an Emax automated microplate reader (Molecular Devices, Chuo-ku, Japan). Measurements were performed in duplicate.

Plasma fluvoxamine assay

The plasma fluvoxamine level was measured using high-performance liquid chromatography according to the method we previously described [13]. In brief, 1 mL of plasma alkalized with 500 μL of 2 M sodium hydrogen carbonate was extracted by hexane (10 mL) after the addition of the internal standard (clomipramine). Shaken horizontally for 20 min and then centrifuged at 2,000 *g* for 10 min, the upper organic layer was removed and dried

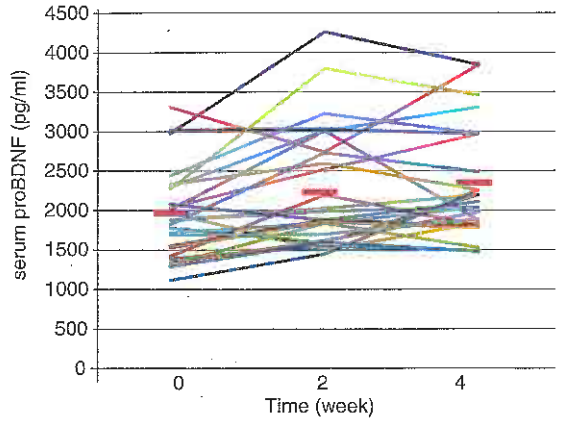

Figure 4 The HAMD17 scores and serum BDNF. Red line shows the mean of value.

under N_2. After being dissolved in 200 μL of mobile phase, a 50-μL aliquot of the final preparation was subjected to HPLC. All experiments were performed in duplicate.

Statistical analyses

Student's *t* test was used to compare two groups (serum levels of proBDNF and BDNF; healthy control vs. MDD). Serum levels of proBDNF and BDNF and plasma fluvoxamine concentrations were measured, and correlations with clinical variables were performed using Peason's correlation. One-way ANOVA was used regarding the time course of proBDNF and BDNF. Power analysis was performed in BDNF (0 week) × HAMD17 (0 week) and BDNF (healthy control, 0 week) × BDNF (MDD, 0 week). A significant value of $p < 0.05$ was judged as statistically significant. All analyses were carried out using SPSS version 19.0 (SPSS Inc, Chicago, IL, USA).

Results

The demographics of the participants are shown in Table 1. Twenty-five of 51 (49%) MDD patients responded to fluvoxamine at least within 4 weeks. Nine of 51 (18%) MDD patients had remission. Serum BDNF of all subjects could be measured. Serum proBDNF of 32 of 51 HC (63%) and 25 of 51 MDD patients (49%) could be assayed. Serum BDNF levels in MDD were significantly lower than those in HC ($t = 3.046$, $p = 0.0018$, 1-β = 82.3%) (Figure 1). On the other hand, no difference was found in serum proBDNF between the MDD patients and the HC ($t = -0.979$, $p = 0.833$) (Figure 2). Twenty-four of 51 MDD patients (47%) responded to fluvoxamine treatment at least within 4 weeks. No difference was found in baseline proBDNF between responders and nonresponders ($t = 1.837$, $p = 0.073$). No difference was also found in baseline BDNF between responders and nonresponders ($t = 1.19$, $p = 0.23$). A trend for negative correlation was found between baseline serum BDNF and baseline HAMD17 scores ($r = -0.183$, $p = 0.071$) (Figure 3). No correlation was however found between the HAMD17 scores and proBDNF at baseline ($r = 0.092$, $p = 0.421$) (Figure 4). No difference was found between serum levels of proBDNF ($r = 0.090$, $p = 0.336$) (Figure 5) and BDNF ($r = -0.084$, $p = 0.730$) (Figure 6) at week 0 in MDD patients. No changes were observed in serum levels of proBDNF at baseline, 2 weeks, and 4 weeks after administrating fluvoxamine ($F = 2.580$, $p = 0.080$) (Figure 7). No changes were also observed in serum levels of BDNF at baseline, 2 weeks, and 4 weeks after administrating fluvoxamine ($F = 0.579$, $p = 0.561$) (Figure 8). Furthermore, no correlation was observed between baseline HAMD17 scores and baseline proBDNF/BDNF in MDD patients ($r = -0.130$, $p = 0.190$) (Figure 9). No correlation was also found between plasma fluvoxamine levels at week 4 and the changes in HAMD17 scores ($r = 0.211$, $p = 1.514$) (Figure 10). No correlation was found

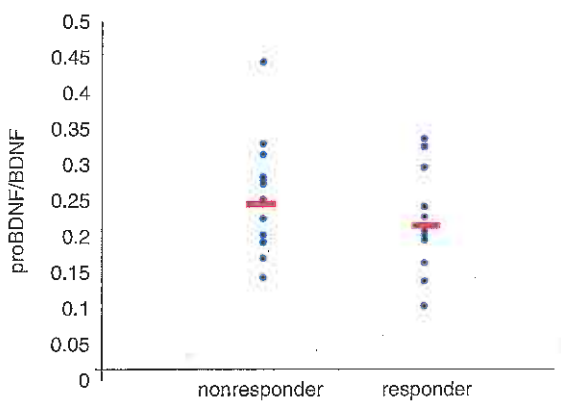

Figure 5 Serum BDNF change during treatment with fluvoxamine.

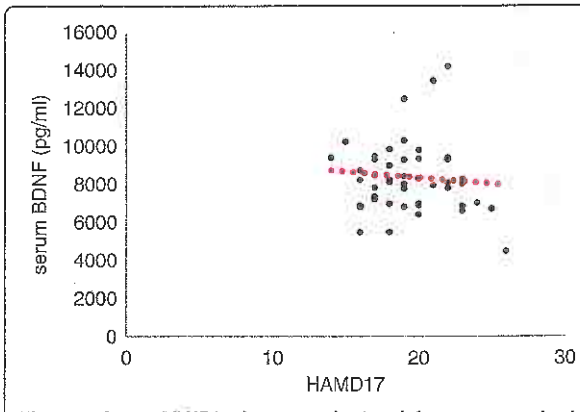

Figure 6 Serum BDNF in the responders' and the nonresponders' treatment with fluvoxamine. Red dot line shows regression line.

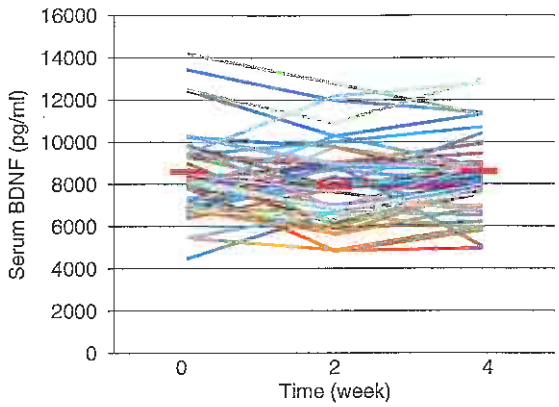

Figure 7 Serum BDNF change during treatment with fluvoxamine. Red line shows the mean of value.

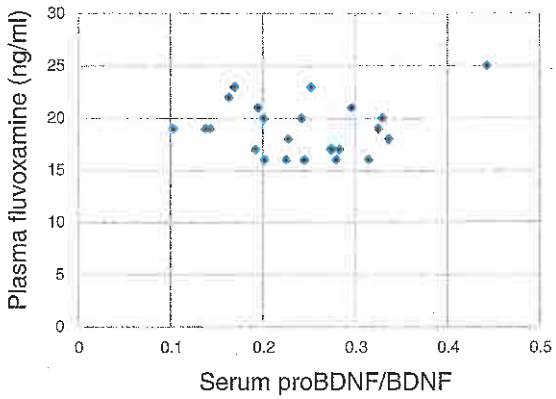

Figure 9 The proBDNF/BDNF ratio and plasma fluvoxamine concentration.

between plasma fluvoxamine levels at 4 weeks and the changes in serum BDNF levels (from 0 to 4 weeks) ($r = 0.117$, $p = 0.691$) (Figure 11).

Discussion

Recent meta-analyses demonstrated that mature BDNF levels in serum in patients with MDD were decreased compared to those in healthy controls. The result in the present study regarding serum BDNF confirms the recent meta-analyses [10,14,15]. Low serum mature BDNF levels increased over the course of antidepressant treatment [10,14,15]. We have previously reported that a significant correlation was found between the HAMD17 score and serum BDNF levels before pharmacotherapy [16]. In the present study, we reconfirmed our previous finding. A correlation was not however observed between serum proBDNF levels and the HAMD17 scores before starting fluvoxamine. In addition, there was no relationship between serum levels of proBDNF/BDNF and HAMD17. Taking these findings into account, the BDNF level, but not proBDNF and proBDNF/BDNF, reflects the severity of MDD. Moreover, no correlation was observed between serum fluvoxamine levels and serum levels of proBDNF/BDNF. The result in the present study suggests that the plasma fluvoxamine level was not independent of proBDNF/BDNF in MDD patients after fluvoxamine treatment. In addition, serum levels of BDNF and proBDNF/BDNF did not change at least 4 weeks after fluvoxamine administration. However, serum proBDNF increased during fluvoxamine treatment but did not reach the statistically significant level. Taking these findings into account, our hypothesis was not confirmed. In other words, the influence of fluvoxamine on serum levels of proBDNF, BDNF, and proBDNF/BDNF is complicated. Another interpretation is that 4 weeks is not enough to alter the dynamics of proBDNF and BDNF. Zhou et al. [17] reported that protein and serum levels of proBDNF were higher in MDD than in

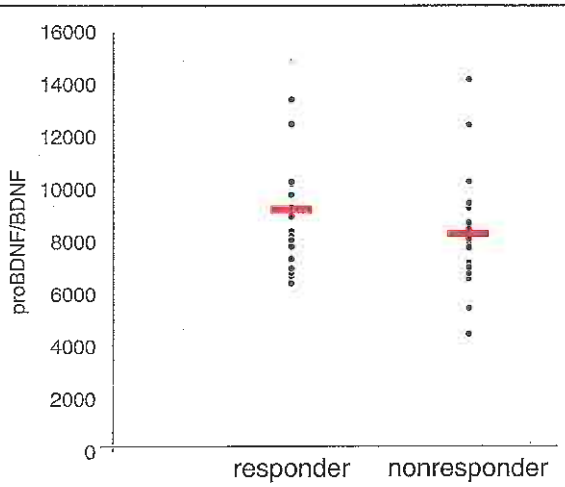

Figure 8 The proBDNF/BDNF ratio between the responders and the nonresponders. Red line shows the mean of value.

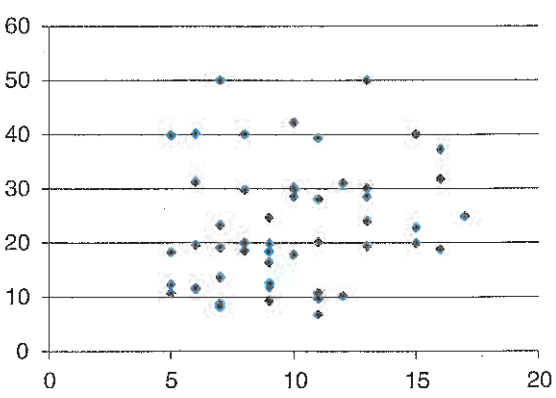

Figure 10 The changes in the HAMD17 (0–4 weeks) and plasma fluvoxamine concentration at week 4.

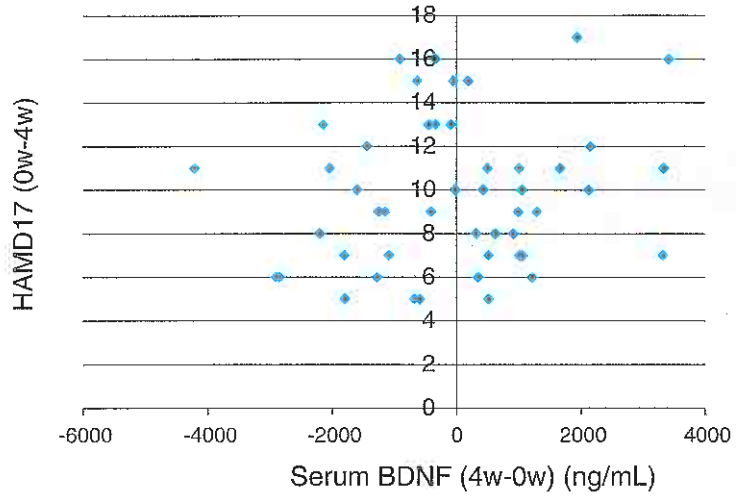

Figure 11 The changes in the HAMD17 (0–4 weeks) and plasma concentration at week 4.

healthy control subjects while BDNF levels were lower. The authors also demonstrated that the levels of BDNF and proBDNF negatively and positively correlated with major depression severity, respectively. These results suggest that the balance between proBDNF and BDNF is disturbed in MDD. Sodersten et al. [18] recently reported a very interesting finding using two independent cohort studies (Sahlgrenska cohort and Karolinska cohort). The authors found that serum MDNF, proBDNF, the ratio of BDNF/proBDNF, and interaction with MMP-9 were different between patients with bipolar disorders and healthy controls. The function of proBDNF however remains precisely elucidated.

