Deletion or inhibition of prolyl oligopeptidase blocks lithium-induced phosphorylation of GSK3β and Akt by activation of protein phosphatase 2A

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Abstract
Alterations in prolyl oligopeptidase (PREP) activity have been connected, for example, with bipolar and major depressive disorder, and several studies have reported that lack or inhibition of PREP blocks the effects of lithium on inositol 1,4,5-triphosphate (IP₃) levels. However, the impact of PREP modulation on other intracellular targets of lithium, such as glycogen synthase kinase 3 beta (GSK3β) or protein kinase B (Akt), has not been studied. We recently found that PREP regulates protein phosphatase 2A (PP2A), and because GSK3β and Akt are PP2A substrates, we studied if PREP-related lithium insensitivity is dependent on PP2A. To assess this, HEK-293 and SH-SY5Y cells with PREP deletion or PREP inhibition (KYP-2047) were exposed to lithium, and thereafter, the phosphorylation levels of GSK3β and Akt were measured by Western blot. As expected, PREP deletion and inhibition blocked the lithium-induced phosphorylation on GSK3β and Akt in both cell lines. When lithium exposure was combined with okadaic acid, a PP2A inhibitor, KYP-2047 did not have effect on lithium-induced GSK3β and Akt phosphorylation. Therefore, we conclude that PREP deletion or inhibition blocks the intracellular effects of lithium on GSK3β and Akt via PP2A activation.

KEYWORDS
bipolar disorder, depression, glycogen synthase kinase 3 beta, lithium, prolyl oligopeptidase

1 | INTRODUCTION AND BACKGROUND

Lithium has been in use for more than 70 years as a mood stabilizer, and it is still commonly used for bipolar disorder.1 It has also beneficial effects on drug-resistant major depression.2 One main mechanism for lithium is considered to be direct inhibition of glycogen synthase kinase 3β (GSK3β) via phosphorylation that has been comprehensively presented in various models (for reviews, see Freland and Beaulieu and Malhi and Outhred3,4) and in patients with bipolar disorder.5 Additionally, dysregulated GSK3β has been connected with several mental disorders where lithium has therapeutic effect, but the results are not unambiguous.6–9 GSK3β is an important kinase in the cell, regulating, for example, glucose response, neuronal plasticity and apoptosis, and GSK3β inhibition by lithium has been shown...
to reduce apoptosis and to induce neuronal plasticity and
growth via brain-derived neurotrophic factor (BDNF; for
review, see Freland and Beaulieu1). Another important
target for biological effects of lithium is protein kinase B
(Akt, PKB),10 and lithium induces the activation of Akt
by phosphorylating it.11 A recent study discovered
hypotensive Akt in prefrontal cortex of bipolar patients
that can in part explain the beneficial effects of lithium
in this disease.12 However, lithium regulates also several
other cellular pathways, and the exact mechanism-
of-action for lithium is not known.13

Although lithium is still an effective treatment for
bipolar disorder, there is still a substantial number of
patients who do not respond to it, particularly in
bipolar depression.14 There are several genetic mutations
(for review, see Pickard15) and intracellular mechanisms
that may explain lithium insensitivity, for example, decreased expression of lymphoid enhancer binding that
leads to dysregulated Wnt/β-catenin signalling16 or alter-
ations in phosphodieseterase 11A expression that contrib-
utes to cyclic nucleotide signalling.17 Another factor that
blocks several effects of lithium in the cellular models is
the lack of a serine protease, prolyl oligopeptidase (PREP,
aka POP). In 1999, Williams et al showed that the lack of
DpoA gene, encoding PREP, causes the loss of lithium
response in dictyostelium.18 After this finding, the authors
showed that PREP deletion or inhibition blocks the
effects of three different mood stabilizing drugs, lithium,
valproic acid and carbamazepine.19 Based on the lack of
genetic association between DpoA gene coding PREP and
GskA gene that codes GSK3 proteins in dictyostelium, it
was concluded that PREP does not cause its lithium-
related effects via GSK3b.18 Williams et al found that
PREP deletion elevated inositol 1,4,5-triphosphate (IP3)
levels in dictyostelium,18 and similar results were seen in
U343 cells after PREP silencing or inhibition.20 As lith-
ium is known to deplete intracellular IP3 levels,4 this was
concluded to be the mechanism behind PREP-related the
lithium resistance. In more detail, the PREP was shown
to regulate to IP3 turnover via multiple inositol
polyphosphate phosphatase (Mipp1).18,21

