Kinesin *Kif3b* mutation reduces NMDAR subunit NR2A trafficking and causes schizophrenia-like phenotypes in mice

キネシン分子モーターKIF3Bの遺伝子異常は
統合失調症の原因となる

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THE EMBO JOURNAL

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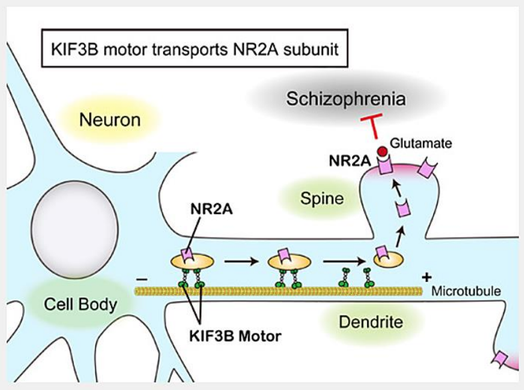
Abstract

The transport of *N*-methyl-D-aspartate receptors (NMDARs) is crucial for neuronal plasticity and synapse formation. Here, we show that KIF3B, a member of the kinesin superfamily proteins (KIFs), supports the transport of vesicles simultaneously containing NMDAR subunit 2A (NR2A) and the adenomatous polyposis coli (APC) complex. *Kif3b*$^{+/-}$ neurons exhibited a reduction in dendritic levels of both NR2A and NR2B due to the impaired transport of NR2A and increased degradation of NR2B. In *Kif3b*$^{+/-}$ hippocampal slices, electrophysiological NMDAR response was found decreased and synaptic plasticity was disrupted, which corresponded to a common feature of schizophrenia (SCZ). The histological features of *Kif3b*$^{+/-}$ mouse brain also mimicked SCZ features, and *Kif3b*$^{+/-}$ mice exhibited behavioral defects in prepulse inhibition (PPI), social interest, and cognitive flexibility. Indeed, a mutation of KIF3B was specifically identified in human SCZ patients, which was revealed to be functionally defective in a rescue experiment. Therefore, we propose that KIF3B transports NR2A/APC complex and that its dysfunction is responsible for SCZ pathogenesis.

Keywords KIF3B; neuronal plasticity; NMDAR; NR2A; schizophrenia
Subject Categories Membranes & Trafficking; Molecular Biology of Disease; Neuroscience
DOI 10.15252/embj.2018101090 | Received 6 November 2018 | Revised 19 October 2019 | Accepted 22 October 2019 | Published online 20 November 2019
The EMBO Journal (2020) 39: e101090

Synopsis



Altered synaptic surface expression of *N*-methyl-d-aspartate receptors (NMDARs) affects neuronal function and has been linked to schizophrenia. Here, the kinesin KIF3B is shown to transport NMDAR subunit 2A (NR2A) into neuronal dendrites, with its disruption causing schizophrenia-like phenotypes in mice.

- *Kif3b*$^{+/-}$ mice exhibit schizophrenia-like behavioral phenotypes.
- KIF3B transports vesicles containing the NR2A complex into neuronal dendrites.
- A mutation in human *Kif3b* gene was identified in schizophrenia patients.
- The patient mutation-carrying KIF3B is functionally defective and cannot rescue *Kif3b*$^{+/-}$ phenotypes.

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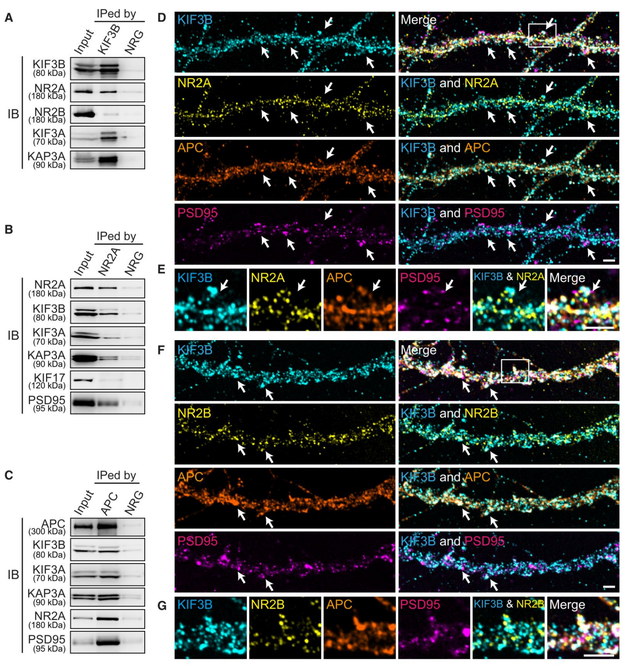