Furthermore, there is little information about the role of serum proBDNF. We know that a controversy exists about the relationship between brain BDNF and peripheral BDNF. A recent study reported that circulating BDNF revealed a positive correlation with hippocampal BDNF, which reinforces the relevance to identify a potentially useful therapeutic biomarker [19].

No correlation was found between the changes in the HAMD17 scores and plasma fluvoxamine levels, which indicates that the effect of plasma fluvoxamine levels is independent of an individual's depressive clinical efficacy in fluvoxamine. In other words, the pharmacodynamic factors of each patient might also be involved in the effects of fluvoxamine. We should consider various factors for predicting the treatment response, and it could be more complicated in the fluvoxamine response. The present study had several limitations: (i) small samples, (ii) assaying serum proBDNF was tricky and the detection rate of serum proBDNF was very low using the ELISA kit, and (iii) we did not measure MMP-9 levels. Thus, we are undergoing reconfirmation of these preliminary results using another ELISA kit or Western blotting method.

Conclusions

We reconfirmed that serum levels of BDNF, but not proBDNF or proBDNF/BDNF ratio, in MDD were lower than those in healthy controls. Fluvoxamine however did not change serum levels of BDNF, proBDNF, and proBDNF/BDNF ratio at least within 4 weeks. Finally, no correlations exist between plasma levels of fluvoxamine and the changes in the HAMD17 scores or serum BDNF levels. In short, there is no association between serum levels of proBDNF, BDNF, or proBDNF/BDNF ratio and fluvoxamine response in MDD patients at least within 4 weeks of treatment. Using a different antidepressant medication on proBDNF/BDNF could be useful to determine the specificity of the effect of fluvoxamine.

Competing interests

Professor Nakamura has received grant support from Dainippon-Sumitomo Pharma Co., Tanabe-Mitsubishi Pharma Co., and Astellas Pharma Co., Ltd in 2013. The other authors declare that they have no competing interests.

Authors' contributions

RY designed the study, measured the serum BDNF, proBDNF, and plasma fluvoxamine, wrote the first draft, and managed the literature searches. TK performed the statistical analyses. HH, KA, AK, WU-N, and AI-S collected the clinical data. NI and JN wrote the final manuscript. All of the authors took part in either drafting the article or revising it critically for important intellectual content and approved the final manuscript.

Acknowledgements

We thank Ms. Kazuko Shimizu for her technical advice.

Author details

[1]Department of Psychiatry, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan. [2]Department of Psychiatry, Fujita Health University, Toyoake 470-1192, Japan.

Received: 11 March 2014 Accepted: 2 June 2014
Published: 9 July 2014

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doi:10.1186/1744-859X-13-19
Cite this article as: Yoshimura *et al.*: Serum proBDNF/BDNF and response to fluvoxamine in drug-naïve first-episode major depressive disorder patients. *Annals of General Psychiatry* 2014 **13**:19.

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ORIGINAL RESEARCH

Plasma levels of 3-methoxy-4-hydroxyphenylglycol are associated with microstructural changes within the cerebellum in the early stage of first-episode schizophrenia: a longitudinal VBM study

Joji Nishimura[1]
Shingo Kakeda[1]
Osamu Abe[2]
Reiji Yoshimura[3]
Keita Watanabe[1]
Naoki Goto[3]
Hikaru Hori[3]
Toru Sato[1]
Hidemasa Takao[4]
Hiroyuki Kabasawa[5]
Jun Nakamura[3]
Yukunori Korogi[1]

[1]Department of Radiology, University of Occupational and Environmental Health School of Medicine, Fukuoka, Japan; [2]Department of Radiology, Graduate School of Medicine, Nihon University, Tokyo, Japan; [3]Department of Psychiatry, University of Occupational and Environmental Health School of Medicine, Fukuoka, Japan; [4]Department of Radiology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; [5]MR Applied Science Laboratory Japan, GE Yokogawa Medical Systems, Hino-shi, Tokyo, Japan

Correspondence: Shingo Kakeda
Department of Radiology, University of Occupational and Environmental Health School of Medicine, 1-1, Iseigaoka, Yahatanishi-ku, Kitakyushu, Japan
Tel +81 93 603 1611
Fax +81 93 692 0249
Email kakeda@med.uoeh-u.ac.jp

Abstract: The aims of this study are to determine how the interval changes of the brain structures in the early stage of first-episode schizophrenia relate to the interval changes in the clinical data, including the clinical symptoms of schizophrenia and catecholaminergic measures (plasma homovanillic acid [HVA] and 3-methoxy-4-hydroxyphenylglycol [MHPG]). Regional brain volumes and fractional anisotropy (FA)/mean diffusivity (MD) with diffusion tensor imaging (DTI) were measured at baseline and 6-month follow-up in a 3T magnetic resonance imaging (MRI) system in a cohort of 16 schizophrenic patients, who were in their first episode at the time of baseline MRI. At the time of baseline and follow-up MRI, all 16 patients underwent evaluations that included a psychopathological assessment (Positive and Negative Syndrome Scale [PANSS]) and peripheral catecholaminergic measures (plasma MHPG or HVA). For interval changes between baseline and follow-up MRI data (morphological change, MD, and FA), the correlation/regression analysis was performed as a series of single regression correlations in Statistical Parametric Mapping 5, with the interval changes in PANSS or plasma HVA and MHPG as the covariates of interest. Positive and inverse correlations contrasts were created, and in this preliminary analysis, a family-wise error-corrected threshold of $P<0.05$ was considered significant. In the correlation/regression analysis, a positive correlation between the FA in the right cerebellar vermis and the MHPG was observed. No significant correlations between the brain volume or MD and any laboratory data (plasma HVA and MHPG) were found. During the 6-month follow-up in the early stage of first-episode schizophrenia, the MHPG changes were correlated with the microstructural FA changes in the cerebellum, which may reflect the functional connections of the noradrenergic system in the cerebellum.

Keywords: 3T MR imaging, apparent diffusion coefficient, fractional anisotropy, mean diffusivity, voxel-based morphometry, homovanillic acid

Introduction

Schizophrenia is a neuropsychiatric disorder that shows detectable evidence of brain structural abnormalities in the majority of afflicted individuals during the chronic stages.[1] The identification of the structural brain abnormalities in patients with schizophrenia, using magnetic resonance imaging (MRI), has become an important area of neuroimaging research in recent years. Several previous studies using a voxel-wise analysis have reported that the morphological changes are detectable on 3D MRI and/or the reduced fractional anisotropy (FA) on diffusion tensor imaging (DTI) in several regions at the first manifestation of schizophrenia (first-episode schizophrenia).[2–5] Use of voxel-wise analysis has also allowed correlation/regression analysis to be performed between the

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patient's MRI data and their clinical data. Recently, many studies have looked at the relationship between structural data (including DTI) and clinical data (including the Positive and Negative Syndrome Scale [PANSS]).[6,7] One study reported inverse correlations of FA values with PANSS positive symptom scores in the left uncinate fasciculus, right sagittal stratum, and the left superior longitudinal fasciculus.[7] In these studies, however, it seems unclear whether the relationship between the structural brain abnormalities and the clinical data really existed, because no study has evaluated the longitudinal changes in patients with first-episode schizophrenia.

The actions of antipsychotic drugs on the dopamine system have led to many examinations of dopaminergic metabolites as possible markers for psychosis and antipsychotic response. However, it has also become clear that the noradrenergic system has extensive interactions with the dopamine system, and may play a role in schizophrenia,[8] and may also have a key role in psychotic relapse.[9] Interactions between the two systems have been well-studied in the cerebrospinal fluid and plasma of schizophrenic patients through their respective metabolites; plasma homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) are the major degradation products of the monoamines dopamine and noradrenaline, respectively. Plasma HVA and MHPG are good predictors of response to antipsychotic treatment;[10–12] the effects of risperidone (a benzisoxazole derivative belonging to a family of atypical antipsychotic drugs) on plasma levels of HVA and MHPG have been related to its clinical efficacy in ameliorating the positive and negative symptoms of schizophrenia, respectively.[11,13] Moreover, recent studies reported that plasma levels of MHPG are decreased in patients in the early stage of first-episode schizophrenia,[10,14] which suggests that the MHPG may be a useful biomarker in the early stages of schizophrenia. Although it is of interest to explore whether and how anatomic deficits relate to clinical manifestations and alterations in brain physiology, we know of no voxel-wise correlation/regression analysis in which correlation between MRI data and peripheral catecholaminergic measures have been shown.

Herein, we report the results of a longitudinal MRI study of first-episode schizophrenia conducted to date, to our knowledge, using well-validated and highly reliable state-of-the-art neuroimaging tools. We measured regional brain volumes, FA, and mean diffusivity (MD) during an average of 6 months using high-resolution MRI with a 3T MRI system in a cohort of schizophrenic patients, who were in their first episode at the time of baseline MRI. The aims of this study were to determine how the interval changes of the brain structures in the early stage of first-episode schizophrenia relate to interval changes in the clinical data, including clinical symptoms of schizophrenia and peripheral catecholaminergic measures (plasma HVA and MHPG).

Materials and methods

Subjects

The diagnosis of schizophrenia, according to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV-TR), requires at least a 6-month duration of signs of the disturbance. In this study, a total of 26 patients who fulfilled the DSM-IV-TR criteria A, B, D, E, and F, were recruited for the study, except for the criterion regarding the disease duration. All subjects underwent baseline magnetic resonance (MR) examinations within 6 months from the date from which the patients fulfilled these criteria. Therefore, all baseline MR examinations were performed during the early stage of first-episode schizophrenia, before a diagnosis of schizophrenia was established.[6] After a 6-month follow-up, a diagnosis of schizophrenia was established in 23 of the 26 patients. The symptoms of the remaining patients had completely remitted spontaneously without any medication use preceding the 6-month follow-up. One of the 23 patients was subsequently excluded from the study, because the image quality was impaired by severe artifacts from dental materials. After a 6-month follow-up, the remaining 22 patients were invited for a repeat MR examination (follow-up MRI). This MRI was unavailable for six patients. Therefore, 16 patients (eight males, eight females; mean age: 29.0±11.6 years; age range: 13–52 years) were finally enrolled in the longitudinal study. All were considered to have been in the early stage of first-episode schizophrenia at the time of the baseline MR examinations. All patients were screened by using the Structured Clinical Interview for DSM-IV-TR Disorders (SCID; New York State Psychiatric Institute 1995), and exclusion criteria for all groups were: 1) current or past serious medical illness such as cancer, diabetes mellitus, epilepsy, and cerebral infarction; 2) dependence on alcohol; and 3) illicit substance use such as methamphetamine, narcotics, and marijuana.

The mean duration from the date from which the patients fulfilled the DSM-IV-TR criteria A, B, D, E, and F to the date of baseline MR examination for the patients was 2.9±2.5 months. The duration of psychosis (DUP) was defined as the day of emergence of several symptoms of psychosis to the day when baseline MR examination was performed. The mean DUP was 6.9±6.4 months; therefore, the mean duration from the date of onset of psychotic symptoms to the date on which the patients fulfilled DSM-IV-TR criteria A, B, D, E, and F was 4.1±6.1 months. These two items were reconfirmed by the

patients themselves and their family members. At baseline MR examination, the total cumulative exposure to a chlorpromazine equivalent dose of antipsychotic drugs was 214±117 mg (mean ± standard deviation [SD]). One of the 16 patients had not received any medications, and the rest of the 15 patients were treated with atypical antipsychotic drugs (risperidone, n=2; aripiprazole, n=6; olanzapine, n=5; quetiapine, n=2). At follow-up MR examination, the total cumulative exposure to a chlorpromazine equivalent dose of antipsychotic drugs was 390±218 mg. All 16 patients were treated with atypical antipsychotic drugs (risperidone, n=2; aripiprazole, n=6; olanzapine, n=5; quetiapine, n=2; perospirone, n=1).

At the time of baseline and follow-up MR examinations, all 16 patients underwent an evaluation, which included a psychopathological assessment (PANSS) and peripheral catecholaminergic measures (plasma HVA and MHPG).

Magnetic resonance imaging acquisition and image processing for voxel-based morphometry

For baseline and follow-up MR examinations, the MRI data were obtained using a 3.0-Tesla scanner with a three-dimensional fast spoiled gradient recalled acquisition with steady state (3D-FSPGR), which was acquired with parameters of 10/4.1/700 (repetition time [ms]/echo time [ms]/inversion time), a flip angle of 10°, a 24 cm field of view (FOV), and 1.2 mm thick sections 0.47×0.47×1.2 mm resolution. All images were corrected for image distortion due to gradient nonlinearity using 'GradWarp'[15] and for intensity inhomogeneity using 'N3'.[16] Image processing for voxel-based morphometry (VBM),[17] a fully automatic technique for computational analysis of differences in regional brain volume throughout the entire brain, was conducted using SPM5 (Statistical Parametric Mapping; v5; Institute of Neurology, London, UK). The 3D-FSPGR images in native space were bias-corrected; spatially normalized; segmented into gray matter, white matter, and cerebrospinal fluid images; and intensity-modulated using SPM5.[18] The DARTEL (Diffeomorphic Anatomical Registration Through Exponential Lie Algebra) toolbox was used in a high-dimensional normalization protocol. DARTEL was proposed by Ashburner as an alternative method of normalization in the SPM package.[19] In an intensity modulation step, voxel values of the segmented images were multiplied by the measure of warped and unwarped structures derived from the nonlinear step of the spatial normalization. This step converted the relative regional gray matter density into absolute gray matter density, expressed as the amount of gray matter per unit volume of brain tissue before spatial normalization. The resulting modulated gray and white matter images were smoothed with an 8 mm Gaussian kernel.

