Updated Guide to Stress, Stress-related Diseases, and Coping. Freeman and Co, New York, 1998, pp 76-79
24. Bray G : Autonomic and endocrine factors in the regulation of food intake. Brain Research Bulletin 14, 505-510, 1985
25. Zukowska-Grojec Z : Neuropeptide Y ; a novel sympathetic stress hormone and more. Annals of the New York Academy of Sciences 771 : 219-233, 1995
26. Morley J : Neuropeptide regulation of appetite and weight. Endocrine Review 8 : 256-287, 1987
27. McEwen B, Sakai R, Spencer R : Adrenal Steroid Effects on the Brain : Versatile Hormones with Good and Bad Effects. Hormonally Induced Changes in Mind and Brain 157-189, 1993
28. Fehm-Wolfsdorf G, Scheible E, Zenz H, Born J, Fehm HL : Taste thresholds in man are differentially influenced by hydrocortisone and dexamethasone. Psychoneuroendocrinol 14 : 433-440, 1989
29. Henkin RL : The effects of corticosteroids and ACTH on sensory systems. Prog Brain Res 32 : 279-294, 1970
30. Greeno C, Wing R, Matthews K, Vitaliano P : Stress predicts overeating and weight gain outside of the laboratory : data from multiple studies. Annals of Behavioral Medicine 20 : S 032, 1998
31. Epel E, Lapidus R, McEwen B, Brownell K : Stress may add bite to appetite in women : a laboratory study of stress-induced cortisol and eating behavior. Psychoneuroendocrinol 26 : 37-49, 2001
32. Klatsky AL, Armstrong MA, Friedman GD : Coffee, tea, and mortality. Ann Epidemiol 3 : 375-381, 1993
33. Heishman SJ, Henningfield JE : Stimulus function of caffeine in humans : relation to dependence potential. Neurosci Biobehav Rev 16 : 273-287, 1992
34. Chou T : Wake up and smell the coffee : caffeine, coffee, and the medical consequences. West J Med 1657 : 544-553, 1992
35. Goldstein A, Kaizer S : Psychotropic effects of caffeine in man, II : alertness, psychomotor coordination, and mood. J Pharmacol Exp Ther 150 : 146-151, 1965
36. Goldstein A, Kaizer S, Whitby O : Psychotropic effects of coffee in man, IV : quantitative differences associated with habituation to coffee. Clin Pharmacol Ther 24 : 243-252, 1978
37. Griffiths RR, Evans SM, Heishman SJ, Preston KL, Sannerrud CA, Wolf B, Woodson PP: Low-dose caffeine discrimination in humans. J Pharmacol Exp Ther 2556 : 1123-1132, 1990
38. Furlong FW : Possible psychiatric significance of excessive coffee consumption. Can J Psychiatry 20 : 577-583, 1975
39. Stephenson PE : Physiologic and psychotropic effects of caffeine on man. J Am Diet Assoc 71: 240-247, 1977
40. Mino Y, Yasuda N, Fujimura T, Ohara H : Caffeine consumption and anxiety and depressive symptomatology among medical students. Jpn J Alcohol Drug Depend 25 : 486-496, 1990
41. Stunkard AJ, Grace WJ, Wolff HG : The night-eating syndrome : a pattern of food intake among certain obese patients. Am J Med 19 : 78-86, 1955
42. Rand CS, Macgregor AM, Stunkard AJ : The night-eating syndrome in the general population and among postoperative obesity surgery patients. Int J Eat Disord 22 : 65-69, 1997
43. Stunkard A, Berkowitz R, Wadden T, Tanrikut C, Reiss E, Young L : Binge eating disorder and the night-eating syndrome. Int J Obes Relat Metab Disord 20 : 1-6, 1996
44. Birketvedt G, Florholmen J, Sundsfjord J, Osterud B, Dinges D, Bilker W, Stunkard A : Behavioral and neuroendocrine characteristics of the night-eating syndrome. J Am Med Assoc 282 : 657-663, 1999
45. Gluck ME, Geliebter A, Satov T : Night eating syndrome is associated with depression, low self-esteem, reduced daytime hunger, and less weight loss in obese outpatients. Obes Res 9 : 264-267, 2001
46. Birketvedt GS, Sundsfjord J, Florholmen JR : Hypothalamic-pituitary-adrenal axis in the night eating syndrome. Am J Physiol 282 : E366-E369, 2002
47. Perrig WJ, Perrig P, Stavelin HB : The relation between antioxidants and memory performance in the old and very old. J Am Geriatr Soc 45 : 718-724, 1997
48. Perkins A, Hendrie HC, Callahan CM, Gao S, Unverzagt FW, Xu Y, Hall KS, Hui SL : Association of antioxidants with memory in a multiethnic elderly sample using the Third National Health and Nutrition examination Survey. Am J Epidemiol 150 : 37-44, 1999
49. Sano M, Ernesto MS, Thomas RG, Klauber MR, Schafer K, Grundman M, Woodbury P, Growdon J, Cotman CW, Pfeiffer E, Schneider LS, Thal LJ : A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. N Engl J Med 336 : 1216-1222, 1997

50. Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen JJ, Bickford PC : Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberries, spinach, or strawberry dietary supplementation. J Neurosci 19 : 8114-8121, 1999

51. Joseph JA, Shukitt-Hale B, Denisova NA, Prior RL, Cao G, Martin A, Taglialatela G, Bickford PC: Long-term dietary strawberry, spinach, or vitamin E supplementation retards the onset of age-related neuronal signal-transduction and cognitive behavioral deficits. J Neurosci 18 : 8047-8055, 1998

特集―統合失調症の新しい治療の展望―I

統合失調症の早期発見・発症予防の可能性

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抄録：本論では，まず精神病未治療期間（DUP）に関する近年の研究成果を紹介した。その中で，DUPが統合失調症の経過を予測する因子の1つである可能性が高いが，現状ではわが国の内外を問わず1年から2年という非常に長いDUPの報告が多いことを述べた。次に，統合失調症の早期発見・発症予防を視野に入れた海外の代表的な活動としてオーストラリアの早期精神病予防・介入センター（EPPIC），ノルウェー・デンマークの精神病の早期治療と発見プロジェクト（TIPS）を挙げ，TIPSによりDUPが大幅に短縮したという報告を紹介した。わが国のプロジェクトとして，小椋らの琉球大学グループ，倉知らの富山医科薬科大学グループ，水野らの慶応大学グループの活動を紹介し，さらに中安の業績について述べた。最後に，①筆者らが経験した「統合失調症の早期発見・発症予防」と関連のある症例を報告し，②筆者らが作成した「統合失調症の予防への寄与を目指すパンフレット」と「日本版バーチャルハルシネーション」に触れた。

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Key words：*schizophrenia, prevention, DUP, EPPIC, TIPS*

I．はじめに

本質的な治療法が確立されていない疾患を含め，すべての病気の臨床において早期発見・早期治療（2次予防）や発症予防（1次予防）が重要な意味を持つことは医学の常識といえよう。とすれば，統合失調症でも予防活動が大切であることは当然至極であるし，実際以前から識者によって指摘され議論されてきた経緯がある。しかしながら，表1に示したような諸事情があったこともあり，統合失調症の予防をめぐる従来の議論には，実践・普及面での実績がやや乏しい卓上の論述という色彩が若干なりともあったように思われる。

しかるに近年，精神病未治療期間（Duration of Untreated Psychosis：DUP）に関する研究が進み，世界各地で早期発見・早期治療，発症予防の実践を目指すプロジェクトが立ち上げられ様々なデータが発表され始めている。統合失調症の予防をめぐる議論は，夢や理念の段階から実践の段階に入ったといえよう。

本論では「統合失調症の早期発見・発症予防の可能性」というテーマに関して，①統合失調症の

Early identification and prevention of schizophrenia.

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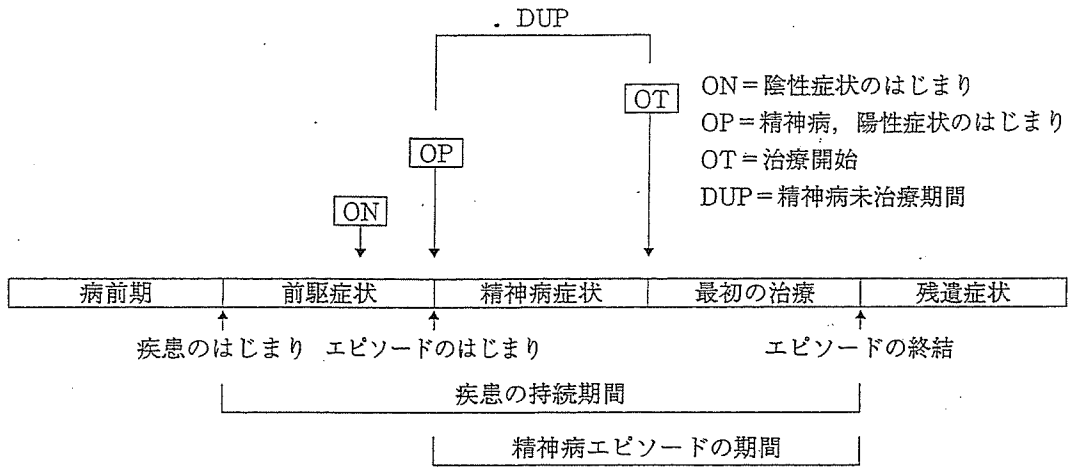

図1　統合失調症の経過とDUP（文献15より引用）

表1　統合失調症の予防が注目されていなかった理由（文献9より引用）

(1)疾病研究の進展が不十分
　→危険因子，防御因子が明らかになっていない
(2)悲観的な疾病感
　→「進行性の病気で予後不良」「予防は無理」という先入観があった
(3)関係者の協力体制が未確立
　→精神科医，コメディカルスタッフ，疫学担当者などの協力が不十分であり，自助グループや家族会との連携もできていなかった
(4)予防活動を評価しない医療経済制度
　→わが国の医療保険制度では，病気の発症や再発を予防しても評価されないシステムになっている
(5)以上の事情が重なり，健康保険の分野で「統合失調症の予防」の重要性が十分認識されていなかった

表2　諸研究によるDUP（文献15より引用）

	n	中間値(週)	平均値(週)	標準偏差
Beiser et al.	72	8	56	148
Birchwood et al.	71		30	
Haas and Sweeny	71		99	
Loebel et al.	70	39	52	82
Moscarelli et al.	20		76	92
Larsen et al.	43	26	114	173
Häfner et al.	165		109	
Moller and Husby	18	18	32	35
McGorry et al.*				
pre-EPPIC	200	30	227	714
post-EPPIC	147	52	175	385

*McGorryらのデータの単位のみ「週」でなく「日」

DUPをめぐる近年の研究成果に触れた上で，②わが国の内外における早期発見・早期治療のプロジェクトを紹介し，③筆者らの試みを記すという順で，論を進める。

Ⅱ．統合失調症の未治療期間（DUP）

DUP（図1）をめぐる研究結果の中で，当初精神医療関係者の耳目を集めたポイントの1つは，「ある程度予想されたことではあるが，実地調査の結果明らかになったDUPがいかに長いか！」という点ではなかろうか。表2に示されているように，報告されているDUPの平均値は30週（7ヵ月半）から114週（2年以上）の間にある[15]。DUPが極端に長い一部症例が平均値を長くしている事情を踏まえても，「DUPを短縮する対策を講じる必要がある現状が如実に示されている」と言わざるをえないデータであろう。そして欧米諸国同様，わが国においても大変長いDUPが報告されている〔水野ら（カッコ内は標準偏差）：17.6（26.9）ヵ月[14]，村上ら[16]：19.6（21.5）ヵ月〕。また，気分障害などの他の精神障害と比較しても，特に統合失調症のDUPが長い可能性が示唆されている[1]。

