おわりに

mTORの機能異常はさまざまな精神神経疾患の病態に関与しており，従来の治療薬とは異なる治療標的になりうる．幸いにもrapamycin，エベロリムス，ケタミンなど既存の薬剤によるエビデンスが，基礎臨床の両面において蓄積されつつある．これら既存の薬剤の臨床応用とともに，mTORを標的とした精神神経疾患の新規治療薬の開発が進むことが期待される．

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総説

自閉症スペクトラム障害の病態解明と治療薬開発を目指して

自閉症スペクトラム障害の分子薬理学的研究

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要約：自閉症スペクトラム障害（以下，自閉症）は，対人相互関係やコミュニケーションの障害を主な特徴とする発達障害である．自閉症の病態解明や治療薬開発のために，現在まで様々な自閉症モデル動物が作製され，解析されてきた．本稿では自閉症の発症に関わる分子の中でも特にmammalian/mechanistic target of rapamycin（mTOR）シグナル系の分子を中心に紹介しながらこれまでの主な自閉症モデル動物を示し，さらに薬物投与によって改善が見られた自閉症モデル動物の研究をあげ，今後のヒトへの応用と治療薬開発に向けた考察を行う．

1．はじめに

自閉症は0.6～1％の割合で発症する小児発達障害の一つであり，発症における男女比は4：1と比較的男子に多く見られる(1,2)．自閉症の特徴的な症状として，対人相互関係の質的障害，コミュニケーション障害，繰り返し行動，常同行動，興味の限局，感覚刺激に対する反応の亢進あるいは低下を示すことがあげられる(3)．また自閉症患者によっては，体の使い方において不器用さを示すことがある(3,4)．このため，自閉症に罹患した子供たちが家庭や学校において快適な生活を送るためには自閉症の特性に配慮した指導や支援を必要とする．また，彼らが就業し地域の中における社会生活を送るためには，周囲の人々の自閉症の特性への理解や支援が重要となってくる．

自閉症は遺伝子における異常や，胎内における感染・発達神経毒への曝露，さらには他の疾患と合併して発症することが知られている(5)．これまで自閉症の要因を調べるために，自閉症関連遺伝子の探索研究が精力的に行われ，シナプスの形成や機能，神経細胞の分化・移動，転写の制御に関連する遺伝子が見出されてきた(6)．自閉症の病態や効果的な治療法を調べるために，自閉症関連遺伝子を改変した自閉症モデル動物が作製され，行動学的，生化学的，電気生理学的な手法などを用いて詳細に調べられてきた．また，神経系以外での表現型を有するが，高い確率で自閉症を合併する自閉症スペクトラム障害関連症候群が存在する．これらの症候群には，脆弱X症候群や結節性硬化症などが含まれる(7)．自閉症スペクトラム障害関連症候群の原因遺伝子の改変動物も作製され，自閉症モデル動物として解析されている．

本稿においては，現在まで扱われている自閉症モデル動物の一部を紹介し，さらに薬理学的な処置によって病態の改善を報告した研究をあげ，自閉症モデル動物を用いた研究から今後の現場におけるヒトへの還元に至るまでを概説していく．

2．自閉症モデル動物

これまで自閉症に関連することが報告されてきた主な分子と自閉症モデル動物について示す（表1）．最初に当研究室において現在研究を推進しているTSC1とTSC2が関与するmTORシグナル系について説明を行い，mTORシグナル系分子に関連した自閉症モデル動物を示す．mTORシグナル系は多くの細胞プロセスに関わり，なかでもTSC1，TSC2のヘテロ欠損によって自閉症関連症候群である結節性硬化症が引き起こされることが知られている(8)．

1）mTORシグナル系分子

mTORとは抗腫瘍薬及び免疫抑制薬であるrapamycin

キーワード：mTORシグナル系分子，自閉症，rapamycin
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表1　自閉症関連因子とモデル動物の一覧

自閉症関連因子	モデル動物
遺伝子異常	
Tsc	*Tsc1*$^{+/-}$，*Tsc2*$^{+/-}$マウス
Pten	*Pten* コンディショナル KO マウス
Nf1	*Nf1*$^{+/-}$マウス
Fmr1	*Fmr1* KO マウス
Nlgn	*Nlgn1* KO マウス
Mecp2	*Mecp2* KO マウス
Shank	*Shank3* KO マウス
神経ペプチド欠損	
Oxytocin	Oxytocin KO マウス
発達神経毒曝露	
Valproic acid	胎生期にバルプロ酸に曝露されたラットあるいはマウスの仔

の標的タンパク質であり，哺乳類で見出されたホモログは mammalian/mechanistic target of rapamycin（mTOR）と名付けられた(9)．mTOR は PI3K ファミリーに属するセリン／スレオニンキナーゼであり，2つの異なる複合体として存在する（図1）．1つは mTOR complex 1（mTORC1）であり，もう一方は mTOR complex 2（mTORC2）である．mTORC1 はタンパク質合成，脂質合成，オートファジー，エネルギー代謝に関与し，mTORC2 は細胞の生存／増殖，細胞骨格の調節に関与している(10)．mTORC1 と mTORC2 に共通するタンパク質は，mTOR，mammalian lethal with sec-13 protein 8（mLST8），DEP domain containing mTOR-interacting protein（DEPTOR）である．regulatory-associated protein of mammalian target of rapamycin（Raptor），proline-rich Akt substrate 40 kDa（PRAS4）は mTORC1 のみが有し，なおかつ mTORC1 は rapamycin に対して感受性がある(9)．rapamycin-insensitive companion of mTOR（Rictor），mammalian stress-activated map kinase-interacting protein 1（mSin1），protein observed with rictor 1 and 2（Protor）は mTORC2 のみが有しており，mTORC2 は rapamycin に対して感受性を持たないと考えられていたが，rapamycin の慢性投与においても mTORC2 の活性を抑えることが報告されている(11)．神経細胞において，*N*-methyl-D-aspartate（NMDA）受容体や tyrosine receptor kinase B（TrkB）受容体からのシグナルは phosphoinositide 3-kinase（PI3K），phosphatidylinositol-dependent kinase 1（PDK1）を介し Akt（protein kinase B）を活性化させる．Akt は tuberous sclerosis complex 2（TSC2）に作用して，これを抑制する．TSC2 は Ras homolog enriched in brain（Rheb）に対する抑制作用を失い，Rheb が脱抑制（＝活性化）

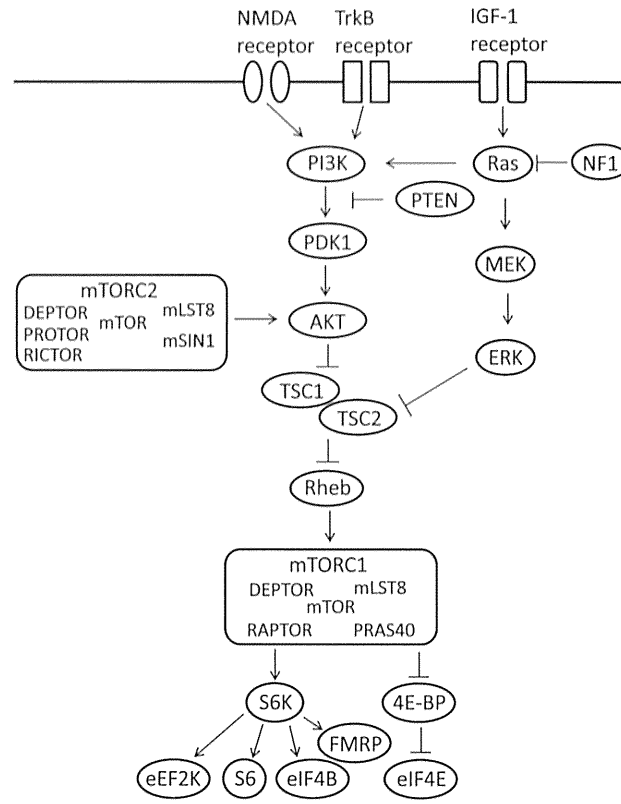

図1　mTOR シグナル系分子
protein kinase B (Akt), DEP domain containing mTOR-interacting protein (DEPTOR), eukaryotic initiation factor 4E (eIF4E)-binding protein (4E-BP), eukaryotic initiation factor 4B (eIF4B), eukaryotic translation initiation factor 4E (eIF4E), eukaryotic translation elongation factor 2 kinase (eEF2K), extracellular signal regulated kinase (ERK), the fragile X mental retardation protein (FMRP), insulin-like growth factor 1 (IGF 1), mitogen-activated protein kinase/extracellular signal regulated kinase kinase (MEK), mammalian lethal with sec-13 protein 8 (mLST8), mammalian stress-activated map kinase-interacting protein 1 (mSin1), mTOR complex 1 (mTORC1), mTOR complex 2 (mTORC2), neurofibromatosis type 1 (NF1), *N*-methyl-D-aspartate (NMDA), phosphatidylinositol-dependent kinase 1 (PDK1), phosphoinositide 3-kinase (PI3K), phosphatase and tensin homolog deleted from chromosome 10 (PTEN), proline-rich Akt substrate 40 kDa (PRAS40), protein observed with rictor 1 and 2 (Protor), regulatory-associated protein of mammalian target of rapamycin (Raptor), RAS homolog enriched in brain (Rheb), rapamycin-insensitive comparison of mTOR (Rictor), ribosomal protein S6 (S6), p70 S6 kinases (S6K), tyrosine receptor kinase B (TrkB), tuberous sclerosis complex 1 (TSC1), tuberous sclerosis complex 2 (TSC2)

