Common exonic missense variants in the C2 domain of the human KIBRA protein modify lipid binding and cognitive performance

K Duning1,10, DO Wennmann1,10, A Bokemeyer1,10, C Reissner2, H Wersching3, C Thomas1, J Buschert1, K Guske1, V Franzke1, A Fiol1,11, H Lohmann4, S Knecht4, S-M Brand5, MP o¨ ter6, U Rescher6, M Missler2, P Seelheim7, C Pro¨ pper8, TM Boeckers8, meta analysis9 and a putative protective association against Alzheimer’s disease,10 the impact of the intronic variant on ninth intron of the human nucleotide polymorphism (SNP; rs17070145, residing in the screen for candidate genes affecting human cognitive performance in different cohorts, 4–6,8 including a confirmed the association between rs17070145 SNP and with superior performance in episodic memory tasks. 5 Although a number of subsequent studies reproduced and also involved in Ca2þ-sensitive lipid-binding motifs in PKC, but are also found in various other proteins including the family of synaptotagmins and phosphoinositide-specific phospholipase C.12 In contrast to other lipid-binding motifs (for example, Pleckstrin homology domains, Bin–Amphipysin–Rvs domains, Fab-YOTB-Vac1-EAA1 domains), C2 domains display a unique structure that lacks a defined lipid-binding pocket and a conserved cationic patch.13 Whereas the majority of known C2 domains have a role in Ca2þ-dependent vesicle membrane association, these protein modules are also involved in Ca2þ-insensitive membrane targeting as well as in intracellular protein–protein interactions.15 Although the function of the KIBRA C2 domain in membrane association has not been analyzed yet, a recent study in null-mutant mice demonstrated that KIBRA is involved in the regulation of the vesicle-based turnover of postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the hippocampus.7 Additional studies suggested that KIBRA may also function in other cellular processes including regulation of endosomal vesicle sorting,14 directed cell migration11,15 and establishment of cell polarity.16

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Introduction

KIBRA (also called WWC1 for WW-and-C2-domain–containing-protein-1) represents a cytoplasmic protein highly expressed in kidney and brain that was recently linked to higher brain functions,1,4,11 presumably based on differences in hippocampal processing.5,7 Intriguingly, a genome-wide screen for candidate genes affecting human cognitive functions and memory formation revealed an intronic single-nucleotide polymorphism (SNP; rs17070145, residing in the ninth intron of the human KIBRA gene) that was associated with superior performance in episodic memory tasks.5 Although a number of subsequent studies reproduced and confirmed the association between rs17070145 SNP and cognitive performance in different cohorts,4–6,8 including a meta analysis9 and a putative protective association against Alzheimer’s disease,10 the impact of the intronic variant on KIBRA protein function remains elusive.

KIBRA is a member of the WWC family of proteins that was initially identified to interact with the actin-binding protein dendrin1 as well as with the atypical protein kinase C ζ (PKCζ).2 KIBRA contains two amino terminal WW domains that are known to bind to PPxY motifs in target molecules (for example, dendrin and synaptopodin), a C2 domain and a carboxyterminal-binding motif for PSD-95/Discs-large/ZO-1 modules.1,4,11 C2 domains were initially identified as Ca2þ-sensitive lipid-binding motifs in PKC, but are also found in various other proteins including the family of synaptotagmins and phosphoinositide-specific phospholipase C.12 In contrast to other lipid-binding motifs (for example, Pleckstrin homology domains, Bin–Amphipysin–Rvs domains, Fab-YOTB-Vac1-EAA1 domains), C2 domains display a unique structure that lacks a defined lipid-binding pocket and a conserved cationic patch.13 Whereas the majority of known C2 domains have a role in Ca2þ-dependent vesicle membrane association, these protein modules are also involved in Ca2þ-insensitive membrane targeting as well as in intracellular protein–protein interactions.15 Although the function of the KIBRA C2 domain in membrane association has not been analyzed yet, a recent study in null-mutant mice demonstrated that KIBRA is involved in the regulation of the vesicle-based turnover of postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the hippocampus.7 Additional studies suggested that KIBRA may also function in other cellular processes including regulation of endosomal vesicle sorting,14 directed cell migration11,15 and establishment of cell polarity.16