さらに，DUPがその後の経過を予測する可能性を支持する報告が相次いだことも，（内容的には意外性はないものの）やはり印象的であった。

表3　長いDUPがもたらす可能性のある悪影響（文献3より引用）

- 寛解までの遅れ，不完全寛解
- より不良な予後
- うつや自殺のリスクの増加
- 心理的，社会的発達の妨害
- 人間関係での緊張
- 家族や社会からの支援の喪失
- 患者の子育てについての技能の崩壊
- 患者の家族内における苦痛や心理的問題の増加
- 勉学や就職の中断
- 物質乱用
- 暴力行為や犯罪行為
- 不必要な入院
- 自尊心や自信の喪失
- マネジメントのためのコストの増大

表4　DUPを短縮するための戦略（文献3より引用）

- 理解を深める
  - —プライマリケアに携わる者への教育
  - —精神病の早期サインへの注意を促す
  - —地域社会の教育
  - —患者やその家族が援助を求めることをためらう原因である精神疾患に対するスティグマを減らす
- 専門医への照会を増やす
  - —利用者に対する反応のよい，親しみやすいサービスを提供する
  - —精神科サービスに対する恐怖感やスティグマを減らす
- 精神科サービスへの容易なアクセスを提供する
  - —すばやい対応
  - —柔軟性のあるアプローチ
  - —積極的訪問

今までに，初発統合失調症患者のDUPの長短と，その後の精神状態・社会適応などの関連が認められるという結果が多く報告されている[2,3,12,15]。現在，統合失調症の治療開始遅延によって生じうると想定されている悪影響には，表3の様々な事項がある。

Ⅲ．統合失調症の早期発見・早期治療プロジェクト

前節で触れたDUPの知見などを踏まえて，「発症後数年間の時期は，統合失調症の治療上非常に重要な期間（臨界期：critical period）[2,12]であり，この時期の介入を逸すると長期化・慢性化しやすいので，当面の臨床上の目標は，①DUPを短縮化するための社会への様々な働きかけ（表4）を行い，②臨界期の患者に適切な薬物療法，精神療法（個人，集団，家族），精神科リハビリテーションを包括的に実施して重篤化・慢性化を予防することにある」という共通認識が持たれるに至っている。そして，その実践のために世界各地で様々なプロジェクトが立ち上げられている。

Birchwoodらの編書[2]では世界各地の26のプロジェクトチームが紹介されているが，代表例としてオーストラリアの早期精神病予防・介入センターの活動（Early Psychosis Prevention and Intervention Center：EPPIC），ノルウェー・デンマークの精神病の早期治療と発見プロジェクト（Early Treatment and Identification of Psychosis：TIPS）などがある（表5）。

オーストラリアの早期精神病予防・介入センター（EPPIC）[3,12]は図2のような多様なチームからなる組織で，「発症を可能な限り早期に発見し，以後18ヵ月の間にそれぞれの病期に見合った特異的な集中治療をするという2つの戦略で早期精神病患者の1次的，2次的な病的状態を軽減すること」を目的としている。また，早期精神病のための認知療法的精神療法（Cognitive-Oriented Psychotherapy for Early Psychosis：COPE）を開発・利用しており，その有用性を示すデータを発表している。

一方，ノルウェー・デンマークの精神病の早期治療と発見プロジェクト（TIPS）[2,3]は，①精神病の早期兆候を一般に広く啓発する，②教師，若年者，家庭医に対して特別な教育をする（図3），③未治療の初回エピソード精神病患者を早期発見し治療を行うチーム（Detection Teams：DTs）を稼働する，などを行ってきた。予備的な報告によれば，TIPSによりDUPが大幅に短縮した（114週→17週）とのことである[2]。また最新の報告[13]によれば，早期発見チームのある地区とない地区の統合失調症のDUPと経過を比較すると，前者

表5　統合失調症の早期発見・早期治療を目指す主なプロジェクト(文献3より引用)

	開始年月日	診断	対象年齢(歳)	経過追跡時間(年)	サービス対象	管轄の人口	年間の新患者数	稼働症例数	評価	運営費	連絡先
EPPIC メルボルン	1992 (入院施設は1984以来)	初回エピソード精神病	15~29	1.5	入院患者, 外来患者, 前兆症状を呈する患者	819,000 (15~29年齢人口で206,259)	255(1997~2000の平均)	400+	経過,効果,6ヵ月,12ヵ月,24ヵ月予後	継続的	www.epic.org.au
TIPS ノルウェーおよびデンマーク	1997	統合失調症圏内,および幻覚妄想を伴う感情障害	18~65	2	外来患者, 前兆を呈する患者	370,000(ログランド) 190,000(オスロ) 95,000(ロスキルデ)	100	n/a	3ヵ月,1,2,5年予後：介入群を2つの非介入群と比較	6年プロジェクト(1997~2002)	www.tips-info.com
EIS バーミンガム,イギリス	1995	初回エピソード精神病	16~30	3	外来患者, 前兆を呈する患者	300,000(2002年3月より100万人)	120	150	1,2,3年予後	継続的	www.iris-initiative.org.uk
EPP カナダ,アルバータ州カルガリィ	1996	非感情病性初回エピソード精神病	16~45	3	外来患者, 前兆を呈する患者	930,000(カルガリィ市)	85	170	3,6,9,12,15,18,21,24ヵ月および3年予後	継続的	www.early-psychosis.com
PEPP ロンドン オンタリオ, カナダ	1996	非感情病性初回エピソード精神病	16~50	2	入院患者, 外来患者	390,000(ロンドンおよびミドルセックス)	50	100	1,2年予後	継続的	www.pepp.ca

のDUPが有意に短く（5週 vs16週），前者の経過が良好であった。

Ⅳ．わが国の早期発見・早期治療プロジェクト

前節で紹介した世界の動きに対応して，わが国でも独自の実践が始まっている。

小椋らの琉球大学のグループ[18]は，1994年から大学生を対象として精神障害の予防活動を行ってきた。大学保健管理センターで健康診断を受ける琉球大学の新入生全員を対象にして質問票を用いたスクリーニングを行い，ハイリスク者に対して精神科医による診断面接，心理テスト（ロールシャッハテスト），神経生理学テスト（event-related potential：ERP）などを施行して介入し，成果を上げてきた。さらに，平松ら[10]は「統合失調症の母親とその子どもに対する積極的支援を出産直後から，あるいは妊娠中から行う」ための専門外来「子作り，子育て支援外来」を琉球大学精神科で開設している。そして平松は，統合失調症の予防に関して「1次予防のポイント：統合失調症者の育児の相談・支援，2次予防のポイント：青少年の援助を行いつつearly psychosisの診断・治療を実践する，3次予防のポイント：服薬遵守＋生活支援・社会参加促進を通じての人生の回復と再発見」と手際よくまとめて述べている。

また，中安[17]は統合失調症の初期症状に関する臨床研究を行い，初期分裂病の疾患概念を提唱している。そして，精神病理学や神経心理学の観点から初期分裂病に関する精緻な考察を行い，薬物療法・精神療法にも言及している。

一方，倉知ら[11]富山医科薬科大学のグループは脳の画像研究をもとに統合失調症の側頭葉-前頭葉2段階発症仮説を提唱し，早期発見・早期治療の可能性を検討している。

さらに，水野[14]ら慶応大学のグループはわが国のDUPに関する実地調査を行い，統合型地域精神科治療プログラム（Optimal Treatment Project：OTP）を展開しつつ，精神障害の早期発見・早期治療を目指す実践も行っている。

また筆者らも，統合失調症の早期発見・早期治

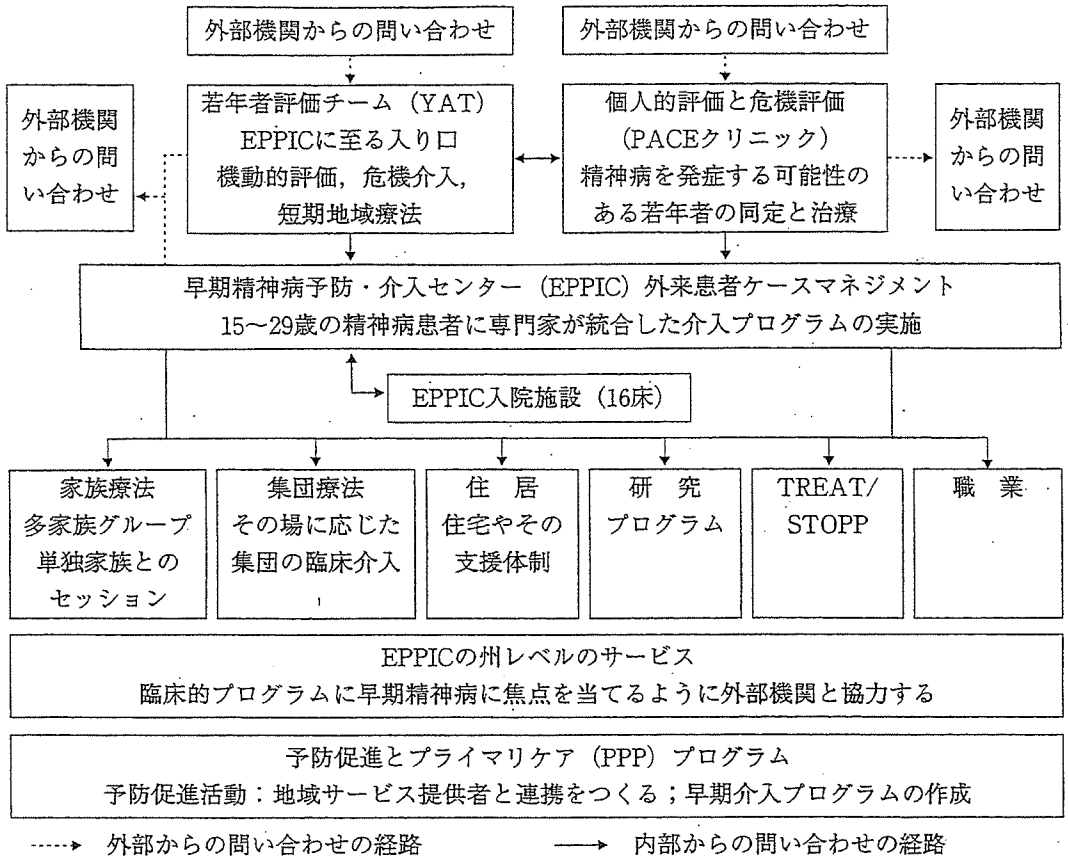

図2　EPPICのサービスモデル（文献3より引用）

療というテーマに対して，独自の立場から関心を抱き実践活動を行ってきたので，次節で一部を紹介させていただく。

V．筆者らの取り組み

1．統合失調症のハイリスク児での早期発見・発症予防

筆者らは岡崎が中心となり，統合失調症患者の子弟（ハイリスク児）の追跡研究を行ってきた[7,8,19]。その過程で様々な知見が得られているが，ここでは本論のテーマ「統合失調症の早期発見・発症予防の可能性」と関連のある1症例を紹介させていただく。