して mTORC1 の活性化に至る．mTORC1 の下流において p70 S6 kinases（S6K）や eukaryotic initiation factor 4E（eIF4E)-binding proteins（4E-BP）のリン酸化を介してタンパク質合成を促進する．また，insulin-like growth factor（IGF-1）受容体からのシグナルは，Ras を介して mitogen-activated protein kinase/extracellular signal regulated kinase kinase（MEK），extracellular signal regulated kinase（ERK）を活性化すると TSC2 をリン酸化し，TSC1 と TSC2 の複合体を不活化する．

①TSC1，TSC2

Tsc1$^{+/-}$マウスと*Tsc2*$^{+/-}$マウスにおいては海馬に関する学習や記憶の障害が恐怖条件付け学習やモリスの水迷路学習を用いて報告された(12, 13)．*Tsc1*$^{+/-}$マウスにおいて，見知らぬマウスと同じケージに15分間入れると，*Tsc1*$^{+/-}$マウスの見知らぬマウスに対する探索行動の時間が野生型マウスの探索行動時間に比べて減少したことから，自閉症の主な特徴である社会性行動の低下が示された(12)．一方で，*Tsc2*$^{+/-}$マウスにおいて3-チャンバー型の社会性行動テストを用いて調べると，見知らぬマウスがいるカップが置かれたチャンバーに滞在する時間は，野生型マウスと有意な差はない(13, 14)．また超音波啼鳴*を指標にした母仔間における社会性を調べた研究では，野生型母マウスと*Tsc2*$^{+/-}$母マウスの仔マウスに対して，母仔分離（1回目の母仔分離→母マウスに再会→2回目の母仔分離）を行うと，野生型母マウスの仔マウス（野生型，*Tsc2*$^{+/-}$）や*Tsc2*$^{+/-}$母マウスの仔マウス（*Tsc2*$^{+/-}$）は1回目より2回目の母仔分離において超音波啼鳴の増加を示したが，*Tsc2*$^{+/-}$母マウスの仔マウス（野生型）のみ増加を示さなかった(15)．また同研究において雌雄別に解析を行うと*Tsc2*$^{+/-}$母マウスのオスの仔マウスのみ，遺伝子型を問わず母仔分離における超音波啼鳴に異常が見られることが示された．この研究から*Tsc2*$^{+/-}$母マウスと仔マウスにおける社会性行動（超音波啼鳴を指標にした母仔間コミュニケーション）は，仔マウスの遺伝子型や性別との相互作用によって影響を受けることが示唆された．これらの*Tsc2*$^{+/-}$マウスを用いた研究から，*Tsc2*$^{+/-}$マウスにおける社会性行動についてはマウスの週齢や測定方法，指標によって異なることが示唆された．私たちの研究室において，ホームケージで見知らぬマウスと出合わせるテストを用いて*Tsc1*$^{+/-}$マウスと*Tsc2*$^{+/-}$マウスの見知らぬマウスに対する探索行動を調べたところ，野生型マウスに比べて*Tsc1*$^{+/-}$マウスと*Tsc2*$^{+/-}$マウスの両マウスにおいて探索行動時間が短縮していることから，*Tsc1*$^{+/-}$マウスと*Tsc2*$^{+/-}$マウスにおいては社会性行動の低下が示されることを見出した(16)．また自閉症ではシナプスにおける興奮／抑制のバランスの破綻が病態の一つとして考えられている(17)．近年の研究においては，*Tsc1*$^{fl/fl}$マウスを作製し，synapsinをプロモーターとしたGFP-IRES-Creをコードするレンチウィルスに感染させ，全ての神経細胞からTSC1を欠損させたマウスから海馬の切片を作製し微小抑制性シナプス後電流を測定したところ，野生型マウスに比べてTSC1を欠損させたマウスにおける微小抑制性シナプス後電流が減少しており，シナプスの興奮性と抑制性のバランスが破綻していることが報告された(18)．Tangらは*Tsc2*$^{+/-}$マウスの社会性を3-チャンバー型の社会性行動テストを用いて調べると，*Tsc2*$^{+/-}$マウスは見知らぬマウスへのsniffing時間が野生型マウスに比べて短く，なじみのあるマウスと新奇なマウスへのsniffing時間を比較すると，野生型マウスが新奇なマウスに対してsniffing時間が増加するのに対して*Tsc2*$^{+/-}$マウスはなじみのあるマウスと新奇なマウスへのsniffing時間が変わらなかった．また同研究において，マウスの頭頂皮質における感覚野と視覚野の第5層における錐体細胞の樹状突起スパインの密度を生後29〜30日齢において比較すると野生型マウスに比べて*Tsc2*$^{+/-}$マウスの密度が高いことを報告している(19)．

②Phosphatase and tensin homolog deleted from chromosome 10（PTEN）

mTORシグナル系においてPI3K-Aktシグナルを抑制するPTENの変異は大頭症を伴う自閉症患者に伴ってみられる(20)．PTENの神経細胞におけるコンディショナルノックアウト（KO）マウスの自閉症様行動が調べられている．このマウスの脳における形態的な特徴は大頭症が見られることである(21, 22)．PTENの神経細胞におけるKOマウスは野生型マウスと比較すると，オープンフィールドテストにおいて中心部滞在時間が減少していることやケージ内の見知らぬマウスに対する探索行動の時間が減少していることから不安の亢進と社会性の低下が示されている(21)．さらに同研究においては，PTENの神経細胞におけるコンディショナルKOマウスは野生型マウスに比べて，海馬の歯状回におけるシナプス数の増加や大脳皮質における樹状突起スパインの密度の増加を示すことも報告されている．またメスの*Pten*$^{+/-}$マウスにおいても，3-チャンバー型の社会性行動テストで見知らぬメスマウスがいるカップがあるチャンバーにおける滞在時間がメスの野生型マウスの滞在時間に比べて減少していることから社会性行動の低下が示されているが，オスの*Pten*$^{+/-}$マウスの社会性行動は障害されていない(23)．

③4E-BP2

mTORC1は4E-BPをリン酸化し，さらにリン酸化された4E-BPがeIF4Eに結合すると，mRNA翻訳の開始を抑制することでタンパク質合成を制御する(10)．4E-BPには3つの類似遺伝子，*4E-BP1*，*4E-BP2*，*4E-BP3*が存在するが，このうち*4E-BP2*は哺乳類の脳に多量に存在する(24)．Gkogkasらは4E-BP2をコードする*Eif4ebp2*をKOしたマウスを作製し解析した(25)．*Eif4ebp2*をKOしたマウスは，3-チャンバー型の社会

性行動テストを用いて社会性行動を測定すると，見知らぬマウスがいるチャンバーにおける滞在時間が野生型マウスの滞在時間に比べて短く，また自己毛づくろい（固執性の行動の指標とされている）をする時間が野生型マウスに比べて長く，さらに超音波啼鳴を測定すると啼鳴の回数，時間が野生型マウスに比べて増加していることが示された．同研究において海馬のCA1におけるシナプスにおいては*Eif4ebp2*をKOしたマウスは野生型マウスに比べて微小興奮性シナプス後電流の振幅と発生頻度が増加していることが示され，シナプスにおける興奮／抑制のバランスの破綻を示した．しかし，このマウスにおいてシナプス接着分子であるNeuroligin1をノックダウンすると，社会性行動が回復しシナプスにおける興奮／抑制のバランスの破綻も回復することが報告されている．このため，4E-BP2–eIF4Eにおけるタンパク質の翻訳調節がNeuroligin1合成に関与し，自閉症の表現型に寄与することが示唆されている．

④ NF1

IGF-1受容体からのシグナルを受けるRasを抑制するNF1の異常は，神経線維腫症を引き起こす．神経線維腫症の患者は認知や学習に障害を有するが，20～30%の患者において自閉症と診断されることが報告されている(26)．*Nf1*$^{+/-}$マウスは，モリスの水迷路学習において学習の障害を示し，海馬における長期増強の障害を示す(27)．また，3-チャンバー型の社会性行動テストにおいて，野生型マウスがなじみのあるマウスがいるチャンバーより，新奇なマウスがいるチャンバーにおける滞在時間が増加するのに対して，*Nf1*$^{+/-}$マウスは双方のチャンバーの滞在時間において有意な差はなかったことが示されている(28)．

⑤ FMR1

脆弱X症候群は，男児に多く発症し，遺伝性が確認されている小児発達障害であり，自閉症に類似した症状を伴う．この症候群はX染色体上のfragile X mental retardation 1（FMR1）遺伝子の5′非翻訳領域のCGG繰り返し配列が異常に延長し，FMR1遺伝子がコードするthe fragile X mental retardation protein（FMRP）（標的mRNAの翻訳を抑制する）を産出できなくなることで発症する(29)．なじみのあるマウスと新奇なマウスへの探索時間において，野生型マウスは新奇なマウスに対してより探索時間が長いが，*Fmr1* KOマウスは新奇なマウスに対する探索時間が野生型マウスに比べて短いことや(30)，なじみのあるマウスと新奇なマウスにおける双方への探索時間に差はないことが示されている(31)．一方で，*Fmr1* KOマウスはミラーチャンバーテストにおいて，センターのミラーにいる割合が少ないことから，野生型マウスに比べて不安が亢進していることや，また社会性行動においては見知らぬマウスに対するsniffing時間が野生型マウスに比べて増加することから社会性行動が亢進していることが報告された(32)．これらの研究から*Fmr1* KOマウスは社会性行動において異常を持つことが示された．さらに*Fmr1* KOマウスの海馬においては，AktやmTORのリン酸化が亢進し，PTENのリン酸化が減少していることが報告されている(33)．