Diffusion tensor images: MRI scanning protocol

All subjects also underwent DTI examinations with the same scanner and at the same time as 3D-FSPGR. The methods of DTI acquisition and data analysis were similar to those in a previous study.[20] In brief, a single-shot, spin-echo planar sequence was used (repetition time/echo time [TR/TE] =12,000/83.3 ms; 4 mm slice thickness; no gap; FOV 26 cm; number of excitations =1, spatial resolution 1.02×1.02×4 mm). The diffusion properties were measured at a b-value of 1,000 s/mm^2 along 25 noncollinear directions. Eddy current correction and patient motion correction were performed on the diffusion-weighted basis images using Functional MRI of the Brain (FMRIB)'s Linear Image Registration Tool (FLIRT) from the FMRIB Software Library (FSL).[21] The images were corrected for image distortion due to gradient nonlinearity using 'GradWarp'. Individual FA and MD maps were calculated using the DTIFIT tool implemented in FSL.

Spatial normalization of DTI for SPM analysis

The echo planar sequence used for the acquisition of the diffusion tensor dataset suffers from inherent geometric distortion from magnetic field inhomogeneities. Moreover, the contrast of the FA map is quite different from that of T1-, T2-, or proton density–weighted template images provided with SPM5. Therefore, an FA template specific to this study was created using the data from all participants. Each T2-weighted echo-planar image was co-registered into the 3D-FSPGR image, and the co-registration parameter was applied to the corresponding FA map. The parameters of the normalization used in the spatial normalization step of the 3D-FSPGR images in native space onto the T1 template were also applied to the co-registered FA map. The normalized FA maps were smoothed with an 8 mm isotropic Gaussian kernel, and a mean image (FA template) was created. Thereafter, all FA maps in native space were transformed onto the stereotactic space by registering each of the images to the customized FA template. The normalized FA map was smoothed with an 8 mm isotropic Gaussian kernel.

Image processing for tract-based spatial statistics

The structural distortion of the diffusion-weighted MR images was corrected based on each T2-weighted echo

planar image (b=0 s/mm^2) by using eddy current correction in the FMRIB Diffusion Toolbox software program (v5.0.4; parts of the FSL). Non-brain tissue of each MR image was deleted using the brain extraction tool. Voxel-wise statistical analysis of the DTI data was performed by using Tract-Based spatial analysis (TBSS; v1.1) software program. The FA volumes were aligned to a target image as follows: 1) apply nonlinear registration of each subject's FA into the FMRIB58_FA_1 mm standard-space image as the target image; and 2) the target image was affine transformed to 1×1×1 mm MNI 152 (Montreal Neurologic Institute, Montreal, QC) space. A mean FA image was created by averaging the aligned individual FA images, and was then thinned to create an FA skeleton representing white matter tracts common to all subjects. For the FA skeleton, a threshold was set at 0.2 to exclude voxels with low FA values, which are likely to include grey matter or cerebrospinal fluid. Individual FA data and voxel-wise statistical results were projected onto this FA skeleton. Subsequently, the MD were projected onto the mean FA skeleton and also compared between groups at the same spatial location.

Statistical analyses of voxel-based morphometry and DTI

In order to examine the interval changes of brain volume, a new set of images (temporal subtracting image [TSI]) was generated by calculating an interval change on MR images for each patient by subtracting the follow-up images from the baseline images.[22] The TSI were created using the following formula: TSI = (baseline image – follow-up image)/0.5 (baseline image + follow-up image). Therefore, the TSI represents the brain volume differences between the follow-up and baseline images for each voxel. Positive voxel values on the image indicate that the baseline MR images had higher intensity values than the follow-up images; negative voxel values on the image indicate that the follow-up images had higher intensity values than the baseline MR images. The same method was applied for creating the TSI on either the FA or MD maps for SPM analysis. We used the FA or MD skeleton to calculate TSI for TBSS.

Measurement of plasma levels of HVA and MHPG

Plasma concentrations of HVA and MHPG were analyzed by high-performance liquid chromatography with electrochemical detection (HPLC-ECD). The plasma HVA levels were analyzed by HPLC-ECD according to the method of Yung et al with a slight modification.[23] The plasma MHPG levels were also analyzed by HPLC-ECD according to the method of Ohnishi et al.[24]

Correlational analysis

The interval changes in clinical or laboratory data were calculated for each subject by subtracting the follow-up data from the baseline data. For each interval change (TSI) between baseline and follow-up MR data (morphological changes, MD, and FA), the correlational analysis was performed as a series of single regression correlations in SPM5, with the interval changes of clinical data (positive and negative PANSS) or laboratory data (plasma HVA and plasma MHPG) as the covariates of interest. Positive and inverse correlations contrasts were created. Family-wise error (FWE) correction was applied. The significance level was set at false discovery rate (FDR)-corrected $P<0.05$. Significant clusters were identified by specific white matter tract through meticulous comparison to the MRI Atlas of Human White Matter.

Results

There was no significant difference in the global brain volume (total gray matter, total white matter, and intracranial volume) between baseline and follow-up MRI (Table 1). Both the positive and negative PANSS at follow-up MRI were significantly lower than those at baseline MRI. The plasma HVA and MHPG at follow-up MRI were significantly lower and higher, respectively, than that at baseline MRI. The mean duration between the baseline and follow-up MRI was 6 months. The laboratory data (plasma HVA and MHPG) were not associated with the dose of antipsychotic drugs both at baseline and after follow-up interval.

In the correlation/regression analysis, a positive correlation between the MHPG and the FA in the right cerebellar vermis was observed in the SPM analysis ([MNI coordinate x, y, z] on DTI map = [–6, –50, –26], FWE-corrected P=0.012, Z=5.05, T=9.80; Figure 1), whereas an inverse correlation between the FA and MHPG was not found in any brain region. The TBSS analysis also showed a positive correlation between the MHPG and FA in the white matter of the right cerebellar vermis ([MNI coordinate x, y, z] on DTI map = [–6, –51, –27], uncorrected $P<0.001$, cluster size =390; Figure 2), although no voxels could survive after correction for multiple comparisons. No significant correlations between the brain volume or MD and any laboratory data (plasma HVA and MHPG) were evident. There were no significant correlations between any MR data (brain volumes, FA, and MD) and PANSS.

Table 1 Demographic and clinical characteristic of the patients at baseline and follow-up MRI

	Baseline MRI	Follow-up MRI	Paired *t*-test
	Mean ± SD	Mean ± SD	*P*-value
Age	29.0±11.6		
Male/female	8/8		
Total intracranial volume (mL)	1,476.6±123.5	1,484.6±131.0	0.21
Total GM volume (mL)	665.2±61.1	665.2±74.4	0.99
Total WM volume (mL)	477.6±57.9	482.3±62.2	0.28
PANSS-P	16.8±5.5	12.4±4.9	0.02
PANSS-N	17.8±5.7	14.3±4.6	0.02
PANSS-G	27.2±3.5	21.0±3.4	<0.01
PANSS-T	61.7±11.8	47.6±10.8	<0.01
MHPG	4.3±2.0	5.2±1.4	0.01
HVA	7.2±2.1	6.3±1.3	<0.01
CPZ (mg)	214±117	390±218	0.01
GAF	36.1±8.4	51.9±11.9	<0.01

Abbreviations: CPZ, chlorpromazine; GAF, Global Assessment of Functioning; GM, gray matter; HVA, homovanillic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol; MRI, magnetic resonance imaging; ; PANSS-P, PANSS positive symptoms subscale; PANSS-N, PANSS negative symptoms subscale; PANSS-G, PANSS general psychopathology subscale; PANSS-T, PANSS total score; SD, standard deviation; WM, white matter.

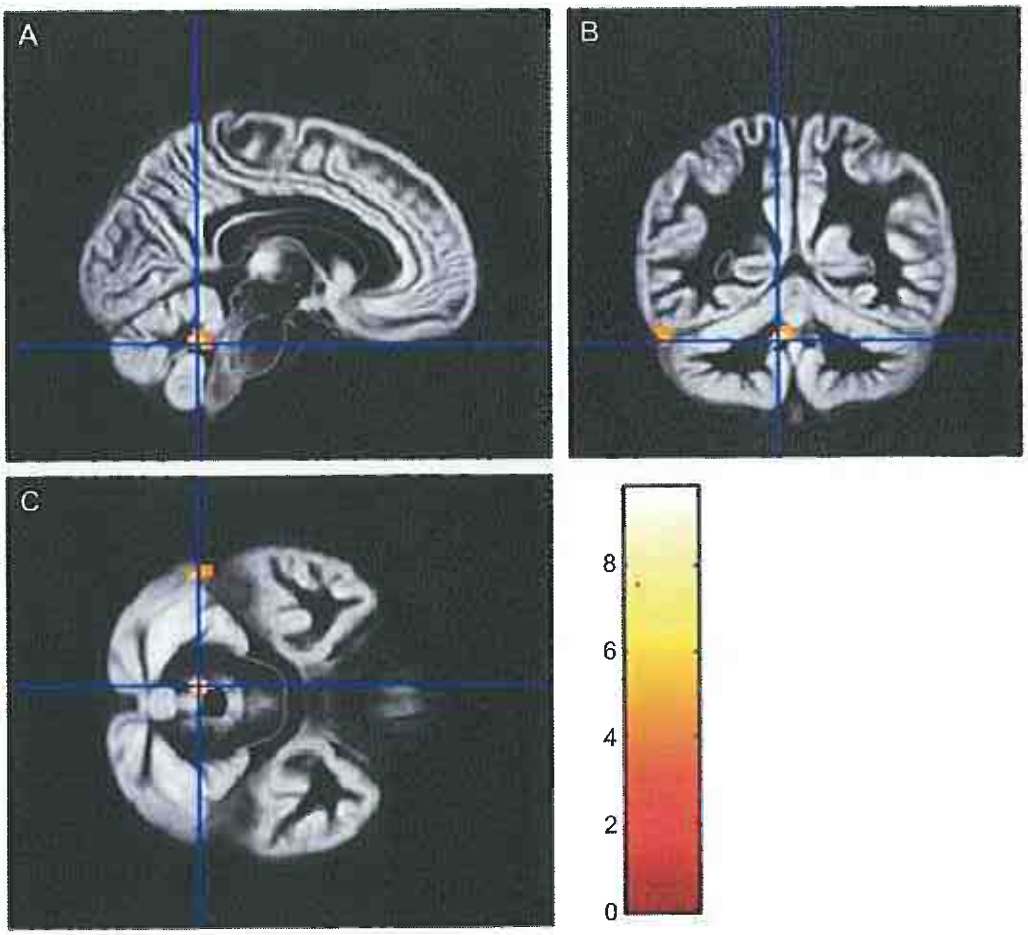

Figure 1 Correlation/regression analysis for 16 patients.
Notes: **(A)** Sagittal image; **(B)** Coronal image; **(C)** Axial image. Positive correlation of FA with plasma MHPG was seen in the cerebellar vermis. Blue lines indicate the most representative position and the color scale shows the T value. FWE-corrected threshold of $P<0.05$.
Abbreviations: FA, fractional anisotropy; FWE, family-wise error; MHPG, 3-methoxy-4-hydroxyphenylglycol.