Figure 1. KIF3B binds to the NR2A complex.
A–C Co-immunoprecipitation (IP) of mouse whole brain extracts with an anti-KIF3B antibody (A); and their vesicle co-IP with an anti-NR2A antibody (B) and an anti-APC antibody (C); with normal rabbit IgG (NRG) as a control, immunoblotted (IB) against the indicated antibodies. *n* = 4. See Source data for Figure 1.
D–I Immunofluorescence microscopy showing the intracellular colocalization of KIF3B, APC, and NR2A (D and E) or NR2B (F and G) in PSD95-positive spines of the dissociated hippocampal neurons with a negative control (H) and statistics (I). Arrows, positions of colocalization. Squares in (D) and (F), magnified regions in (E and G), respectively. Scale bars, 2 μm. Center and error bars show mean ± SEM. *n* = 10. ***P < 0.001; Student's *t*-test.
Source data are available online for this figure.

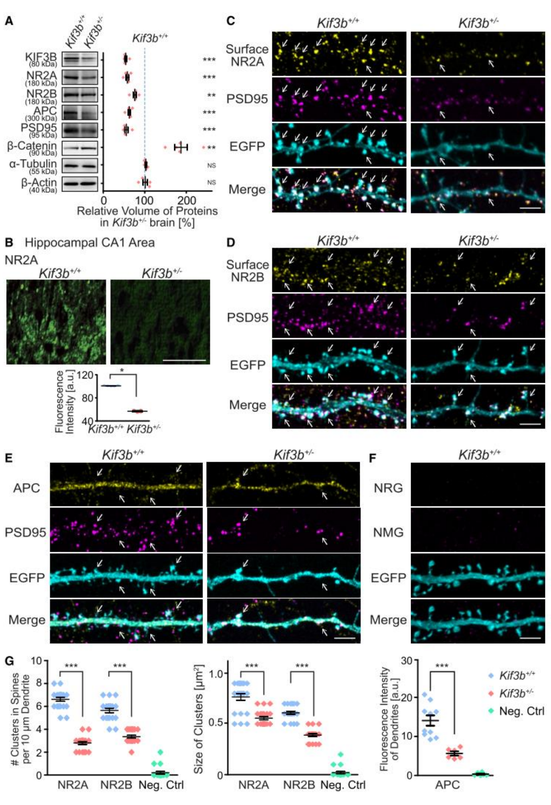

Figure 2. Decreased expression of NR2A, NR2B, and APC in *Kif3b*$^{+/-}$ hippocampal neurons.
A Immunoblotting of adult mouse lysates of *Kif3b*$^{+/+}$ and *Kif3b*$^{+/-}$ mice using the indicated antibodies with statistics. Protein volumes in *Kif3b*$^{+/-}$ brains were normalized with those in *Kif3b*$^{+/+}$ brains and are expressed as the % of the control. Note that the expression levels of plasticity-related proteins (NR2A, NR2B, and PSD95) and a KIF3-associated protein (APC) were decreased, whereas that of β-catenin was increased. Center and error bars show mean ± SEM. *n* = 5. NS, *P* ≥ 0.05; **P < 0.01; ***P < 0.001; one-way ANOVA. See Source data for Figure 2.
B Immunofluorescence histochemistry of the hippocampal CA1 apical area of *Kif3b*$^{+/+}$ and *Kif3b*$^{+/-}$ mouse brain coronal sections using an anti-NR2A antibody and statistics for the fluorescence intensity. Scale bar, 50 μm. Center and error bars show mean ± SEM. *n* = 5. *P < 0.05; paired *t*-test.
C–G Representative micrographs of distal dendrites of dissociated hippocampal neurons at DIV 16–18, immunostained against surface NR2A (C), surface NR2B (D), and cytoplasmic APC (E), accompanied by a negative control with normal rabbit and mouse IgGs (NRG and NMG, respectively in F), and statistics for the density and size of the surface NR2A- and NR2B-positive clusters and the fluorescence intensity of APC (G). Neurons were permeabilized only in APC staining (E). Note that in *Kif3b*$^{+/-}$ neurons, the cluster size and density of both NR2A and NR2B, and the APC expression level were affected compared with *Kif3b*$^{+/+}$ neurons. Arrows, signals at PSD95-positive spines. Scale bars, 5 μm. Center and error bars show mean ± SEM. *n* = 20–21. ***P < 0.001; Student's *t*-test. See Fig EV1.
Source data are available online for this figure.