症例：初診時17歳，女性[7]

家族歴：父親が統合失調症で通院・服薬中。母親は一時期うつ病になり，通院・服薬をしていたことがある。

生活歴：2人姉妹の第1子，長女として出生。学業成績は上位で友人も多く，小学校～高校を通して学校生活への適応は良好であった。

現病歴：X年9月（本人は高校3年生），大学入試の重圧に加えて，父親の統合失調症が再発して母親のうつ病も悪化した。X年10月初旬，「両親の声がぼんやり聞こえてくる。何を言っているのかはわからないが，声が聞こえてくる」「昔のいやな記憶がどんどん出てきて止まらない」体験が出現。それを知った母親に勧められて，X年10月16日に初診となった。

治療経過：初診時より薬物療法（fluphenazine 1 mg/日）を開始するとともに，幻聴に対する心理教育を行った。約2週間で幻聴と自生記憶想起は消褪した。

X＋1年4月，大学に入学。大学生活になじむまでの約1年間投薬を継続した後，薬物療法を中止した。その後，現在（X＋10年）までのところ精神状態は一貫して安定しており，社会適応も良好である。

図3　TIPSで用いられている学生用教育パンフレット（文献2より引用）

コメント：統合失調症（父親）とうつ病（母親）の家族歴があり，筆者らが追跡していた個体が「大学受験，父親の統合失調症の再発，母親のうつ状態の悪化」というストレス条件下で幻聴，自生記憶想起を体験したが，治療に反応して速やかに症状が消褪した症例である。薬物療法を中止してから8年以上経過しているが，現在までのところ一貫して安定している。本論のテーマ「統合失調症の早期発見・発症予防の可能性」と重なる部分の大きい症例と思われるため，紹介させていただいた。なお本症例の診断は，一過性精神病性障害を考えている。

2．予防への寄与を目指す疾患教育の試み

筆者らは，統合失調症の子弟（ハイリスク児）の追跡研究[8]や統合失調症患者の病前行動特徴の研究[4]から，統合失調症の1次・2次予防の実現には3つのポイントがあると考えている（表6）。

1つ目のポイントは，病前特徴への働きかけである。ハイリスク児研究や病前特徴研究の結果から，統合失調症患者は発症前から「消極的，自信がない，対人緊張が強い，非社交的で孤立しがち」などの性格・行動上の特徴を示す場合が多く，発症しない人は「自己肯定的で自己評価が安定，積極的で自主性がある，対人関係が円満」などの対照的な特徴を示す場合が多いことが明らかになった。発症者で乏しく発症しない人で認められやすい「対人関係能力，問題処理技能，自己評価」は，発症に防御的に働く抗罹病効果を持つ可能性がある。そこで予防のポイントの1つ目は，これらの特性を自分の個性にあった方法で学習し身につける大切さを本人，家族，教師などに伝え，習得方法の例を紹介することとなる。

表6　統合失調症の1次・2次予防実現のための3つのポイント（文献9より引用）

①病前特徴への働きかけ
　対人関係能力
　問題処理技能
　自己評価　など
②ライフイベントに関する心理教育
③2次予防実現のために役立つ情報提供
　・精神病理現象（前駆症状，初期症状，精神病体験）の現れ方
　・精神科の治療の内容
　・早期治療の必要性，有効性
　・受診・相談を行う際に利用できる社会資源など

表7　心の病を予防するためのパンフレット―心の健康を守り育てるための9章―（文献9より引用）

①心の病について知っておく利点
②心の病とは？
　―代表的な心の病「統合失調症」のアウトライン―
③統合失調症でよくみられる「空耳」について
④統合失調症でよくみられる「空耳」の内容と影響力
⑤統合失調症でよくみられる「勘繰り」について
⑥統合失調症が起こるきっかけになりやすい生活環境
　―ストレスによるピンチ―
⑦ピンチに陥った時の上手な対応法
　―逆境の受け止め方，しのぎ方―
⑧ピンチに陥らないために役立つこと
　―「転ばぬ先の杖」になりうる事柄―
⑨心の病が出てきた時の対処法と精神科の治療の説明
　―利用できる社会資源の紹介―

表8　予防教育受講者を対象としたアンケート調査の結果（文献9より引用）

質問内容	結果
(1)講義内容への興味・関心の有無	「たいへん興味を持った」または「少し興味を持った」＝88%
(2)パンフレットの内容の理解の可否	「よく理解できる」または「一部理解できる」＝81%（第9項目）～97%（第1項目）
(3)講義内容の有用性の有無	「有用性を感じた」＝80%
(4)講義内容を一般教育の場で扱う必要性	「もっと早く知っておいた方がよい」＝70% 「今頃で（＝大学または専門学校入学時）丁度よい」＝14%

予防の2つ目のポイントは，ライフイベントに関する心理教育である。従来から，統合失調症発症前に様々なライフイベントがみられる場合が多いと知られていたが，筆者らのハイリスク児研究でも同様の結果が得られた。そこで，ライフイベントに関する説明を行い，困難に陥った際の対処法を伝える心理教育が予防に役立つ可能性があると思われる。

3つ目のポイントは，（逆説的であるが）1次予防実現の困難さである。ハイリスク児研究を通して，親の精神状態が比較的安定していて精神科医が日常的に相談に乗っていても，1次予防が容易には実現できないことが明らかになった。そこで，2次予防の重要性があらためてクローズアップされることになる。そしてその実現には，「精神病体験の実際の現れ方や悪影響，精神科の治療の内容と必要性・有効性，相談・治療のために利用できる社会資源」などの情報伝達が役立つ可能性がある。

筆者らは，以上の「3つのポイント」の内容などを一般者向けにわかりやすく解説したパンフレットを作成した[5]。パンフレットの表題は「心の病を予防するためのパンフレット―心の健康を守り育てるための9章―」であり，表7に全9章の表題を示した。

筆者らは，このパンフレットを用いて青年期の一般者を対象とした予防教育を実践し，受講者の関心の度合いや理解の程度などを調べるために，講義終了時に無記名でアンケート調査を行った[6]。表8に結果の概要を示したが，この結果から予防教育がある程度受講者の興味・関心を引いて内容の一部を伝達でき，有用性も一定程度感じとってもらえたとみなせるのではないか，と考えている。

図4　日本版バーチャルハルシネーション用のパンフレット

また，筆者らは統合失調症でよくみられる幻覚症状を疑似体験できる装置「日本版バーチャルハルシネーション（VH）」の精神医学面の監修に携わり，VHの解説パンフレット（図4）を作成した。VHを統合失調症や薬物乱用の予防教育で活

用しうる可能性を考え，試行を始めたところである。

Ⅵ. おわりに

本論では，統合失調症のDUPをめぐる研究結果に触れ，わが国の内外で行われている早期発見・早期治療のためのプロジェクトを紹介した。今後統合失調症の予防をめぐる議論・実践がさらに広がり，統合失調症のDUP短縮が実現し，発症予防に関する臨床研究が進むことが望まれる。

文　献

1) Beiser, M., Erickson, D., Fleming, J.A.E. et al.: Establishing the onset of psychotic illness. Am. J. Psychiatry, 150; 1349–1354, 1993.
2) Birchwood, M., Fowler, D., Jackson, C.(eds.): Early Intervention in Psychosis. A Guide to Concepts, Evidence and Interpretations. Wiley, New York, 2000.
3) Edwards, J. and McGorry, P.D. (eds.): Implementing Early Intervention in Psychosis: A Guide to Establishing Early Psychosis Services. Martin Dunitz, London, 2002. (水野雅文, 村上雅明監訳: 精神疾患早期介入の実際—早期精神病治療サービスガイド. 金剛出版, 東京, 2003.)
4) 原田誠一, 岡崎祐士, 増井寛治ほか: 精神分裂病患者の病前行動特徴. 精神医学, 29; 705–715, 1987.
5) 原田誠一, 岡崎祐士, 増井寛治ほか: 一般者を対象とした精神分裂病に関する疾患教育プログラムの作成（第1報)—分裂病の1次・2次予防への寄与を目指す疾患教育パンフレットの紹介. 精神医学, 41; 811–819, 1999.
6) 原田誠一, 岡崎祐士, 増井寛治ほか: 一般者を対象とした精神分裂病に関する疾患教育プログラムの作成（第2報)—疾患教育の受講者を対象にしたアンケート調査の結果. 精神医学, 41; 937–945, 1999.
7) 原田誠一, 岡崎祐士: 日常臨床における精神分裂病の早期発見と早期治療—ハイリスク児の追跡研究から. 精神経誌, 101; 916–922, 1999.
8) 原田誠一, 岡崎祐士: ハイリスク児研究からみた分裂病の病因と予防. 臨床精神医学, 29; 367–374, 2000.
9) 原田誠一, 岡崎祐士: 統合失調症の早期発見・早期治療. 精神科, 2: 303–310, 2003.
10) 平松謙一: 統合失調症ハイリスク者の相談. 岡崎祐士編: 新世紀の精神科治療1—統合失調症の診療学, 中山書店, 東京, p.249–264, 2002.
11) 倉知正佳, 川崎康弘: 統合失調症の病態形成と脳の発達. 岡崎祐士編: 新世紀の精神科治療1—統合失調症の診療学, 中山書店, 東京, p.30–39, 2002.
12) McGorry, P.D. and Jackson, H.J. (eds.): The recognition and management of early psychosis. A preventive approach. Cambridge University Press, Cambridge, 1999. (鹿島晴雄監修, 水野雅文, 村上雅昭, 藤井康男監訳: 精神疾患の早期発見・早期治療. 金剛出版, 東京, 2001.)
13) Melle, I, Larsen, T.K., Haahr, U. et al.: Reducing the duration of untreated first-episode psychosis: Effects on clinical presentation. Arch. Gen. Psychiatry, 61; 143–150, 2004.
14) 水野雅文, 山澤涼子, 三浦勇太ほか: 日本における初発分裂病の精神病未治療期間（DUP）について. 小椋力編: 精神障害の予防をめぐる最近の進歩, 星和書店, 東京, p.154–155, 2002.
15) 水野雅文, 山澤涼子: 初回エピソード分裂病の未治療期間（DUP）と治療予後. Schizophrenia Frontier, 3; 35–39, 2002.
16) 村上忠, 福治康秀, 宮里洋ほか: 沖縄県における精神分裂病の未治療期間（DUP）に関する予備的調査. 小椋力編: 精神障害の予防をめぐる最近の進歩, 星和書店, 東京, p.156–157, 2002.
17) 中安信夫: 初期分裂病. 星和書店, 東京, 1990.
18) 小椋力: 精神障害をいかに予防するか. 精神科, 2; 297–302, 2003.
19) 岡崎祐士: 発病前ないし早期分裂病への介入. 臨床精神薬理, 2; 1113–1120, 1999.

