

Enzyme activity of the *PIP4K2A* gene product polymorphism that is implicated in schizophrenia

Jonathan H. Clarke · Robin F. Irvine

Received: 11 September 2013 / Accepted: 14 September 2013 / Published online: 1 October 2013
© The Author(s) 2013. This article is published with open access at Springerlink.com

Dear Editor,

Genome-wide association studies (GWAS) provide evidence for a potentially large polygenic component in schizophrenia risk (Purcell et al. 2009), and meta-analyses of polymorphisms have recently been used in the attempt to identify true candidate genes (Allen et al. 2008). One gene identified as a susceptibility candidate by linkage analysis is *PIP4K2A*, coding for the phosphatidylinositol 5-phosphate 4-kinase, PI5P4K α (Bakker et al. 2007; Schwab et al. 2006). Although GWAS and meta-analysis have not confirmed *PIP4K2A* as contributory to disease risk, the contradictory association studies listed on the SzGene database (Allen et al. 2008) may indicate genetic or phenotypic heterogeneity in an allelic variant of small effect size (Schwab and Wildenauer 2009). Although multiple DNA sequence variants have been reported for *PIP4K2A*, only one non-synonymous SNP in the coding region has been identified (rs10828317), causing an allelic change of asparagine to serine (N251S). This gene variant was found to be significantly associated with schizophrenia in two separate case studies (Bakker et al. 2007; Schwab et al. 2006).

PI5P4K α is one of the three isoforms present in mammalian cells that convert the membrane lipid phosphatidylinositol 5-phosphate (PI5P) to phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂). The main route for PI(4,5)P₂ synthesis in the cell is the conversion of the alternative substrate, phosphatidylinositol 4-phosphate (PI4P), by the phosphatidylinositol 4-phosphate 5-kinases (Di Paolo and De Camilli 2006). Consistent with substrate abundance in cells (in a typical cell relative PI5P levels are approximately 2 % of PI4P levels), the minor contribution of the conversion of PI5P

to PI(4,5)P₂ suggests that the PI5P4Ks function to attenuate a signalling role of PI5P itself or to generate a localised (compartmentalised) pool of the PI(4,5)P₂ second messenger molecule (Clarke et al. 2007).

Phosphoinositides have been shown to regulate a number of different channels and transporters, and recent evidence published in this journal suggests that the schizophrenia-associated PI5P4K α N251S variant may be involved indirectly in modulating neuronal excitability. PIP₂ regulates neuronal KCNQ potassium channels, which determine membrane potential sensitive to muscarinic receptor signalling (M current). This suppresses activity of dopaminergic neurons, and a recent study links PI5P4K α N251S to this (Fedorenko et al. 2008). The PI5P4K α variant has also been shown to be involved with the decreased glutamate uptake by the excitatory amino acid transporter, EEAT3, and the altered glutamate metabolism induced by EEAT3 has also been implicated in schizophrenia (Fedorenko et al. 2009). These studies suggested that the activity of PI5P4K α N251S might be impaired in comparison to the wild-type enzyme, although no direct enzymic measurements have yet been made. Earlier studies also noted that functional activity measurements of the PI5P4K schizophrenia variant were required (Schwab et al. 2006).

Here, we report on the relative intrinsic activities of the PI5P4K α protein variants. Human *PIP4K2A* was cloned into a bacterial expression vector (PI5P4K α WT). Site-directed mutagenesis was performed to make a base change in codon 251 (AAC to AGC), recreating the sequence for the allelic SNP (PI5P4K α N251S). Site-directed mutagenesis was also used to produce a kinase-dead version of PI5P4K α (D273K). Full-length protein from all forms was purified by proteolytic cleavage from the expressed affinity tag.

To determine whether the N251S variant was a catalytically disabled enzyme, the ATPase activity of the kinase was tested. This monitors the ability of the enzyme to bind and utilise ATP in the absence of lipid substrate as intrinsic futile cycling.

J. H. Clarke (✉) · R. F. Irvine
Department of Pharmacology, University of Cambridge,
Tennis Court Road, Cambridge CB2 1PD, UK
e-mail: jhc30@cam.ac.uk

ATP utilisation was assayed using the Transcreener ADP² fluorescence polarisation method for a range of enzyme concentrations and the relationship plotted using nonlinear regression analysis, as depicted in Fig. 1a. In comparison to the kinase-dead mutant, both the wild-type and N251S PI5P4K α variants showed appreciable ability to process ATP, indicating that both are functional kinases.

Lipid kinase activity was assessed by incubation of purified protein with PI5P micelles in the presence of [γ -³²P]ATP, as described previously (Clarke et al. 2001). Radiolabelled lipid product (PI(4,5)P₂) was extracted by acidic phase separation and isolated by thin-layer chromatography. Counts from these radioactive lipid spots were converted to specific activity units using integral plate controls. Figure 1b shows the comparative activity of wild-type PI5P4K α against the negative control kinase-dead mutant. The difference in observed activity between PI5P4K α WT and PI5P4K α N251S was not statistically different using an unpaired *t* test.