これらのmTORシグナル系分子の異常を有するマウスの研究から，mTORシグナル系における異常が自閉症の病態に寄与する可能性が考えられる．

2）その他の研究されている分子

(1) シナプス関連分子

① Neuroligin

Neuroligin（Nlgn）はシナプス後膜にある接着分子であり，シナプスの形成や維持に関与しており，自閉症の原因遺伝子として最初に同定された．Nlgnの中でも，自閉症との関連では特にNlgn3とNlgn4が調べられている．*Nlgn3*（R451C）ノックインマウスでは，微小抑制性シナプス後電流の頻度が増加することが示されている(34)．*Nlgn3* KOマウスはオープンフィールドテストや高架式十字迷路テストにおいて，野生型マウスに比べて総移動距離や速度が増加し，ホールボードテストにおける穴の探索回数が増加した．恐怖条件付け学習においてはすくみ応答の減弱を示した．さらに，超音波啼鳴の回数が野生型マウスに比べて減少していることが示されている(35)．*Nlgn4* KOマウスでは，社会性行動が減少し，超音波啼鳴の回数が野生型マウスに比べて減少していることも報告された(36)．

② SHANK

SHANKは興奮性シナプス後肥厚部に存在する足場タンパク質であり3つのアイソフォーム，SHANK1，SHANK2，SHANK3が存在する(37)．22q13.3欠失症候群は自閉症様の特徴を示すが，*SHANK3*はこの22q13.3に存在する遺伝子であり(38)，前述したNlgnと結合することが知られている(39)．*Shank3* KOマウスにおいては，自己毛づくろい行動が野生型マウスに比べて増加し，3-チャンバー型の装置を用いた社会性行動の測定においては，初めて出会うマウスのいるチャンバーにおける滞在時間が野生型マウスに比べて短いことから，社会性行動が減少していることが示された(40)．Shank1，Shank2においてもKOマウスが作製され，社会性行動の障害や自己毛づくろいの増加など自閉症様行動を示すことが報告されている(41, 42)．

③ Neurexin

Neurexin（Nrxn）はシナプス前膜にある接着分子であり，前述したNlgnと結合し，シナプスの形成や維持に関与している．自閉症患者においてミスセンス変異が見つけられている(43)．*Nrxn1α* KOマウスは，自己毛づくろい行動の増加や巣作り行動の障害が見られ，回転棒課題における運動学習においては，野生型マウスに比べて回転棒から落下するまでの時間が長いことが示された(44)．同研究において，*Nrxn1α* KOマウスは，海馬CA1における微小興奮性シナプス後電流の減少を示し，シナプス伝達機能に異常をもつ．

(2) Oxytocin

Oxytocinは下垂体後葉から分泌され，9つのアミノ酸からなるペプチドホルモンであり，授乳期の成熟した乳腺に作用して射乳を起こし，成熟肥大した子宮筋に作用して収縮の強さや頻度を増大することが知られている．自閉症との関連においてはヒトの臨床研究より，自閉症児の血漿中のoxytocinの濃度が健常児に比べて低いことが報告されている(45)．野生型マウスが初めて出会ったマウスに対する探索行動時間が徐々に減少するのに対して，oxytocinを欠損したマウスにおいては探索行動の減少が見られないことから社会性の記憶の障害が示された(46)．oxytocin受容体を欠損したマウスにおいても，幼若期における超音波啼鳴の回数が減少していることや，新奇なマウスと出会ったことのあるマウスへの探索行動時間が，野生型マウスは新奇なマウスへの時間が長いのに対して，oxytocin受容体を欠損したマウスは両マウスに対する探索時間に差はなく社会性行動の障害が報告されている(47)．

CD38は膜タンパク質でありリンパ球の増殖を活性化するが，oxytocinの放出にも関与する(48)．哺育中の仔マウスを3方向に離れて配置すると，野生型母マウスは仔マウスをすばやく寄せ集め仔マウスの上にしゃがみ込むが，CD38遺伝子を欠損した母マウスは，仔マウスを寄せ集めるのに野生型母マウスより時間がかかり，仔マウスの上にしゃがみ込む時間が短いことが示された(49)．また，野生型のオスマウスは出会ったことのあるメスマウスに再び曝露されると，そのメスマウスに対する探索行動時間が減少するが，CD38を欠損したオスマウスは，探索行動時間が減少しなかった(49)．これらのことから，oxytocinの放出に関与するCD38の欠損は，マウスの哺育行動や社会性認知の異常に関与することが示された．

(3) その他

ヒトにおける染色体15q11-13の重複は，自閉症における細胞遺伝的異常として高頻度なものの一つである(50)．マウスにおいてヒトの15q11-13の相同領域である染色体7cを6.3 Mbに渡って重複したマウス（*patDp/+*マウス）が作製された．3-チャンバー型の装置を用いた社会性行動の測定において，野生型マウスはマウスのいないチャンバー側より，見知らぬマウスのいる側のチャンバーにおける滞在時間が長いが，*patDp/+*マウスはいずれのチャンバーにおける滞在時間に差はなかった(51)．

Rett症候群は女児にのみ生じ，自閉症に類似した症状を伴い，遺伝子発現抑制に関与するMECP2の機能不全により生じる(52)．*Mecp2*の308番目のコドンの後にストップコドンとネオマイシン耐性遺伝子を挿入した$Mecp2^{308}$マウスにおいて，巣作り行動に異常が見られ，ケージ内に見知らぬマウスを入れたところ，野生型マウスに比べて探索時間が短く，社会性行動の低下を示した(53)．*Mecp2 null*マウスの脳においてS6やAktのリン酸化が減少していることから，mTORシグナルが抑制されていることが報告されている(54)．

3) 環境による自閉症モデル

Valproic acid（VPA）は気分障害や抗てんかん薬として用いられているが，妊娠中VPAに曝露された子は生後自閉症と診断される率が高くなる(55)．このため，妊娠中のラットやマウスにVPAを投与することで生まれてきた仔を自閉症モデル動物として扱う研究が行われている．ラット妊娠12.5日にVPAを600 mg/kg投与し，生まれてきた仔の運動機能，痛覚応答，情動，社会性行動が調べられた．コントロールのラットに比べて，VPAを投与されたラットは運動機能の低下が見られ，また痛覚刺激に対する応答が早く，さらに不安の亢進や社会性行動の低下が示されている(56)．ラットと同様にマウスに関しても運動機能の低下，不安の亢進，社会性行動の低下が示されている(57, 58)．またコントロールマウスに比べて，VPAを投与されたマウスは海馬や体性感覚野におけるNlgn3のmRNAが増加していることや(59)，VPAを投与されたラットの体性感覚野においてNMDA受容体の数が増加し，長期増強が亢進されていることも報告されている(60)．

4) 近交系による自閉症モデル

近交系のマウスには自閉症様行動を示すマウスが存在する．BTBRT+tf/J（BTBR）マウスは，見知らぬマウスに対する探索行動時間がC57BL/6Jマウスの探索行動時間に比べて減少し，自己毛づくろい行動の増加，オープンフィールドにおける総移動距離の増加やゼロ迷路テストにおけるオープンアームにおける滞在時間が増加することが報告されている(61)．C58/Jマウスにおいても，社会性行動の減少，自己毛づくろい行動

の増加が報告されている(62)．

3. 自閉症モデル動物を用いた薬理学的効果の検討

これまで示されてきた自閉症モデル動物に薬剤投与を用いて，異常な行動や病態が改善された研究の一部を以下に示す．

1) Rapamycin

mTORシグナル系における*TSC1*や*TSC2*遺伝子のいずれかのヘテロ欠損を有することで結節性硬化症に罹患することが知られている．結節性硬化症は，皮膚症状，神経症状及び全身の過誤腫からなる難治性疾患の一つであるが患者の20～60%で自閉症を合併し(63)，患者の社会生活を著しく困難なものにしている．また，結節性硬化症における自閉症の発症率に男女差はない．mTOR阻害薬は結節性硬化症の腫瘍の治療に効果的であることから(64)，結節性硬化症のモデル動物の自閉症様病態に有効性が検討された．*Tsc2*$^{+/-}$マウスは海馬が関与する学習や記憶の障害が示されているが，mTOR阻害薬であるrapamycinを1または5 mg/kgを投与することで学習や記憶の障害が回復することが報告された(13)．さらに3～7ヵ月齢の*Tsc1*$^{+/-}$マウスと*Tsc2*$^{+/-}$マウスの両マウスにrapamycinを5 mg/kg，2日間投与し，最終投与24時間後に見知らぬマウスに対する探索行動時間を測定したところ，投与前に比べて探索時間が増加したことが示された(16)．また行動における効果のみならずrapamycin投与後*Tsc2*$^{+/-}$マウスにおいて，S6Kのリン酸化レベルが野生型マウスと同レベルまで抑制されていた．またTangらは4～5週齢の*Tsc2*$^{+/-}$マウスは社会性行動の低下が見られ，なおかつ海馬におけるシナプスの数が野生型マウスより増加するが，rapamycinを3 mg/kg，8日間投与することによって社会性行動の改善とシナプスの刈り込みが生じ，シナプス数が減少することを示した(19)．

