Intracellular localization of GGA accessory protein p56 in cell lines and central nervous system neurons

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ABSTRACT

Adaptor protein complex-1 (AP-1) and Golgi associated, γ-adaptin ear containing, Arf binding proteins (GGAs) are clathrin adaptors that regulate membrane trafficking between the trans-Golgi network (TGN) and endosomes. p56 is a clathrin adaptor accessory protein that may modulate the function of GGAs in mammalian cell lines. However, the precise relationship between p56 and the three GGAs (GGA1–3), as well as the physiological role of p56 in tissue cells, remain unknown. To this end, we generated an antibody against p56 and determined its cellular localization. In ARPE-19 cells and mouse embryonic fibroblasts, p56 was found to be localized as fine puncta in the TGN. Interestingly, the depletion of each clathrin adaptor by RNAi revealed that this localization was dependent on the expression of GGA1, but not that of GGA2, GGA3, or AP-1. Using immunohistofluorescence microscopy in the mouse central nervous system (CNS), p56 was clearly detected as scattered cytoplasmic puncta in spinal motor neurons, cerebellar Purkinje cells, and pyramidal neurons of the hippocampus and cerebral cortex. Moreover, double labeling with organellar markers revealed that the majority of these puncta were closely associated with the TGN; however, a small fraction was associated with endosomes or lysosomes in spinal motor neurons. Collectively, these results indicate a functional association of p56 with GGA1, suggesting an important role of p56 in larger CNS neurons.
cargo sorting (5, 7, 37).

Clathrin adaptor functions may be regulated by several accessory proteins that bind the ear domain of γ-adaptin of AP-1 and/or the GAE domains of GGAs. These include γ-synergin (11, 29, 36), afnaprilin (10, 23), γ-Bar/Gadkin (28), entrophoprin/Clint1/epsinR (12, 13, 24, 39), rabaptin-5 (4, 11, 22, 34), GAK (14), and p56 (3, 20, 21). Studies have shown that afnaprilin, γ-Bar/Gadkin, GAK, and p56 regulate cathepsin D sorting (10, 14, 21, 28), and that afnaprilin and γ-synergin regulate the secretion of the von Willebrand factor (19). In addition, p56 is one of the GGA accessory proteins, and it has been suggested that p56 plays a role in the long-range movement of GGA/clathrin-containing transport carriers in certain cell lines (20, 21). However, these studies utilized only cell lines, and a tissue-based study has yet to be performed. Therefore, in this study, we focused on p56 and initially generated an antibody against this accessory protein. We then investigated the GGA-dependency of p56 in its membrane recruitment and the localization of p56 in CNS neurons.

MATERIALS AND METHODS

Antibodies and cells. The p56 rabbit polyclonal antibody was generated by injecting a unique peptide [amino acids 1–15 (MDDDFGGEAAETF)] of human p56 into rabbits. The antiserum was then used for Western blot analyses, and the affinity-purified antibody was used for immunostaining in both cell lines and tissues. The other antibodies used in this study were as follows: rabbit antibodies against GGA1 (Santa Cruz Biotechnology; sc-30102), p44/42 MAP kinase (Cell Signaling Technology; 4695); mouse antibodies against GGA2 (BD transduction laboratories; 612612), GGA3 (BD transduction laboratories; 612310), γ-adaptin (BD transduction; 610385), EEA1 (BD transduction laboratories; 610456), LAMP1 (Santa Cruz Biotechnology; sc-19992), GAPDH (Santa Cruz Biotechnology; sc-22077), LBPA (a gift from Dr. Toshihide Kobayashi, RIKEN), a sheep antibody against human TGN46 (Novus Biologicals; NB10-60520), and a goat antibody against mouse TGN38 (Santa Cruz Biotechnology; sc-27681).

ARPE-19 cells were purchased from ATCC. Mouse embryonic fibroblasts (MEFs) were kindly provided by Dr. Masaaki Komatsu from Niigata University.

Knockdown by siRNA. The siRNAs used in the present study were as follows; human GGA1 (5'-GGC AAGUUCGCUUUCUCAAC-3'), human GGA2 (5'-GGAGUUCUGCUUUCUCAACAG-3'), human GGA3 (5'-CUGCAGUGCCAAGUAAUGA-3'), human γ-adaptin [ON-TARGET plus SMARTpool (Dharmacon; L-019183-00-0005)], and human p56 [ON-TARGET plus SMARTpool (Dharmacon; L-018638-01-0005)]. ARPE-19 cells were transfected with the indicated siRNA using Lipofectamine RNAiMAX (Invitrogen). Three days later, cells were processed for immunostaining or Western blotting.