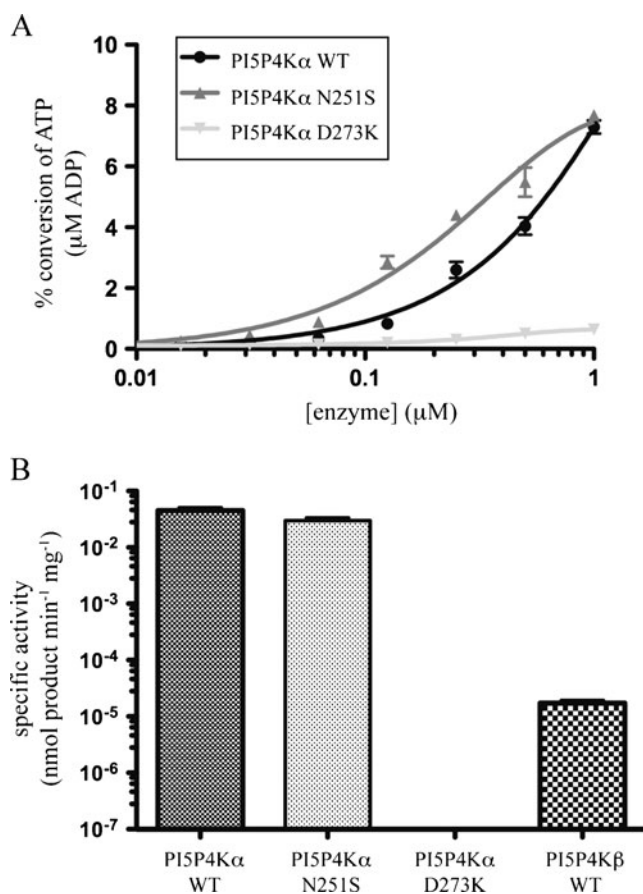


Fig. 1 ATPase and lipid kinase activities of different PI5P4K isoforms. **a** Intrinsic ATPase activities of wild-type PI5P4K α (WT), PI5P4K α carrying the allelic SNP change found in schizophrenia patients (N251S) and kinase-dead PI5P4K α (D273K) using a fluorescence polarisation assay ($N=3$, error bars \pm SEM). **b** Specific lipid kinase activities for conversion of PI5P into PI(4,5)P₂ by wild-type, N251S and kinase-dead PI5P4K α and also wild-type PI5P4K β (WT) for comparison ($N=4$, error bars \pm SEM)

These data suggest that PI5P4K α N251S does not significantly differ in activity from the wild-type enzyme. Furthermore, alignment of the PI5P4K isoform sequences show that the corresponding amino acid in the PI5P4K β sequence is also a serine, and this isoform is still active (Fig. 1b).

A recent observation suggesting that PI5P4K α may be upregulated (based on mRNA levels) in non-neuronal cells of schizophrenia patients (Saggers-Gray et al. 2011) implies that the enzyme titre or localisation may be more relevant than the activity. So rather than a consequence of impaired PI5P4K activity, the PI5P4K α N251S variant may be impaired in its ability to directly associate with ion channels or to regulate the trafficking of the channels to the plasma membrane. Indeed, a defect in such trafficking associated with the N251S mutation was experimentally demonstrated by Fedorenko et al. (2009). Thus our observation of unimpaired activity of PI5P4K α N251S throws a new light on its association with schizophrenia, which warrants further mechanistic investigation.

Acknowledgments The authors would like to acknowledge the Medical Research Council for funding this research [grant number MR/J001120/1].

Conflict of interest The authors declare no conflicts of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

- Allen NC, Bagade S, McQueen MB, Ioannidis JP, Kavvoura FK, Khoury MJ, Tanzi RE, Bertram L (2008) Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. *Nature genetics* 40:827–834
- Bakker SC, Hoogendoorn ML, Hendriks J, Verzijlbergen K, Caron S, Verduijn W, Seltens JP, Pearson PL, Kahn RS, Sinke RJ (2007) The PIP5K2A and RGS4 genes are differentially associated with deficit and non-deficit schizophrenia. *Genes, brain, and behavior* 6:113–119
- Clarke JH, Letcher AJ, D'Santos CS, Halstead JR, Irvine RF, Divecha N (2001) Inositol lipids are regulated during cell cycle progression in the nuclei of murine erythroleukaemia cells. *Biochem J* 357:905–910
- Clarke JH, Richardson JP, Hinchliffe KA, Irvine RF (2007) Type II PtdInsP kinases: location, regulation and function. *Biochem Soc Symp* 74:149–59
- Di Paolo G, De Camilli P (2006) Phosphoinositides in cell regulation and membrane dynamics. *Nature* 443:651–657
- Fedorenko O, Strutz-Seeböhm N, Henrion U, Ureche ON, Lang F, Seeböhm G, Lang UE (2008) A schizophrenia-linked mutation in PIP5K2A fails to activate neuronal M channels. *Psychopharmacology (Berl)* 199:47–54
- Fedorenko O, Tang C, Sopjani M, Foller M, Gehring EM, Strutz-Seeböhm N, Ureche ON, Ivanova S, Semke A, Lang F, Seeböhm G, Lang UE (2009) PIP5K2A-dependent regulation of excitatory

- amino acid transporter EAAT3. *Psychopharmacology (Berl)* 206: 429–435
- Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF, Sklar P (2009) Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* 460:748–752
- Saggers-Gray L, Wildenauer DB, Schwab SG (2011) Expression of PIP4K2A in lymphocyte cell lines from a sample of schizophrenia patients with previous evidence for association. *Schizophrenia research* 130:295–296
- Schwab SG, Wildenauer DB (2009) Update on key previously proposed candidate genes for schizophrenia. *Curr Opin Psychiatr* 22:147–153
- Schwab SG, Knapp M, Sklar P, Eckstein GN, Sewekow C, Borrmann-Hassenbach M, Albus M, Becker T, Hallmayer JF, Lerer B, Maier W, Wildenauer DB (2006) Evidence for association of DNA sequence variants in the phosphatidylinositol-4-phosphate 5-kinase II alpha gene (PIP5K2A) with schizophrenia. *Mol Psychiatr* 11:837–846