In this study, we describe the identification of two novel adjacent SNPs in the human KIBRA gene that results in

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structural changes within the C2 domain of the encoded protein. Genetic analysis demonstrated a striking linkage between the exonic SNPs affecting the C2 domain and the well-characterized intronic SNP known to be associated with human cognitive performance. A biochemical characterization revealed a Ca^{2+}-dependent-binding specificity of the KIBRA C2 domain for monophosphorylated phosphatidylinositols. Finally, the identified exonic SNPs were shown to affect the lipid-binding specificities of the C2 domain accompanied by changes in the structural dynamics of the protein motif. In conclusion, our data elucidate the function of the KIBRA C2 domain in membrane association and present for the first time a linkage between natural KIBRA protein variants and cognitive performance in humans.

Materials and methods

Population-based cohort study and neuropsychological testing. We used clinical, neuropsychological and genetic data of 545 participants aged above 50 years from the population-based SEARCH Health study (Systematic evaluation and alteration of risk factors for cognitive health). Details of the study protocol were described elsewhere.8,17

Identification and genotyping of KIBRA polymorphisms. Genomic DNA was extracted from white blood cells using QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). We screened 8773 bp for genetic variants using single-strand conformation polymorphism analysis and subsequent direct sequencing (ABI 3730). As both exon 15 SNP variants (rs3822660 and rs3822659) were in complete linkage disequilibrium with one another (tested by direct sequencing in 100 individuals), only the frequency of exonic SNP variant rs3822660 in the study population was determined by restriction fragment length polymorphism. For rs3822660, genomic DNA of all individuals of the study population was extracted with 3% bovine serum albumin (BSA) in TRIS-buffered saline.

Statistical analysis. As both exon 15 SNP variants (rs3822660 and rs3822659) were in complete linkage disequilibrium with one another, only KIBRA rs3822660 GT (heterozygote for the variant) and TT (homozygote for the variant) genotypes were combined and tested against KIBRA rs3822660 GG (‘wild type’, homozygote) genotype. Effects of the KIBRA rs3822660 on the cognitive domains were tested with multivariable analyses of variance using the general linear model procedure in SAS 9.2 (www.sas.com/software/sas9/). The model included the following covariates: age, gender, education, history of arterial hypertension, intake of antihypertensive medication, history of diabetes, lifestyle index, history of depression and intake of antidepressive medication. Allelic associations of KIBRA rs3822660 and rs17070145 were tested using a χ² test.

Reagents. Standard highest-grade reagents were from Sigma-Aldrich (Deisenhofen, Germany).

Antibodies. Monoclonal antibodies directed against glutathion-S-transferase (GST) were purchased from Sigma-Aldrich. Horseradish peroxidase-conjugated secondary antibodies were purchased from Dianova (Hamburg, Germany).

Preparation of GST fusion proteins. Human KIBRA cDNA1 (Acc. No. AF506799) fragments were cloned into a modified pGEX-KG vector. The constructs pGEX-KG, pGEX-KG-human KIBRA C2 wild-type (WT; aa 661–776), pGEX-KG-KIBRAC2 var. (I734/A735) were used for the isopropyl β-D-1-thiogalactopyranoside-induced expression of GST fusion proteins in Escherichia coli BL21 as previously described.13 GST and C2 fusion proteins were purified from bacterial lysates using glutathione sepharose beads (Amersham Bioscience, Freiburg, Germany) according to the manufacturer’s instructions.

Western blotting. Western blotting experiments were performed using standard techniques.11 Antibodies were diluted in blocking buffer containing 5% skim milk powder in phosphate-buffered saline (PBS)/Tween. After washing, the membranes were incubated with respective secondary antibodies coupled to horseradish peroxidase. Finally, the membranes were washed and developed using the Lumi-Light chemiluminescence detection kit (Roche, Mannheim, Germany) and an X-ray film developer.