Mice. Two 8-week-old C57 BL/6J mice were purchased from CLEA Japan, Inc. For immunohistochemistry, mice were fixed by perfusion according to the standard protocol using 4% paraformaldehyde (PFA), 4% sucrose, and 0.1 M phosphate buffer (PB; pH 7.4). Tissues were excised and treated with 4–20% sucrose for cryoprotection, and further embedded in OCT compound. Cryosections were prepared and immunostained with the antibodies above. For Western blotting, excised organs were washed with cold phosphate-buffered saline (PBS) and frozen with liquid nitrogen. This study was approved by the Ethics Committee for Animal Research of Fukushima Medical University (approval numbers: 29007, 27016, 25003, 23007, and 21104). All animal experiments were performed according to the guidelines of Fukushima Medical University.

Western blot analyses. Frozen organs were homogenized in lysis buffer (PBS containing 1% Triton X-100 and protease inhibitor [Roche]). ARPE-19 cells were washed three times with cold PBS and lysed using the same lysis buffer. The protein concentrations of the lysates were measured by using the BCA Protein Assay Reagent (Pierce Biotechnology, Inc). For SDS-PAGE, 5–10 μg/lane of protein was applied and separated, followed by transferring to a PVDF membrane. The membrane was blocked with PBS containing 5% skim milk and 0.1% Tween 20 for more than 30 min, followed by incubation with the primary antibodies detailed above. For the secondary antibodies, HRP-conjugated anti-rabbit and anti-mouse antibodies were used. For signal detection, the ECL prime reagent and LAS4000 mini (GE Healthcare) were used.

Immunofluorescence and immunohistochemistry. Cultured cells were fixed with 4% PFA in 0.1 M PB (pH 7.4), and processed for permeabilization and blocking with PBS containing 0.1% Triton X-100 and 0.4% BSA for more than 30 min. The cryosections prepared above were blocked with 5%
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reported previously (21). p56 was also detected as a diffuse signal in the cytosol (Fig. 1B). Importantly, the specificities of both the TGN and cytosolic signals were confirmed by their drastic decreases in p56-depleted cells by siRNA (Fig. 1B). A similar pattern of p56 localization was also observed in MEFs (Fig. 1C).

p56 has been shown to interact with GGAs and the γ-adaptin subunit of the AP-1 complex in vitro (20). However, the TGN localization of p56 has been shown to be dependent on GGAs, but not on AP-1, in HeLa cells (20, 21). Therefore, we reevaluated this dependency in ARPE-19 cells. Using Western blotting, the suppression of GGA1, GGA2, GGA3, and γ-adaptin by each siRNA did not influence the amount of p56 (Fig. 2). Similarly, the depletion of p56 did not affect the expressions of each clathrin adaptor (Fig. 2). By immunofluorescence, GGA1-depletion caused a disappearance of the p56 signal from the TGN region, whereas GGA2-depletion slightly increased the signal in the TGN (Fig. 3A and B). The depletion of GGA3 or γ-adaptin had no effect on p56 localization (Fig. 3C and D). When

normal donkey serum in PBS for 60 min. They were then immunostained with primary antibodies. For secondary antibodies, donkey anti-rabbit, -mouse, or -goat antibodies conjugated with Alexa Fluor fluorescent dye were used. Fluorescent images were then obtained using a confocal microscope FV1000 (Olympus).

RESULTS

To investigate the intracellular localization of the p56 protein in both cell lines and tissues, an antibody against p56 was generated using a synthesized peptide corresponding to amino acids 1–15 of human p56. The sequence of this peptide was identical to mouse p56. The antibody was first examined in ARPE-19 cells, which are derived from human retinal pigment epithelial cells. Western blot analyses showed a strong band for p56 in control cells, which disappeared when p56 expression was suppressed by siRNA (Fig. 1A). Immunofluorescence microscopy revealed that p56 was localized in the TGN46-positive TGN (Fig. 1B), consistent with what has been reported previously (21). p56 was also detected as a diffuse signal in the cytosol (Fig. 1B). Importantly, the specificities of both the TGN and cytosolic signals were confirmed by their drastic decreases in p56-depleted cells by siRNA (Fig. 1B). A similar pattern of p56 localization was also observed in MEFs (Fig. 1C).