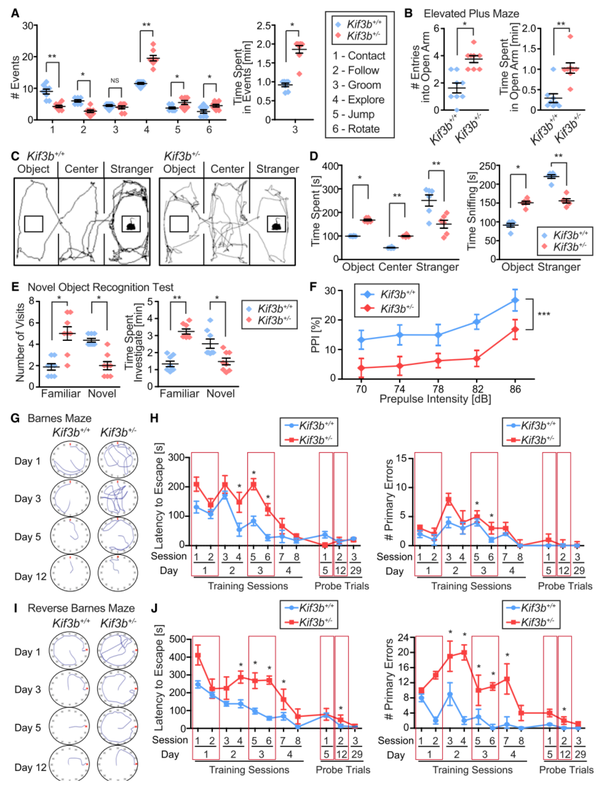

Figure 5. *Kif3b*$^{+/-}$ mice exhibit schizophrenia-like behaviors.
A Social interaction test using two unfamiliar adult male mice. The numbers of indicated events (#1–6) in a 10-min trial were manually counted. Note that *Kif3b*$^{+/-}$ mice exhibited impaired social interest. Center and error bars show mean ± SEM. *n* = 8. ^{NS}*P* ≥ 0.05; *P < 0.05; **P < 0.01; Student's *t*-test.
B Elevated plus-maze test. Note that *Kif3b*$^{+/-}$ mice exhibited decreased anxiety and elevated activity. Center and error bars show mean ± SEM. *n* = 8. *P < 0.05; **P < 0.01; Student's *t*-test.
C, D Three-chambered sociability test with an object, center, and a stranger mouse in the same object areas (C), accompanied by statistics (D). Note that *Kif3b*$^{+/-}$ mice spent less time in the stranger area. Black lines in area (C), representative traces of mouse movement in 10 min of the trial. Center and error bars show mean ± SEM. *n* = 6. *P < 0.05; **P < 0.01; Student's *t*-test.
E Novel object recognition test. Note that *Kif3b*$^{+/-}$ mice exhibited less interest to the novel object compared with the *Kif3b*$^{+/-}$ mice. Center and error bars show mean ± SEM. *n* = 8. *P < 0.05; **P < 0.01; Student's *t*-test.
F Prepulse inhibition (PPI) test. Note that *Kif3b*$^{+/-}$ mice exhibited decreased inhibition in the startle response. Pulse intensity was set to 115 dB. *n* = 28. ***P < 0.001; two-way ANOVA. See Fig EV3.
G–J Barnes maze test showing spatial reference memory (G and H) and reverse Barnes maze test showing learning flexibility (I and J) of both genotypes, with representative traces of the mouse movements (G and I) and statistics for latency to escape and number of primary errors during the training sessions and probe trials (H and J). Note that *Kif3b*$^{+/-}$ mice required a longer period of time to acquire spatial memory but showed a similar ability to sustain memory compared with *Kif3b*$^{+/+}$ mice. Red boxes in H and J, corresponding to the trial traces presented in (G) and (I). Error bars show mean ± SEM. *n* = 6. *P < 0.05; Student's *t*-test.