Liposome co-sedimentation assay. Lipid-binding assay was performed as described earlier.18 In brief, brain lipid extracts (total bovine brain lipids, Folch fraction I, Sigma, Munich, Germany) were resuspended at 2 mg ml⁻¹ in binding buffer (20 mM HEPES, pH 7.4, 150 mM NaCl, 1 mM dithiothreitol) at 50 °C, and liposomes were formed using a Liposofast mini extruder (Avestin, Mannheim, Germany). Subsequently, 0.6 mg ml⁻¹ liposomes were incubated with purified proteins (0.5 μM) in 100 μl buffer for 15 min at 37 °C and were centrifuged at 140 000 g for 15 min at 4 °C. After removing the supernatants, pellets were washed once with binding buffer and subjected to SDS-polyacrylamide gel electrophoresis. Sedimented GST or GST-KIBRA C2 proteins were detected using blotting analysis with anti-GST antibodies.

Phospholipid overlay (phosphatidylinositolphosphate (PIP) strip) assay. For the PIP strip assays, phospholipid membranes (Echelon, Salt Lake City, UT, USA) were blocked with 3% bovine serum albumin (BSA) in TRIS-buffered saline.
containing 0.2% Tween-20 (TBST) for 1 h at room temperature. Afterwards, membranes were incubated in buffer containing GST–C2 fusion proteins or GST alone (each 0.5 μg ml⁻¹) in the presence or absence of calcium (1 mM CaCl₂ or 5 mM ethylene glycol tetraacetic acid) in 3% BSA/TBST at 4 °C overnight.

Unbound protein was washed away with TBST (three times for 10 min). For the detection of bound protein, anti-GST antibodies were used. Blots were incubated for 1 h at room temperature with primary antibodies diluted in 3% BSA/TBST followed by incubation with appropriate secondary antibodies coupled to horseradish peroxidase. After three washing steps with TBST the immunoreactivity was visualized with the enhanced chemiluminescence detection system.

**Lipid plate-binding assays.** Wells of 96-well microtiter plates (Immuno Plate Maxisorp, Nunc) were coated with 100 ng per well of the respective lipid and air-dried overnight. After blocking the wells with 5% BSA in PBS for 1 h, purified GST-C2 proteins (500 ng per well) in 1 mM Ca²⁺-containing PBS was added for 1 h at room temperature. Subsequently, wells were washed three times with PBS containing 0.05% Tween-20 in an ELISA plate washer (Tecan, Crailsheim, Germany). The amount of bound complex was determined by a colorimetric reaction using GST-specific antibodies (Sigma), peroxidase-coupled secondary antibodies (Dia-nova) and tetramethylbenzidine substrate (Thermo Scientific, Dreieich, Germany) measured at 450 nm in an ELISA plate reader (MWG-Biotech, Ebersberg, Germany).

**Structural analysis.** We used the recently deposited coordinates of the calcium- and ligand-free C2 structure of KIBRA (protein data bank (PDB) Id: 2Z0U) at the PDB to generate structural models of the single (M734I, S735A) and double variant (M734I + S735A). Two calcium ions have been placed according to the predictions of 3DLigandSite and the calcium coordination found in rabphilin-3A (PDB Id: 2K3H). Stabilities of wild-type and variant structures were compared by calculating difference binding energies ΔΔG using FoldX. Dynamic structures were generated using the method of normal and geometric parameters that have been determined with DynDom. Model structures were visualized using pymol (http://www.pymol.org).