PTENのKOマウスは野生型マウスと比較すると，不安の亢進や社会性の低下が見られた(23)．しかし，rapamycinを10 mg/kg，連続5日間の投与（月～金）を4週間行うと不安の亢進と社会性行動の低下が回復することが見られ，さらに体重で補正した頭の重さの割合も投与前に比べて顕著に減少し，Aktのリン酸化も減少することが報告されている(23)．

BTBRマウスは社会性行動の低下が見られるが，rapamycinを10 mg/kg，連続4日間投与し，投与最終日において投与から60分後に社会性行動を測定すると，社会性行動が増加することが報告されている(65)．

2) Oxytocin

Oxytocinを欠損したマウスの，出会ったことのあるマウスに対する探索行動時間は，野生型マウスの探索行動時間より長いことから，社会性認知の欠損が示されていたが，1 ngのオキシトシンを脳室内にカニュレーションを用いて投与すると，社会性認知の成績が改善され海馬や体性感覚野における神経活動の増加がc-FOS陽性細胞の増加によって確認された(66)．CD38遺伝子を欠損した母マウスの哺育行動の異常や，CD38遺伝子を欠損したオスマウスの社会性認知の異常が示されていた(49)．しかし同研究において，CD38遺伝子を欠損した母マウスやCD38遺伝子を欠損したオスマウスにoxytocinを投与すると，両マウスの行動に改善が見られたことが報告されている．さらに，CD38が産出するthe formation of cyclic ADP-ribose（cADPR）がカルシウム放出に関与し，CD38を欠損したマウスにおいてはカルシウム放出がないことで，oxytocinの放出が妨げられていたことが示された(49)．

3) Metabotropic glutamate receptor（mGluR）のアロステリック調節薬と拮抗薬

Tsc2$^{+/-}$マウスは海馬におけるmGluR依存的な長期抑圧が特異的に減弱しており，この減弱は*Tsc2*$^{+/-}$マウスにおけるmTORシグナルの亢進によりタンパク質合成が抑制されたためと考えられた(67)．さらに，*Fmr1* KOマウスはmGluR依存的な長期抑圧やタンパク質合成が亢進しているが，mGluR5のシグナル減少によりmGluR依存的な長期抑圧やタンパク質合成の亢進が減少する(68)．このため，Auerbachらはmglur5のポジティブアロステリック調節薬である3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl) benzamide（CDPPB）を*Tsc2*$^{+/-}$マウスに10 mg/kg投与したところ，タンパク質合成が増加し，恐怖条件付け学習におけるすくみ行動に改善が見られたことを報告した(67)．一方で，Potterらは結節性硬化症の患者の大脳皮質結節においてmGluR5の発現が高まることから(69)，*Tsc2*$^{+/-}$マウスの海馬におけるmGluR5の発現量を調べたところ，野生型マウスの発現量は若齢時に比べ成熟時において減少するが，*Tsc2*$^{+/-}$マウスの発現量は減少せず，野生型マウスの発現量より高いことが示された(70)．このため，mGluR5の拮抗薬である2-methyl-6-phenylethynyl-piridine（MPEP）を実験開始30分前に30 mg/kgを*Tsc2*$^{+/-}$マウスに投与すると，radial arm water mazeテストにおいて最初に覚えた安全なプラットフォームのあるアームへ行く回数が減少することから，固執性の行動が改善されたことを報告している(70)．これらの研究は，mGluR5のポジティブアロステリック調節薬

と拮抗薬のいずれでも，*Tsc2*$^{+/-}$マウスにおいて改善効果が示されたことを報告している．今後，mGluR5の作用を亢進，あるいは阻害する薬剤を処置したマウスを用いて，様々な行動における効果を調べる必要がある．

プレパルス抑制テストにおいて*Fmr1* KOマウスは野生型マウスに比べ，プレパルスによる驚愕反応抑制の減少が見られるが，MPEPを20 mg/kg投与するとプレパルスによる驚愕反応抑制が増加し，増加していた海馬の神経細胞におけるフィロポディアの密度の減少が示された(71)．

胎生期にVPAに曝露されたマウスの自己毛づくろい行動やガラス玉覆い隠しテストを行うと，コントロールマウスに比べて自己毛づくろいの行動が増加し，隠すガラス玉の個数が増加するが，MPEPを20 mg/kg投与して測定を行うと，自己毛づくろい行動やガラス玉覆い隠しテストにおいて改善が見られることが報告されている(72)．

BTBRマウスにおいて，自己毛づくろい行動の増加が見られるが，mGluR5のネガティブアロステリック調節薬であるGRN-529を1あるいは3 mg/kg投与すると，自己毛づくろい時間の減少が見られ，社会性行動において見知らぬマウスがいるチャンバーにおける滞在時間が増加したことが示されている(73)．またMPEPを投与することでsniffing時間の増加や自己毛づくろいの時間が減少することが報告されている(74)．

これらの報告から，mTORシグナル系分子に異常を有する自閉症モデルマウスや一部の近交系マウスにおいてはrapamycinが効果的なことが示され，oxytocinは治療薬として，またmGluRは治療薬の標的として有効なことが示されている．

4. 今後の展望

自閉症関連遺伝子の探索，自閉症モデル動物の作製と解析，自閉症モデル動物への薬剤投与による効果の検討が病態解明や治療薬開発のために行われてきた．これまでの自閉症患者における治療薬の処方は症状に応じた対処療法であったため，根本的な薬物治療には至っていなかった．本稿で示したrapamycinは結節性硬化症のモデルマウスに合併する自閉症様病態に有効なことが示された．さらに幼若期のマウスにおける効果の有無を調べることや結節性硬化症のモデル動物以外への効果も充分に検討する必要があると考えられる．mTOR阻害薬はすでにヒトで使用されており，結節性硬化症患者のてんかんの改善効果やそれに伴う生活の質の改善も報告されている(75)．Rapamycinは結節性硬化症に合併する自閉症への有効な治療薬の候補として考えられるが，長期投与における影響なども検討していく必要がある．脳移行率は不明ながら，oxytocinを点鼻薬として自閉症患者に投与する臨床研究が実施されている．また近年では，グリア細胞も治療薬の標的として調べられており，今後も効果的な治療薬開発のために有効なモデル動物の作製と解析が求められる．さらに自閉症患者は薬物治療のみならず，家庭や病院において療育や行動療法も行っているのが現状である．このため，それらの療法の効果における分子メカニズムも調べ薬物治療との相乗効果の可能性も検討する必要があると考えられる．

*自閉症モデルマウスの超音波啼鳴を測定した研究においては，超音波啼鳴の増加を示すと刺激に対する反応性の増大や不安が亢進されていること，超音波啼鳴の低下を示すと社会性が障害されていることが考えられている(15, 35, 36, 47)．しかしながら，超音波啼鳴は回数や長さのみならず，10種類に分類されることが見出されている(76)．22q11.2欠失症候群は自閉症を発症することがあるが，Hiromotoらは22q11.2にある遺伝子のうち*Tbx1*をヘテロ欠損したマウスを作製し，超音波啼鳴を調べた(77)．超音波啼鳴10種類のうち5種類において，*Tbx1*をヘテロ欠損したマウスは野生型マウスの超音波啼鳴頻度より減少を示し，4種類においては超音波啼鳴の長さが減少することを示したが，他の種類において差はみられなかった(75)．これらの超音波啼鳴の種類についての生理的な意味は現在のところ明確にはなっていない．

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Research Article Open Access

Association between Genetic Polymorphism rs2952768, Close to the *METTL21A* and *CREB1* Genes, and Intellectual Ability in Healthy Subjects

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Abstract

Objective: Human intelligence, which represents a set of cognitive abilities, is assumed to be a highly heterogenic trait. The Intelligence Quotient (IQ) is the most widely used index for characterizing human intelligence in psychometric studies, and knowledge of the genes associated with IQ has continuously grown. Several previous reports indicated that IQ may be associated with addictive behaviors or the use of addictive substances, although the trend toward an association is not straightforward and depends on the substances abused. To explore the genetic factors that contribute to IQ, we conducted an association study of a genetic polymorphism, rs2952768. The rs2952768 single-nucleotide polymorphism (SNP) was recently reported to be associated with human opioid sensitivity and shown to be associated with the efficacy of opioid analgesics, severity of substance dependence, and mRNA expression levels of a neighboring gene, *CREB1*.

Methods: The present study used data from 298 biologically unrelated Japanese subjects. Psychiatrically, medically, and neurologically healthy subjects were evaluated using the Structured Clinical Interview for the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, Non-Patient Edition (SCID-I/NP), to exclude individuals who had substance-related disorders, who had received psychiatric medications, or who had first- or second-degree relatives with psychiatric disorders. Genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 6.0. The rs2952768 SNP close to the *METTL21A* gene was extracted from this dataset. Multiple linear regression analysis was performed to compare intellectual ability among rs2952768 SNP genotypes.

Results: A significant effect of the SNP genotype was observed on current IQ (β = -2.27, p = 0.026). The number of non-risk major C allele for drug and alcohol dependence was correlated with higher IQ scores.

Conclusion: The present results suggest that the rs2952768 SNP, which was identified as a potent SNP associated with human opioid sensitivity, is also one of the genetic factors that contribute to human intellectual ability.