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Evidence of novel neuronal functions of dysbindin, a susceptibility gene for schizophrenia

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Genetic variation in dysbindin (DTNBP1: dystrobrevin-binding protein 1) has recently been shown to be associated with schizophrenia. The dysbindin gene is located at chromosome 6p22.3, one of the most promising susceptibility loci in schizophrenia linkage studies. We attempted to replicate this association in a Japanese sample of 670 patients with schizophrenia and 588 controls. We found a nominally significant association with schizophrenia for four single nucleotide polymorphisms and stronger evidence for association in a multi-marker haplotype analysis ($P = 0.00028$). We then explored functions of dysbindin protein in primary cortical neuronal culture. Overexpression of dysbindin induced the expression of two pre-synaptic proteins, SNAP25 and synapsin I, and increased extracellular basal glutamate levels and release of glutamate evoked by high potassium. Conversely, knockdown of endogenous dysbindin protein by small interfering RNA (siRNA) resulted in the reduction of pre-synaptic protein expression and glutamate release, suggesting that dysbindin might influence exocytotic glutamate release via upregulation of the molecules in pre-synaptic machinery. The overexpression of dysbindin increased phosphorylation of Akt protein and protected cortical neurons against neuronal death due to serum deprivation and these effects were blocked by LY294002, a phosphatidylinositol 3-kinase (PI3-kinase) inhibitor. SiRNA-mediated silencing of dysbindin protein diminished Akt phosphorylation and facilitated neuronal death induced by serum deprivation, suggesting that dysbindin promotes neuronal viability through PI3-kinase-Akt signaling. Genetic variants associated with impairments of these functions of dysbindin could play an important role in the pathogenesis of schizophrenia.

INTRODUCTION

Schizophrenia is a complex genetic disorder characterized by profound disturbances of cognition, emotion and social functioning. It affects ~1% of the general population worldwide. Chromosome 6p is one of the most consistently replicated susceptibility regions in linkage studies of schizophrenia (1). A recent study implicated a gene on chromosome 6p, dysbindin (DTNBP1: dystrobrevin-binding protein 1), as a susceptibility locus in the Irish pedigrees (2). Since then, four studies have reported evidence supporting the association between genetic variants in dysbindin and schizophrenia in

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German, Chinese, Swedish and Irish populations (3–6), while one study failed to replicate positive association in an Irish case–control design (7). In the present study, we attempted to perform an independent association study in a Japanese population of schizophrenic cases and controls.

The pathophysiology of schizophrenia is still unclear; however, this disease is believed to involve genetic abnormalities in developmental processes leading to abnormal synaptic plasticity, including glutamatergic transmission (8,9). Several genes, e.g. dysbindin, neuregulin 1, G72, D-aminoacid oxidase, the regulator of G-protein signaling-4, GRM3 and PPP3CC are described as susceptibility genes for schizophrenia, and those genes may have convergent effects on glutamatergic synapses (10,11). Neuregulin affects the expression and plasticity of the *N*-methyl-D-aspartate (NMDA) receptor (12,13). D-aminoacid oxidase metabolizes D-serine, an endogenous modulator of the NMDA receptor (14), and G72 is probably an activator of D-aminoacid oxidase (15). The regulator of G-protein signaling-4 is the negative regulator of G-protein-coupled receptors, including metabotropic glutamate receptors (16). GRM3 encodes the mGlu3 receptor gene. PPP3CC, the calcineurin γ-subunit, is critical for certain types of NMDA-mediated plasticity. However, no evidence of a role in glutamatergic transmission has been imputed to dysbindin, although dysbindin is believed to play a role in synaptic plasticity and signal transduction. Although dysbindin has recently been cloned as a dystrobrevin-binding protein in mouse (17), little is known about the functions in neurons. Here, we examined neuronal functions of dysbindin and found two novel actions: (1) increased glutamate release with upregulation of pre-synaptic proteins and (2) neurotrophic effect through Akt signaling pathway.

RESULTS

Genetic association analysis

We genotyped six single nucleotide polymorphisms (SNPs) in dysbindin in 670 schizophrenic patients and 588 controls in a Japanese population. The genotype distributions of the six SNPs for the schizophrenic patients and the control subjects were in Hardy–Weinberg equilibrium (data not shown). Allele frequencies of the six SNPs among the patients and controls are shown in Table 1. A significant difference in allele frequency was observed between cases and controls for four SNPs, but not for the remaining two SNPs (Table 1). The G allele of P1635 was in excess in our cases when compared with controls ($\chi^2 = 10.3$, df = 1, $P = 0.0013$, odds ratio = 2.71, 95% CI 1.46–5.79, corrected $P = 0.0078$).

To further analyze the haplotype structure in our sample, we computed the linkage disequilibrium (LD) between the SNPs using D'. D' values ranged between 0.5 and 1.0 and indicated strong to intermediate LD between the markers. Thus, adjacent combinations of up to six markers were examined for association with schizophrenia. Global and individual *P*-values corresponding to haplotypes consisting of adjacent markers and estimated haplotype frequencies in patients and controls are shown in Table 2. All haplotype combinations were significantly associated with schizophrenia, except the P1320–P1763 haplotype. Given this result, we tested the contribution of individual haplotypes to the global result. The G–G haplotype (P1635–P1325), including the G allele of P1635, which was significantly more frequent in our cases (Table 2), was enriched in patients with schizophrenia when compared with controls (estimated frequencies: patients 3.0% versus controls 0.9%, *P*-value = 0.00028, corrected $P = 0.0042$).

Functional analysis in dysbindin-overexpressing cultured neurons

To clarify the function of dysbindin in the central nervous system, we focused on the pre-synaptic machinery in neuronal transmission, as dysbindin is primarily expressed in axonal terminals of the mouse brain (17). Pre-synaptic machinery for exocytotic transmitter release is composed of membrane proteins, cytoskeletal proteins and synaptic vesicle proteins (18). SNAP25 (25 kDa synaptosomal associated protein) and syntaxin are membrane proteins implicated in the docking, priming and fusion of the vesicles. Synapsin I is a cytoskeletal protein associated with the synaptic vesicles in the reserve pool. Synaptotagmin is a synaptic vesicle protein, which has been identified as a calcium sensor protein. Thus, we examined the expression of these synaptic associated molecules after overexpression of dysbindin with virus-mediated gene transfer system. Infected neuronal cultures were doubly stained with GFP signal and immunostaining signal by anti-MAP2 (a neuronal dendritic marker) antibody (Fig. 1A). Approximately 80% of MAP2-positive cells in either control (GFP-infected) or dysbindin-overexpressing (dysbindin- and GFP-infected) cultures were GFP-positive, indicating that the majority of neurons were infected. As shown in Figure 1B, SNAP25 and synapsin I expression tended to be upregulated in dysbindin-overexpressing cultures compared with control (49 and 57%, respectively), whereas the changes of synaptotagmin and syntaxin expression were not observed (data not shown). The levels of class III β-tubulin (TUJ1, a neuronal marker) were not altered in the three conditions (Fig. 1B). We confirmed the overexpression of dysbindin (~17-fold when compared with control) in dysbindin-infected cultures and the expression of GFP in both control and dysbindin-overexpressing cultures (Fig. 1B).

Upregulation of synapsin I and SNAP25 raised the possibility that release of neurotransmitter might be increased by the overexpression of dysbindin. Therefore, we measured the release of glutamate, which is the principle neurotransmitter in these neurons. As expected, the amount of basal glutamate from dysbindin-infected cortical cultures was significantly increased when compared with the uninfected or control cultures (Fig. 1C), indicating that dysbindin overexpression resulted in an elevation of extracellular glutamate. Furthermore, high KCl (HK^+)-evoked exocytotic release of glutamate was enhanced in dysbindin-infected cultures. These results suggest that dysbindin might be one of the regulator proteins in the excitatory neurotransmission.

We then investigated the effects of dysbindin on neuronal viability. Interestingly, it was found that the phosphorylation of Akt, a molecule in the phosphatidylinositol 3-kinase (PI3-kinase) pathway, was significantly enhanced by 67% in the dysbindin-overexpressing cultures, whereas total Akt protein levels were unchanged (Fig. 2A). As the activation of Akt is

Table 1. Allele frequencies of six dysbindin SNPs between the patients with schizophrenia and controls

Marker name	dbSNP ID	Polymorphism major/minor	Location	Minor allele frequency		*P*-value	Odds ratio (95% CI)
				Controls	Patients		
P1655	rs2619539	G/C	Int 5	0.311	0.317	0.748	1.03 (0.87–1.22)
P1635	rs3213207	A/G	Int 4	0.011	0.030	0.0013	2.71 (1.46–5.79)
P1325	rs1011313	G/A	Int 4	0.153	0.166	0.372	0.91 (0.72–1.15)
P1320	rs760761	C/T	Int 3	0.071	0.095	0.027	1.38 (1.04–1.83)
P1763	rs2619522	T/G	Int 1	0.070	0.095	0.022	1.40 (1.05–1.86)
SNPA	rs2619538	T/A	Promoter	0.024	0.040	0.025	1.69 (1.05–2.86)

Table 2. Estimated haplotype frequencies and case–control haplotype results

Markers	*P*-value		Haplotype	Haplotype frequency	
	Global	Individual		Controls	Patients
P1655–P1635	0.0026	0.0003	G–G	0.011	0.030
P1635–P1325	0.00041	0.00028	G–G	0.009	0.030
P1325–P1320	0.0074	0.013	G–T	0.069	0.096
P1320–P1763	0.06	0.02	C–T	0.929	0.904
P1763–SNPA	0.025	0.0047	G–A	0.009	0.025
P1655–P1635–P1325	0.0055	0.001	G–G–G	0.011	0.030
P1635–P1325–P1320	0.0006	0.0009	G–G–T	0.010	0.027
P1325–P1320–P1763	0.027	0.029	G–T–G	0.068	0.095
P1320–P1763–SNPA	0.05	0.0045	T–G–A	0.009	0.025
P1655–P1635–P1325–P1320	0.011	0.0038	G–G–G–T	0.011	0.027
P1635–P1325–P1320–P1763	0.0015	0.001	G–G–T–G	0.010	0.027
P1325–P1320–P1763–SNPA	0.015	0.0019	G–T–G–A	0.007	0.025
P1655–P1635–P1325–P1320–P1763	0.025	0.0028	G–G–G–T–G	0.011	0.027
P1635–P1325–P1320–P1763–SNPA	0.003	0.0016	G–G–T–G–A	0.009	0.026
P1655–P1635–P1325–P1320–P1763–SNPA	0.024	0.0012	G–G–G–T–G–A	0.010	0.026

Case–control haplotype analysis were performed using the permutation method to obtain empirical *P*-values. Global *P*-values and individual *P*-values (lowest *P*-values among the haplotypes) are indicated. Estimated frequency for the haplotype with significant association in controls and patients were shown.

regulated by phosphorylation, overexpression of dysbindin resulted in the activation of Akt. LY294002, a PI3-kinase inhibitor, completely blocked the activation of Akt by the dysbindin overexpression, with no alteration of the expression levels of Akt and TUJ1 proteins (Fig. 2A). As the PI3-kinase pathway is involved in neuronal function and survival (19), we examined the viability of cortical neurons with our virus infection system (Fig. 2B). The overexpression of dysbindin protein itself did not alter neuronal viability when compared with control. However, dysbindin overexpression significantly blocked the reduced viability of cortical cultures by serum deprivation. Additionally, LY294002 significantly inhibited the protective effects of dysbindin, suggesting that the PI3-kinase pathway was involved in the dysbindin-dependent viability promoting effects.