Decreased PREP activity has been found from the
plasma of bipolar disorder patients that were on lithium
treatment,22 although lithium does not directly inhibit
PREP.23 This suggests that the decreased PREP activity
could be related to the pathophysiology of bipolar disor-
der. Additionally, PREP activity is reduced in the pitu-
tary gland of the major depressive disorder patients.24
Similar findings of the reduced PREP activity were done
in the major depressive disorder patient plasma by Maes
et al.,25,26 but these studies did not control the role of
fibroblast activating protein that cleaves similar peptides
as PREP in plasma,22 and therefore, the results need to
be interpreted with caution. However, to support these
findings, PREP knock-out (PREPko) mouse shows vari-
ous mood disturbances having reduced anxiety, impaired
prepulse inhibition and elevated locomotor activity and
aggressiveness.27,28 A study looking for single nucleotide
polymorphism (SNP) in PREP gene in bipolar disorder
patients did not find any correlation,29 but PREP very
rarely shows any genetic association on diseases based on
GWAS databases.30 Moreover, the regulation of PREP gene
or protein expression PREP or genes that PREP
regulates is very poorly known, and therefore, the lack of
genetic association between PREP and GSK3 in
dictyostelium does not exclude the possibility that modifi-
cations of PREP could regulate, for example, GSK3b indi-
rectly. Svarchbahs et al recently discovered that PREP
negatively regulates protein phosphatase 2A (PP2A) via
direct protein–protein interactions and that the lack of
PREP or PREP inhibition elevates PP2A activity in cells
and in vivo.31 PP2A is one of the main phosphatases in
cell, and GSK3b and Akt are substrates for PP2A.32,33 On
the basis of on this, we wanted to clarify if PREP could
block the intracellular effects of lithium via PP2A. Our
results show that PREP inhibition or deletion blocks the
lithium-induced phosphorylation of GSK3b and Akt, and
this effect is dependent on PP2A activity.

2  |  MATERIALS AND METHODS

The study was conducted in accordance with the Basic &
Clinical Pharmacology & Toxicology policy for experi-
mental and clinical studies.34

2.1  |  Reagents

Reagents were purchased from Sigma-Aldrich
(St. Louis, MO) if not otherwise specified. The PREP
inhibitor, KYP-2047 (4-phenylbutanoyl-l-prolyl-2(S)-
cyanopyrrolidine), was synthesized for us in the Division
of Pharmaceutical Chemistry, University of Helsinki, as
described in Jarho et al.35

2.2  |  Cell cultures

Human embryonic kidney (HEK-293; RRID:CVCL_0045)
cells and HEK-293 PREPko cells were cultured in full
Eagle’s medium (Dulbecco’s modified Eagle medium
[DMEM]; #D6429, Sigma) with an additional
10% (v/v) (20% with PREPko cells) foetal bovine
serum (FBS; #16000-044, ThermoFisher Scientific),
1% (v/v) l-glutamine–penicillin–streptomycin solution
(#15140122, ThermoFisher Scientific). Human neuroblastoma cells (SH-SY5Y) and SH-SY5Y PREPko cell lines were cultured with DMEM (DMEM-Glutamax; #31966021, ThermoFisher Scientific) containing 15% FBS (ThermoFisher Scientific) for wild-type (wt) and 30% FBS for SH-SY5Y PREPko cells, 1% non-essential amino acids (NEAAs; #11140050, ThermoFisher Scientific) and 50 μg/ml gentamycin (15750-045, ThermoFisher Scientific). Higher FBS concentration is required for PREPko cell cultures due to the higher basal autophagy levels.31,36 Generation of the PREPko cell cultures with CRISPR-cas9 plasmid is described earlier in Svarcbahs et al,31,36 and the validation of the PREPko cell cultures is presented in the Figure S1.

2.3 | Cell treatments

Briefly, cells were plated on 6-well plates (400 000 cells per well) and allowed to attach overnight. For PREP inhibitor experiments, concentration of 1- and 10-μM KYP-2047 was used based on previous studies.31,37,38 KYP-2047 was diluted to cell culture medium from 100-mM stock in 100% DMSO, and corresponding concentration of DMSO (0.01% or 0.001%) was used as a vehicle control. Okadaic acid (OA; O8010), a PP2A inhibitor, was diluted to DMSO as 10-μM stock and then diluted to 10 nM in cell medium for assays. Lithium chloride (LiCl; L9650) was dissolved in PBS as 10-M LiCl stock and diluted to cell medium in concentrations of 10- or 20-mM LiCl for assays. The concentration of the lithium was based on earlier PREP and lithium studies.18