Keywords: Intelligence Quotient (IQ); Opioids; Addictive substances; Substance dependence; Single-nucleotide polymorphism (SNP); Cyclic adenosine monophosphate response element binding protein 1 (*CREB1*); Methyltransferase like 21A (*METTL21A*)

Introduction

Human intelligence, which represents a set of cognitive abilities, such as thinking, remembering, reading, learning, problem solving, and using language, is assumed to be a highly heterogenic trait. Intelligence Quotient (IQ) is the most widely used index for characterizing human intelligence in psychometric studies. It can be used to assess intellectual ability in not only healthy subjects but also in patients with disorders such as schizophrenia, autism, depression, and anxiety [1–3]. Among the well-examined genes are those involved in brain functions related to mechanisms of learning and memory, and genetic variations in such genes associated with IQ have been identified [4–6]. Knowledge of the genes associated with IQ has increased. A publicly available database explores IQ-associated human genes [7], revealing that IQ-associated genes are significantly enriched in multiple signaling events, especially those related to cognitive systems.

Several previous reports suggested that IQ can affect and also be affected by addictive behaviors or the use of addictive substances. For example, people with lower IQ scores are more likely to become cigarette smokers [8,9]. In a longitudinal study that assessed marijuana's impact on IQ, current marijuana use was found to be significantly and dose-dependently correlated ($p < 0.05$) with a decline in IQ over the ages studied [10]. High childhood IQ has generally been linked to alcohol dependence and more frequent alcohol consumption [11,12]. In a study that investigated demographic profiles related to estimations

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of Wechsler Adult Intelligence Scale-Revised (WAIS-R) Full Scale IQs (DP Estimated IQs), the DP Estimated IQ was found to be significantly related to the duration of opioid addiction, and a higher estimated IQ was associated with a shorter duration [13]. However, few studies have focused on genes or their functional involvement in the mechanism of addiction in the context of investigating genes related to human intelligence or IQ scores.

To explore the genetic factors that contribute to IQ, we conducted an association study of a genetic polymorphism, rs2952768. The potent rs2952768 single-nucleotide polymorphism (SNP) was recently associated with human opioid sensitivity and shown to be associated with the efficacy of opioid analgesics, severity of substance dependence, and mRNA expression levels of a neighboring gene, *CREB1* [14].

Materials and Methods

Subjects

The data from 298 healthy subjects (40.9% male [122/176]; mean age ± SD: 36.8 ± 12.4 years) were used in the present study. The subjects were all biologically unrelated and Japanese. The subjects were recruited through local advertisements at Osaka University. Psychiatrically, medically, and neurologically healthy subjects were evaluated using the Structured Clinical Interview for the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV), Non-Patient Edition (SCID-I/NP), to exclude individuals who had substance-related disorders, who had received psychiatric medications, or who had first- or second-degree relatives with psychiatric disorders. Additionally, subjects were excluded from the study if they had neurological or medical conditions that could potentially affect their central nervous system, such as atypical headaches, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, active cancer, cerebrovascular disease, epilepsy, seizures, or mental retardation. Written informed consent was obtained from all of the subjects after the procedures were explained. This study was performed in accordance with the World Medical Association's Declaration of Helsinki and approved by the Osaka University Research Ethics Committee.

Measurement of intellectual ability

Current low IQ may or may not be a determinant of drug and alcohol dependence or the use of addictive substances, and the tendency toward an association may be different between abused substances [8-13]. Based on our evidence that a genetic variant close to the methyltransferase like 21A (*METTL21A*) gene, rs2952768, is related to the severity of drug and alcohol dependence, we investigated the association between the rs2952768 genotype for drug and alcohol dependence and current IQ in healthy Japanese subjects. To assess current intellectual ability, we used verbal IQ from the Japanese version of the Wechsler Adult Intelligence Scale, 3rd edition (WAIS-III) [15]. The subjects were assessed by trained clinical psychologists to obtain verbal IQ scores on the WAIS-III.

Single-nucleotide polymorphism genotyping

Venous blood was collected from the subjects, and genomic DNA was extracted from whole blood according to standard procedures. Genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA) as previously described [16]. The rs2952768 SNP close to the *METTL21A* gene was extracted from this dataset. No deviation from Hardy-Weinberg equilibrium (HWE) in the examined SNP was detected ($p = 0.10$).

Statistical analysis

Differences in clinical characteristics between the genotype groups were analyzed using the χ^2 tests for categorical variables and Kruskal-Wallis test for continuous variables using PASW Statistics 18.0 software (SPSS Japan, Tokyo, Japan). Deviation from HWE was tested using the χ^2 test for goodness-of-fit using SNPAlyze 5.1.1 Pro software (DYNACOM, Yokohama, Japan). Multiple linear regression analysis was performed to compare intellectual ability among rs2952768 SNP genotypes (the number of major alleles: 0, 1, or 2) using PASW software. Intellectual ability may be influenced by sex and years of education, and these variables were corrected for as covariates. We did not include age as a covariate because IQ score was already corrected for age. All p values were two tailed, and statistical significance was defined as $p < 0.05$.

Results

Influence of the rs2952768 genotype on current intellectual ability

Demographic variables, mean age, sex, and years of education are shown in Table 1. The mean age and years of education did not differ significantly between the genotype groups ($p > 0.59$), whereas the sex ratio differed significantly between groups ($p = 0.015$). We examined the possible effect of the rs2952768 genotype on intellectual ability. A significant effect of the SNP genotype was observed on current IQ ($\beta = -2.27$, $p = 0.026$). The number of C allele was correlated with higher IQ scores (Figure 1).

	Total	C/C	T/C	T/T	
Variables	(*N* = 298)	(*N* = 45)	(*N* = 123)	(*N* = 130)	*p* (*H*)
Age (years)	36.8 ± 12.4	36.3 ± 13.6	37.0 ± 11.7	36.8 ± 12.6	0.83 (0.38)
Sex (male/female)	122/176	11/34	60/63	51/79	**0.015 (8.35)**[a]
Education (years)	14.9 ± 2.3	15.0 ± 2.3	14.8 ± 2.3	15.0 ± 2.3	0.59 (1.06)

Means ± SD are shown. $p < 0.05$ is in boldface and underlined [a]χ^2 test.

Table 1: Demographic variables for subjects included in this study.

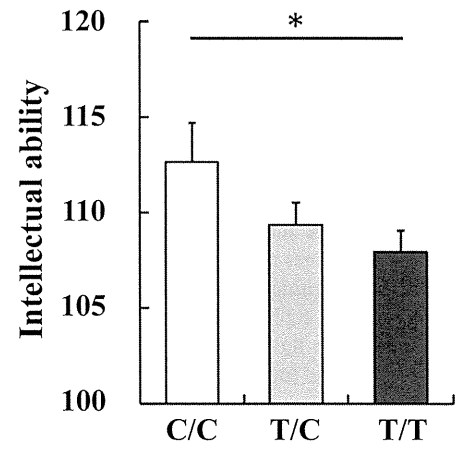

Figure 1: Impact of the rs2952768 genotype on intellectual ability
White, gray, and black bars represent individuals with the C/C genotype, T/C genotype, and T/T genotype, respectively. Error bars represent the standard error. The asterisk indicates $p<0.05$.

Discussion

We conducted an association study between verbal IQ and the rs2952768 SNP, which was recently identified as a potent SNP associated with opioid sensitivity that affects both the efficacy of opioid analgesics and liability to severe substance dependence. A significant effect of the SNP genotype was observed on current IQ (β = -2.27, p = 0.026), and the number of non-risk major C allele for severe drug and alcohol dependence was correlated with higher IQ scores (Figure 1), suggesting that the rs2952768 SNP is one of the genetic factors that contribute to human intellectual ability.

Several previous reports suggested associations between IQ score and addictive behaviors or the use of addictive substances, but the trend toward an association is not straightforward or easily understood [8-13]. Several reports indicated that people with lower IQ scores are more likely to become cigarette smokers [8,9]. Another report found that higher estimated IQ was significantly related to a shorter duration of opioid addiction [13]. High childhood IQ generally has been linked with alcohol dependence and more frequent alcohol consumption, and a 1 SD (15-point) increase in IQ score was found to be associated with an increased risk of illegal drug use in women, such as the use of cannabis, cocaine, amphetamines, amyl nitrate, and "magic mushrooms" [11,12]. The outcome in the present study that the number of non-risk major C allele for substance dependence in the rs2952768 SNP was correlated with higher IQ scores (Figure 1) is seemingly consistent with the results reported by Chastain et al. [13]. Although the rs2952768 SNP was identified as an opioid sensitivity-related SNP, the association was also found in the same direction with the severity of substance dependence, including alcohol dependence, methamphetamine dependence, and eating disorder [14]. Much more studies will be required to make definitive conclusions about the correlations or causal associations between IQ and the use of various addictive substances and vulnerability to or severity of dependence, since the fundamentally important pre-condition, the relationship of rs2952768 with severe drug dependence, has not been well-established.

In our previous study, the homozygote of the non-risk C allele for severe drug and alcohol dependence of the rs2952768 SNP was significantly associated with the elevated expression of a neighboring gene, cyclic adenosine monophosphate response element binding protein 1 (*CREB1*), which encodes a transcription factor that is a member of the leucine zipper family of DNA binding proteins. CREB plays various roles as a transcription factor in many cells, including neuronal cells, and it is also involved in the molecular mechanisms that couple synaptic activity to long-term changes in neuronal plasticity, which is thought to underlie learning and memory [17]. Therefore, the elevated expression of the *CREB1* gene may promote the transcription levels of some target genes related to both human intellectual ability and addiction, leading to alterations in the neural mechanisms that are involved in both increasing intelligence and decreasing the rewarding effects of addictive substances. However, such speculative statements should be avoided before much more extensive studies are conducted in the future, and the precise mechanism by which elevated *CREB1* expression generally affects human opioid sensitivity requires further study.