**Results**

**Identification of genomic KIBRA variants.** To search for novel genetic variations that may have a functional impact on the KIBRA protein, we performed a screening of the human KIBRA gene by PCR/single stranded conformation polymorphism and subsequent direct sequencing, and identified 21 genetic variants (Figure 1a). Importantly, among the four missense SNPs identified in our screen, two variants (rs3822660 and rs3822659) were located in exon 15, leading to adjacent amino acid substitutions (M734I and S735A) within the KIBRA C2 domain (Figure 1b). In addition, we observed that both SNPs were in complete linkage disequilibrium with one another (see Materials and Methods for details). To evaluate a putative effect of these exonic SNPs on human cognition, we next performed cognitive testing in a large cohort of volunteers (SEARCH Health study, n = 545), and genotyped the entire study population for rs3822660 (as described in Materials and Methods). Of the 545 participants studied, 485 carried rs3822660 GG (‘C2 wild

![Figure 1](image-url)
arrangement of two four-stranded β sheets (β1-8) that are connected by three loops (loop 1–3) and a single short α helix (Figure 2a). Sequence alignment of the C2 domains from human KIBRA, rabphilin-3a and synaptotagmin (Figure 2a) and with KIBRA from other species (Supplementary Figure 1A) and related WW2 and WW3 proteins (Supplementary Figure 1B) demonstrated the presence of conserved amino acid residues in the β sheet and loop regions (Figure 2a), and revealed that the position of our exonic variants coincides with a moderately conserved region preceding the α helix (Figure 2a; Supplementary Figure 1A and B). To test whether these common amino acid substitutions affect the function of the KIBRA C2 domain, we first analyzed the membrane-binding capacity of the wild-type domain. We purified recombinant GST-KIBRA C2 fusion proteins (hereafter named GST-C2 WT, see Materials and Methods for details) and used them in a co-sedimentation assay with purified liposomes from bovine brain (Folch fraction I). Although GST as a negative control did not precipitate in the presence of Folch liposomes (Figure 2b, upper row, lane 4), a significant amount of GST-C2 WT was found in the liposome-containing pellet fraction after ultracentrifugation (Figure 2b, lower row, lane 4). Precipitation of GST-C2 WT was not observed in samples without liposomes (Figure 2b, lower row, lane 2), demonstrating that the KIBRA C2 domain possesses binding activity toward brain liposomes. Next, we assessed the lipid-binding specificity of the recombinant GST-C2 WT protein using a commercially available array of common cellular phospholipids (PIP strips).

In this assay, GST-C2 WT protein bound specifically and strongly to phosphatidylinositol-3-phosphate (PI(3)P) but not to other poly-phosphorylated PIPs (for example, PI(4,5)P2 or phosphatidylinerine and phosphatidylycholine (Figure 2c). GST alone as a negative control showed no binding to the phospholipids spotted on the membranes (Figure 2c). To confirm the results from the PIP strip assays, we established an ELISA-based lipid-binding assay for KIBRA C2 domain. For this, defined concentrations of PI(3)P, PI(4)P, PI(5)P or PI(4,5)P2 were immobilized and incubated with recombinant GST or GST-C2 WT. Quantification of bound GST fusion proteins mostly validated our data from the PIP strip experiments because it revealed a binding preference of GST-C2WT for PI(3)P. However, using this highly sensitive assay, we also detected a weaker interaction between GST-C2 WT and PI(4)P, PI(5)P and PI(4,5)P2 (Figure 2d). These data give evidence for a function of the KIBRA C2 domain as Ca2+-sensitive lipid-binding domain (Figure 2c). To finally confirm the results from the PIP strip assays, we established an ELISA-based lipid-binding assay for KIBRA C2 domain. For this, defined concentrations of PI(3)P, PI(4)P, PI(5)P or PI(4,5)P2 were immobilized and incubated with recombinant GST or GST-C2 WT. Quantification of bound GST fusion proteins mostly validated our data from the PIP strip experiments because it revealed a binding preference of GST-C2WT for PI(3)P. However, using this highly sensitive assay, we also detected a weaker interaction between GST-C2 WT and PI(4)P, PI(5)P and PI(4,5)P2 (Figure 2d). These data give evidence for a function of the KIBRA C2 domain as Ca2+-sensitive lipid-binding domain with preference for PI(3)P but also raised the question if this function was altered by the amino acid substitutions described above.