Figure 3. KIF3B transports NR2A, but not NR2B.
A–F Time-lapse imaging of NR2A-ECFP (A–C) and NR2B-ECFP (D–F) along dendrites of dissociated hippocampal neurons, represented by the kymographs (A and D), and statistics for the velocity (B and E) and directionality of the vesicles (C and F). Note that the transport of NR2A vesicles, but not that of NR2B vesicles, was affected in *Kif3b*$^{+/-}$ neurons. Scale bars, 5 μm. P, proximal; D, distal region of the dendrite. Arrows, anterogradely moving vesicles; arrowheads, non-mobile or diffusing vesicles. Boxplots in (B and E) represent median, first, and third quartiles, and whiskers represent minimum to maximum. *n* = 240–316 vesicles from 18 neurons. ^{NS}*P* ≥ 0.05; *P < 0.05; ***P < 0.001; Student's *t*-test (B and E) and chi-square test (C and F).
G–N Time-lapse fluorescence images of NR2A (G and K)- or NR2B-PA-GFP-expressing dissociated hippocampal neurons (I and M), in the absence (G and I) or in the presence of proteasome inhibitor, MG132 (K and M), and statistics showing the time course of degradation along dendrites at 1-h intervals after photoactivation at time 0 (H, J, L, and N). Note that this proteasomal degradation of NR2B-PA-GFP, but not NR2A-PA-GFP, in *Kif3b*$^{+/-}$ dendrites, occurred in a significantly faster rate than in *Kif3b*$^{+/+}$ dendrites. Orange arrows in micrographs, degrading clusters stimulated by photoactivation; red arrows in statistics, time point of photoactivation. Scale bars, 5 μm. Error bars show mean ± SEM. *n* = 10. ***P < 0.001; two-way ANOVA. See Fig EV2.