The *CREB1* and *METTL21A* genes are both located within a linkage disequilibrium block that spans 2q33.3–2q34 [14]. Although these genes were not contained in the publicly available database that explores IQ-associated human genes [7], the chromosomal region 2q33 was included in the linkage regions, indicating that this region may be an IQ-associated region. Furthermore, chromosomal abnormalities, such as duplication and deletion of 2q33.3–2q34, were reported in patients with developmental delay and mental retardation [18,19], the severity of which may be related to IQ [20]. Despite the fact that the responsible genes within this region for IQ should be further clarified in future studies, these previous reports support the results of the present study, in which SNPs in this region may be associated with intellectual ability.

In conclusion, we identified a significant effect of the SNP genotype on current IQ, and the number of non-risk major C allele for drug and alcohol dependence was correlated with higher IQ scores. Although we should not over-interpret the present finding and the precise underlying mechanisms remain to be clarified in future studies, the results of the present study suggest that this SNP may be one of the genetic factors that contribute to human intellectual ability.

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Factors that Affect Intravenous Patient-Controlled Analgesia for Postoperative Pain Following Orthognathic Surgery for Mandibular Prognathism

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Abstract

The predictors of postoperative pain and analgesic consumption were previously found to include preoperative pain, anxiety, age, type of surgery, and genotype, but remaining unclear was whether intraoperative factors could predict postoperative pain. In the present study, we investigated the time-course of fentanyl consumption using intravenous patient-controlled analgesia records from patients who underwent orthognathic surgery for mandibular prognathism and analyzed the influence of anesthesia methods and surgical methods together with sex on the time course. A significant difference in the time course of fentanyl administration was found ($P<0.001$). No significant difference in the time course of fentanyl administration was found between males and females ($P=0.653$), with no interaction between time course and sex ($P=0.567$). No significant difference in the time course of fentanyl administration was found among anesthesia methods, such as fentanyl induction followed by fentanyl maintenance, fentanyl induction followed by remifentanil maintenance, and remifentanil induction followed by remifentanil maintenance ($P=0.512$), but an interaction between time course and anesthesia method was observed ($P=0.004$). A significant difference in the time course of fentanyl administration was found between surgical methods, such as bilateral mandibular sagittal split ramus osteotomy (BSSRO) and BSSRO combined with Le Fort I osteotomy (bimaxillary; $P=0.008$), with no interaction between time course and surgical method ($P=0.535$). Total postoperative 24 h consumption associated with the bimaxillary procedure was significantly higher than with BSSRO ($P=0.008$). The present results indicate that administration patterns and total 24 h consumption were different among the three groups of anesthesia methods and between the two groups of surgical methods, respectively. Although more research on patient-controlled analgesia patterns and consumption is necessary, the present study will contribute to adequately relieving individual patients from postoperative pain.

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Introduction

Every year, 234.2 million major surgical procedures are performed worldwide [1]. These patients experience postoperative pain, and the range of pain varies from mild to severe. Postoperative pain management is very important to reduce distress caused by pain itself, contribute to cardiovascular stability [2] and proper respiratory function [3], and enable early recovery [3]. Postoperative pain is frequently controlled by opioids, which are especially heavily used in the United States [4]. Postoperative pain is reportedly affected by preoperative pain, anxiety, age, and type of surgery, and postoperative analgesic consumption is affected by type of surgery, age, and psychological distress [5]. Clarification and the control of preoperative and intraoperative factors will provide patients with more effective pain management.

In the present study, we investigated the time course of fentanyl consumption using the intravenous patient-controlled analgesia (IV-PCA) records of patients who underwent orthognathic surgery for mandibular prognathism and analyzed the factors (e.g., sex, anesthesia method, and surgical method) that may influence postoperative pain management. We found that the time course of IV-PCA was associated with the anesthesia method (i.e., time course × anesthesia method interaction) and surgical method (i.e., main effect).

Materials and Methods

1. Patients

The study protocol was approved by the Institutional Review Boards of Tokyo Dental College and the Tokyo Metropolitan Institute of Medical Science. Written informed consent was obtained from all of the patients and from parents if the patient was under 20 years old. Enrolled in the study were 143 healthy patients (American Society of Anesthesiologists Physical Status I

[ASA PS I], 15–53 years old, 56 males and 87 females) who were scheduled to undergo orthognathic surgery for mandibular prognathism at Tokyo Dental College Suidobashi Hospital. Patients were excluded preoperatively if they had a history of acute or chronic kidney injury, drug abuse, or chronic pain or were unable to use the IV-PCA device.

2. Anesthesia

The groups comprised consecutive patients who underwent cosmetic orthognathic surgery for mandibular prognathism and received fentanyl induction and maintenance (F-F group) over a half-year period prior to 2010, consecutive patients who underwent the same surgery and received fentanyl induction and remifentanil maintenance (F-R group) in the first half of 2010, and consecutive patients who underwent the same surgery and received remifentanil induction and maintenance (R-R group) in the second half of the year 2010. All of the groups were orally premedicated with 5 mg diazepam and 150 mg famotidine 90 min before the induction of anesthesia.

In the F-F group, the patients were inducted with 2 μg/kg fentanyl. General anesthesia was performed with propofol at a target blood concentration of 4–6 μg/ml using a target-controlled infusion (TCI) pump (TE-317, Terumo, Tokyo, Japan). Vecuronium (0.1 mg/kg) was administered to facilitate nasotracheal intubation (Portex; inner diameter, 6.5–8.0 mm; Smiths Medical Japan, Tokyo, Japan) and maintained at 0.08 mg/kg/h during surgery. Whenever systolic blood pressure or heart rate increased more than 20% over baseline during surgery, fentanyl was intravenously administered at 1 μg/kg.

In the F-R group, the patients were inducted with 2 μg/kg fentanyl. General anesthesia was performed with propofol at a target blood concentration of 4–6 μg/ml using a TCI pump. Recuronium (0.6 mg/kg) was administered to facilitate nasotracheal intubation (Portex; inner diameter, 6.5–8.0 mm; Smiths Medical Japan, Tokyo, Japan) (Portex; inner diameter, 6.5–8.0 mm; Smiths Medical Japan, Tokyo, Japan). General anesthesia was maintained with 0.125–0.5 μg/kg/min remifentanil and 7 μg/kg/min recuronium during surgery. The patients received 100 μg fentanyl as a transitional opioid at the end of surgery.

In the R-R group, the patients were inducted with 0.5 μg/kg/min remifentanil. General anesthesia was performed with propofol at a target blood concentration of 4–6 μg/ml using a TCI pump. Recuronium (0.6 mg/kg) was administered to facilitate nasotracheal intubation (Portex; inner diameter, 6.5–8.0 mm; Smiths Medical Japan, Tokyo, Japan). General anesthesia was maintained with 0.125–0.5 μg/kg/min remifentanil and 7 μg/kg/min recuronium during surgery. The patients received 100 μg fentanyl as a transitional opioid at the end of surgery.

In the three groups, the lungs were ventilated with oxygen-enriched air. All of the patients received local anesthesia at the surgical sites with 8 ml of 2% lidocaine that contained 12.5 μg/ml epinephrine.

3. Surgery

Sagittal split osteotomy described by Obwegeser [6] is likely the most frequently used procedure for osteotomy to correct mandibular anomalies, including hypoplasia, hyperplasia, and asymmetries. Le Fort I osteotomy, first described by Wassmund [7] and later standardized by Obwegeser [8] and Bell [9], has also become the most frequently used procedure for osteotomy in the maxilla [10]. In the present study, the surgical methods for mandibular prognathism included bilateral mandibular sagittal split ramus osteotomy (BSSRO) and BSSRO combined with Le Fort I osteotomy (bimaxillary). Bimaxillary surgery was performed for patients who had been deemed to present only marginal improvements in mandibular prognathism after BSSRO alone.

4. Postoperative pain management

At the end of surgery, 50 mg rectal diclofenac sodium and 8 mg intravenous dexamethasone were administered to prevent postoperative orofacial edema/swelling. After emergence from anesthesia and tracheal extubation, 1.25 mg droperidol was intravenously administered to prevent nausea/vomiting, and IV-PCA with 20 μg/ml fentanyl commenced using a CADD-Legacy PCA pump (Smiths Medical Japan, Tokyo, Japan). Droperidol (0.1 mg/ml) was co-administered with fentanyl to prevent nausea/vomiting because of a high incidence (up to 30%) of nausea/vomiting with PCA fentanyl in young females [11]. A bolus dose of fentanyl of 20 μg on demand and a lockout time of 10 min were set. Continuous background infusion was not employed. Patient-controlled analgesia was continued for 24 h postoperatively. In the case of refractory adverse effects or inadequate analgesia, PCA with fentanyl was discontinued, and 50 mg rectal diclofenac sodium was prescribed as a rescue analgesic as required.

The PCA pump recorded all of the administration events, providing the researchers with the administration times, number of administrations, dose of the administrations, and number of attempts without administration. The number of administrations was converted to consumption every 2 h after the end of anesthesia. Consumption every 2 h was standardized by body weight. Total postoperative 24 h consumption was calculated as the sum of consumption every 2 h. In 63 of the 143 cases, the intensity of spontaneous pain was assessed 3 and 24 h postoperatively using a 100 mm visual analog scale (VAS), with 0 mm indicating no pain and 100 mm indicating the worst pain imaginable.