To evaluate the impact of rs3822660 and rs3822659 on protein function, we amplified the DNA encoding the C2 domain from an individual carrying both minor alleles, and

**Lipid-binding specificity of KIBRA C2 domain variants.**

Our discovery of two common exonic SNPs allows us to relate the polymorphisms to testable defects in protein function. Sequence analysis of the KIBRA C2 domain that is affected by the exonic variants shows the prototypical

| Table 1 | Association of rs3822660 genotype with cognitive domains |
| --- | --- |
| | Verbal memory | Word fluency | Working memory | Executive functions/ psychomotor speed |
| All participants | | | | |
| (n = 545) | β = 0.08 | P = 0.53 | β = 0.01 | P = 0.04 |
| | (n = 53) | | | |
| Women | | | | |
| (n = 234) | β = 0.09 | P = 0.56 | β = 0.25 | P = 0.11 |
| | (n = 101) | | | |
| Men | | | | |
| (n = 211) | β = 0.03 | P = 0.09 | β = 0.29 | P = 0.25 |

**Table 2** | Association of rs3822660 SNP (‘exonic variant’) with rs17070145 (‘intronic variant’) |
| --- | --- |
| rs17070145 | rs3822660 |
| (n = 485) | (n = 56) | (n = 4) |
| GG | 232 (47.84%) | 3 (3.57%) | 0 (0.0%) |
| GT | 196 (40.41%) | 37 (66.07%) | 1 (25.0%) |
| TT | 57 (11.75%) | 17 (30.36%) | 3 (75.0%) |

**Abbreviation:** SNP, single-nucleotide polymorphism. Frequencies shown in absolute numbers and percentage of the rs3822660 genotype. See Materials and Methods for details.
expressed this fragment as a GST-C2 I734/A735 fusion protein (hereafter named GST-C2 var). We first compared the lipid-binding specificity of the variant C2 domain with the wild-type C2 domain (M734/S735). PIP strip assays revealed that the GST-C2 var binds to PI(3)P at similar levels as the GST-C2 WT (Figure 3a), suggesting that I734 and A735 did not simply disrupt the integrity of the domain. However, we also observed that in contrast to the wild type the variant C2 domain interacts stronger with PI(4)P and even more so with PI(5)P (Figure 3a). These findings were then confirmed by the quantitative ELISA-based lipid-binding assay indicating that the two amino-acid substitutions within the KIBRA C2 domain in fact altered its binding preference for monophosphorylated phosphatidylinositols (Figure 3b).

**Structural analysis of the KIBRA C2 domain variants.** To uncover the molecular determinants of the altered phospholipid-binding profile caused by the novel SNP variants (Figures 2 and 3), we first analyzed the wild-type KIBRA C2 domain (PDB ID: 2Z0U), which has a fold similarity to other C2 domains in, for example, rabphilin, synaptotagmin or PKC. Unlike most C2 domains, the KIBRA C2 structure exists as a homophilic dimer that is covalently linked by a cysteine bridge between the C771 residues of different molecules (Figure 4a). The Ca$^{2+}$ chelator ethylene glycol tetraacetic acid (EGTA) completely abolished PI(3)P binding of GST-C2 WT. Abbreviations: LPA, lysophosphatidic acid; LPC, lysophosphocholine; PI, phosphatidylinositol; PI4P, PtdIns(4)P; PI5P, PtdIns(5)P; PI(3,4)P2, PtdIns(3,4)P2; PI(3,5)P2, PtdIns(3,5)P2; PI(4,5)P2, PtdIns(4,5)P2; PI(3,4,5)P3, PtdIns(3,4,5)P3; PA, phosphatic acid; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; S1P, sphingosine 1-phosphate; WB, Western blotting. (d) Lipid binding of the KIBRA C2 domain determined in an ELISA-based assay. GST C2 WT displays a binding preference for PI(3)P and a moderate affinity for PI(4)P, PI(5)P and PI(4,5)P2. Binding assays were performed eight times. Bar graphs indicate the mean value ± s.e.m. *Significant; **highly significant.
KIBRA (Figure 2c) suggests a mechanistic scenario similar to synaptotagmin, in which the negatively charged surface of the Ca$^{2+}$-free structure of the C2A domain repels association to phospholipid membranes but gets attracted by binding to one or more Ca$^{2+}$ ions because of an electrostatic switch. However, in contrast to synaptotagmin that contains two positionally flexible C2 domains (PDB ID: 1DQV), the conformation of the C2 dimer of KIBRA is constrained through a cysteine bridge of C771. Consequently, the KIBRA C2 is only able to bind to phospholipid membranes in cis configuration (Supplementary Figure 3A) or with a single C2 domain (Supplementary Figure 3B).