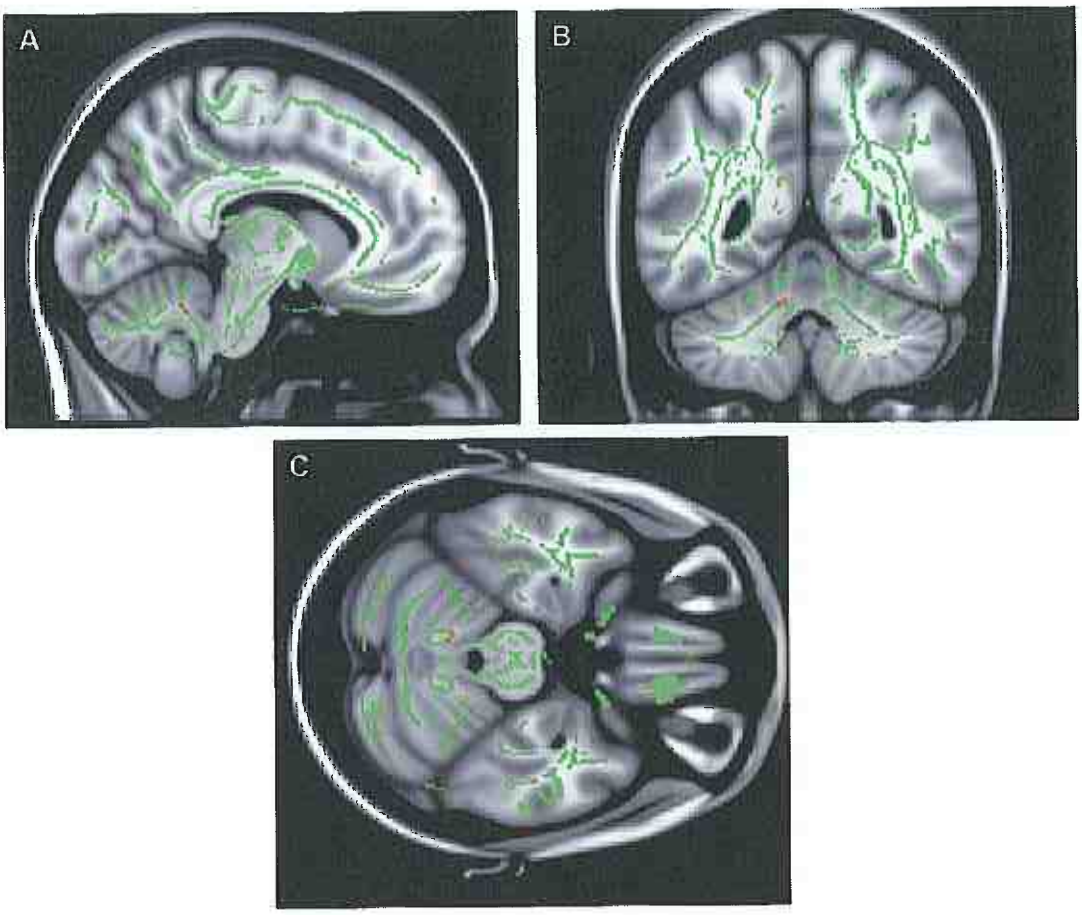

Figure 2 Correlation/regression analysis for 16 patients.
Notes: **(A)** Sagittal image; **(B)** Coronal image; **(C)** Axial image. Positive correlation of FA with plasma MHPG was seen in the white matter of the cerebellar vermis (red voxels). Green lines show an alignment-invariant tract representation (the "mean FA skeleton"). Uncorrected threshold of $P<0.001$.
Abbreviations: FA, fractional anisotropy; MHPG, 3-methoxy-4-hydroxyphenylglycol.

At the time of baseline and follow-up, no correlations were found in any brain region between any of the MR data (brain volumes, FA, and MD) and the laboratory data (plasma HVA and MHPG).

Discussion

Schizophrenia usually shows a prepsychotic phase of illness in which a change from premorbid functioning occurs.[23] This period is characterized by various mental state features, including nonspecific symptoms such as depressed mood and anxiety as well as subthreshold or attenuated psychotic symptoms. There is increasing interest in the potential for early detection and intervention during the early phase of a psychotic disorder.[25] Previous studies have reported that early detection and intervention in schizophrenia may offer a promising opportunity to redirect the illness' negative course.[26,27] In this study, all patients underwent the baseline MR examinations within 6 months from the time they fulfilled DSM-IV-TR criteria A, B, D, E, and F. Therefore, all baseline MR examinations were performed during the early stage of first-episode schizophrenia, before a diagnosis of schizophrenia was established. This is the first longitudinal MRI study to perform VBM analysis and voxel-based analysis of MD and FA maps computed from DTI obtained during the early stage of first-episode schizophrenia.

In the present study, we assessed the interval changes of MRI data and peripheral catecholaminergic measures during an average of 6 months using voxel-wise correlation/regression analysis, because there are individual variations in response to antipsychotic treatment; a positive correlation was observed between the FA in the right cerebellar vermis and MHPG. The reduced FA could be associated with microstructural alteration or damage involving the myelin sheath and/or directional coherence of fiber tracts. Some studies

assessed first-episode schizophrenia and reported lower FA values in the patients as compared to controls at widespread brain regions, including the cerebral peduncle.[2,3] Some studies conducted in schizophrenic patients demonstrated significantly lower FA than in controls in the cerebellum.[28,29] In the present study, plasma MHPG was significantly higher at follow-up than at baseline. This indicates that the FA value in the cerebellum, which has a positive correlation with plasma MHPG, may have increased over the follow-up interval, probably due to the effect of antipsychotic drug treatment.

The neurodegenerative hypothesis in schizophrenia suggests that pathologic alterations in brain morphology may occur after onset of the illness, mainly in the early stages, and may be associated with the illness' course and severity.[30] This hypothesis is supported by both longitudinal clinical and neuroimaging studies after onset.[1,31] Several prospective longitudinal studies in first-episode schizophrenia found progressive volume reduction at the initial stage of schizophrenia in the frontal and temporal areas, especially the superior temporal gyrus, Heschl's gyrus, and amygdala–hippocampal complex;[1,32,33] more pronounced volume reduction in these areas was associated with poor outcome, negative symptoms, and a decline in neuropsychological performance.[31,33] Furthermore, progressive reduction in cerebellum volume has also been reported in first-episode schizophrenia. In contradiction to this previous study, our results indicate that the FA changes (microstructural tissue alteration) within the cerebellum can be normalized during the early stage of first-episode schizophrenia.

Moreover, our results may also suggest that microstructural changes (FA changes) within the cerebellum relate to norepinephrine activity during the early stage of first-episode schizophrenia. Many regions of the brain are supplied by the noradrenergic systems. The principal centers for noradrenergic neurons are the locus coeruleus and the caudal raphe nuclei. The ascending nerves of the locus coeruleus project to the frontal cortex, thalamus, hypothalamus, and limbic system. Noradrenaline is also transmitted from the locus coeruleus to the cerebellum. Our positive correlation between the FA in the cerebellar vermis and norepinephrine metabolite MHPG may reflect these functional connections in the cerebellum as noradrenergic systems.

Traditionally, the emphasis of studies on cerebellar function has been on the coordination of somatic motor function, control of muscle tone, and equilibrium. However, the cerebellum also shares bidirectional connections with a large portion of the limbic lobe and the associated subcortical nuclei, the amygdaloid complex, the septal nuclei, and various hypothalamic and thalamic nuclei, which are regions of interest to psychiatry through their association with emotional processing. There is accumulating evidence of a cognitive role of the cerebellum,[30] including executive function and working memory, which are impaired in schizophrenia.[31] The previous neuroimaging studies by the resting-state functional MRI[34,35] and DTI method[28,29] suggest that the connectivity of the cerebellum is impaired in schizophrenia. Collin et al[34] demonstrated that, compared to healthy control subjects, schizophrenia patients showed impaired functional connectivity between the cerebellum and several cerebral regions, including the hippocampus, thalamus, middle cingulate gyrus, triangular part of the inferior frontal gyrus, supplementary motor area, and lingual gyrus. Furthermore, Liu et al[35] found that in schizophrenic patients, the bilateral cerebellum showed reduced functional connectivities to some regions compared to controls, such as the left middle temporal gyrus, bilateral middle cingulate cortex, right paracentral lobule, right thalamus, and bilateral cerebellum, and the FA of the left superior cerebellar peduncles was significantly reduced in patients. These results also support the opinion that the cerebellum might play a key role in schizophrenia.

Andreasen et al and Wiser et al proposed that disruption of a cortico–cerebellar–thalamic–cortical circuit (CCTCC) may underlie the combination of symptoms observed in schizophrenia.[36,37] This model of schizophrenia as secondary to disrupted development in the CCTCC has been termed 'cognitive dysmetria',[37] referring to incoordination in the processing, prioritization, retrieval, and expression of information. Our finding that the microstructural changes in the cerebellum occurred during 6-month follow-up in patients with first-episode schizophrenia may be consistent with theoretical accounts of schizophrenia as a disorder of functional integration, and with the cognitive dysmetria hypothesis, which posits a disconnection within the CCTCC as a fundamental abnormality in schizophrenia.

Recent studies have reported the relationship between MRI data and the PANSS. One study reported that a small area of white matter near the right insula showed a positive correlation between the PANSS negative symptoms and apparent diffusion coefficient.[38] Another study demonstrated that the positive correlation of FA values with positive symptom scores were seen in the white matter adjacent to the right lateral ventricle, and also found an inverse correlation between FA values in the same brain region and negative symptom scores.[6] To the best of our knowledge, however, no previous MRI studies have reported

the longitudinal relationship between MRI data and the PANSS in schizophrenic patients. Although the PANSS was improved significantly over the follow-up interval by ongoing antipsychotic drug treatment, correlations between longitudinal MR data and PANSS changes were not found in any brain regions. Therefore, clinical improvement may not necessarily be related to the microstructural changes of the brain. Whitford et al have reported the evidence of progressive white matter atrophy over the first 2–3 years of illness in patients with first-episode schizophrenia, although the psychotic symptoms (PANSS) in these patients improved over this interval.[39] The results from these previous studies may support our negative data.

There were several limitations to this study. Firstly, the number of patients was small. Although previous MR studies have reported the cerebellar volume abnormalities in schizophrenia patients,[40,41] our study did not show any correlation between brain volume and laboratory data in any brain region. Furthermore, in this study, the correlations between the longitudinal MR data and PANSS changes were not found in any brain regions. Therefore, our small sample size might limit the statistical power in these analyses. Secondly, our patients received various kinds of antipsychotic medication at baseline MR examinations, which may have affected the MR data and peripheral catecholaminergic measures. The DUP classically refers to the duration of untreated psychosis. In this sense, the value of DUP in the present study may not be precise, because the patients had been administered antipsychotic drugs before they underwent baseline MRI. However, it could be ethically problematic to follow the psychotic patients without any antipsychotic drugs until performing an MR examination. Thirdly, the initial symptoms of schizophrenia might start before the patients and their family notice a problem. Finally, the heterogeneity of our sample population may exist by the inclusion of a wide age range or clinical variables, such as disorganization score and intelligence quotient (IQ), in comparison with a previous study.[5,30,42–44] Although DeLisi et al reported that approximately 60% of plasma MHPG is derived from the brain,[42] it is currently believed that about one-third of plasma MHPG is of brain origin.[45] In short, we should note with caution the use of plasma MHPG as an indicator of activities in central noradrenergic neurons as it is predominantly derived from the periphery.

Conclusion

We found evidence that patients with first-episode schizophrenia exhibit a positive correlation of interval changes between the FA in the right cerebellar vermis and the norepinephrine metabolite MHPG during 6-month follow-up; this may reflect both anatomic and functional connections within the cerebellum to the prefrontal cortex, the subcortical limbic structures, and monoamine-producing brainstem nuclei. Our findings also suggest that microstructural FA changes could be reversible during the early stage of first-episode schizophrenia, and that plasma MHPG might therefore be a sensitive marker for the detection of this change.

Disclosure

The authors report no conflicts of interest in this work.

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Neuropsychiatric Disease and Treatment

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ORIGINAL RESEARCH

COMT Val158Met, but not *BDNF* Val66Met, is associated with white matter abnormalities of the temporal lobe in patients with first-episode, treatment-naïve major depressive disorder: a diffusion tensor imaging study

Kenji Hayashi[1]
Reiji Yoshimura[1]
Shingo Kakeda[2]
Taro Kishi[3]
Osamu Abe[4]
Wakako Umene-Nakano[1]
Asuka Katsuki[1]
Hikaru Hori[1]
Atsuko Ikenouchi-Sugita[1]
Keita Watanabe[2]
Satoru Ide[2]
Issei Ueda[2]
Junji Moriya[2]
Nakao Iwata[3]
Yukunori Korogi[2]
Marek Kubicki[5]
Jun Nakamura[1]

[1]Department of Psychiatry, [2]Department of Radiology, University of Occupational and Environmental Health, Kitakyushu, Japan; [3]Department of Psychiatry, Fujita Health University, Toyoake, Japan; [4]Department of Radiology, Nihon University School of Medicine, Tokyo, Japan; [5]Psychiatry Neuroimaging Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Correspondence: Reiji Yoshimura
Department of Psychiatry, University of Occupational and Environmental Health, Kitakyushu, 8078555, Japan
Tel +81 93 691 7253
Fax +81 93 692 4894
Email yoshi621@med.uoeh-u.ac.jp

Abstract: We investigated the association between the Val158Met polymorphism of the *catechol-O-methyltransferase* (*COMT*) gene, the Val66Met polymorphism of the *brain-derived neurotrophic factor* (*BDNF*) gene, and white matter changes in patients with major depressive disorder (MDD) and healthy subjects using diffusion tensor imaging (DTI). We studied 30 patients with MDD (17 males and 13 females, with mean age ± standard deviation [SD] =44±12 years) and 30 sex- and age-matched healthy controls (17 males and 13 females, aged 44±13 years). Using DTI analysis with a tract-based spatial statistics (TBSS) approach, we investigated the differences in fractional anisotropy, radial diffusivity, and axial diffusivity distribution among the three groups (patients with the *COMT* gene Val158Met, those with the *BDNF* gene Val66Met, and the healthy subjects). In a voxel-wise-based group comparison, we found significant decreases in fractional anisotropy and axial diffusivity within the temporal lobe white matter in the Met-carriers with MDD compared with the controls ($P<0.05$). No correlations in fractional anisotropy, axial diffusivity, or radial diffusivity were observed between the MDD patients and the controls, either among those with the *BDNF* Val/Val genotype or among the *BDNF* Met-carriers. These results suggest an association between the *COMT* gene Val158Met and the white matter abnormalities found in the temporal lobe of patients with MDD.