5. Statistical analysis

All of the data are expressed as mean ± SD or median (range) and were statistically analyzed using SPSS 19.0 software (SPSS, Chicago, IL, USA). Differences between groups and within time courses were assessed using mixed-design analysis of variance (ANOVA; one-way for independent groups and repeated measures with Huynh-Feldt correction). When a significant overall effect was detected, Bonferroni's test and Scheffe's test were used to compare the mean values of the groups and time courses, respectively. Differences between groups in total postoperative 24 h consumption were analyzed using one-way ANOVA. The threshold for statistical significance was $P<0.05$. The sample size for the present data was higher than the estimated size that possesses statistical power (1 minus type II error probability) of 98% for the Cohen's conventional "medium" effect size of 0.3. Power analyses were performed using G*Power v.3.1.5 [12].

Results

The attributes of the patients are shown in Table 1.

1. Sex

A significant difference was found in the time course of fentanyl administration ($F_{6.435,907.356}=24.211$, $MSe=0.166$, $P<0.001$, Huynh-Feldt). In the time course, 2 h consumption significantly decreased from 6 h to 24 h after the end of anesthesia compared with consumption in the first 2 h (Fig. 1). No significant difference was found in fentanyl administration between males and females ($F_{1,141}=0.204$, $MSe=0.593$, $P=0.653$, Huynh-Feldt), with no time course × sex interaction ($F_{6.435,907.356}=0.814$, $MSe=0.166$, $P=0.567$, Huynh-Feldt). No significant difference was found in

Table 1. Number of patients, age, sex, anesthesia methods, and surgical methods.

Sex	*n*, Age: median (range)	Anesthesia	*n*, Age: median (range)	Surgery	*n*, Age: median (range)
Male	56, 22.5 (16–53) years	F-F	44, 25.5 (16–53) years	BSSRO	94, 23.0 (15–49) years
		F-R	40, 22.0 (15–47) years		
Female	87, 25.0 (15–50) years			Bimaxillary	49, 25.0 (16–53) years
		R-R	59, 25.0 (16–50) years		
Total	143, 25.0 (15–53) years	Total	143, 25.0 (15–53) years	Total	143, 25.0 (15–53) years

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total postoperative 24 h consumption between males and females (Table 2; $F_{1,141} = 0.204$, $MSe = 7.115$, $P = 0.653$).

2. Anesthesia methods

A significant difference was found in the time course of fentanyl administration ($F_{6.661,932.489} = 24.653$, $MSe = 0.157$, $P<0.001$, Huynh-Feldt). In the time course, 2 h consumption significantly decreased from 6 h to 24 h after the end of anesthesia compared with consumption in the first 2 h (Fig. 2). No significant difference was found in fentanyl administration among the anesthesia methods ($F_{2,140} = 0.672$, $MSe = 0.592$, $P = 0.512$, Huynh-Feldt), but a significant time course × anesthesia method interaction was observed ($F_{13.321,932.489} = 2.359$, $MSe = 0.157$, $P = 0.004$, Huynh-Feldt). Consumption in the first 2 h in the R-R group was significantly higher than in the F-F group, but 8 h consumption in the R-R and F-R groups was significantly lower than in the F-F group. Nevertheless, total postoperative 24 h consumption was not significantly different among the three groups (Table 2; $F_{2,141} = 0.672$, $MSe = 7.108$, $P = 0.512$).

3. Surgical methods

A significant difference was found in the time course of fentanyl administration ($F_{6.482,913.936} = 24.144$, $MSe = 0.165$, $P<0.001$, Huynh-Feldt). In the time course, 2 h consumption significantly decreased from 4 h to 24 h after the end of anesthesia compared with consumption in the first 2 h. A significant difference was found in fentanyl administration between the surgical methods

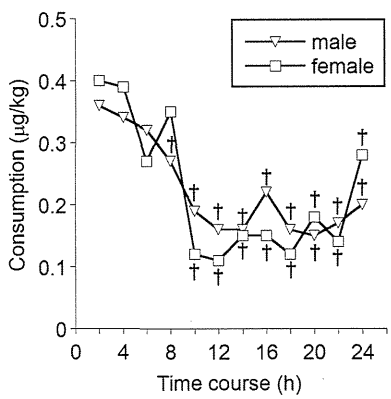

Figure 1. Differences in the time course of fentanyl administration between males and females. Main effects, interactions, and differences within time courses were analyzed using mixed-design ANOVA (one-way for independent groups and repeated-measures with Huynh-Feldt correction). The values indicate the medians. †$P<0.05$, compared with fentanyl consumption in the first 2 h.
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($F_{1,141} = 7.237$, $MSe = 0.565$, $P = 0.008$, Huynh-Feldt), but no time course × surgical method interaction was observed ($F_{6.482,913.936} = 0.855$, $MSe = 0.165$, $P = 0.535$, Huynh-Feldt). Consumption in the first 2 h was higher in the bimaxillary group than in the BSSRO group (Fig. 3). Total postoperative 24 h consumption in the bimaxillary group was significantly higher than in the BSSRO group (Table 2; $F_{1,141} = 7.237$, $MSe = 6.778$, $P = 0.008$, Huynh-Feldt).

4. Visual analog scale

The attributes of the patients are shown in Table S1. No significant difference was found in VAS scores between the anesthesia methods (F-F and F-R groups) at 3 h ($t_{61} = -0.713$, $P = 0.478$) and 24 h ($t_{61} = -0.098$, $P = 0.992$). A significant positive correlation was found between total postoperative 24 h consumption and VAS scores at 3 h, but the correlation coefficient was relatively small ($r = 0.295$, $P = 0.019$). No significant positive correlation was found between total postoperative 24 h consumption and VAS scores at 24 h ($r = 0.240$, $P = 0.058$). A significant positive correlation was found between VAS scores at 3 and 24 h, and the correlation coefficient was relatively large ($r = 0.667$, $P<0.001$).

Discussion

The predictors of postoperative pain were previously found to include preoperative pain, anxiety, age, type of surgery [5], and genotype [11,13–15]. We investigated orthognathic patients in whom these predictive factors are considered to be relatively similar. They had been treated by a few orthodontists in the hospital over several years. Their anxiety appeared to be much less than patients who presented in the emergency room. Almost all of the patients were young (mean age = 23.16 years, SD = 0.696 years) and healthy (ASA PS I). Orthognathic surgery was performed after body growth ceased. Orthognathic procedures, such as BSSRO and BSSRO combined with Le Fort I osteotomy, have been well established. The patients were subjected to uniform invasiveness by these typical operations. Postoperative pain after BSSRO has been reported to be more intense than after soft tissue surgery [16]. Thus, these patients had no preoperative pain (e.g., inflammatory pain), had less anxiety, were young, and had similar levels postoperative pain; therefore, they were deemed to be suitable for inclusion as subjects to investigate the factors that influence the time course of IV-PCA.

Young patients were reported to be more sensitive to postoperative pain than older patients [5]. We did not analyze the association between age and postoperative fentanyl consumption because our data were collected mainly from young patients.

Generally, sex is not associated with postoperative pain [5], although postoperative pain in patients who underwent impacted third molar extraction was associated with sex [17]. Pain assessed

Table 2. Total postoperative 24 h consumption.

Subjects	*n*	Total 24 h consumption (μg/kg)	
		median	range
Sex			
Male	56	2.88	0.00–10.00
Female	87	2.40	0.00–11.34
Total	143	2.59	0.00–11.34
Anesthesia method			
F-F	44	2.64	0.00–10.54
F-R	40	2.28	0.00–9.07
R-R	59	2.70	0.00–11.34
Total	143	2.59	0.00–11.34
Surgical method			
BSSRO	94	2.29	0.00–9.07
Bimaxillary	49	3.16*	0.00–11.34
Total	143	2.59	0.00–11.34

*$p<0.05$, significant difference between BSSRO and bimaxillary groups.
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by a visual analog scale (VAS) during the first 24 h in females was significantly higher than in males, but the VAS score after the first 24 h was not significantly different between males and females [17]. The initially higher level of pain in females may be attributable to a smaller and thinner mandible in females [17]. The surgical site in the present study was similar to impacted third molar extraction; thus, we analyzed the association between sex and postoperative fentanyl consumption. However, our data did not show a significant difference in fentanyl consumption between males and females. Impacted third molar extraction might cause more micro-bone fractures in females than in males. Because osteotomy is not a micro-bone fracture but rather an artificial fracture, postoperative fentanyl consumption might not have been affected by sex differences in the structure of the mandible in the present study.