2.4 | Western blot

Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (50-mM Tris HCl pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 150-mM NaCl) containing phosphatase inhibitor (#87786, ThermoFisher Scientific) and protease inhibitor cocktails (#78430, ThermoFisher Scientific). Samples were sonicated 3 × 1 s and centrifuged at 16 000 ×g for 15 min. Protein concentration was measured from supernatant with bicinchoninic acid (BCA; #23225, ThermoFisher Scientific). Standard SDS-PAGE techniques were used, and 30 μg of sample was loaded to 12% gel (#4561044, Bio-Rad, Hercules, CA). Gels were transferred by Trans-Blot Turbo Transfer System (#1704150, Bio-Rad) onto Trans-Blot Turbo Midi PVDF (#1704157, Bio-Rad) or nitrocellulose (#1704159, Bio-Rad) membranes. Membranes were incubated at +4°C overnight in 5% skim milk in Tris-buffered saline with 0.05% Tween-20 (TBS-T). A list and details of primary antibodies and respective concentrations are presented in Table 1. After overnight incubation, the membranes were washed and incubated in secondary antibody for 2 h in room temperature (1:2000 in TBS-T; HRP-conjugated goat-anti rabbit (#31463, ThermoFisher Scientific)). The images were captured using the ChemiDoc XRS+ (Bio-Rad). To verify that bands are in the linear range of the detection, increasing exposure time and automatic detection of saturated pixels in Image Lab software (version 6.01, Bio-Rad) was used. Thereafter, the images were converted to 8-bit grayscale format, and the optical densities (ODs) of the bands were measured by ImageJ (histogram area analysis; version 1.48; National Institute of Health, Bethesda, MD). The OD obtained from each band was normalized against the corresponding vinculin band (or to beta-actin in Figure S1). For the negative control (NC) group, the OD values were averaged between the technical replicates in the same plate (in SH-SY5Y PREP-KO cell culture assays) and thereafter normalized to this value. For other treatments, OD value of the NC value was set to 100%. Treatment effects were then correlated to the control value. In the Western blot experiments, 1–2 replicates (technical replicates) of each treatment were used in the well plate with SH-SY5Y PREP-KO assays and one of each treatment with the PREP inhibitor and OA assays. Thereafter, we performed 4–6 individual experiments for each treatment (biological replicates) and immunoblots to have sufficient number of repeats. Samples were not reused to immunoblot same proteins.

TABLE 1  Details of primary antibodies

| Antibody                | Species | Product code and manufacturer | Dilution used |
|-------------------------|---------|-------------------------------|--------------|
| GSK3b                   | Rabbit  | ab32391, Abcam                | 1:1000       |
| pGSK3b                  | Rabbit  | ab75814, Abcam                | 1:1000       |
| Akt                     | Rabbit  | ab8805, Abcam                 | 1:1000       |
| pAkt                    | Rabbit  | ab8933, Abcam                 | 1:500        |
| Vinculin (loading control) | Rabbit   | ab129002, Abcam               | 1:10 000     |
| Beta-actin (loading control, Figure S1) | Rabbit | ab8227, Abcam                | 1:2500       |
2.5 | Data and statistical analyses

Data are expressed as mean values ± standard error of the mean (mean ± SEM), and NC average was set as 100% on each assay to reduce variability between repeats. When having two variables, two-way ANOVA followed by Sidak’s post hoc comparison was used if ANOVA test gave statistical significance ($P < 0.05$). With one variable, one-way ANOVA was followed by Tukey’s post hoc comparison if ANOVA test gave statistical significance ($P < 0.05$). In all cases, $P$ values of <0.05 were considered to be significant. Statistical analysis was performed using PRISM GraphPad statistical software (version 6.07, GraphPad Software, Inc., San Diego, CA).