Knockdown analysis of endogenous dysbindin in cultured neurons

We further examined the endogenous dysbindin function in cortical cultures using small interfering RNA (siRNA) for dysbindin. Previously, we reported siRNA-dependent down-regulation of endogenous protein expression in primary cultured neurons (20). Here, we performed transfection of siRNA for dysbindin and confirmed the robust decrease (83%) of endogenous dysbindin protein (Fig. 3A). The protein expression levels of SNAP25 and synapsin I and the phosphorylation level of Akt protein was significantly suppressed after dysbindin-siRNA transfection (43, 37 and 52% of reduction, respectively), although the expression levels of TUJ1 and Akt proteins were not altered (Fig. 3A). Thus, we investigated dysbindin function on glutamate release and neuronal viability under this condition. The amount of basal and released glutamate from dysbindin-siRNA-transfected cortical cultures significantly decreased when compared with the control (scramble) cultures (Fig. 3B), indicating that endogenous dysbindin protein plays a role in the excitatory neurotransmission. The neuronal viability was not changed by dysbindin-siRNA transfection in the presence of horse serum (Fig. 3C). However, dysbindin-siRNA transfection significantly facilitated neuronal death when horse serum was deprived (Fig. 3C), suggesting that the endogenous dysbindin protein has a promoting effect on survival.

DISCUSSION

In the present study, we report a significant association between genetic variation of dysbindin and schizophrenia in a Japanese population. In previous studies, highly significant

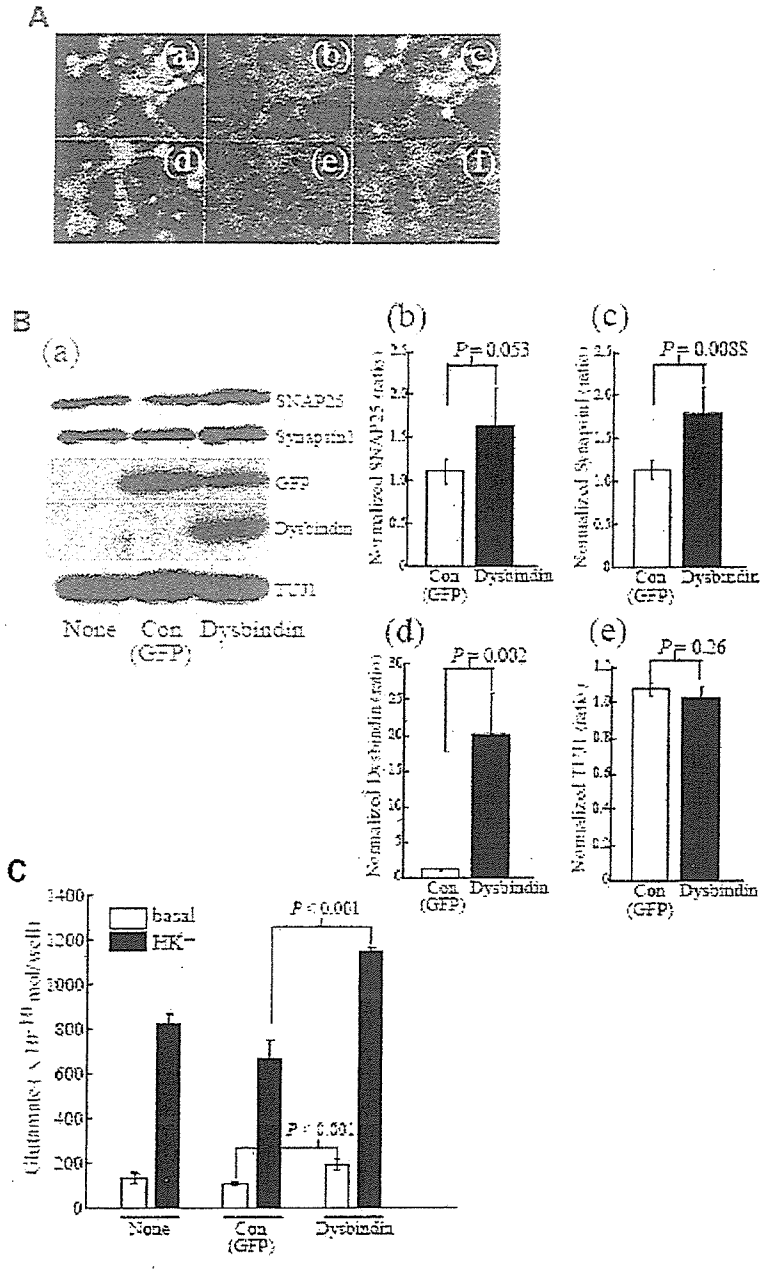

Figure 1. Dysbindin increases the expression of pre-synaptic proteins and glutamate release. (**A**) Double-staining of GFP and MAP2. Cortical cultures (6 days *in vitro*, DIV6) were prepared with viral infection of GFP only (a–c) or with viral infection of GFP and dysbindin (d–f) at DIV4. Images were obtained with GFP (a, d; green) and with immunostaining of anti-MAP2 antibody (b, e; red). Merged images (c, f; yellow) were also shown. (**B**) (a) Upregulation of pre-synaptic proteins. Cortical cultures (DIV6) were prepared without viral infection (None), with viral infection of GFP (Con) or with viral infection of GFP and dysbindin (Dysbindin) at DIV4. The cell lysates were collected at DIV6 and SNAP25, synapsin I, GFP, dysbindin and TUJ1 were detected by western blotting. The immunoblots shown are representative of four independent experiments. (b–e) Quantification of the immunoreactivity of SNAP25, Synapsin I, dysbindin and TUJ1. Data represent mean ± SD of the immunoreactivity from four independent experiments. (**C**) Increase of the released glutamate in dysbindin-overexpressing cortical cultures. Cortical cultures were prepared without viral infection (None), with viral infection of GFP (Con) or with viral infection of GFP and dysbindin (Dysbindin) at DIV4. Basal or HK^+ (50 mM KCl)-evoked release of glutamate was measured at DIV6 (after 48 h from infection). Data represent mean ± SD ($n = 4$).

associations were found for SNPs in introns 4–6, which is consistent with our results. The G allele of P1635, which was significantly in excess in our cases (3.0%), was also over-transmitted in Irish samples (10.2%) (2), whereas this allele was under-transmitted in German samples (17.6%) (3), suggesting that this SNP might be a marker rather than a polymorphism responsible for giving susceptibility. Notably, a high-risk haplotype in our samples was the G–G–T–G

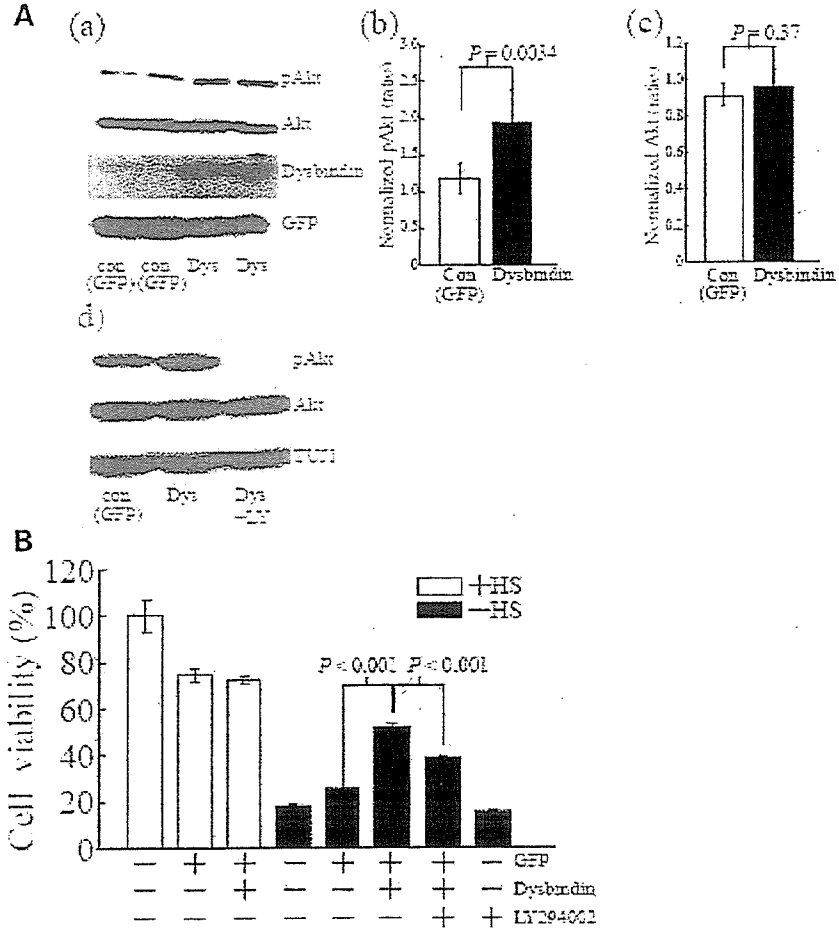

Figure 2. Dysbindin protects cortical neurons through PI3-kinase-Akt signaling. (**A**) (a) The activation of PI3-kinase pathway in dysbindin-overexpressing cultures. Cortical cultures after DIV4 were treated with viral infection of GFP (Con) or with viral infection of GFP and dysbindin (Dys) for 48 h. (b, c) Quantification of the immunoreactivity of pAkt and total Akt proteins. Data represent mean ± SD of the immunoreactivity from four independent experiments. (d) The inhibitory effect of LY294002 on activation of Akt. Cortical cultures at DIV4 were treated with viral infection of GFP (Con), with viral infection of GFP and dysbindin (Dys) or with viral infection of GFP and dysbindin in the presence of LY294002 (1.0 μM) (Dys + LY) for 48 h. Cortical cultures were harvested at DIV6 for western blotting for pAkt, Akt, dysbindin, GFP or TUJ1. The immunoblots shown are representative of four independent experiments. (**B**) Neuroprotective effects of dysbindin against serum deprivation. Cortical cultures after DIV4 were treated with viral infection of GFP (Con), with viral infection of GFP and dysbindin (Dysbindin) or with LY294002 (1.0 μM) for 48 h. Deprivation of horse serum (HS) at DIV5 24 h after viral infection is indicated as −HS. Cell viability was determined using the MTT assay at DIV6 48 h after the viral infection and/or 24 h after HS deprivation. Data represent mean ± SD ($n = 8$).

haplotype (P1635–P1325–P1320–P1763), which includes the high-risk haplotype (G–G–G–G–T–G–C–C; P1635–P1325–P1765–P1757–P1320–P1763–P1578–P1792) reported in an Irish sample (6). The frequency of our high-risk haplotype (2.7% in cases versus 1.0% in controls) is lower than that in an Irish population (6%). Novel schizophrenia risk and protective haplotypes (C–A–T, C–A–A, G–G–T; P1655–P1635–SNPA) were recently identified in Cardiff and Dublin samples (21). We also analyzed these haplotypes in our sample and obtained evidence for a significant association with a different haplotype (global P-value = 0.0086, individual P-value = 0.005; G–G–A). Furthermore, the estimated frequencies of C–A–A and G–G–T haplotypes in our sample were <0.1%, although the overall frequencies in Cardiff and Dublin were 33 and 1.4%, respectively. We failed to find a significant association for the C–A–T haplotype (overall frequency, Cardiff and Dublin versus ours, C–A–T: 18 versus 32%). These differences of the haplotype frequencies might be based on the different ethnicity. A false-positive association owing to population stratification could not be excluded in our case–control study, despite the precaution of ethnic matching of this study.