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cause the pattern of the signal was somewhat different from that observed in cultured cells (Fig. 1), detailed localizations were determined in spinal cord motor neurons using markers for the TGN (TGN38), early endosomes (EEA1), late endosomes (LBPA), and late endosomes/lysosomes (LAMP1). As shown in Fig. 6A, a large part of the p56-positive puncta was present in the vicinity of the reticular TGN structure, and the extent of colocalization with the TGN marker appeared lower than that in the cell lines (Fig. 1). This difference might be due to larger TGN in neurons compared with perinuclear compacted TGN in the cell lines, and/or lower occupancy of the clathrin coated pits on the TGN membrane in neurons, which should be clarified in future. In addition, a small fraction of the p56-positive puncta was associated with structures positive for EEA1, LBPA, and LAMP1 (Fig. 6B–D).

**DISCUSSION**

p56 has the ability to bind to both the GAE domains of GGAs and the ear domain of γ-adaptin *in vitro* (20). However, previous studies have clearly indicated that both the expression and the Golgi localization of p56 are not affected in μ1A-adaptin-deficient fibroblasts (20) or HeLa cells treated with siRNA for γ-adaptin (21). Similarly, our study using ARPE-19 cells showed no alteration in the expression and localization of p56 by the depletion of γ-adaptin, which strengthens previous findings that p56 functions independently of AP-1. On the other hand, our results of the present study demonstrated that the TGN localization of p56 was affected by the depletion of GGA1, but not of GGA2 or GGA3. Importantly, this GGA1 depletion did not affect the protein expression of p56. These observations are slightly different from those previously reported by Mardones et al., where both the expression and the TGN localization of p56 were partially suppressed by the depletion of each GGA (21). While this discrepancy cannot be fully explained, differences in the cell lines and/or RNAi procedures utilized may have influenced the results. It should be noted that in the brains of GGA1-, GGA2-, GGA3-, or GGA1/3 knockout mice, the protein levels of p56 are unchanged (5), consistent with our results. It would be interesting to know the intracellular distribution of p56 in those mice. In conclusion, our findings confirmed the preference of p56 as an accessory protein for GGAs, and suggest the importance of GGA1 in mediating its function.

So, what is the function of p56? It has been pre-
Fig. 3  Immunofluorescence analysis of p56 and each clathrin adaptor in the siRNA experiment. (A–D) Dependency of p56 on clathrin adaptors. ARPE-19 cells were treated with control siRNA (si-Ctrl), or each siRNA against GGA1 (A), GGA2 (B), GGA3 (C), or γ-adaptin (D; si-γ-ad), followed by fixation. They were immunostained with the anti-GGA1 or anti-p56 antibody (A), or double-immunostained with a combination of the anti-p56 antibody and either anti-GGA2 (B), anti-GGA3 (C), or anti-γ-adaptin (D; anti-γ-ad) antibodies. Thus, only (A) shows different fields for the two antibodies. Note that only GGA1-depletion caused the disappearance of the TGN signal for p56 (A). Bars, 20 μm. (E–H) Dependency of clathrin adaptors on p56. ARPE-19 cells were treated with control siRNA (si-Ctrl), or siRNAs against p56. They were then immunostained with the anti-GGA1 or the anti-p56 antibody (E), or double-immunostained with a combination of the anti-p56 antibody and either, the anti-GGA2 (F), anti-GGA3 (G), or anti-γ-adaptin (H; anti-γ-ad) antibodies. Thus, only (E) shows different fields for the two antibodies. p56-depletion did not affect the localizations of four clathrin adaptors. Bars, 20 μm.
Fig. 4 Western blot analysis of p56 in the mouse brain, lung, spleen, and testis. Lysates of the mouse brain, lung, spleen, and testis were analyzed by Western blotting using the anti-p56 antibody (p56). GAPDH and p44/p42 MAPK were used as loading controls. Molecular weights (kDa) are shown on the right.

Fig. 5 Immunohistological localization of p56 in the mouse CNS. Cryosections of the cerebral cortex (cc, A), hippocampus (hippo, B), cerebellum (cerebel, C), and spinal cord (sc, D) were immunostained using the anti-p56 antibody (anti-p56) or normal rabbit IgG as a control (cont). The boxed regions in the middle column are magnified on the right (enlarged). Note that p56-positive puncta are discerned mainly in the perikarya of large neurons. Bars, 100 μm (left and middle column) and 20 μm (right column).
Previously shown that RNAi-mediated depletion of p56 in HeLa cells causes the missorting of the lysosomal enzyme, cathepsin D, and decreases the mobility of GGA-containing transport carriers, suggesting that p56 supports the long-range transport of carriers containing lysosomal enzymes (21). This is consistent with our observations that p56-positive puncta were primarily detected in large-sized neurons, such as hippocampal pyramidal cells, cerebellar Purkinje cells, and spinal cord motor neurons. It is also conceivable that p56 contributes to the efficient delivery of lysosomal enzymes in such large neurons.