3 | RESULTS

At first, we wanted to assess the effect of lithium on GSK3b phosphorylation in PREPko cells because the lack of PREP was connected to lithium resistance in earlier studies. In the assays, we used 10 and 20 mM of LiCl as these concentrations have been used to assess the impact of lithium on the intracellular kinases in the HEK-29339–41 and SH-SY5Y cell cultures.32–34 However, it needs to be noted that the concentrations are significantly higher than the therapeutic plasma concentration of lithium in patient use (0.8–2 mM).45 Phosphorylation of GSK3b at Ser9 (pGSK3b) is well-characterized effect of lithium, and both in the wt HEK-293 and SH-SY5Y cells, 24-h incubation with 20-mM lithium significantly elevated the levels of pGSK3b. However, this was not seen in PREP-KO cells (Figure 1B, F; SH-SY5Y cells: $F_{2,28} = 4.134; P = 0.0267$ wt vs. PREP-KO cells; HEK-293 cells: $F_{2,28} = 4.537; P = 0.0254$ wt vs. PREP-KO cells; $P < 0.05$ 20-mM LiCl compared with NC in wt cells; two-way ANOVA with Sidak’s multiple comparison test); 24 h was selected as the time point since 4-h incubation with 10- or 20-mM lithium did not alter the levels of pGSK3b (Figure S2).

We also studied the changes in Akt phosphorylation (pAkt), as it has been indicated as one of the main targets for lithium in the cell. Similar to pGSK3b, 20-mM lithium significantly elevated the phosphorylation of Akt in both wt cell cultures but not in PREP-KO cells (Figure 1D,G; SH-SY5Y cells: $F_{2,26} = 11.03; P = 0.0003$ wt vs. PREP-KO cells; HEK-293 cells: $F_{2,26} = 13.49; P = 0.006$ wt vs. PREP-KO cells; $P < 0.05$ 20-mM LiCl compared with NC in wt cells; two-way ANOVA with Sidak’s multiple comparison test). The levels of total GSK3b or Akt were not significantly changed by the treatments (Figure 1A,C,E,G).

PREP inhibition by a small-molecular inhibitor has been shown to block the effects of lithium and other mood stabilizers.19 On the basis of this, we assessed if PREP inhibition by KYP-2047 has similar effect as PREP deletion on lithium-induced phosphorylation of GSK3b and Akt. The results showed that simultaneous incubation of SH-SY5Y and HEK-293 cells with 20-mM lithium and 1- or 10-μM KYP-2047 blocked the lithium-induced phosphorylation of GSK3b (Figure 2B,F; SHSY: $F = 6.216, P = 0.0018; P < 0.01$ 20-mM LiCl vs. 20-mM LiCl + 10-μM KYP-2047. HEK-293: $F = 10.02, P = 0.0001; P < 0.01$ 20-mM LiCl vs. 20-mM LiCl + 1- and 10-μM KYP-2047; one-way ANOVA with Tukey’s multiple comparison test). Similar to PREP-KO cells, pAkt was also significantly reduced by 1- and 10-μM KYP-2047 when incubated with LiCl (Figure 2D,H; SHSY: $F = 4.159, P = 0.0158; P < 0.05$ 20-mM LiCl vs. 20-mM LiCl + 1- and 10-μM KYP-2047. HEK-293: $F = 4.635, P = 0.0062; P < 0.01$ 20-mM LiCl vs. 10-μM KYP-2047; $P < 0.01$ 20-mM LiCl vs. 1-μM KYP-2047; one-way ANOVA with Tukey’s multiple comparison test). LiCl and KYP-2047 caused fluctuation in the protein levels of unphosphorylated GSK3b and Akt, but the changes were not significant (Figure 2A,C,E,G).

Finally, we wanted to test if the impact of PREP modifications on LiCl effects is dependent on PP2A activation. To study this, the cells were incubated with a PP2A inhibitor, OA (10 nM), together with 20-mM LiCl and 20-mM LiCl + 10-μM KYP-2047 in HEK-293 cells. As expected, PP2A inhibition by OA elevated significantly pGSK3b and pAkt levels (Figure 3B,D; pGSK3b: $F = 4.365, P = 0.0228; P < 0.05$ NC vs. 10-nM OA; pAkt: $F = 3.386, P = 0.0362; P < 0.05$ NC vs. 10-nM OA one-way ANOVA with Tukey’s multiple comparison test). When LiCl was added with OA, the levels of pGSK3b and pAkt were not significantly altered but slight decrease in phosphorylation levels were seen (Figure 3B,D); 10-μM KYP-2047 did not have effect on the phosphorylation of GSK3b or Akt in the presence of the OA, suggesting that PREP inhibition effect is dependent on PP2A activity (Figure 3B,D; no significance compared with 10-nM OA or 20-mM LiCl + 10-nM OA, one-way ANOVA with Tukey’s multiple comparison test). The levels of total GSK3b or Akt were not significantly altered by OA or other treatments (Figure 3A,C).