We next addressed the question how our newly discovered variants from SNPs rs3822660G/T (M734I) and rs3822659T/G (S735) may affect the C2 domain structure and their properties. As both variant residues are located distant to the PIP- and Ca$^{2+}$-binding sites and to the membrane-binding loops 1 and 3 (Supplementary Figure 3), the effect of the double variant on the binding profile for phospholipids (Figure 3) must result from indirect modifications of the PIP-binding site. To investigate this possibility, we modeled a KIBRA C2 structure carrying the double variant M734I and S735A, and calculated the change of free binding energy ($\Delta$G) using FOLDX. Interestingly, we observed that the amino-acid variant I734/A735 reduces protein stability because of a sterically non-favored hydrophobic interaction of I734 (Supplementary Figure 4). Although M734 of the wild-type C2 structure is in hydrophobic contact to the cysteine bridge of C705 and C711 (Figure 4d) that connects $\beta$-strands $\beta$6 and $\beta$7 and exposes the two conserved PIP-binding residues R699 and R714, the I734 variant structure lacks this hydrophobic contact (Figure 4d). As PIP2 binding to PKC changes the curvature of the C2 domain, we propose that the M734I variant alters the connectivity of the interior hydrophobic cluster, which would change the dynamics of the curvature and the geometry of residues of the basic belt toward a preference for PI(4,5)P2. To test this idea, we performed an additional analysis of the protein dynamics of wild-type and variant C2 domains using the elastic normal mode method, and indeed observed a change in the dynamics within the concave PIP-binding region and the Ca$^{2+}$-binding site (C$\alpha$-C$\alpha$-difference distance L712-L756 doubles for variant C2; and Supplementary Movie 1).

Although nuclear magnetic resonance or electron spin resonance determination of C2 structures are desirable to further elucidate the exact residues that participate in PIP coordination and substrate specificity, an aim that has defied experimental efforts thus far for any C2 domain, our modeling-based analysis revealed a logical structural explanation for the effect of SNPs rs3822660G/T and rs3822659T/G in KIBRA.

**Discussion**

Earlier studies have clearly demonstrated a linkage between KIBRA expression and higher brain functions. However, the impact of KIBRA on molecular processes within neurons remains elusive. Our biochemical and structural analyses demonstrated that the KIBRA C2 domain has a *bona fide* lipid-binding capacity with a preference toward PI(4,5)P2. To test this idea, we performed an additional analysis of the protein dynamics of wild-type and variant C2 domains using the elastic normal mode method, and indeed observed a change in the dynamics within the concave PIP-binding region and the Ca$^{2+}$-binding site. Although nuclear magnetic resonance or electron spin resonance determination of C2 structures are desirable to further elucidate the exact residues that participate in PIP coordination and substrate specificity, an aim that has defied experimental efforts thus far for any C2 domain, our modeling-based analysis revealed a logical structural explanation for the effect of SNPs rs3822660G/T and rs3822659T/G in KIBRA.