The TGN localization of p56 in cultured cells indicates the function of this molecule at the TGN membranes. However, we found that a small but significant fraction of p56 was associated with endocytic structures in spinal cord motor neurons, suggesting that it may also function in relation to GGAs at the endosomes in vivo. In fact, accumulating evidence indicates that GGAs function at endosomes by regulating the recycling and/or degradation of physiologically and pathologically important cargoes. These include BACE1 (6, 15, 17) and LR11/SorLA (7), which are involved in Alzheimer’s disease pathology, TrkA (18), c-Met through the Crk adaptor under HGF stimulation (30), the α2B-adrenergic receptor (40), Polycystin-1,2 (16), β1-integrin (33), and EGFR (32, 37). In light of our findings that p56 is specifically recruited by GGA1, GGA1-specific cargoes may prove to be linked to p56. Among the studies above, LR11/SorLA (7) has been reported as a GGA1-specific cargo because the depletion of GGA1, but not GGA2 or GGA3, decreases LR11/SorLA protein levels. Importantly, it has been shown that the expression of GGA1 protein is decreased in the brains of Alzheimer’s disease patients (38). Therefore, although future studies are warranted, p56 may have a function in regulating the transport of LR11/SorLA protein levels.

Fig. 6 Intracellular localization of p56 with reference to organelle markers in spinal motor neurons. Spinal cord cryosections were double-immunostained with combinations of the anti-p56 antibody (anti-p56) and anti-TGN38 (A), -EEA1 (B), -LBPA (C), or -LAMP1 antibodies (D). They were then labeled with appropriate secondary antibodies. In the right two columns, the green and red colors indicate the signals for p56 and each marker, respectively. Boxed regions in the merged images are magnified and shown in the enlarged images. Note that the p56-positive puncta are associated not only with the TGN (A), but also with endosomes/lysosomes (arrowheads in B, C, and D). Bars, 10 μm.
SorLA in neurons and may be implicated in Alzheimer’s pathology.

Unexpectedly, we found that a fraction of the p56-positive puncta was associated with LAMP1-positive puncta in spinal motor neurons, which may represent late endosomal structures (31). However, it cannot be excluded that p56 plays unknown roles in the lysosomes of CNS neurons.

Genome-wide association studies have associated the CCDC91 gene that encodes p56 with several diseases and clinical imaging parameters. To date, CCDC91 has been identified as one of the susceptible genes for the ossification of the posterior longitudinal ligament of the spine (25) and bipolar disorder (2). Moreover, a SNP in the CCDC91 gene was associated with fractional anisotropy observed by MRI imaging, which reflects a combination of myelin thickness, fiber coherence and axon integrity (35).

The latter two studies support the role of the p56 gene in the CNS. In conclusion, our findings in the present study, together with those previously reported, warrant further investigation of this clathrin accessory protein in the CNS.