It is of interest to study how genetic variation affects dysbindin function/expression. We do not know that any of the SNPs in our haplotypes are functional. Very little is known about the potential function of specific intronic sequences with regard to protein binding, stability and splicing efficacy. A recent study showed the functional possibility of intronic SNPs on gene expression. For example, an intronic SNP affects the transcriptional efficiency of SLC22A4 *in vitro*, owing to an allelic difference in affinity to Runt-related transcription factor 1, and this SNP is associated with rheumatoid arthritis, one of

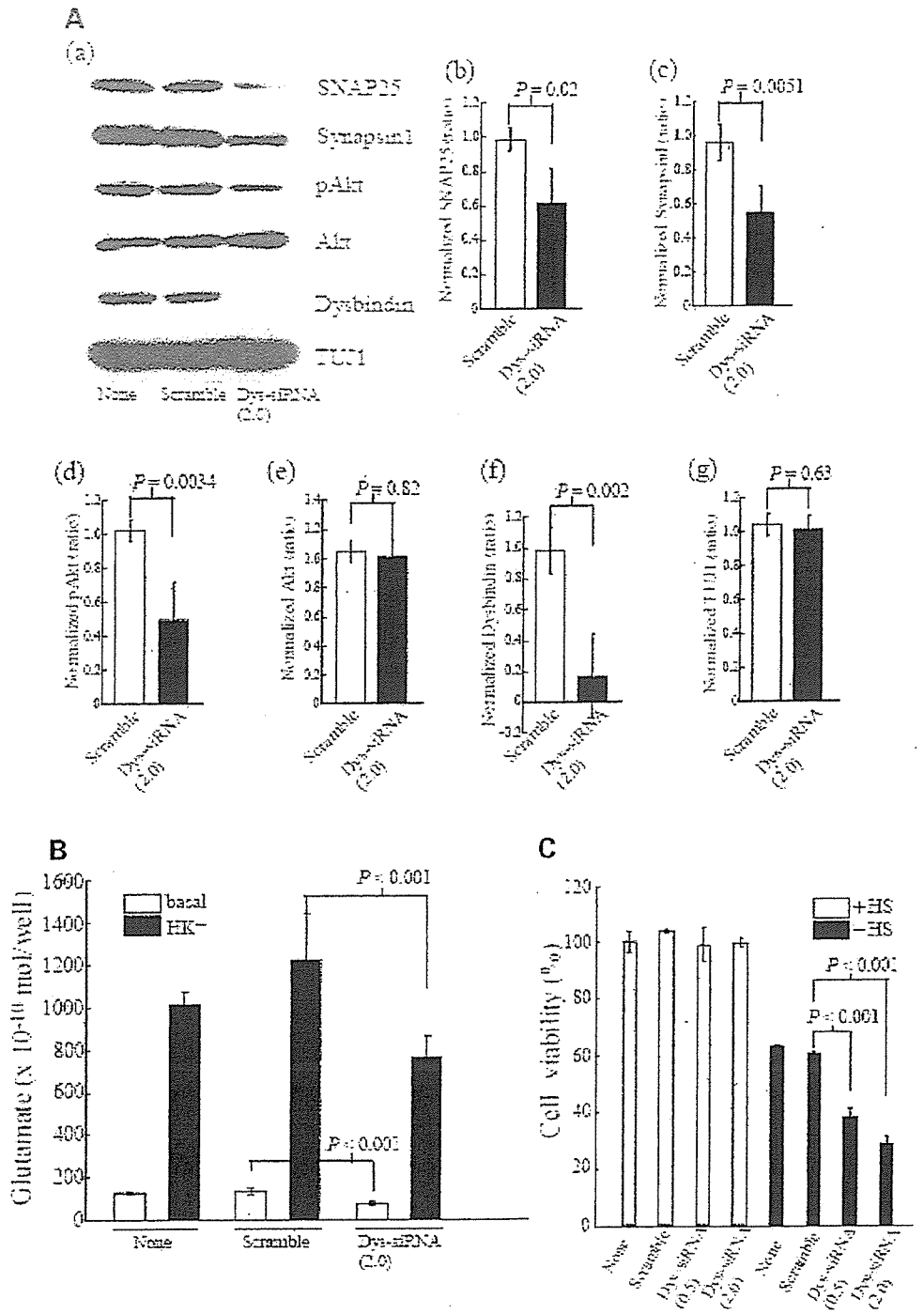

Figure 3. siRNA inhibition of endogenous dysbindin protein modulates protein expression, glutamate release and cell viability. (**A**) (a) Suppression of the pre-synaptic proteins and the phosphorylation of Akt in dysbindin-siRNA-transfected cultures. Cortical cultures after DIV4 were treated with siRNA for dysbindin (dys-siRNA; 2 mg/ml) or control (scramble; 2 mg/ml) for 72 h. Cortical cultures were harvested at DIV7 for western blotting for SNAP25, Synapsin I, pAkt, Akt, dysbindin or TUJ1. The immunoblots shown are representative of four independent experiments. (b–g) Quantification of the immunoreactivity of SNAP25, synapsin I, pAkt, total Akt, dysbindin and TUJ1. Data represent mean ± SD of the immunoreactivity from four independent experiments. (**B**) The reduced glutamate release in dysbindin-siRNA-transfected cultures. Cortical cultures were prepared without transfection (None), with transfection of control siRNA (Scramble; 2 mg/ml) or with transfection of siRNA for dysbindin (dys-siRNA; 2 mg/ml) at DIV4. Basal or HK^+ (50 mM KCl)-evoked release of glutamate was measured at DIV7 (after 72 h from transfection). Data represent the mean ± SD ($n = 6$). (**C**) Facilitation of neuronal death after serum deprivation by dysbindin-siRNA transfection. Cortical cultures after DIV4 were treated without transfection (None), with transfection of control siRNA (Scramble; 0.5 or 2 mg/ml) or with transfection of siRNA for dysbindin (dys-siRNA; 0.5 or 2 mg/ml) for 72 h. Deprivation of horse serum (HS) at DIV6 48 h after transfection is indicated as −HS. Cell viability was determined using the MTT assay at DIV7 72 h after the transfection and/or 24 h after HS deprivation. Data represent mean ± SD ($n = 8$).

the complex genetic diseases like schizophrenia (22). Alternatively, an unknown functional polymorphism, which is in LD with the SNPs and/or haplotypes, may be responsible for providing susceptibility to schizophrenia.

To date, association of dysbindin with schizophrenia has been confirmed across diverse populations. In addition, decreased expression of dysbindin mRNA and protein levels has been observed in prefrontal cortex and hippocampus of postmortem brain in schizophrenic patients (23–25). As dysbindin is distributed at least in part in axonal terminals (17), we focused on the possible role of dysbindin in neuronal transmission. We used two techniques, overexpression and knockdown, to investigate neuronal function of dysbindin. As the overexpression levels of dysbindin using sindbis virus were quite high when compared with the control level (~17-fold), the results could have non-physiological effects. However, the results from the knockdown experiments of the endogenous dysbindin protein were consistent with those from overexpression experiments. Our experiments suggest that dysbindin regulates the expression of SNAP25 and synapsin I proteins in the pre-synaptic machinery and is associated with increased glutamate release. SNAP25 is one of the fundamental molecular components of the SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors) protein complex, which is involved in intracellular vesicle trafficking and neurotransmitter release (18). Synapsin I is localized to the synaptic vesicles that are both docked and located away from the plasma membrane (18). Reduction of SNAP25 protein has been observed in frontal cortex of schizophrenia patients (26) and synapsin I protein was found to be reduced in the hippocampus of patients with schizophrenia (27). Hypofunction of glutamatergic system has been implicated in the neuropathology in schizophrenia (8). The abuse of phencyclidine, an NMDA receptor antagonist, results in positive symptoms, negative symptoms and cognitive impairments, similar to schizophrenic patients. The postmortem brain studies suggested impaired glutamatergic systems, e.g. reduced glutamate level, decreased AMPA receptor binding and expression and reduced NMDA receptor expression in several brain areas, including frontal cortex and hippocampus.

Our experiments also suggest the survival effect of dysbindin protein on cortical neurons against serum deprivation through the PI3-kinase-Akt signaling pathway. Thus, dysbindin might play an important role in neuronal vulnerability. Impaired PI3-kinase-Akt signaling in schizophrenia has been reported recently (28). Dysbindin expression in the brain of schizophrenic patients was reduced (23–25) and our data suggested that the downregulation of dysbindin expression suppressed the phosphorylation levels of Akt. Taken together, impaired PI3-kinase-Akt signaling in the schizophrenic brain might be due, in part, to the decreased expression of dysbindin. As dysbindin may affect neuronal viability through Akt activation, dysbindin-Akt signaling might be involved in early disruptions producing long-term vulnerability that leads to the onset of schizophrenia symptoms. As PI3-kinase-Akt signaling is activated by several growth factors such as brain-derived neurotrophic factor, nerve growth factor and insulin-like growth factors through tyrosine kinase receptors (19), the regulation of this system might be associated with dysbindin.

The Hermansky–Pudlak syndrome defines a group of autosomal recessive disorders characterized by deficiencies in lysosome-related organelles complex-1 (BLOC-1). Hermansky–Pudlak type-7 is caused by a nonsense mutation of dysbindin, which is a component of the BLOC-1 (29). Biological roles of BLOC-1 are still unknown; however, it might be involved in vesicle docking and fusion. Sandy mouse, which has a deleted dysbindin gene, expresses no dysbindin (29). Thus, this mouse could be a powerful tool for investigating brain function of dysbindin *in vivo*. It is of interest to examine the pre-synaptic protein expression, glutamate release, Akt phosphorylation and neuronal vulnerability *in vivo* using this mouse.

We have demonstrated the additional support for the genetic association between dysbindin and schizophrenia in a relatively large sample and the evidence of novel functions of dysbindin in cultured neurons. Our results suggest that an abnormality of dysbindin might influence glutamatergic systems and Akt signaling. Further investigation is necessary to elucidate the mechanisms of Akt activation and upregulation of pre-synaptic molecules by dysbindin.

MATERIALS AND METHODS

Subjects

Subjects for the association study were 670 patients with schizophrenia [males: 50.6%, mean age of 44.2 years (SD 14.6)] and 588 healthy comparison subjects [males: 48.7%, mean age of 36.2 years (SD 12.4)]. All the subjects were biologically unrelated Japanese patients. Consensus diagnosis was made for each patient by at least two psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria. Control subjects were healthy volunteers who had no current or past contact to psychiatric services. After description of the study, written informed consent was obtained from every subject. The study protocol was approved by institutional ethical committees (Fujita Health University School of Medicine, Showa University School of Medicine and National Center of Neurology and Psychiatry).