Anesthesia methods were analyzed by two-way ANOVA without sex as a covariate because no significant difference was found between males and females in the present study. A time course × anesthesia method interaction was observed, in which consumption in the first 2 h was higher than 4 h consumption in the R-R and F-R groups, but 2 h consumption was lower than 4 h consumption in the F-F group. The context-sensitive half-life of remifentanil is extremely less than fentanyl [18]. The recovery of psychomotor function after total intravenous anesthesia (TIVA) with remifentanil, which does not use any inhalational agents, was 30–120 min faster than TIVA with fentanyl [19]. Orthognathic patients who were maintained with TIVA with remifentanil had

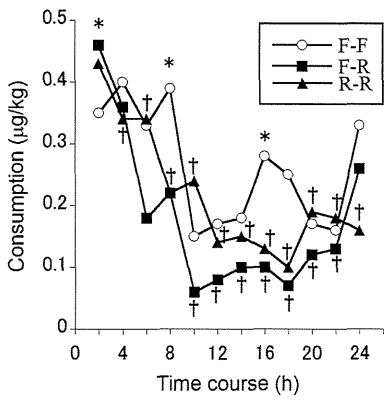

Figure 2. Differences in the time course of fentanyl administration among anesthesia methods. Main effects, interactions, and differences within time courses were analyzed using mixed-design ANOVA (one-way for independent groups and repeated-measures with Huynh-Feldt correction). F-F, fentanyl induction followed by fentanyl maintenance; F-R, fentanyl induction followed by remifentanil maintenance; R-R, remifentanil induction followed by remifentanil maintenance. The values indicate the medians. †$P<0.05$, compared with fentanyl consumption in the first 2 h; *$P<0.05$, significant difference among the three groups in 2 h fentanyl consumption.
doi:10.1371/journal.pone.0098548.g002

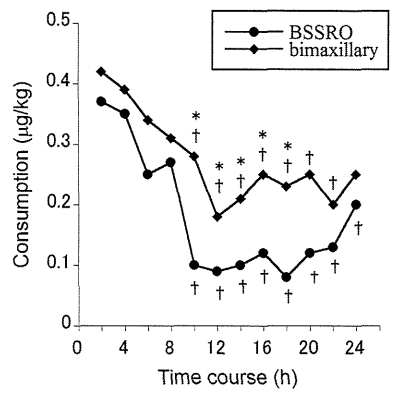

Figure 3. Differences in the time course of fentanyl administration between surgical methods. Main effects, interactions, and differences within time courses were analyzed using mixed-design ANOVA (one-way for independent groups and repeated-measures with Huynh-Feldt correction). The values indicate the medians. †$P<0.05$, compared with fentanyl consumption in the first 2 h; *$P<0.05$, significant difference between the BSSRO and bimaxillary groups.
doi:10.1371/journal.pone.0098548.g003

significantly higher pain scores within the first 4 h postoperatively [20]. Thus, the groups that were maintained with remifentanil (i.e., the F-R and R-R groups) may recognize postoperative pain father than the group that was maintained with fentanyl (i.e., the F-F group). Faster psychomotor recovery and the faster recognition of pain might explain why the groups that were maintained with remifentanil had higher fentanyl consumption in the first 2 h than the group that was maintained with fentanyl. Interestingly, the administration pattern was different between the remifentanil and fentanyl groups, but total postoperative 24 h consumption was not different among the three groups of anesthesia methods. Additionally, the 3 and 24 h VAS scores were mostly less than 50 mm and not different between anesthesia methods (F-F and F-R groups), indicating that subjective pain was appropriately controlled in both the F-F and F-R groups.

We had empirically known that bimaxillary surgery is experimentally more painful than BSSRO. Postoperative pain following BSSRO and Le Fort I osteotomy is conveyed from the surgical sites to supraspinal sites by the third and second branches of the trigeminal nerve, respectively. Thus, postoperative pain following bimaxillary surgery was conveyed from the surgical sites to supraspinal sites by both the second and third branches of the trigeminal nerve. Our results suggest that postoperative pain increased because of the increase in the number of branches of the trigeminal nerve from the surgical site. Further studies of single Le Fort I osteotomy (second branch of the trigeminal nerve) are required to determine whether the increase in postoperative pain is caused by synergistic or additive effects.

The present study involved healthy patients who underwent oral surgery. The influence of perioperative factors on IV-PCA was controlled. The results showed that the administration patterns and total 24 h consumption were different among the three groups of anesthesia methods and between the two groups of surgical methods, respectively. Although more research on patient-controlled analgesia patterns and consumption is necessary, the present study will contribute to adequately relieving individual patients from postoperative pain.

Supporting Information

Table S1 Frequency of patients and VAS scores.
(DOCX)

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Author Contributions

Conceived and designed the experiments: YA KI KF. Performed the experiments: YA KY DN SK. Analyzed the data: YA DN. Contributed reagents/materials/analysis tools: KY TI KI KF. Wrote the paper: YA DN KI KF.

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—Original—

Stress Sensitivity in Patients with Atopic Dermatitis in Relation to the Translocator Protein 18 kDa (TSPO)

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Abstract

Atopic dermatitis (AD) is a chronic inflammatory skin disease, characterized by pruritic and eczematous skin lesions and dermatitis that worsens under stressful conditions. However, the relation of these symptoms to an individual's stress sensitivity is not well understood. On the other hand, expression of the translocator protein (18 kDa) (TSPO), formerly known as the peripheral-type benzodiazepine receptor, has been used as a biological marker of trait anxiety and stress sensitivity. The present study was designed to address this issue by examining TSPO in patients with AD. Fifty-two patients with AD (30 male and 22 female) and 163 healthy volunteers (89 male and 74 female) participated in this study. State-Trait Anxiety Inventory (STAI) scores were significantly higher in patients with AD, especially male patients, than in healthy subjects. The expression of platelet TSPO, as determined with a binding assay with [^{3}H] PK11195, was also significantly higher in patients with AD, indicating that AD is a stress-responsive disease. In genomic analysis using lymphocytes, a single-nucleotide polymorphism of the human TSPO gene at exon 4 (485G>A), which is presumably associated with an individual's stress sensitivity, showed significantly lower frequencies of G/G and higher frequencies of G/A in patients with AD than in healthy subjects. The severity of AD, as determined with the Scoring of Atopic Dermatitis index, was correlated with TSPO expression in male patients with the G/A phenotype. In conclusion, the present study provides new evidence that variation in the TSPO gene affects susceptibility to AD.
(J Nippon Med Sch 2014; 81: 148–156)

Key words: atopic dermatitis, stress sensitivity, translocator protein 18 kDa (TSPO), genomic analysis

Introduction

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease of persons with the predisposing factor of atopy. In 2006, mutations in the gene for the production of filaggrin were found to strongly increase the risk of AD[1]. Genetic

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mutations in filaggrin often reduce the barrier function of skin, elevate immunoglobulin E levels, and lead to the pathogenesis of chronic dermatitis[2].

Many patients with AD live under stressful conditions, in which intense unavoidable itching disturbs their sleep and markedly reduces their quality of life[3]. Repeated scratching can cause erythema, pigmentation, and lichenification. Due to these cosmetic problems, the patients are exposed to psychological stress as well as physical stress. Frequent scratching as a means of escaping the intolerable stress worsens the pruritic and eczematous skin lesions.

AD can lead to psychological disturbances, such as stigmatization, social isolation, and discrimination[4]. Patients with AD have been reported to exhibit anxiety, depression, and emotional excitability[5,6]. Psychological stress and symptoms of AD appear to form a vicious cycle[7]. It remains unclear, however, how stress affects AD.

On the other hand, there has been a growing interest in *the translocator protein (18 kDa)* (TSPO), formerly known as *the peripheral-type benzodiazepine receptor* (*PBR*), in the subjects of steroidogenesis, apoptosis, and immunomodulation[8–10]. The TSPO is involved in the regulation of several major stress systems, *i.e.*, the hypothalamic-pituitary-adrenal axis, the sympathetic nervous system, the renin-angiotensin axis, and the neuroendocrine-immune axis[8].

Our previous studies have found that the expression of TSPO on platelets is significantly correlated with the trait anxiety score in healthy human subjects[11]. The evidence for TSPO as a promising biological marker of stress has prompted us to investigate the stress response of TSPO at the genomic level.

A 485G>A single nucleotide polymorphism (SNP) in a coding region of exon 4 of the TSPO gene was found to affect susceptibility to panic disorder (PD)[12]. Before the onset of PD, individuals with the G/G genotype showed high anxiety sensitivity and an increase in TSPO. Our study suggests that individuals with the G/G genotype are at increased risk for stress-related disorders.

The present study was designed to examine how the symptoms of AD are related to individual's stress sensitivities by analyzing the density of platelet TSPO together with the genetic variation of TSPO.

Materials and Methods

Subjects

Fifty-two patients with AD (30 male and 22 female) and 163 healthy volunteers (89 males and 74 female) participated in this study. The participants were given the State-Trait Anxiety Inventory (STAI), a self-reported measure of anxiety. For patients with AD, the Scoring of Atopic Dermatitis (SCORAD) index was performed. The SCORAD index is a well-established severity-scoring tool for AD which is widely used in dermatology. The SCORAD index consists of the interpretation of the extent of the disorder (A: according to the rule of nines; score 0–20), the intensity composed of 6 items (B: erythema, edema/papules, effect of scratching, oozing/crust formation, lichenification and dryness; score 0–63; each item has 4 grades: 0, 1, 2, and 3), and symptoms (C: itch, sleeplessness; score 0–20). All subjects were fully informed about the nature of the study and gave their written consent. This study was approved by the Ethics Committees of Nippon Medical School and Tokyo Metropolitan Institute of Medical Science.

Preparation of Platelet Membranes

Blood samples (20 mL) were collected, and platelets were isolated with our standard procedures[11]. In brief, blood samples (20 mL) were obtained from the subjects in the morning between 9:00 a.m. and 10:00 a.m. The samples were collected in plastic-walled, evacuated blood collection tubes (Venoject II, Terumo Corp., Tokyo, Japan) and spun twice at 180 x *g* for 15 minutes at 4℃. Platelet-rich plasma was collected and spun at 1,500 x *g* for 15 minutes at 4℃. The platelet-containing pellet was frozen at −80℃.

Before the binding assay, the samples were thawed, and each pellet was homogenized in 10 mL of ice-cold Tris-HCl buffer (50 mM, pH 7.4) in a homogenizer (Polytron PT-10, Thermo Fisher