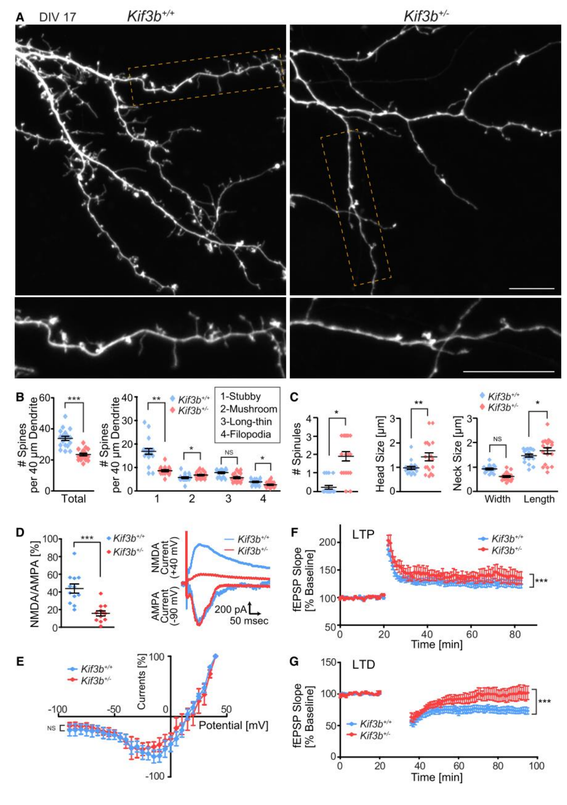

Figure 4. KIF3B is essential for synaptic plasticity.
A–C Fluorescence micrographs of EGFP-expressing *Kif3b*$^{+/+}$ and *Kif3b*$^{+/-}$ dendrites of dissociated hippocampal neurons at DIV 17 (A), accompanied by the statistics for the total and classified spine numbers per 40 μm dendrite (B) and morphological features including the number of spinules, head size, and neck size (C). Note that the number of stubby and filopodial spines was decreased and that of mushroom spines was increased in *Kif3b*$^{+/-}$ neurons. Scale bars, 10 μm. Center and error bars show mean ± SEM. *n* = 446–642 spines of 18 neurons from five mice. ^{NS}*P* ≥ 0.05; *P < 0.05; **P < 0.01, ***P < 0.001; Student's *t*-test.
D–G Electrophysiological analysis of Schaffer collateral–CA1 synapses in acute hippocampal slices from *Kif3b*$^{+/+}$ and *Kif3b*$^{+/-}$ mice. (D and E) Synaptic NMDA currents over AMPA currents recorded at +40 mV and −90 mV EPSCs, respectively with the representative traces (D) and current–voltage relationships of NMDAR channel currents recorded in *Kif3b*$^{+/+}$ and *Kif3b*$^{+/-}$ slices (E). Current amplitudes were normalized to the values at +40 mV EPSC. Note that the synaptic NMDA currents were reduced in *Kif3b*$^{+/-}$ slices. Center and error bars show mean ± SEM. *n* = 12 cells from six mice. ^{NS}*P* ≥ 0.05; ***P < 0.001; Student's *t*-test (D) and two-way ANOVA (E). (F and G) Synaptic plasticity indicated by time course of the fEPSP slopes, with induction of LTP by high-frequency stimulation (F) and of LTD by low-frequency stimulation (G). Error bars show mean ± SEM. *n* = 9 slices from nine mice. ***P < 0.001; two-way ANOVA.