SNP genotyping

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to the standard procedures. Six SNPs (P1655, P1635, P1325, P1320, P1763 and SNPA) adopted in the work of Straub *et al.* (2) and Williams *et al.* (21) were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay, described previously (30,31). Briefly, the probes and primers for detection of the SNP were as follows. P1655: forward primer 5′-AGTTTTTAT CACTAATCAAAATGAAACAGCCTTT-3′, reverse primer 5′-CTCATTCTGTTATAACTAGTCTGACATGGT-3′, probe 1 5′-VIC-TATTAGCTATGATAGTGTTTTAT-MGB-3′ and probe 2 5′-FAM-ATTAGCTATGATAGTCTTTTAT-MGB -3′; P1635: forward primer 5′-GGAACTTTTCTTTGAAGA CTTCCTTTCG-3′, reverse primer 5′-ACCACTAACAACC AAAAAGAAAACAAACA-3′, probe 1 5′-VIC-TAAAGCC AATAATTACC-MGB-3′ and probe 2 5′-FAM-AGCCAG

TAATTACC-MGB-3′; P1325: forward primer 5′-GATATG ACTCCTTAATTCACAGGCTACAG-3′, reverse primer 5′-GTTACTGCACACAAGCAACTGTTAA-3′, probe 1 5′-VIC -AATGGATGTTGCATTAGT-MGB-3′ and probe 2 5′-FAM -ATGGATGTTGCGTTAGT-MGB-3′; P1320: forward primer 5′-CCAATCCATTCTTTTATTGACATGGAGTTT-3′, reverse primer 5′-TGATTTTGACCAAGTCCATTGTGTCT -3′, probe 1 5′-VIC-AAAAGCACAAACAACAAG-MGB-3′ and probe 2 5′-FAM-AAAAGCACAAATAACAAG-MGB-3′; P1763: forward primer 5′-GGCAGAAGCAGTGAGTGAGA-3′, reverse primer 5′-TGGGCTCTTATGTCTACCTTTCCTAAA -3′, probe 1 5′-VIC-TCACCTGGATGTCAGC-MGB-3′ and probe 2 5′-FAM-ACCTGGCTGTCAGC-MGB-3′; SNPA: forward primer 5′-TCTGTTATGTGCCATTCACTGTTTT-3′, reverse primer 5′-TAGGGCTGGGATTGGATGA-3′, probe 1 5′-VIC-AGCAGTTTACATCTGGG-MGB-3′ and probe 2 5′-FAM-AGCAGTTTACATCAGGG-MGB-3′. PCR cycling conditions were 95°C for 10 min, 45 cycles of 92°C for 15 s and 60°C for 1 min.

Cell culture

Dissociated cortical cultures were prepared from postnatal 2- or 3-day-old rat (SLC, Shizuoka, Japan) cortex, as described previously (32,33). Briefly, cells were gently dissociated with a plastic pipette after digestion with papain (90 U/ml, Sigma) at 37°C. The dissociated cells were plated at a final density of 5×10^5 per cm^2 on polyethyleneimine-coated 12- or 24-well plates (4 and 2 cm^2 surface area/well, respectively; Corning, NY, USA) or cover glasses (Matsunami, Osaka, Japan) attached to flexiperm (VIVASCIENCE, Gottingen, Germany). The culture medium consisted of 5% precolostrum newborn calf serum, 5% heated-inactivated horse serum and 90% of a 1 : 1 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's F-12 medium containing 15 mM HEPES buffer, pH 7.4, 30 nM Na_2SeO_3 and 1.9 mg/ml of $NaHCO_3$.

Sindbis virus

A bicistronic vector plasmid (pSinEGdsp) was provided by Dr Kawamura (Niigata University, Japan). The plasmid was derived from pSinRep5 (Invitrogen, USA) and had two subgenomic promoters followed by a multiple cloning site for arbitrary gene insertion and an EGFP open reading frame, thus the virus can produce arbitrary protein and EGFP independently in the infected cell, as previously described (34). Dysbindin cDNAs amplified by RT–PCR with specific primer pairs (forward 5′-ACGCGTCAATGCTGGAGACCCTTCG-3′ and reverse 5′-GCATGCCAATTTAAGAGTCGCTGTCC-3′) were inserted at the *Mlu*1 and *Sph*1 sites of the plasmid. Each plasmid was cleaved with *Pac*1, and used as a template for mRNA transcription *in vitro* using mMESSAGE mMACHINE kit (Ambion, USA). Pseudovirions were produced according to the experimental procedure of Invitrogen. Baby hamster kidney (BHK) cells were transfected with each mRNA and 26S helper mRNA (Invitrogen) by electroporation (1250 V/cm, 50 μF, single pulse) using Gene Pulser2 (BioRad, USA). The cells were incubated with DMEM supplemented with 10% FCS for 24 h at 37°C, the supernatants were collected as pseudovirion-containing solutions.

Immunocytochemistry

Cultured neurons were fixed with 4% paraformaldehyde for 20 min and then rinsed three times with PBS. Subsequently, cultured cells were permeabilized with 0.2% Triton X-100 in PBS for 5 min at room temperature. The primary antibodies (anti-MAP2; Sigma) with 3% skim milk in PBS were applied overnight at 4°C. After washing, cells were incubated with secondary antibodies (Alexa Fluor, Molecular Probes) for 1 h at room temperature. Fluorescent images were captured by an inverted microscope (Axiovert 200, Zeiss) with a CCD (cool SNAPfx) purchased from Zeiss. Monochrome images were turned into color and analyzed using software (Slide BookTM 3.0, Intelligent Imaging Innovations, Inc., Denver, CO, USA). The images of GFP were analyzed with the same software.

Immunoblotting

Cells were lysed in SDS lysis buffer containing 1% SDS, 20 mM Tris–HCl (pH 7.4), 5 mM EDTA (pH 8.0), 10 mM NaF, 2 mM Na_3VO_4, 0.5 mM phenylarsine oxide and 1 mM phenylmethylsulfonyl fluoride. Lysates were centrifuged at 15 000 rpm for 60 min at 4°C, and the supernatants were collected for analysis. Samples were heat denatured with the standard SDS sample buffer. Immunoblottings were carried out as described previously (35). Briefly, immunoblottings were carried out with anti-SNAP25 antibody (1:3000, mouse monoclonal, Synaptic System, Gottingen, Germany), anti-synapsin I antibody (1:1000, rabbit anti-serum, Chemicon), anti-synaptotagmin antibody (1:1000, mouse monoclonal, BD Transduction Laboratory), anti-syntaxin antibody (1:3000, mouse monoclonal, Sigma), anti-GFP antibody (1:1000, rabbit polyclonal, MBL, Nagoya, Japan), anti-dysbindin antibody (23) (1:100, rabbit polyclonal), anti-TUJ1 antibody (1:5000, mouse monoclonal, Berkeley antibody company, CA, USA), anti-Akt antibody (1:1000, rabbit antiserum, Cell Signaling) and anti-phospho-Akt antibody (Ser473, 1:1000, rabbit anti-serum, Cell Signaling) in TBS containing 1% non-fat dried milk. The immunoblotting experiments were performed four times and they were quantitatively analyzed by capturing images on films using a scanner (Epson, Tokyo, Japan) in conjunction with the Lane and Spot Analyzer software (version 6.0, ATTO, Tokyo, Japan).

Anti-dysbindin antibody was produced as described previously (36). Briefly, the peptide synthesized (QSDEEEVQVDTALC: 320–333 amino acid residue of human dysbindin, with no homology in any mammalian protein) was conjugated with maleimide-activated keyhole limpet hemocyanin and immunized to two rabbits. The titer was measured by ELISA and sera of high titer against the peptide were obtained from both rabbits. The sera were affinity purified by a column conjugated with the immunized peptide.

Detection of glutamate release

The amount of glutamate released from the cultures was measured as previously reported (33,35). The glutamate released into the modified HEPES-buffered Krebs–Ringer assay buffer (KRH; 130 mM NaCl, 5 mM KCl, 1.2 mM

NaH_2PO_4, 1.8 mM $CaCl_2$, 10 mM glucose, 1% bovine serum albumin and 25 mM HEPES, pH 7.4) were measured by HPLC (Shimadzu, Kyoto, Japan) with a fluorescence detector (excitation wavelength, 340 nm; emission wavelength, 445 nm, Shimadzu). For stimulation of cortical neurons, we used a HK^+ KRH solution consisting of 85 mM NaCl, 50 mM KCl, 1.2 mM NaH_2PO_4, 1.8 mM $CaCl_2$, 10 mM glucose, 1% bovine serum albumin and 25 mM HEPES, pH 7.4. Before exposing the cultures to HK^+ solution (1 min), basal fractions were collected. The glutamate release experiments were performed three times with independent cultures to confirm reproducibility.

MTT assay

To examine the cell viability, the metabolic activity of mitochondria was estimated by measuring the mitochondrial-dependent conversion of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma). We performed the viral infection or transfection of siRNA and then, the serum was deprived from culture medium. MTT (0.5 mg/ml in PBS) was added to each well at 24 h after serum deprivation. MTT was incubated for 1.5 h at 37°C. Then, the medium was carefully aspirated, and 200 μl of acidified isopropyl alcohol was added to solubilize the colored formazan product. Absorbance was determined at 550 nm on a scanning multi-well plate reader (Bio-Rad) after agitating the plates for 5 min on a shaker.

siRNA transfection

We used 23 nt siRNA duplexes with two 3′ overhanging nucleotides targeting position 182–204 (aagugacaagucaaga gaagcaa) of human dysbindin mRNA. Scrambled sequence (aacgaugagaacgaucaagaaga), which had no homology to any mammalian mRNA, was used as a control siRNA. Both sense and antisense strands were synthesized by Dharmacon Research Inc (Lafayette, PA, USA). SiRNA duplexes in the 2′-ACE deprotected and desalted form were dissolved in a 1× universal buffer (Dharmacon Research Inc). Transfection of both siRNAs was performed using NeuroPORTER™ (Gene Therapy Systems, Inc., San Diego, CA, USA), as reported (20).

Statistical analysis

Statistical analysis of association studies was performed using SNPAlyse (DYNACOM, Yokohama, Japan). The presence of Hardy–Weinberg equilibrium was examined by using the χ^2-test for goodness of fit. Allele distributions between patients and controls were analyzed by the χ^2-test for independence. The measure of LD, denoted as D', was calculated from the haplotype frequency using the expectation–maximization algorithm. Case–control haplotype analysis was performed by the permutation method to obtain the empirical significance (37). The global P-values represent the overall significance using the χ^2-test when the observed versus expected frequencies of all the haplotypes are considered together. The individual haplotypes were tested for association by grouping all others together and applying the χ^2-test with 1 df. P-values were calculated on the basis of 10 000 replications. Statistical analysis of neurobiological assays was performed by Students' t-test. All P-values reported are two tailed. Statistical significance was defined at $P < 0.05$. To be conservative, Bonferroni corrections were applied for multiple comparisons, e.g. number of analyzed SNPs and haplotypes, although SNPs were in LD.