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REFERENCES

1. Bonifacino JS (2004) The GGA proteins: adaptors on the move. Nat Rev Mol Cell Biol 5, 23–32.
2. Chen X, Li X, Wang P, Liu Y, Zhang Z, Zhao G, Xu H, Zhu J, Qin X, Chen S, Hu L and Kong X (2010) Novel association strategy with copy number variation for identifying new risk Loci of human diseases. PLoS One 5, e12185.
3. Collins BM, Praefcke GJ, Robinson MS and Owen DJ (2003) Structural basis for binding of accessory proteins by the appendage domain of GGAs. Nat Struct Mol Biol 10, 607–613.
4. Doray B and Kornfeld S (2001) Gamma subunit of the AP-1 adaptor complex binds clathrin: implications for cooperative binding in coated vesicle assembly. Mol Biol Cell 12, 1925–1935.
5. Govero J, Doray B, Bai H and Kornfeld S (2012) Analysis of Gga null mice demonstrates a non-redundant role for mammalian GGA2 during development. PLoS One 7, e30184.
6. He X, Li F, Chang WP and Tang J (2005) GGA proteins mediate the recycling pathway of memapsin 2 (BACE). J Biol Chem 280, 11696–11703.
7. Herskowitz JH, Offe K, Deshpande A, Kahn RA, Levey AI and Lah JJ (2012) GGA1-mediated endocytic traffic of LR11/ SorLA alters APP intracellular distribution and amyloid-beta production. Mol Biol Cell 23, 2645–2657.
8. Hinners I and Tooze SA (2003) Changing directions: clathrin-mediated transport between the Golgi and endosomes. J Cell Sci 116, 763–771.
9. Hirsh J, Borner GH, Antrobus R, Peden AA, Hodson NA, Sahlender DA and Robinson MS (2012) Distinct and overlapping roles for AP-1 and GGAs revealed by the “knock-sideways” system. Curr Biol 22, 1711–1716.
10. Hirsh J, Borner GH, Harbour M and Robinson MS (2005) The afthophilin/p200/gamma-synergin complex. Mol Biol Cell 16, 2554–2565.
11. Hirsh J, Lui WW, Bright NA, Totty N, Seaman MN and Robinson MS (2000) A family of proteins with gamma-adaptin and VHS domains that facilitate trafficking between the trans-Golgi network and the vacuole/lysosome. J Cell Biol 149, 67–80.
12. Hirsh J, Motley A, Harasaki K, Peak Chew SY and Robinson MS (2003) EpsinR: an ENTH domain-containing protein that interacts with AP-1. Mol Biol Cell 14, 625–641.
13. Kalthoff C, Groos S, Kohl R, Mahrhold S and Ungewickell EJ (2002) Clint: a novel clathrin-binding ENTH-domain protein at the Golgi. J Biol Chem 277, 4060–4073.
14. Kametaka S, Moriyama K, Burgos PV, Eisenberg E, Greene LE, Mattera R and Bonifacino JS (2007) Canonical interaction of cyclin G associated kinase with adaptor protein 1 regulates lysosomal enzyme sorting. Mol Biol Cell 18, 2991–3001.
15. Kang EL, Cameron AN, Piazza F, Walker KR and Tesco G (2010) Ubiquitin regulates GGA3-mediated degradation of BACE1. J Biol Chem 285, 24108–24119.
16. Kim H, Xu H, Yao Q, Li W, Huang Q, Outeda P, Cebotaru V, Chiaravalli M, Boletta A, Piontek K, Germino GG, Weinman EJ, Watnick T and Qian F (2014) Ciliary membrane proteins traffic through the Golgi via a Rabep1/GGA1/Arl3-dependent mechanism. Nat Commun 5, 5482.
17. Koh YH, von Arnim CA, Hyman BT, Tanzi RE and Tesco G (2005) BACE is degraded via the lysosomal pathway. J Biol Chem 280, 32499–32504.
18. Li X, Lavigne P and Lavoie C (2015) GGA3 mediates TrkA endocytic recycling to promote sustained Akt phosphorylation and cell survival. Mol Biol Cell 26, 4412–4426.
19. Lui-Roberts WW, Ferraro F, Nightingale TD and Cutler DF (2008) Afthophilin and gamma-synergin are required for secretagogue sensitivity of Weibel-Palade bodies in endothelial cells. Mol Biol Cell 19, 5072–5081.
20. Lui WW, Collins BM, Hirsh J, Motley A, Millar C, Schu P, Owen DJ and Robinson MS (2003) Binding partners for the COOH-terminal appendage domains of the GGAs and gamma-adaptin. Mol Biol Cell 14, 2385–2398.
21. Mardones GA, Burgos PV, Brooks DA, Parkinson-Lawrence E, Mattera R and Bonifacino JS (2007) The trans-Golgi network accessory protein p56 promotes long-range movement of GGA/clathrin-containing transport carriers and lysosomal enzyme sorting. Mol Biol Cell 18, 3486–3501.
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5-Rabex-5 complex. *EMBO J* **22**, 78–88.

23. Mattera R, Ritter B, Sidhu SS, McPherson PS and Bonifacino JS (2004) Interactions of the consensus motif recognized by gamma-adaptin ear domains. *J Biol Chem* **279**, 8018–8028.

24. Mills IG, Praefke GJ, Vallis Y, Peter BJ, Olesen LE, Gallop JL, Butler PJ, Evans PR and McMahon HT (2003) EpsinR: an AP1/clathrin interacting protein involved in vesicle trafficking. *J Cell Biol* **160**, 213–222.