**Figure 3** Different lipid-binding specificity of the KIBRA C2 domain isoforms. (a) Lipid-binding specificities of glutathion-S-transferase (GST)-KIBRA variant (var) determined in a phosphatidylinositolphosphate strip assay. The GST-C2 var protein interacts with phosphatidylinositol-3-phosphate (PI(3)P), but also with PI(4)P and PI(5)P, respectively. Similar to GST-C2 wild-type (WT), lipid binding of GST-C2 var was completely abolished in the presence of ethylene glycol tetraacetic acid (EGTA). (b) Comparison of the lipid-binding specificities of KIBRA C2 WT and KIBRA C2 var in the ELISA-based assay revealed a binding preference of GST-C2 WT for PI(3)P and a moderate affinity for PI(4)P, PI(5)P and PI(4,5)P2. Compared with GST-C2 WT, the GST-C2 var displays a strong binding to PI(3)P and also to PI(4)P, PI(5)P or PI(4,5)P2. Bar graphs indicate the mean value of ± s.e.m. calculated from eight performed binding assays. *Significant; **highly significant.
at postsynaptic sites. 30 Mutations in the human gene encoding the PI(3)P-phosphatase myotubularin 2 cause Charcot-Marie-Tooth disease type 4B1, an autosomal-recessive peripheral neuropathy associated with an impaired synaptic transmission, 31,32 implying that normal PI(3)P levels are crucial for human brain functions. KIBRA binds to protein-interacting-with-C-kinase-1, which is known to regulate the sorting of AMPA receptors from early endosomes to a specialized recycling compartment. 18 Consistently, the deletion of KIBRA expression in mice accelerated the recycling of

Figure 4  Structural analysis of wild-type and variant KIBRA C2 domains. (a) Structure of the homophilic dimeric C2 domain of human KIBRA (residues 658–785, PDB ID: 2Z0U) that represents the first C2 domain that is covalently linked (C771-C771). Two additional cysteine bridges stabilize helical loop z2 (C705-C711) and the Ca2+ -binding loop 3 (C749-C759). The proposed Ca2+ - (red) and phosphatidylinositolphosphate (PIP; blue) -coordinating residues are indicated. The single-nucleotide polymorphism variations M734I/S735A (Var) are located in a region of unknown function. (b) Ca2+ coordination in KIBRA C2 modeled in analogy to rabphilin-3A (PDB ID: 2K3H); Loop 3 is conserved with D752 binding to two and E758 to one Ca2+ ion, whereas loop 1 is in unique conformation because of the extended hydrophobic helical loop a1 (a) and a triple Q insertion. The highly conserved D693 binds to the second Ca2+ ion. (c) Proposed PIP (green) binding at the concave basic surface and to Ca2+. In ligand-free KIBRA C2, the two-faced loop 3 is oriented toward the concave surface allowing E757 to make a side chain interaction to R714 (left panel). Ca2+ coordination requires a conformational change of loop 3 that opens the concave surface and exposes R714 to bind PIP2 (right panel). Phosphatidylserine or PIP2 may also bind to the conserved residues R753 or H755 and coordinate Ca2+ via a water molecule as determined for cPLA2.35 Note that PIP to C2 interactions are Ca2+ dependent (Figure 2c). (d) Side chain geometry of wild-type (PDB ID: 2Z0U, left) and variant (model, right) KIBRA C2 domains. S735 makes hydrogen bonds to K673 that are released in the A735 variant. M734 fills a hydrophobic pocket, whereas I734 does not perfectly fit and makes non-favored sterical contacts (see also Supplementary Figure 4).
postsynaptic AMPA receptors in neurons indicating a role in retaining cytosolic AMPA receptor vesicles. Based on these findings and our current data, we hypothesize that KIBRA may act as a local, postsynaptic docking station for AMPA receptors on PI(3)P-containing endosomes. A regulated anchoring of these vesicles probably depends on an KIBRA-mediated linkage between PI(3)P-bearing endosomal membranes and components of the postsynaptic cytoskeleton represented by the known KIBRA-interacting proteins dendrin and synaptopodin.

In this model, the lipid-binding capacity of the Ca\(^{2+}\)-sensitive KIBRA C2 domain may allow a fine-tuning of the constitutive docking-and-release process of endosomes at postsynaptic sites. Differences in the C2 domain lipid-binding specificity as demonstrated for our exonic SNPs may cause an altered AMPA receptor turnover in activated neurons and lead to changes in synaptic transmission, which in turn determines cognitive performance.

Conflict of interest

The authors declare no conflict of interest.

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