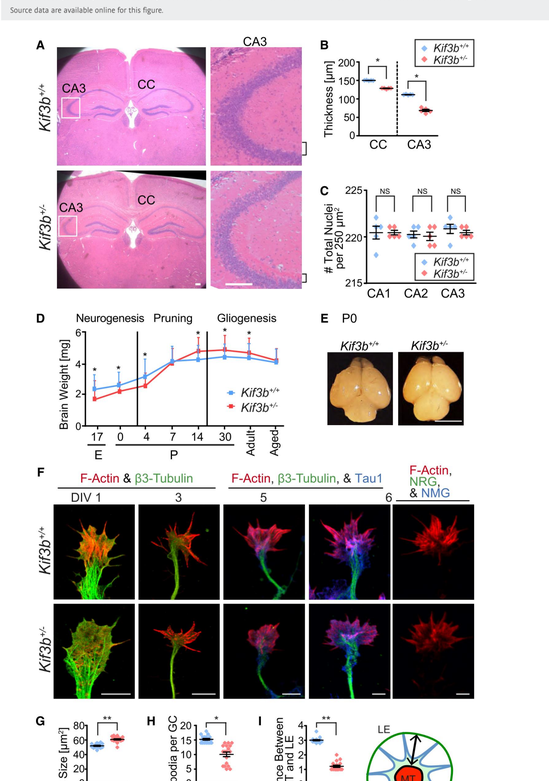

Figure 6. Abnormal histology of *Kif3b*$^{+/-}$ mouse brains.
A–C Hematoxylin–eosin (HE) staining of adult mouse brain coronal sections and magnified images of hippocampal CA3 area of the indicated genotypes (A), accompanied by statistics (B and C). Squares in the left panels, magnified regions in the right panels; CC, corpus callosum; brackets, stratum radiatum in hippocampal CA3 area. Scale bars, 100 μm. Center and error bars show mean ± SEM. *n* = 5. ^{NS}*P* ≥ 0.05; *P < 0.05; Student's *t*-test.
D, E Developmental profile of mouse brain weight throughout the neurogenesis, pruning, and gliogenesis phases, from embryonic day 17 (E17) to aged (D), and representative appearance of the brains at postnatal day 0 (P0; E). Scale bar, 5 mm. Error bars show mean ± SEM. *n* = 5. *P < 0.05; Student's *t*-test.
F–I Immunofluorescence merged micrographs stained against F-actin (red) and β3-tubulin (green), focusing on the morphology and development of the axonal growth cone (GC) from DIV 1, 3, 5, and 6 of the dissociated hippocampal neurons (F), accompanied by statistics (G–I). Arrow in (I), measured distance between MT domain and LE. MT, microtubule; LE, leading edge; NRG, normal rabbit IgG; NMG, normal mouse IgG. Scale bars, 5 μm. Center and error bars show mean ± SEM. *n* = 18. *P < 0.05; **P < 0.01; Student's *t*-test. See Fig EV4 for the individual color images.

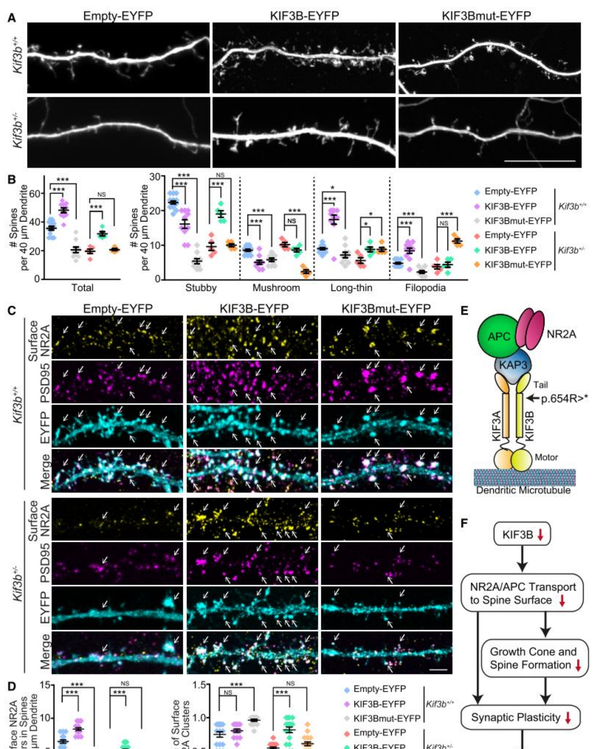

Figure 7. The expression of KIF3B, but not mutated KIF3B, restores spine morphology and NR2A surface expression.
A, B Fluorescence micrographs of Empty-EYFP, KIF3B-EYFP, or KIF3Bmut-EYFP vector-expressing *Kif3b*$^{+/+}$ and *Kif3b*$^{+/-}$ dendrites in dissociated hippocampal neurons at DIV 16–18 (A), accompanied by statistics for the total and classified spine number per 40 μm dendrite (B). Scale bars, 10 μm. Center and error bars show mean ± SEM. *n* = 98–535 spines of 5–15 neurons from three mice. ^{NS}*P* ≥ 0.05; *P < 0.05; ***P < 0.001; Student's *t*-test. See Fig EV5.
C, D Immunofluorescence micrographs showing surface NR2A along *Kif3b*$^{+/+}$ and *Kif3b*$^{+/-}$ dendrites of the dissociated hippocampal neurons at DIV 16–18, transfected with Empty-EYFP, KIF3B-EYFP, or KIF3Bmut-EYFP vector. Arrows, signals at PSD95-positive spines. Scale bar, 5 μm. Center and error bars show mean ± SEM. *n* = 16–17. ^{NS}*P* ≥ 0.05; ***P < 0.001; Student's *t*-test. See Fig EV1.
E, F Schematic view of the SCZ-related human KIF3B mutation in KIF3/KAP3/APC/NR2A complex (E) and the working hypothesis of SCZ pathogenesis related with KIF3B dysfunction (F). See Fig EV5 and Table EV1.