25. Nakajima M, Takahashi A, Tsuji T, Karasugi T, Baba H, Uchida K, Kawabata S, Okawa A, Shindo S, Takeuchi K, Taniguchi Y, Maeda S, Kashihi M, Seichi A, Nakajima H, Kawaguchi Y, Fujibayashi S, Takahata M, Tanaka T, Watanabe K, Kida K, Kanchiku T, Ito Z, Mori K, Kaito T, Kobayashi S, Yamada K, Takahashi M, Chiba K,Matsumoto M, Furukawa K, Kubo M, Toyama Y and Ikegawa S (2014) A genome-wide association study identifies susceptibility loci for ossification of the posterior longitudinal ligament of the spine. *Nat Genet* **46**, 1012–1016.

26. Nakatsu F and Ohno H (2003) Adaptor protein complexes as the key regulators of protein sorting in the post-Golgi network. *Cell Struct Funct* **28**, 419–429.

27. Nakayama K and Wakatsuki S (2003) The structure and function of GGAs, the traffic controllers at the TGN sorting crossroads. *Cell Struct Funct* **28**, 431–442.

28. Neubrand VE, Will RD, Mobius W, Poustka A, Wiemann S, Schu P, Dotti CG, Pepperkok R and Simpson JC (2005) Gamma-BAR, a novel AP-1-interacting protein involved in post-Golgi trafficking. *EMBO J* **24**, 1122–1132.

29. Page LJ, Soverby PJ, Lui WW and Robinson MS (1999) Gamma-synergin: an EH domain-containing protein that interacts with gamma-adaptin. *J Cell Biol* **146**, 993–1004.

30. Parachoniak CA, Luo Y, Abella JV, Keen JH and Park M (2011) GGA3 functions as a switch to promote Met receptor recycling, essential for sustained ERK and cell migration. *Dev Cell* **20**, 751–763.

31. Pols MS, van Meel E, Oorschot V, ten Brink C, Fukuda M, Swetha MG, Mayor S and Klumperman J (2013) hVps41 and VAMP7 function in direct TGN to late endosome transport of lysosomal membrane proteins. *Nat Commun* **4**, 1361.

32. Puertollano R and Bonifacino JS (2004) Interactions of GGA3 with the ubiquitin sorting machinery. *Nat Cell Biol* **6**, 244–251.

33. Ratcliffe CD, Sahgal P, Parachoniak CA, Ivaska J and Park M (2016) Regulation of cell migration and betal integrin trafficking by the endosomal adaptor GGA3. *Traffic* **17**, 670–688.

34. Shibay T, Takatsu H, Shin HW and Nakayama K (2002) Gamma-adaptin interacts directly with Rabaptin-5 through its ear domain. *J Biochem* **131**, 327–336.

35. Sprooten E, Knowles EE, McKay DR, Goring HH, Curran JE, Kent JW, Jr., Carless MA, Dyer TD, Drigalenko EI, Olvera RL, Fox PT, Almasy L, Duggirala R, Kochunov P, Blangero J and Ghah DC (2014) Common genetic variants and gene expression associated with white matter microstructure in the human brain. *Neuroimage* **97**, 252–261.

36. Takatsu H, Yoshino K and Nakayama K (2000) Adaptor gamma ear homology domain conserved in gamma-adaptin and GGA proteins that interact with gamma-synergin. *Biochem Biophys Res Commun* **271**, 719–725.

37. Uemura T, Kametaka S and Waguri S (2018) GGA2 interacts with EGFR cytoplasmic domain to stabilize the receptor expression and promote cell growth. *Sci Rep* **8**, 1368.

38. Wahle T, Thal DR, Sastre M, Rentmeister A, Bogdanovic N, Fanulok M, Heneka MT and Walter J (2006) GGA1 is expressed in the human brain and affects the generation of amyloid beta-peptide. *J Neurosci* **26**, 12838–12846.

39. Wasiak S, Legendre-Guillemin V, Puertollano V, Blondeau F, Girard M, de Heuvel E, Boismenu D, Bell AW, Bonifacino JS and McPherson PS (2002) Enthoprotin: a novel clathrin-associated protein identified through subcellular proteomics. *J Cell Biol* **158**, 855–862.

40. Zhang M, Huang W, Gao J, Terry AV and Wu G (2016) Regulation of alpha2B-adrenergic receptor cell surface transport by GGA1 and GGA2. *Sci Rep* **6**, 37921.