Keywords: catechol-O-methyltransferase, brain-derived neurotrophic factor, 3-methoxy-4-hydroxyphenylglycol, homovanillic acid

Introduction

Catecholamines play an important role in the pathogenesis of major depressive disorder (MDD).[1] Catechol-O-methyltransferase (COMT) is a methylation enzyme that plays a role in the degradation of noradrenaline and dopamine, by catalyzing the transfer of a methyl group from S-adenosylmethionine. Biochemical research has established that the enzyme activities in patients with MDD differ from those of nondepressed subjects.[2] The *COMT* gene is located at 22q 11.21. In a multicenter European study, an association was found between the *COMT* gene Val158Met (G324A) functional polymorphism and MDD.[3] The Val allele has been reported to result in three- to fourfold higher activity than the Met allele.[4] One report suggests that there is an association between higher activity of the *COMT* gene Val158Val-type and a poor antidepressant

treatment response.[5] The Met-variants of *COMT* gene Val-158Met were shown to be risk variants for depressed mood and low motivation in depressive Swedish men.[6]

Brain-derived neurotrophic factor (BDNF) is a molecular substrate of stress; data have demonstrated that BDNF expression is reduced by stress (an important risk factor for MDD and posttraumatic stress disorder)[7] and correlates to hippocampus volume in patients.[8] The levels of BDNF and its receptor, tropomyosin-related kinase B (TrkB) receptor, are decreased in regions of the hippocampus in postmortem tissue taken from suicide victims and patients with MDD, and in the serum of MDD patients.[9–11] Researchers have investigated the *BDNF* gene for a single nucleotide polymorphism (SNP) that might be linked to MDD. The most common *BDNF* SNP in humans is at codon 66, resulting in the Val66Met protein variant, which prevents the activity-dependent release of BDNF.[12] Men homozygous for the mutation might be at greater risk for MDD.[13] It has been hypothesized that monoamine and BDNF are associated with the pathogenesis of MDD.[14]

The white matter (WM) abnormalities constitute one element of the pathogenesis of MDD.[15–17] Various fiber tract alterations have been seen in MDD patients.[18–22] Magnetic resonance imaging (MRI) is a noninvasive method used to examine WM abnormalities. Diffusion tensor imaging (DTI) is an MRI technique that can study the orientation and integrity of WM fiber tracts in vivo.[23] DTI-based quantitative measures, such as fractional anisotropy (FA), represents intact myelin and axons, and has been shown to be a useful marker of the microstructural changes in WM.

Although several studies using a voxel-based DTI analysis demonstrated lower FA values in the frontal, temporal, and parietal lobes and the cerebellum of MDD patients,[24–28] such an analysis is not a mainstream of statistical parametric mapping and is not officially supported. Therefore, there has not been a consensus about the optimal method to spatially normalize FA images and the size of the smoothing kernel. A voxel-wise approach of tract-based spatial statistics (TBSS) has been introduced. The TBSS method projects all subjects' FA data onto an average FA tract skeleton before applying voxel-wise cross-subject statistics, and it minimizes the misalignment effects and is more robust and sensitive than voxel-based DTI analyses.[18]

The findings from individual reports of WM abnormalities in MDD patients indicate a widespread pattern of alterations, and the extent of WM abnormalities might be associated with clinical features. Indeed, the severity of illness and poorer treatment outcomes have been associated with increased WM pathology, indicating that patients with a greater illness burden are more likely to have microstructural damages.[29] The most pronounced WM FA reductions have been observed in the main body and genu of the corpus callosum, consistent with some, but not all, DTI reports in MDD. FA values of the WM in the right frontal lobe, right fusiform gyrus, left frontal lobe, and right occipital lobe were also demonstrated to be reduced. Fiber tracking has shown that the main fascicles involved were the right inferior longitudinal fasciculus, right inferior fronto-occipital fasciculus, right posterior thalamic radiation, and interhemispheric fibers running through the genu and body of the corpus callosum.[30]

Carballedo et al[31] recently reported that they observed a significant interaction, in the uncinated fasciculus, between a *BDNF* allele and diagnosis: patients carrying the *BDNF* Met allele had lower FA values in the uncinated fasciculus compared with healthy subjects carrying the Met allele. Kim et al[32] reported an association between altered WM connectivity and *COMT* gene Val158Met polymorphism in panic disorder patients. We hypothesize that the *COMT* gene and the *BDNF* gene are associated with WM connectivity in MDD patients.

In the present study, therefore, we compared the status of polymorphism of the *COMT* gene or *BDNF* gene and DTI findings between drug-naïve MDD patients and age- and sex-matched healthy controls.

Subjects and methods

Subjects

Thirty first-episode, right-handed, treatment-naïve outpatients were recruited. Major depressive episodes were diagnosed using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) according to the DSM-IV, text revision (TR) criteria. The severity of depression was evaluated using the 17-item Hamilton Rating Scale for Depression (HAMD17). Only those patients with a HAMD17 score ≥14 were eligible for the study. Exclusion criteria were: any history of neurological disease or other physical disease, and comorbidity with other mental disorders (no evidence of schizoaffective disorder, bipolar disorder, Axis II personality disorders, or mental retardation). In all, 17 subjects were male, and 13 were female. The age range was from 20 to 67 years, with a mean ± standard deviation (SD) age of 44±12 years. Similarly, 30 right-handed healthy subjects, 17 male and 13 female, with mean age 44±13 years were recruited from the community.

The DTI scans for all 60 subjects were performed on the day when each subject was enrolled. The 30 control subjects

were interviewed by the same psychiatrists that interviewed the MDD patients, using the Structured Clinical Interview for DSM-IV, nonpatient edition. None of the control subjects had a history of serious medical or neuropsychiatric illness or a family history of major psychiatric or neurological illness in their first-degree relatives, and all were well matched with the patients in terms of age, sex, and years of education. All subjects were given complete information about the procedures. Written informed consent was obtained from all subjects via forms approved by the local Ethics Committee of the University of Occupational and Environmental Health, Kitakyushu, Japan.

Methods

Diffusion tensor images: MRI scanning protocol

All MRI examinations were performed using a 3T MRI system (Signa® EXCITE™ 3T; GE Healthcare, Little Chalfont, UK) with an eight-channel brain phased-array coil. DTIs were acquired by a single-shot, spin-echo planar sequence, with the following parameters: TR/TE =12,000/83.3 msec; 4 mm slice thickness; no gap; field of view =26 cm; number of excitations =1, spatial resolution =1.02×1.02×4 mm. Diffusion gradients (b-value of 1,000 sec/mm^2) were always applied on two axes simultaneously around the 180-degree pulse. The diffusion properties were measured along 25 noncollinear directions. The spatial distortion of diffusion-weighted MRIs was corrected based on each T2-weighted echo-echo planar image (b=0 sec/mm^2)[33] using registration functional MRI of the brain (FMRIB) tools.

Image processing

Maps of FA were computed for all subjects from the DTIs, after eddy current correction and automatic brain extraction using the FMRIB Diffusion Toolbox, which is part of the FMRIB Software Library (The Oxford Centre for Functional MRI of the Brain, Oxford, UK).[34] We performed a voxel-wise statistical analysis of the DTI data using TBSS[35] (implemented in the FMRIB Software Library 4.1.6). The FA, radial diffusivity (RD), and axial diffusivity (AD) were created by fitting a tensor model to the raw diffusion data. Brain extraction was then performed using the Brain Extraction Tool 2.1.[36]

The FA data of all subjects were aligned into a common space by means of nonlinear registration.[37] Next, a mean FA image was created and thinned to create a mean FA skeleton representing the centers of all tracts common to the group. This skeleton was thresholded at FA >0.2. Each subject's aligned FA data were then projected onto this skeleton, and the resulting data were fed into a voxel-wise cross-subject statistical analysis. Subsequently, other relevant DTI output images (AD and RD) were projected onto the mean FA skeleton so that other diffusivity values could be compared between groups in the same spatial location.

We compared the DTI metrics between the MDD and control groups using a TBSS analysis.

Genotyping and serum catecholamine metabolites assay

Genomic DNA was extracted from peripheral leukocytes using a QIAamp® DNA Blood Kit (Qiagen, Venlo, the Netherlands) and was stored at −20°C until used for analysis. Genotyping for the presence of the *BDNF* Val66Met and *COMT* Val158Met polymorphisms was performed using direct sequencing in the region.

We analyzed the subjects' plasma concentrations of homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) by high-performance liquid chromatography with electrochemical detection (HPLC-ECD). The plasma HVA levels were analyzed by HPLC-ECD according to the method of Yeung et al,[38] with slight modification. In brief, each cyano-bonded solid-phase extraction cartridge was preconditioned with methanol, followed by glass-distilled water. To each cartridge we added 0.3 mL of plasma sample or standard, and 0.1 mL of working internal standard solution (5 ng of 5-hydroxyindolecarboxylic acid in 0.01 M KH_2PO_4, pH 7.2). The samples were deproteinized with 1 mL of acetonitrile. After mixing by vortex and centrifugation (1,760× *g*, 4°C for 10 minutes), an aliquot (5 µL) of supernatant was allowed to pass through the cartridge slowly, under a mild vacuum (15 mmHg). The cartridge was washed with 0.2 mL of distilled water and extracted containing 1 mL of ethylacetate, and then an aliquot was evaporated to dryness under nitrogen gas. After dissolution in mobile phase (200 µL), a 10 µL portion of this solution was injected into the HPLC system. The detection limit was 0.5 ng/mL, and the calibration curve was linear up to 40 ng/mL. The intra- and interassay coefficients of variation were 6% and 8%, respectively. The recovery rate was more than 80%.

The subjects' plasma MHPG levels were also analyzed by HPLC-ECD, according to the method of Minegishi and Ishizaki.[39] In brief, the plasma was separated by centrifugation at 600× *g* at 4°C. Extraction was performed under a vacuum using Bond-Elut columns (Varian Medical Systems, Inc., Palo Alto, CA, USA) prepacked with 100 mg of C18-bonded silica (40 µm) in a 1 mL capacity disposable syringe. The columns, which were inserted into a vacuum chamber connected to an

aspirator, were prepared by washing with 1 mL methanol followed by 1 mL of water. After the addition of 50 µL of a solution of vanillyl alcohol (internal standard equivalent to 5 ng/mL) to 1 mL of plasma, the samples were passed through the columns, followed by 0.75 mL of water to rinse off both residual samples and easily eluted hydrophilic compounds.

The adsorbed materials were eluted with 200 µL of methanol to a 0.1 M phosphate buffer (pH 4.8) mixture (40:60, v/v [volume/volume]). A 20 µL portion of this solution was injected into the HPLC system. The detection limit was 0.5 ng/mL, and the calibration curve was linear up to 40 ng/mL. The intra- and interassay coefficients of variation were 6% and 8%, respectively. The recovery rate was more than 80%.

Statistical analyses

The significance threshold for between-group differences was set at family-wise error (FWE)-corrected $P<0.05$; this was corrected for multiple comparisons across voxels by using the threshold-free cluster-enhancement option. The number of permutations was set to 20,000 in all voxel-wise analyses. The chi-square test was used to compare the number of patients with the *COMT* or *BDNF* genotype Val/Val, and the number of Met-carriers in both the MDD patient and the control groups. The unpaired *t*-test was used to compare the items of the HAMD17 scores, and the plasma levels of MHPG and HVA between the Val/Val group and Met-carriers in the MDD patient group. The unpaired *t*-test was also used to compare serum BDNF levels between the MDD patients and the healthy controls. A significance level of $P<0.05$ was used. Statistical procedures were performed using the Japanese version of SPSS, version 15 (SPSS Inc., Chicago, IL, USA).

Results

The genotype distributions of the *COMT* Val158Met polymorphism were determined in both the MDD patients and the control subjects, as shown in Table 1. The table provides the allele and genotype distributions as well as the chi-square and *P*-values of Hardy–Weinberg equilibria. As can be seen in Table 2, there were no significant differences among the MDD patients in each item of the HAMD17, with the exception that the responses to item 16 (weight loss) differed significantly between the *COMT* Val/Val group and the *COMT* Met-carriers.

The analysis of the plasma levels of catecholamine metabolites (MHPG and HVA) revealed no significant differences between the MDD patients and the controls (MHPG was 5.3±1.0 ng/mL for MDD and was 5.4±1.2 ng/mL for controls [$P=0.23$]; HVA was 5.5±1.4 ng/mL for MDD and was 5.1±0.8 ng/mL for the controls [$P=0.13$]). In addition, no significant differences between the Val/Val group and the Met-carriers were found in plasma MHPG, a major metabolite of norepinephrine (5.0±1.4 ng/mL [Val/Val group]; 4.9±1.5 [Met-carriers] [$P=0.85$]) or in plasma HVA, a major metabolite of dopamine (8.5±2.7 ng/mL [Val/Val group]; 8.7±2.7 ng/mL [Met-carriers] [$P=0.81$]). The serum BDNF levels were significantly lower in the MDD patients (4.8±0.4 ng/mL) compared with the controls (5.6±0.5 ng/mL) ($P=0.044$).