プレスリリース　キネシン分子モーターKIF3Bの遺伝子異常は統合失調症の原因となる

発表のポイント

- ヒトの統合失調症患者の遺伝子の検索により、細胞内で物質を輸送するキネシン分子モーターKIF3Bに機能的欠損をもたらす遺伝子変異が同定された。
- *Kif3b*遺伝子が欠損したマウスには、社会性の低下や驚愕反応の異常など統合失調症の表現型が現れ、*Kif3b*遺伝子が欠損したマウスの海馬神経細胞には、シナプス形態とその機能に大きな異常が見られた。
- この理由として、シナプス伝達に重要な役割を果たすNMDA型グルタミン酸受容体（NMDAR）のNR2Aサブユニットが KIF3Bによって輸送されることを発見した。

発表概要

統合失調症は日本人の100人に1人が罹る精神疾患であるが、その生物学的な病態にはまだ不明な点が多く、それに基づいた治療薬もまだ開発の途上にある。今回、東京大学大学院医学系研究科分子細胞生物学専攻の廣川信隆特任教授、アルサバンアシュワック特任研究員（研究当時）、森川桃特任研究員、田中講介講師、武井陽介准教授（研究当時、現・筑波大教授）らの研究チームは、キネシン分子モーターKIF3B［注1］の異常が統合失調症の分子的基盤になることを発見した。チームはまず患者さんの遺伝子データから、KIF3Bの遺伝子異常を同定した。次に、KIF3Bの発現が半分に減っているKif3bヘテロ欠損遺伝子操作マウスを作って解析してみると、記憶・学習能力の低下や聴覚驚愕応答のプレパルスによる減弱の低下という統合失調症［注2］に特有の表現型が観察された。さらに、このマウスの神経細胞ではNMDA型グルタミン酸受容体の樹状突起スパイン表面における発現量が減少し、記憶・学習の基盤となるシナプス可塑性［注3］の変化が観察された。そこで、患者さんの変異を導入したKIF3Bタンパク質と野生型のKIF3Bタンパク質をそれぞれKif3bヘテロ欠損神経細胞に導入して活性を調べたところ、確かに患者さんではKIF3Bタンパク質の機能が低下していることがわかった。本研究から、統合失調症に対する新規治療法開発の基盤となる、細胞レベルでの新しい病態が解明された。

発表内容

キネシンスーパーファミリータンパク質（KIFs）は細胞内の物質輸送を担う分子モーターであり、機能分子の局在や活性を制御することで脳の高次機能や個体の発生の基盤となる、細胞の生命や機能を維持する重要なタンパク質群である。KIF3モーターはKIF3A・KIF3B・KAP3の三つのタンパク質からなるユニークな複合体モーターであり、東京大学大学院医学系研究科分子細胞生物学専攻の廣川信隆特任教授のこれまでの研究から、胎児の内臓の左右軸の決定に重要な役割を担っていることが知られていたが、神経・精神疾患との関連はほとんど知られていなかった。今回、廣川特任教授、アルサバン特任研究員（研究当時）、森川特任研究員、田中講師、武井准教授（研究当時）らの研究チームはAMED脳科学研究戦略推進プログラム（融合脳）の支援を受け、ヒトの統合失調症の新たな原因としてKIF3Bタンパク質を変異する遺伝子異常を特定した。

まずチームは、統合失調症患者ゲノムからKIF3Bの654番目のアミノ酸であるアルギニンが終止コドンに変異しているミスセンス変異（p.R654Ter）を同定した。そこでKIF3Bタンパク質の精神疾患への影響を確かめるため、*Kif3b*遺伝子がヘテロ欠損した遺伝子操作マウスの行動解析を行った。すると、オープン・フィールド試験と高架式十字迷路で*Kif3b*遺伝子ヘテロ欠損マウスは過活動の傾向を示し、スリー・チェンバー社会性実験では他個体への興味の低下を示した。また、バーンズ迷路試験では記憶・学習能力の低下と、記憶の書き換えの柔軟性の低下が見られた。聴覚驚愕応答のプレパルスによる減弱は有意に低下していた。これら全ての行動解析の結果から、*Kif3b*遺伝子ヘテロ欠損マウスは統合失調症様の表現型を持つことが分かった。