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REFERENCES

1. O'Donovan, M.C., Williams, N.M. and Owen, M.J. (2003) Recent advances in the genetics of schizophrenia. *Hum. Mol. Genet.*, **12**, R125–R133.
2. Straub, R.E., Jiang, Y., MacLean, C.J., Ma, Y., Webb, B.T., Myakishev, M.V., Harris-Kerr, C., Wormley, B., Sadek, H., Kadambi, B. *et al.* (2002) Genetic variation in the 6p22.3 gene *DTNBP1*, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. *Am. J. Hum. Genet.*, **71**, 337–348.
3. Schwab, S.G., Knapp, M., Mondabon, S., Hallmayer, J., Borrmann-Hassenbach, M., Albus, M., Lerer, B., Rietschel, M., Trixler, M., Maier, W. *et al.* (2003) Support for association of schizophrenia with genetic variation in the 6p22.3 gene, dysbindin, in sib-pair families with linkage and in an additional sample of triad families. *Am. J. Hum. Genet.*, **72**, 185–190.
4. Tang, J.X., Zhou, J., Fan, J.B., Li, X.W., Shi, Y.Y., Gu, N.F., Feng, G.Y., Xing, Y.L., Shi, J.G. and He, L. (2003) Family-based association study of DTNBP1 in 6p22.3 and schizophrenia. *Mol. Psychiatr.*, **8**, 717–718.
5. Van Den Bogaert, A., Schumacher, J., Schulze, T.G., Otte, A.C., Ohlraun, S., Kovalenko, S., Becker, T., Freudenberg, J., Jonsson, E.G., Mattila-Evenden, M. *et al.* (2003) The *DTNBP1* (dysbindin) gene contributes to schizophrenia, depending on family history of the disease. *Am. J. Hum. Genet.*, **73**, 1438–1443.
6. van den Oord, E.J., Sullivan, P.F., Jiang, Y., Walsh, D., O'Neill, F.A., Kendler, K.S. and Riley, B.P. (2003) Identification of a high-risk haplotype for the dystrobrevin binding protein 1 (*DTNBP1*) gene in the Irish study of high-density schizophrenia families. *Mol. Psychiatr.*, **8**, 499–510.
7. Morris, D.W., McGhee, K.A., Schwaiger, S., Scully, P., Quinn, J., Meagher, D., Waddington, J.L., Gill, M. and Corvin, A.P. (2003) No evidence for association of the dysbindin gene [*DTNBP1*] with schizophrenia in an Irish population-based study. *Schizophr. Res.*, **60**, 167–172.
8. Tsai, G. and Coyle, J.T. (2002) Glutamatergic mechanisms in schizophrenia. *Annu. Rev. Pharmacol. Toxicol.*, **42**, 165–179.
9. Weinberger, D.R., Egan, M.F., Bertolino, A., Callicott, J.H., Mattay, V.S., Lipska, B.K., Berman, K.F. and Goldberg, T.E. (2001) Prefrontal neurons and the genetics of schizophrenia. *Biol. Psychiatr.*, **50**, 825–844.
10. Moghaddam, B. (2003) Bringing order to the glutamate chaos in schizophrenia. *Neuron*, **40**, 881–884.
11. Harrison, P.J. and Owen, M.J. (2003) Genes for schizophrenia? Recent findings and their pathophysiological implications. *Lancet*, **361**, 417–419.
12. Buonanno, A. and Fischbach, G.D. (2001) Neuregulin and ErbB receptor signaling pathways in the nervous system. *Curr. Opin. Neurobiol.*, **11**, 287–296.
13. Stefansson, H., Sigurdsson, E., Steinthorsdottir, V., Bjornsdottir, S., Sigmundsson, T., Ghosh, S., Brynjolfsson, J., Gunnarsdottir, S.,

Ivarsson, O., Chou, T.T. *et al.* (2002) Neuregulin 1 and susceptibility to schizophrenia. *Am. J. Hum. Genet.*, **71**, 877–892.
14. Mothet, J.P., Parent, A.T., Wolosker, H., Brady, R.O., Jr, Linden, D.J., Ferris, C.D., Rogawski, M.A. and Snyder, S.H. (2000) D-serine is an endogenous ligand for the glycine site of the *N*-methyl-D-aspartate receptor. *Proc. Natl Acad. Sci. USA*, **97**, 4926–4931.
15. Chumakov, I., Blumenfeld, M., Guerassimenko, O., Cavarec, L., Palicio, M., Abderrahim, H., Bougueleret, L., Barry, C., Tanaka, H., La Rosa, P. *et al.* (2002) Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. *Proc. Natl Acad. Sci. USA*, **99**, 13675–13680.
16. De Blasi, A., Conn, P.J., Pin, J. and Nicoletti, F. (2001) Molecular determinants of metabotropic glutamate receptor signaling. *Trends Pharmacol. Sci.*, **22**, 114–120.
17. Benson, M.A., Newey, S.E., Martin-Rendon, E., Hawkes, R. and Blake, D.J. (2001) Dysbindin, a novel coiled-coil-containing protein that interacts with the dystrobrevins in muscle and brain. *J. Biol. Chem.*, **276**, 24232–24241.
18. Turner, K.M., Burgoyne, R.D. and Morgan, A. (1999) Protein phosphorylation and the regulation of synaptic membrane traffic. *Trends. Neurosci.*, **22**, 459–464.
19. Brunet, A., Datta, S.R. and Greenberg, M.E. (2001) Transcription-dependent and -independent control of neuronal survival by the PI3K-Akt signaling pathway. *Curr. Opin. Neurobiol.*, **11**, 297–305.
20. Numakawa, T., Nakayama, H., Suzuki, S., Kubo, T., Nara, F., Numakawa, Y., Yokomaku, D., Araki, T., Ishimoto, T., Ogura, A. *et al.* (2003) Nerve growth factor-induced glutamate release is via p75 receptor, ceramide, and Ca(2+) from ryanodine receptor in developing cerebellar neurons. *J. Biol. Chem.*, **278**, 41259–41269.
21. Williams, N.M., Preece, A., Morris, D.W., Spurlock, G., Bray, N.J., Stephens, M., Norton, N., Williams, H., Clement, M., Dwyer, S. *et al.* (2004) Identification in 2 independent samples of a novel schizophrenia risk haplotype of the dystrobrevin binding protein gene (*DTNBP1*). *Arch. Gen. Psychiatr.*, **61**, 336–344.
22. Tokuhiro, S., Yamada, R., Chang, X., Suzuki, A., Kochi, Y., Sawada, T., Suzuki, M., Nagasaki, M., Ohtsuki, M., Ono, M. *et al.* (2003) An intronic SNP in a RUNX1 binding site of SLC22A4, encoding an organic cation transporter, is associated with rheumatoid arthritis. *Nat. Genet.*, 35, 341–348.
23. McClintock, W., Shannon Weickert, C., Halim, N.D., Lipska, B.K., Hyde, T.M., Herman, M.M., Weinberger, D.R., Kleinman, J.E. and Straub, R.E. (2003) Reduced expression of dysbindin protein in the dorsolateral prefrontal cortex of patients with schizophrenia. *Program No. 317.9. 2003 Abstract Viewer/Itinerary Planner.* Washington, DC, Society for Neuroscience (Online).
24. Talbot, K., Eidem, W.L., Tinsley, C.L., Benson, M.A., Thompson, E.W., Smith, R.J., Hahn, C.G., Siegel, S.J., Trojanowski, J.Q., Gur, R.E. *et al.* (2004) Dysbindin-1 is reduced in intrinsic, glutamatergic terminals of the hippocampal formation in schizophrenia. *J. Clin. Invest.*, **113**, 1353–1363.
25. Weickert, C.S., Straub, R.E., McClintock, B.W., Matsumoto, M., Hashimoto, R., Hyde, T.M., Herman, M.M., Weinberger, D.R. and Kleinman, J.E. (2004) Human dysbindin (*DTNBP1*) gene expression in normal brain and in schizophrenic prefrontal cortex and midbrain. *Arch. Gen. Psychiatr.*, **61**, 544–555.
26. Honer, W.G., Falkai, P., Bayer, T.A., Xie, J., Hu, L., Li, H.Y., Arango, V., Mann, J.J., Dwork, A.J. and Trimble, W.S. (2002) Abnormalities of SNARE mechanism proteins in anterior frontal cortex in severe mental illness. *Cereb. Cortex*, **12**, 349–356.
27. Vawter, M.P., Thatcher, L., Usen, N., Hyde, T.M., Kleinman, J.E. and Freed, W.J. (2002) Reduction of synapsin in the hippocampus of patients with bipolar disorder and schizophrenia. *Mol. Psychiatr.*, 7, 571–578.
28. Emamian, E.S., Hall, D., Birnbaum, M.J., Karayiorgou, M. and Gogos, J.A. (2004) Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. *Nat. Genet.*, **36**, 131–137.
29. Li, W., Zhang, Q., Oiso, N., Novak, E.K., Gautam, R., O'Brien, E.P., Tinsley, C.L., Blake, D.J., Spritz, R.A., Copeland, N.G. *et al.* (2003) Hermansky–Pudlak syndrome type 7 (HPS-7) results from mutant dysbindin, a member of the biogenesis of lysosome-related organelles complex 1 (BLOC-1). *Nat. Genet.*, **35**, 84–89.
30. Hashimoto, R., Yoshida, M., Ozaki, N., Yamanouchi, Y., Iwata, N., Suzuki, T., Kitajima, T., Tatsumi, M., Kamijima, K. and Kunugi, H. (2004) Association analysis of the −308G>A promoter polymorphism of the tumor necrosis factor alpha (TNF-alpha) gene in Japanese patients with schizophrenia. *J. Neural Transm.*, **111**, 217–221.
31. Hashimoto, R., Straub, R.E., Weickert, C.S., Hyde, T.M., Kleinman, J.E. and Weinberger, D.R. (2004) Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. *Mol. Psychiatr.*, **9**, 299–307.
32. Numakawa, T., Matsumoto, T., Adachi, N., Yokomaku, D., Kojima, M., Takei, N. and Hatanaka, H. (2001) Brain-derived neurotrophic factor triggers a rapid glutamate release through increase of intracellular Ca(2+) and Na(+) in cultured cerebellar neurons. *J. Neurosci. Res.*, **66**, 96–108.
33. Numakawa, T., Yamagishi, S., Adachi, N., Matsumoto, T., Yokomaku, D., Yamada, M. and Hatanaka, H. (2002) Brain-derived neurotrophic factor-induced potentiation of Ca(2+) oscillations in developing cortical neurons. *J. Biol. Chem.*, **277**, 6520–6529.
34. Kawamura, M., Namba, H., Otsu, Y., Hayashi, Y., Takei, N. and Nawa, H. (2003) Characterization of novel bicistronic sindbis virus vectors, SinEGdsp and SinIRES-EG, in cultured neurons. *Recent Res. Dev. Neurochem.*, **6**, 105–120.
35. Numakawa, T., Yokomaku, D., Kiyosue, K., Adachi, N., Matsumoto, T., Numakawa, Y., Taguchi, T., Hatanaka, H. and Yamada, M. (2002) Basic fibroblast growth factor evokes a rapid glutamate release through activation of the MAPK pathway in cultured cortical neurons. *J. Biol. Chem.*, **277**, 28861–28869.
36. Hashimoto, R., Nakamura, Y., Komai, S., Kashiwagi, Y., Tamura, K., Goto, T., Aimoto, S., Kaibuchi, K., Shiosaka, S. and Takeda, M. (2000) Site-specific phosphorylation of neurofilament-L is mediated by calcium/calmodulin-dependent protein kinase II in the apical dendrites during long-term potentiation. *J. Neurochem.*, **75**, 373–382.
37. Good, P. (2000) *Permutation Tests. A Practical Guide to Resampling Methods for Testing Hypothesis.* 2nd edn. Springer-Verlag, New York.