Table 1 Gene distribution of *COMT* and *BDNF* in patients with MDD and healthy controls

	Control	χ^2	*P*-value	Number of patients	χ^2	*P*-value
***COMT* Val158Met**						
GG	10			9		
GA	18	2.566	0.1099	16	0.222	0.5377
AA	2			5		
***BDNF* Val66Met**						
GG	18			15		
GA	10	0.14	0.7082	8	1.12	0.2900
AA	2			7		

Abbreviations: *BDNF, brain-derived neurotrophic factor; COMT, catechol-O-methyltransferase.*

Table 2 Between-group comparisons of scores on each item of the HAMD17, in MDD patients with the *BDNF* gene polymorphism

HAMD 17-item	Val/Val	Met-carrier	*P*-value
1	2.53±0.96	2.60±0.88	0.84
2	0.8±0.75	0.87±0.72	0.81
3	1.40±0.80	1.93±1.18	0.17
4	1.33±0.60	1.07±0.57	0.23
5	1.07±0.25	0.93±0.44	0.33
6	1.13±0.60	1.20±0.65	0.76
7	2.80±0.91	2.93±0.93	0.7
8	0.87±0.65	1.00±0.73	0.6
9	0.80±0.65	0.67±0.70	0.6
10	1.67±1.14	1.67±1.01	1
11	1.53±0.81	1.40±0.80	0.66
12	1.07±0.68	1.07±0.25	1
13	0.8±0.54	1.00±0.52	0.32
14	1.07±0.57	1.47±0.62	0.08
15	0.73±0.77	0.60±0.80	0.65
16	0.53±0.72	0.80±0.91	0.039
17	0.20±0.40	0.47±0.72	0.23
Total	19.50±6.35	21.70±4.91	0.31

Notes: 1, Depressed mood; 2, Feeling of guilt; 3, Suicide; 4, Insomnia early; 5, Insomnia middle; 6, Insomnia late; 7, Work and activity; 8, Retardation; 9, Agitation; 10, Anxiety (psychological); 11, Anxiety (somatic); 12, Somatic symptoms (gastrointestinal); 13, Somatic symptoms (general); 14, Genital symptoms; 15, Hypochondriasis; 16, Loss of weight; 17, Insight.
Abbreviations: *BDNF, brain-derived neurotrophic factor;* HAMD17, 17-item Hamilton Rating Scale for Depression; MDD, major depressive disorder.

Regarding *BDNF* Val66Met, no significant differences between the Val/Val group and the Met-carriers were observed in plasma MHPG (5.0±1.4 ng/mL [Val/Val group]; 4.9±1.5 [Met-carriers] [P=0.85]), plasma HVA (5.3±1.1 ng/mL [Val/Val group]; 5.0±1.3 [Met-carriers] [P=0.54]), or serum BDNF (5.4±1.2 ng/mL [Val/Val group]; 4.8±1.6 [Met-carriers] [P=0.39]). No differences were observed in any items of the HAMD17 between the Val/Val genotype and the Met-carriers (Table 3).

In the voxel-wise-based group comparison, no significant differences were observed regarding FA, AD, or RD, in all patients compared with the controls. We found a significant FA decrease (P<0.05) within the temporal lobe WM in the Met-carriers among the MDD patients compared with those of the healthy controls (Figure 1A–C), on the basis of the Johns Hopkins University (JHU) white-matter tractography atlas and the International Consortium for Brain Mapping DTI-81 WM labels (part of the FMRIB Software Library package). In the voxel-wise-based group comparison, there was no significant difference in FA, AD, or RD, between the MDD patients and the healthy controls.

After dividing the MDD patients into genotype subgroups, we found a significant FA decrease (P<0.05) in the temporal lobe among the MDD patients who were Met-carriers compared with the corresponding values among the healthy controls (Figure 1A–C). Significantly decreased AD in the temporal lobe (P<0.05) was also found in the Met-carrier MDD patients compared with the healthy controls (Figure 1D–F). Significantly decreased AD in the temporal lobe (P<0.05) was also found in the MDD patients compared with the controls (Figure 1D–F). Moreover, the genotype–diagnostic interaction effect on FA was seen in the same position (uncorrected P<0.05), although no voxels could survive after the correction for multiple comparisons (FWE <0.05). The results of the image analyses are shown in Table 4.

No significant differences were observed in FA or AD, at any brain regions, between the MDD patients with the *COMT*

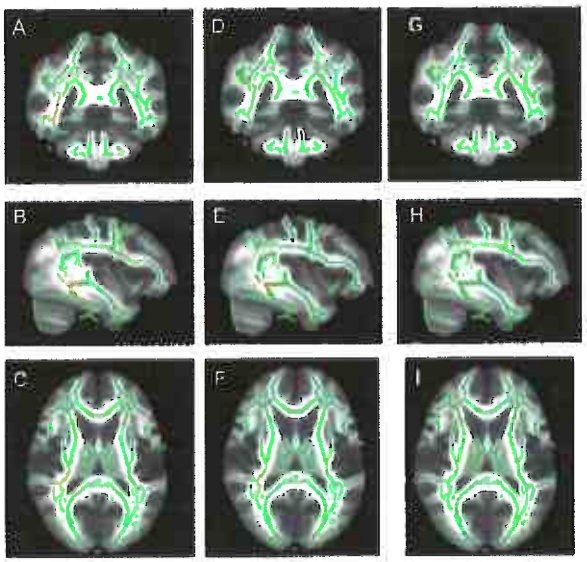

Figure 1 Corrected *P*-maps for the *COMT* gene polymorphism.
Notes: These maps show the regions where FA is reduced in the red voxels (**A–C**), where AD is reduced in the red voxels (**D–F**), and where RD shows no change (**G–I**). The FA, AD, and RD skeletons are projected in green on the MNI 152 template (Montreal Neurological Institute, Montreal, QC, Canada) average brain section.
Abbreviations: AD, axial diffusivity; *COMT*, *catechol-O-methyltransferase*; FA, fractional anisotropy; RD, radial diffusivity.

Table 3 Between-group comparisons of scores on each item of the HAMD17 for the two groups of patients with MDD

HAMD 17-item	Val/Val	Met-carrier	*P*-value
1	2.11±0.34	2.32±0.64	0.71
2	0.73±0.75	0.80±0.69	0.65
3	1.67±0.59	1.81±1.03	0.19
4	1.48±0.76	1.29±0.49	0.18
5	1.02±0.29	1.09±0.38	0.30
6	1.13±0.60	1.20±0.65	0.76
7	2.49±0.67	2.99±1.08	0.52
8	0.91±0.72	1.14±0.81	0.53
9	0.92±0.54	0.71±0.52	0.49
10	1.82±1.02	1.52±0.94	0.78
11	1.23±0.74	1.57±0.79	0.72
12	0.98±0.68	1.12±0.43	0.92
13	0.87±0.63	1.14±0.61	0.43
14	1.21±0.62	1.44±0.78	0.11
15	0.65±0.52	0.63±0.79	0.59
16	0.53±0.69	0.78±0.88	0.13
17	0.37±0.41	0.52±0.51	0.49
Total	20.33±7.29	20.97±5.84	0.51

Notes: 1, Depressed mood; 2, Feeling of guilt; 3, Suicide; 4, Insomnia early; 5, Insomnia middle; 6, Insomnia late; 7, Work and activity; 8, Retardation; 9, Agitation; 10, Anxiety (psychological); 11, Anxiety (somatic); 12, Somatic symptoms (gastrointestinal); 13, Somatic symptoms (general); 14, Genital symptoms; 15, Hypochondriasis; 16, Loss of weight; 17, Insight.
Abbreviations: HAMD17, 17-item Hamilton Rating Scale for Depression; MDD, major depressive disorder.

Table 4 The results of image analyses

Anatomical regions	Cluster size	*P*-value (FWE-corrected)	MNI coordinate x	y	z
FA analysis (HS > MDD in Met carriers)					
Right temporal	81	0.046	54	87	84
lobe	10	0.049	53	91	80
AD analysis (HS > MDD in Met carriers)					
Right temporal	151	0.046	58	90	87
lobe	9	0.049	51	81	71

Abbreviations: AD, axial diffusivity; FA, fractional anisotropy; FWE, family-wise error; HS, healthy subjects; MD, mean diffusivity; MDD, major depressive disorder; MNI, Montreal Neurological Institute.

Val/Val genotype and the healthy controls with the *COMT* Val/Val genotype. No significant difference was observed in RD between the MDD patients and the healthy controls, both among the *COMT* Val/Val group and the *COMT* Met-carriers (Figure 1G–I). In addition, there were no significant differences regarding FA, AD, or RD between the Val/Val group and the Met-carriers among the MDD patients, and no correlations in FA, AD, or RD were observed, at any regions of the brain, between the MDD patients and the healthy controls, both those with the *BDNF* Val/Val genotype and the *BDNF* Met-carriers (Table 4).

Discussion

In the genotype comparison (significant genotype–diagnosis interactions), we found that the reduction of the FA values in the temporal lobe was significantly larger in the MDD patients compared with the healthy subjects. FA has been shown to have an increased sensitivity to WM damage, as its decrease has been reported in the normal-appearing WM of patients with MDD.[24] The use of other DTI parameters, such as AD, which is related to axonal loss, and RD, which is associated with demyelination,[37,40] may increase the specificity of DTI to particular microstructural abnormalities. The most noteworthy finding in the present study was that the FA and AD, but not the RD, in the temporal lobe in the Met-carriers with MDD were significantly decreased compared with those in the healthy controls. These results may indicate that neuronal degeneration (axonal loss) can occur in the temporal lobe of Met-carriers with MDD.

In contrast, Seok et al[41] recently reported that FA reduction in the temporal lobe was significant only in the MDD patients with the Val/Val group of *COMT* Val158Met polymorphism. This finding indicates that MDD patients with a homozygote Val gene might have further abnormalities and brain pathological changes. Taken together, the above-described findings show that it is controversial whether the *COMT* Val158Met polymorphism is associated with structural changes of WM in the temporal lobe.

However, no significant differences were found in the plasma levels of MHPG and HVA between the present MDD patients and the control subjects. Depression is a heterogeneous condition characterized by multiple symptoms and subtypes. The different symptoms and subtypes are likely mediated by different neurocircuitry, and neurotransmitters such as noradrenaline, dopamine, and serotonin, and they might or might not be present in any particular individual with MDD. MDD might be characterized by an increase or a decrease in certain symptoms.

We reported that MDD patients with high plasma MHPG demonstrated severe anxiety and agitation, whereas those with low MHPG and/or HVA demonstrated severe psychomotor retardation.[42,43] We suspect that this is one of the reasons that no significant between-group differences were found in the catecholamine metabolites, in the present study.

In addition, each component of depressive symptoms might be related to the some brain regions and neurocircuits. Anhedonia, for example, has been found to be positively correlated with the ventromedial prefrontal cortex activity and negatively correlated with amygdala/ventral striatal activity in response to "happy" stimuli, using functional MRI (fMRI).[44] Psychomotor symptoms have been associated with frontal and caudate abnormalities in depression.[45] A recent fMRI study has shown that vulnerability to MDD is associated with temporofrontolimbic decoupling that is selective for self-blaming feelings.[46]

According to the meta-analysis of Liao et al[30] using DTI, there are four consistent locations of decreased FA in patients with MDD: WM in the right frontal lobe, the right fusiform gyrus, the left frontal lobe, and the occipital lobe. Fiber tracking showed that the main fascicles involved were the right inferior fronto-occipital fasciculus, the right posterior thalamic radiation, and interhemispheric fibers running through the genu and the body of the corpus callosum.

Taken together, these results indicate that *COMT* gene Val158Met polymorphism did not reflect the plasma and cerebrospinal fluid levels of catecholamine metabolites. The weight loss item scores of the HAMD17 were significantly lower in the present *COMT* Val/Val group than in the Met-carriers. It might be possible that the higher activity of COMT leads to reduced physical activity by influencing the catecholaminergic pathways.

The specificity of WM hyperintensities to age-associated vascular depression[47] reinforces the notion that MDD is a heterogeneous disorder. Although the data suggest that T2-weighted WM is related to late-onset MDD, findings suggestive of microstructural WM changes, as evinced by DTI, in young adults with MDD were reported by Li et al.[48] The age-associated relationship between WM and MDD may preclude the use of this trait to identify young individuals at risk of developing MDD. Nevertheless, an understanding of the mechanisms by which microvascular lesions lead to depression may help elucidate important pathophysiological pathways and facilitate the development of new treatments. In the present study, however, no correlations were found with the *BDNF* Val/Met polymorphism in patients with MDD.

On the other hand, Carballedo et al[31] reported that they observed a significant interaction between *BDNF*

alleles in the uncinate fasciculus and diagnosis. In short, their patients with the *BDNF* Met allele had smaller FA in the uncinate fasciculus compared with the patients in the Val/Val group and compared with healthy controls with Met allele. In the present study, we did not examine the regions of the temporal lobe in detail. One of the reasons for the discrepancy between the results of Carballedo et al[31] and those of the present study was that our MDD patients were at the early stage of depressive state and were drug-naïve.