一方、このKIF3複合体の神経細胞における機能に注目して免疫沈降法により結合タンパク質を検索したところ、スパイン表面に局在して可塑性に重要な役割を担うNMDARのサブユニットNR2AとKIF3Bが結合し、樹状突起内を輸送することを発見した。NR2Aの輸送を担う分子モーターの同定は世界で初めての報告である。

さらに、上記の免疫沈降法ではNR2Aの他にAPCやPSD95がKIF3複合体と結合する小胞に含まれていることが予想されたため、*Kif3b*遺伝子ヘテロ欠損マウスの胎児から海馬初代培養を樹立しセミ超高解像レーザー顕微鏡を用いて免疫細胞化学的な観察を行ったところ、KIF3Bの減少により、スパイン表面におけるNMDARとPSD95の局在量、スパイン内のAPCの量が有意に減少していることが分かり、KIF3複合体がこれらを輸送していることが示唆された。

また海馬初代培養におけるスパインをセミ超高解像レーザー顕微鏡にて観察したところ、KIF3Bの減少によりスパインの数が減少することも分かった。蛍光タンパク質を結合させたNR2Aを海馬初代培養細胞に導入しライブイメージングを行ったところ、*Kif3b*遺伝子ヘテロ欠損細胞ではNR2Aの輸送が特異的に障害されており、KIF3BがNR2Aの輸送分子モーターとして機能していることが確認された（図）。

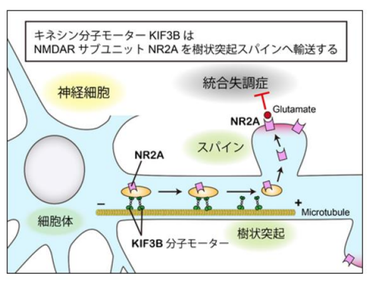

図. KIF3BによるNR2Aの樹状突起スパインへの輸送

NMDARはシナプス可塑性に重要な役割を担っていることが知られているため、研究チームはKIF3Bの減少が神経回路網にどのような影響をもたらすのかを調べるため、海馬の急性スライスを作成して電気生理学的に解析した。まず海馬の単一神経細胞にてパッチクランプ法を用いてAMPA型グルタミン酸受容体（AMPAR）応答とNMDAR応答の比率を計測することにより、KIF3Bの減少によってNMDARの応答が減弱していることが分かった。これはスパイン表面に発現するNMDARの量がKIF3Bの輸送能低下により減少したためだと推測される。さらに海馬急性スライスで可塑性を誘導する刺激を与えたところ、*Kif3b*遺伝子ヘテロ欠損海馬では野生型に比べてシナプス長期増強（LTP）が亢進し、シナプス長期抑圧（LTD）が減弱していることが分かった。これらの電気生理学的特徴は、統合失調症の表現型としてこれまでに報告されたものとよく一致し、またNMDARの機能異常は統合失調症に関与していることが提唱されている。

そこでヒトの統合失調症患者のR654Ter遺伝子変異が本当にKIF3Bタンパク質の機能低下をもたらしているかを、*Kif3b*遺伝子ヘテロマウスから初代培養した神経細胞に、全長のKIF3Bタンパク質を遺伝子工学的に強制発現する手法で調べた。まず、野生型のKIF3Bタンパク質を導入すると、スパイン表面のNR2A量を回復することができ、スパインの数も増加した。ところが、患者さんから同定されたヒト変異を導入したKIF3Bでは、どちらも回復することができなかった。したがってこの変異はまさしくKIF3Bの機能的欠損をもたらしていることが示唆された。

統合失調症は100人に1人が発症すると言われているが、発症メカニズムの全容が解明されていないため、その根本的な治療法はまだ確立していない。廣川特任教授らによる本研究成果は、KIF3複合体がNR2A/APC複合体を輸送することで神経細胞シナプスの形態や可塑性を維持し、その機能的欠損が統合失調症をもたらすという新規の分子メカニズムを提唱するものであり、NMDARが関与する統合失調症患者の治療法開発の基盤となる。