Our finding that the serum BDNF levels in the MDD patients were lower than those in the healthy controls is in agreement with previous reports.[11,49–51] We also found that the *BDNF* gene Val66Met polymorphism was not associated with serum BDNF levels in patients with MDD and that the *BDNF* Val66Met polymorphism is independent of the WM disturbance in MDD. Taken together, these results indicate that the *BDNF* gene Val66Met polymorphism is not critical for WM disturbances in patients with MDD.

The present study has several limitations. The sample size was too small to allow a second statistical analysis. The sample was heterogeneous, and the severity of illness was relatively moderate. A replication study that accounts for these limitations should be performed to confirm our preliminary results. Since the *COMT* gene Met-carriers showed more decreased body weight (Table 2), the possibility that the finding reflected the changed distribution in the brain could not be completely ruled out.

In conclusion, we observed an association between the *COMT* gene Val158Met polymorphism and the reduction of FA and AD, but not RD, in the temporal lobe of patients with MDD.

Acknowledgment

Professor Jun Nakamura was supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan (grant number 21591494).

Disclosure

Professor Jun Nakamura has received grant support from Astellas Pharma, Tanabe-Mitsubishi Pharmaceutical Co Ltd, Otsuka Pharmaceutical Co Ltd, Eli Lilly and Company, Pfizer, Inc., and GlaxoSmithKline plc. The authors report no other conflicts of interest in this work.

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Abnormal White Matter Integrity in the Corpus Callosum among Smokers: Tract-Based Spatial Statistics

Wakako Umene-Nakano[1]*, Reiji Yoshimura[1], Shingo Kakeda[2], Keita Watanabe[2], Kenji Hayashi[1], Joji Nishimura[2], Hidehiko Takahashi[3], Junji Moriya[2], Satoru Ide[2], Issei Ueda[2], Hikaru Hori[1], Atsuko Ikenouchi-Sugita[1], Asuka Katsuki[1], Kiyokazu Atake[1], Osamu Abe[4], Yukunori Korogi[2], Jun Nakamura[1]

1 Department of Psychiatry, University of Occupational and Environmental Health, Kitakyushu, Japan, **2** Department of Radiology, University of Occupational and Environmental Health, Kitakyushu, Japan, **3** Department of Psychiatry, Kyoto University Graduate School of Medicine, Kyoto, Japan, **4** Department of Radiology, School of Medicine, Nihon University, Tokyo, Japan

Abstract

In the present study, we aimed to investigate the difference in white matter between smokers and nonsmokers. In addition, we examined relationships between white matter integrity and nicotine dependence parameters in smoking subjects. Nineteen male smokers were enrolled in this study. Eighteen age-matched non-smokers with no current or past psychiatric history were included as controls. Diffusion tensor imaging scans were performed, and the analysis was conducted using a tract-based special statistics approach. Compared with nonsmokers, smokers exhibited a significant decrease in fractional anisotropy (FA) throughout the whole corpus callosum. There were no significant differences in radial diffusivity or axial diffusivity between the two groups. There was a significant negative correlation between FA in the whole corpus callosum and the amount of tobacco use (cigarettes/day; $R = -0.580$, $p = 0.023$). These results suggest that the corpus callosum may be one of the key areas influenced by chronic smoking.

Citation: Umene-Nakano W, Yoshimura R, Kakeda S, Watanabe K, Hayashi K, et al. (2014) Abnormal White Matter Integrity in the Corpus Callosum among Smokers: Tract-Based Spatial Statistics. PLoS ONE 9(2): e87890. doi:10.1371/journal.pone.0087890

Editor: Gianluigi Forloni, "Mario Negri" Institute for Pharmacological Research, Italy

Received September 30, 2013; **Accepted** January 5, 2014; **Published** February 7, 2014

Funding: Professor Jun Nakamura has received grant support from Dainippon Sumitomo Pharma Co. Ltd. and Otsuka Pharmaceutical Co. Ltd in 2012. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have the following interests: This study was supported by Dainippon Sumitomo Pharma Co. Ltd. and Otsuka Pharmaceutical Co. Ltd. There are no patents, products in development or marketed products to declare. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials, as detailed online in the guide for authors.

* E-mail: wakako-u @med.uoeh-u.ac.jp

Introduction

The most prevalent substance dependence issue worldwide is tobacco smoking. Smoking leads to serious public health problems and preventable early deaths [1]. In neuroimaging researches, smoking has been associated with large-scale structural brain alterations [2,3,4]. Particularly, recent voxel-based morphometry (VBM) study, smoking was associated with reductions in cerebral grey matter in the prefrontal, anterior cingulate, parietal, and temporal cortices and the cerebellum [3] [4]. There was an inverse relationship between cortical volume or cortical thickness and exposure to smoking [3] [5]. Furthermore, lower gray matter density in the prefrontal cortex and higher density in the insula have also been observed in smokers [6]. In short, the results of previous studied were consistent.

Diffusion tensor imaging (DTI) is sensitive to water diffusion characteristics and has been developed as a tool for investigating the local properties of brain white matter [7]. There are three diffusion metrics derived from DTI data: fractional anisotropy (FA), which reflects the directionality of water diffusion and the coherence of white matter fiber tracts; axial diffusivity (AD), which measures the magnitude of diffusivity along the principle diffusion direction; and radial diffusivity (RD), which reflects the magnitude of diffusivity perpendicular to the principle diffusion direction [8] [9]. Furthermore, the mean diffusivity (MD) was also calculated from DTI data. In a previous study, higher FA was measured in the corpus callosum of smokers compared with age-matched nonsmokers [10]. Jacobsen et al. [11] examined FA among adolescent smokers and nonsmokers with and without prenatal exposure to maternal smoking. In this study, both prenatal exposure and adolescent exposure to tobacco smoke was associated with increased FA in the anterior cortical white matter. Adolescent smoking was also associated with increased FA in regions of the internal capsule that contain auditory thalamocortical and corticofugal fibers. Thus, the effect of smoking on FA was inconsistent, and the regions affected were various.

The novel voxel-wise approach of tract-based spatial statistics (TBSS) was recently introduced [12], restricting the evaluation of diffusion parameters to a white matter skeleton common to all subjects. Voxel-based DTI analysis is not a mainstream of SPM (Statistical Parametric Mapping; Institute of Neurology, London, UK) and not officially supported. Therefore, there has not been a consensus about the method to spatially normalize FA images and the size of the smoothing kernel. To overcome these problems TBSS has been proposed [12].

To date, only two studies existed investigated white matter in smokers using TBSS. First, Zhang et al. [13] investigated FA using TBSS analysis methods in smokers with schizophrenia and healthy

age-matched control subjects. The authors reported lower FA in the prefrontal white matter of a subsample of highly nicotine-dependent smokers and a negative correlation between FA and nicotine dependence, as measured by the Fagerström score. Second, Lin et al. [14] also reported that compared with nonsmokers, heavy smokers had lower FA in the left anterior corpus callosum. The results of two former studies were controversial, and the influence of smoking on white matter still remains unclear.

The objectives of the current study were 1) to investigate differences between smokers and nonsmokers in white matter, and 2) to examine relationships between white matter integrity and nicotine dependence parameters in the smokers.

Methods

Participants

Twenty smokers met the DSM-IV criteria for nicotine dependence; the score on the Tobacco Dependence Screener Scale (TDS) was used to diagnose nicotine dependence [15] The exclusion criteria included a current or past history of any comorbid neurological disorder, significant medical conditions, abnormal results on laboratory screening tests, or addiction to alcohol or other substances (with the exception of nicotine). We also performed the Mini International Neuropsychiatric Interview (MINI) [16] to rule out past or present history of comorbid psychiatric disorders. Finally, nineteen males were enrolled of this study. As part of the initial clinical evaluation, all smokers were asked to complete baseline questionnaires that assessed detailed demographic information and smoking-related clinical variables. The expiratory carbon monoxide (CO) levels of the smokers were measured using the Smokerlyzer system (Bedfont Scientific Ltd., Rochester, UK). Nicotine dependence levels were assessed with the Fagerström Test for Nicotine Dependence (FTND) [17]. Twenty age-matched nonsmokers with no current or past psychiatric history were included as controls. Finally, eighteen males were enrolled of this study. Alcohol use levels were assessed with the Alcohol Use Disorders Identification Test (AUDIT) [18] [19]. This study was approved by the Ethics Committee of the University of Occupational and Environmental Health. All the participants gave written informed consent to participate in the study.

Diffusion Tensor Images: Magnetic Resonance Imaging Scanning Protocol

All MR examinations were performed with a 3T MR system (Signa EXCITE 3T; GE Healthcare, Milwaukee, WI) with an 8-channel brain-phased array coil. The brain volume data were obtained with a three-dimensional fast spoiled gradient recalled acquisition with steady state (3D-FSPGR), which was acquired with parameters of 10/4.1/700 (repetition time msec/echo time msec/inversion time msec), a flip angle of 10 degree, a 24-cm field of view, and 1.2-mm-thick sections with $0.47\times0.47\times1.2$ mm resolution. Diffusion tensor images were acquired using a single-shot, spin-echo echo-planar sequence with the following parameters: TR/TE = 12000/83.3 msec, 4-mm slice thickness, no gap, 26-cm field of view; number of excitation = 1, and a spatial resolution of $1.02\times1.02\times4$ mm. Diffusion gradients (b value of 1,000 sec/mm^2) were always applied on each axis simultaneously around the 180-degree pulse. The diffusion properties were measured along 25 noncollinear directions. The structural distortion of diffusion-weighted MR images was corrected based on each T2-weighted planar image (b = 0 sec/mm^2) [20].

Volume Imaging Processing

All images were corrected for image distortion caused by gradient nonlinearity using "GradWarp" [21] and for intensity inhomogeneity using "N3" [22]. Image processing for VBM, a fully automatic technique for the computational analysis of differences in regional brain volume throughout the entire brain, was conducted using SPM8. The 3D-FSPGR images in native space were spatially normalized, segmented into grey matter (GM), white matter (WM), and cerebrospinal fluid images, and intensity-modulated using the Diffeomorphic Anatomical Registration Through Exponential Lie Algebra (DARTEL) toolbox on SPM8 [23]. Age was treated as confounding covariates. The DARTEL was proposed by Ashburner as an alternative method of normalization in the SPM package [24]. In an intensity modulation step, the voxel values of the segmented images were multiplied by the measure of warped and unwarped structures derived from the nonlinear step of the spatial normalization. This step converted the relative regional GM and WM density into absolute volume, expressed as the amount of per-unit volume of brain tissue before spatial normalization. The resulting modulated GM and WM images were smoothed with an 8-mm Gaussian kernel. An analysis of covariance model was used to examine group differences in GM and WM, with age as a covariate. These analyses yielded statistical parametric maps {SPM (t)} based on a voxel-level height threshold of $p<0.001$. Topological false discovery rate (FDR) correction was applied. The significance level was set at FDR-corrected $p<0.05$.

DTI Image Processing using TBSS

FA maps were computed for all subjects from the diffusion-tensor images after eddy current correction and automatic brain extraction using the FMRIB Diffusion Toolbox, which is part of the Functional MR Imaging of the Brain Software Library (FMRIB) Software Library [25]. The data were corrected for spatial distortion caused by gradient nonlinearity using grad_unwarp [21]. A voxel-wise statistical analysis of the DTI data was performed using TBSS [12] and implemented in the FMRIB Software Library 4.1.6 (University of Oxford, Oxford, UK) [25]. The FA, AD, RD and MD images were created by fitting a tensor model to the raw diffusion data. Brain extraction was performed using the Brain Extraction Tool 2.1 [26]. The FA data for all the subjects were aligned into a common space via nonlinear registration [27]. Next, a mean FA image was created and thinned to create a mean FA skeleton representing the centers of all tracts common to the group. This skeleton had a threshold of FA>0.20. Each subject's aligned FA data were then projected onto this skeleton, and the resulting data were fed into a voxel-wise cross-subject statistical analysis. Subsequently, other relevant DTI output images (AD, RD, MD) were projected onto the mean FA skeleton to compare other diffusivity values between groups in the same spatial location.

Diffusion tensor metrics were compared between groups with unpaired t test adjusted for the subject's age using TBSS analysis. The significance threshold for between-group differences was set at $p<0.05$; this was corrected for multiple comparisons across voxels using the threshold-free cluster-enhancement option. The number of permutations was set to 20,000 in all voxel-wise analyses.

DTI Imaging Process Tract-Specific Analysis

FA maps were computed using dTV II and VOLUMEONE1.72, developed by Masutani et al. [28] (University of Tokyo; diffusion tensor visualizer available at http://www.volume-one.org/). Diffusion tensors were computed, and fiber tracts were created by interpolation along the z-axis to obtain data

(voxel size $2.0\times2.0\times3.0$ mm^3). Color coded maps were created using 26 sets of images (25 sets with b = 1,000 s/mm^2, 0 set with b = 0 s/mm^2). Fiber tracts were based on a fiber assignment that was made using the continuous tracking approach [29] to obtain a three-dimensional tract reconstruction. The fiber tracts were initiated by placing a seed area in the anatomical regions through which the particular fibers were expected to course [26]. Tract measurements of the corpus callosum were performed by one of the authors (K.W.), who was blind to the status of each subject. The seed ROI was placed manually, including the entirety of the corpus callosum, on a reconstructed midsagittal image with a non-diffusion-weighted image (b = 0 s/mm^2). Tractographic images of the corpus callosum were generated with threshold values of line-tracking termination FA>0.18. Voxelization along the corpus callosum was then performed. To reduce the partial volume effect of the peripheral portion of the tract and to eliminate small incidental artifactual lines, we used a shape-processing technique based on mathematical morphology [28]. In this shape-processing technique, we dilated the voxels once and eroded them twice. The FA values in coregistered voxels were calculated.

Statistical Analysis

All statistical analyses were conducted using SAS Version 9.3 (SAS Institute, Cary, North Carolina, USA). The Mann-Whitney U test and the chi-squared test were used to compare the variables between the smokers and nonsmokers. Pearson's correlation coefficient was used to examine the relationship between FA levels and smoking-related clinical variables. The level of significance was set at $p<0.05$.

Results

Subject Demographics

Table 1 shows the backgrounds of the smokers (N = 19) and the nonsmokers (N = 18). All were males and there was no significant difference in the distributions of age (Smokers: 40.5±8.6, Nonsmokers: 40.5±8.6) Detailed information about smoking-related clinical variables and the AUDIT scores was not available for three subjects (N = 16). The mean AUDIT score for the smokers was significantly higher compared to that of the nonsmokers (smokers: 9.1±6.3, nonsmokers: 4.9±4.4; $p = 0.022$).

Total Volumes

There was no significant difference in the global brain volume (total gray matter and total white matter) between the smokers and the nonsmokers (Table 1). The regional volumetric analysis showed no significant group difference in any regions.

TBSS and Tract-Specific Analysis Results

Compared with the nonsmokers, the smokers exhibited a significant decrease in FA throughout the corpus callosum (Figure 1). There were no significant differences in AD or RD. In addition, there was a significant negative correlation between FA throughout the whole corpus callosum and the amount of tobacco use (cigarettes/day) covariant age ($R = -0.580$, $p = 0.023$; Figure 2), but there were no significant correlations between the amount of tobacco use and AD, RD or MD ($p = 0.522$, $p = 0.678$, $p = 0.850$). No significant correlations were observed between FA and the duration of tobacco use, the Brinkman index, the TDS scores, the FTND scores, CO levels, or the mean AUDIT scores.

Discussion

The current study provides novel evidence of decreased FA throughout the whole corpus callosum in smokers. Based on this result, we evaluated the relationship between FA levels of corpus callosum and smoking-related clinical variables using tract-specific analysis. This analysis has the advantage of detecting abnormalities in specific white matter tracts, whereas TBSS can influence the outcome by multiple comparisons [30]. We found a significant inverse correlation between FA levels and the amount of tobacco consumptions. No significant differences regarding AD, RD or MD in any region of brain were observed. Our finding is concordant with the results of Lin et al. [14], which observed decreased FA in the left anterior corpus callosum, but not concordant with a significantly decreased AD and a significantly increased RD. It is generally believed that AD mainly reflects axonal integrity [31] and RD is more related to the integrity and thickness of the myelin sheaths covering the axons [32]. Animal

Table 1. Background of smokers and non-smokers.

	Smokers (N = 19)*	Non-Smokers (N = 18)	P
Age (years)	40.5±8.6 (27–54)	36.4±8.0 (30–61)	0.094
AUDIT	9.1±6.3	4.9±4.4	0.022
Age of first use (years)	19.6±1.4		
Duration of use (years)	19.8±8.5		
Amount of use (cigarettes/day)	18.8±7.4		
Brinkman index (daily number of cigarettes × year)	394.1±271.0		
Scores of TDS	6.9±1.7		
Scores of FTND	3.7±1.5		
CO levels (ppm)	16.4±12.8		
Total gray matter volume	718.5±52.9	738.5±52.1	0.186
Total white matter volume	541.2±46.8	545.2±39.5	0.750

Abbreviation. AUDIT: Alcohol Use Disorders Identification Test; TDS: Tobacco Dependence Screener Scale; FTND: Fagerström Test for Nicotine Dependence; CO; carbon monoxide.

*Detailed information about the smoking-related clinical variables and the AUDIT scores of three subjects (N = 16) were not available. The mean AUDIT scores of the smokers (N = 16) was significantly higher than that of the nonsmokers (N = 18; smokers: 9.1±6.3; nonsmokers: 4.9±4.4; p = 0.022).

doi:10.1371/journal.pone.0087890.t001

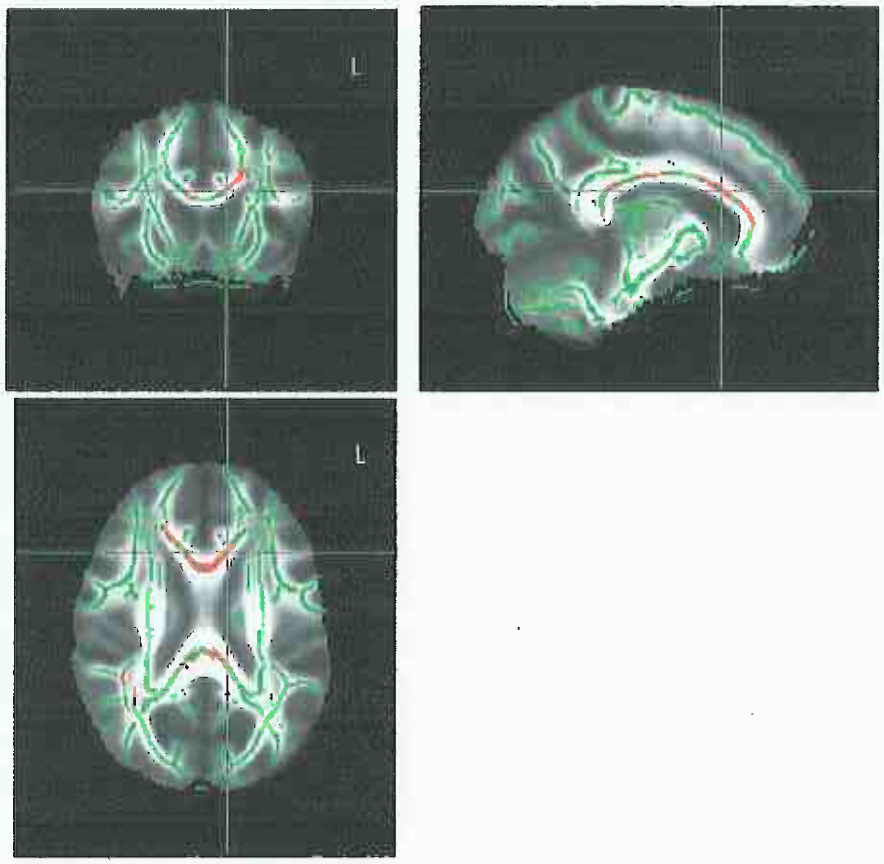

Figure 1. TBSS and tract-specific analysis results. The red voxels represent the areas where the fractional anisotropy (FA) levels of smokers were significantly reduced with respect to those of nonsmokers. Compared with nonsmokers, the smokers exhibited a significant decrease in FA throughout the whole corpus callosum.
doi:10.1371/journal.pone.0087890.g001

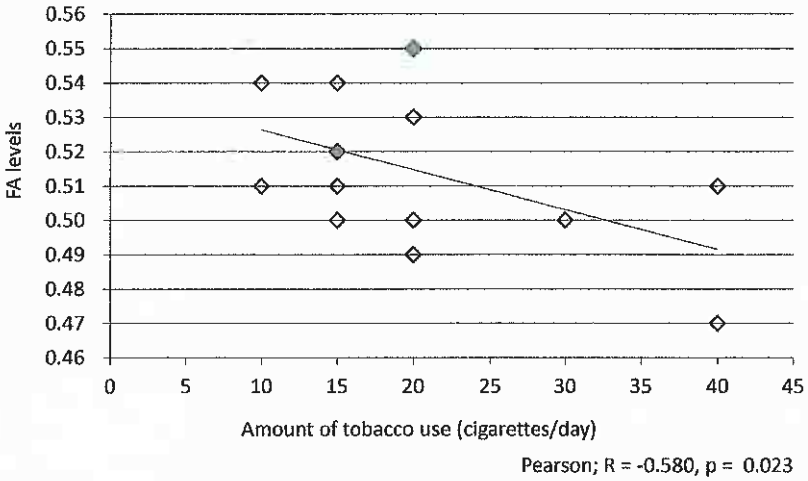

Figure 2. The correlation between FA levels throughout the whole corpus callosum and the amount of tobacco use (N = 16). There was a significant negative correlation between FA levels throughout the whole corpus callosum and the amount of tobacco use covariant age (cigarettes/day; R = − 0.580, $p = 0.023$). The number of spots was decreased from 16 to 13 because three spots represented the same data (three represented the FA level = 0.52 and N = 15, and two represented the FA level = 0.55 and N = 20; these spots are shown in gray spots).
doi:10.1371/journal.pone.0087890.g002

DTI studies have demonstrated that decreased AD with the degeneration, and increased RD with demyelination [32,33]. And the decreased FA in corpus callosum of smokers is likely the manifestation of axonal damage and disrupted myelin integrity in the region. Our subjects were lower FTND score rather than Lin et al. [14], which suggests our samples were not heavy smokers. In other words, not much amount of smoking might influence only in FA.

Zhang et al. [13] also reported lower-than-normal FA in a section of the left prefrontal white matter in a subsample of highly dependent smokers, and no smoking-related differences in other white matter areas. This inconsistency may arise from the comorbidity of psychiatry disorder in a part of our samples both smokers and healthy controls.

The corpus callosum connects the brain hemispheres and facilitates communication between left- and right-side brain structures; it is the largest white matter fiber tract connecting the neocortex of the two hemispheres [34]. The anterior parts of the corpus callosum connect the frontal cortices, while the body and the splenium connect the parietal, temporal, and occipital homotopic regions [35]. Compromised fiber connectivity within the corpus callosum has been previously reported in subjects with substance addictions [36]. Decreased FA in the genu, body and splenium of the corpus callosum has been associated with alcohol-dependent subjects [37]. Significant reductions in FA in the genu and rostral body [38] and in the body and splenium of the corpus callosum [39] have been reported in cocaine-dependent subjects. Methamphetamine abusers showed reduced white matter integrity in the genu [40] and the rostral body [41] of the corpus callosum. There was a significant reduction in FA in the genu and the isthmus of the corpus callosum in opiate-dependent subjects [42]. Qiu et al. [43] recently reported that heroin-dependent individuals had significantly lower FA throughout the brain, including the genu and the isthmus of the corpus callosum, compared with controls. Taken together, these findings suggest that substance abuse may be associated with reduced FA levels that reflect less microstructural integrity. In contrast, Paul et al. [10] reported that smokers exhibited higher levels of FA in the corpus callosum than nonsmokers did. The authors hypothesized that either nicotine-induced cytotoxic cell swelling secondary to nicotine-induced osmotic imbalances or vasogenic swelling characterized by plasma fluid leaking into the parenchymal interstitial space might lead to alterations in the white matter. According to Hudkins et al. [44], FA may rise during the early years of smoking and subsequently decline with continued smoking in later years. Negative correlations between the age at which the subjects began smoking and FA have been observed [41]. Our finding in the present study is in accordance with that of Hudkins et al. [41]. In short, it is plausible that nicotine bidirectionally influences FA in the corpus callosum based on smoking duration or/and smoking amount. The subjects of Lin et al. [14] were also older ages. The reason of discrepancy about location of corpus callosum between whole corpus callosum in this study and anterior corpus callosum in Lin et al. [14] study might be differences sample characteristics (our subject was only male), level of cigarette smoking (our subject was lower FTND score) and alcohol consumption.

There were several limitations to our study. First, the sample size was small and this might lead absence of finding a change in the frontal cortex in the RD and AD Second, we did not evaluate healthy controls using any structured interviewed. Therefore, we might not completely exclude comorbidity psychiatric disorders among both samples. Third, the results may have been influenced by alcohol intake, given the higher AUDIT scores among the smokers (Table 1) and we could not evaluate comparing two groups covariant with scores of AUDIT. The level of score of AUDIT among smokers did not correspond to alcoholism, and there was not a significant correlation between FA level and mean AUDIT scores. Taking together, it is not ruled out that cigarette smoking increases alcohol consumptions, which might influence the results. Forth, we did not measure plasma levels of nicotine and cotinine, a metabolite of nicotine. We are currently undergoing the new study taking account of above problems.

In conclusion, we found that FA in smokers was significantly decreased compared with that of nonsmokers. Moreover, an inverse correlation between the amount of cigarette smoking and FA throughout the whole corpus callosum, which may shed light on the vulnerability of the region after long-term exposure in nicotine.

Author Contributions

Conceived and designed the experiments: WU-N RY SK HT. Performed the experiments: WU-N SK KH HH AI-S AK KA J. Nishimura. Analyzed the data: WU-N SK KW. Contributed reagents/materials/analysis tools: WU-N RY SK KW KH JM SI IU. Wrote the paper: WU-N RY SK KW HT OA YK J. Nakamura.

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