4 | DISCUSSION

In the late 1990s and early 2000s, the lack of PREP was found to block the lithium response in *dictyostelium* by regulating the IP₃ turnover by Mippi.18,19 Later, the same effect was shown by PREP inhibitors in rat primary neurons and in U343 cells, and the impact of PREP on GSK3b was excluded based on genetic association.18–20
However, in the current study, we show that the lack of PREP or PREP inhibition blocks the lithium-induced phosphorylation of GSK3b and Akt, and this effect is PP2A dependent.

After its discovery in 1971,46 PREP has been studied particularly in the context of neuropeptide catabolism and neurodegenerative diseases, and this was also the rationale to develop PREP enzyme inhibitors to combat memory and cognitive deficits, for example, in Alzheimer’s disease (for review, see Garcia-Horsman et al47). However, the effect of PREP and its inhibition on neuropeptide levels in vivo remained unclear,48,49 and a phase II clinical trial testing PREP inhibitor S-17092 as a memory enhancer failed.50 Therefore, the finding that PREP could regulate IP₃ levels in the cells was opening new avenues for PREP studies in early 2000s.18,19 Lithium regulates IP₃ levels by blocking the inositol monophosphatase (IMPase) and inositol polyphosphate 1-phosphatase (IPPase).4,51 This leads to decreased inositol levels and eventually to reduced IP₃ production in the phosphoinositide synthesis. Based on this, it is interesting how PREP deletion or inhibition can elevate IP₃ levels via reduced turnover of IP₃ to IP₅, if the whole IP cascade is already depleted after lithium exposure. The effect of
PREP inhibition on cellular IP₃ levels seems to be also cell line dependent since Schulz et al showed that PREP inhibition elevated IP₃ levels only in U343 cells but not in the SH-SY5Y or in LN405 cell cultures.²⁰ Additionally, Jalkanen et al⁵² did not see changes in the IP₃ levels in rat brain after 4-day PREP inhibition, but the changes in IP₃ levels in vivo are difficult to measure due the rapid degradation of IP₃. Therefore, it is possible that there are also other causes behind the elevated IP₃ levels after PREP modifications. Nevertheless, it is interesting how the impact of PREP on other intracellular targets of lithium, such as GSK3b, was eliminated in the early phase of the studies because a double mutant combining gskA and DpoA mutations showed no genetic interaction.¹⁸

**FIGURE 2** Prolyl oligopeptidase (PREP) inhibition by KYP-2047 blocks the impact of lithium on GSK3b and Akt phosphorylation; 1- and 10-μM KYP-2047 blocked the effect of 20-mM lithium chloride (LiCl) on GSK3b (pGSK3b; 1 μM was not significant in SH-SY5Y cells) and Akt phosphorylation (pAkt) in both cell cultures (B,D,F,H). No significant changes were observed in total protein levels by any treatment (A,C,E,G). *P < 0.05; **P < 0.01; ***P < 0.001; one-way ANOVA with Tukey’s post-test.
Later, the connection between PREP, IP₃, and lithium has been studied only a little, but in 2005, Sarkar et al showed that the PREP inhibition by Z-pro-prolinal can block the lithium-induced autophagy. The positive effect of lithium on autophagy was related to decreased IP₃ levels in the cell in this study, and simultaneous incubation of cells with lithium and PREP inhibitor blocked the IP₃ depletion⁵³ as shown in the studies by Williams et al IP₃ has several pathways to regulate autophagy. Elevated IP₃ initiates Ca²⁺ release from the endoplasmic reticulum that leads to the decreased autophagic flux by reducing the maturation of autophagosomes.⁵⁴ Moreover, the IP₃ regulates mTOR-dependent autophagy by inhibiting 5’adenosine monophosphate-activated protein kinase (AMPK).⁵⁴ Another connection is between IP₃ receptor and Bcl2–Beclin1 complex, where IP₃ receptor forms an interaction with nutrient-deprivation autophagy factor-1 (NAF-1) and thus regulates Bcl2.⁵⁵ Bcl2 blocks autophagy when interacting with Beclin1, and IP₃ receptor–NAF1 complex can enhance the interaction between Bcl2 and Beclin1 in endoplasmic reticulum⁵⁵ where PREP is also located.⁵⁶ Interestingly, other studies have shown that the PREP inhibition or deletion induces autophagy, and this occurs via PP2A-DAPK1-Beclin1 pathway.⁵¹,⁵⁶–⁶⁰ Sarkar et al did not show the impact of PREP inhibition on autophagy alone in their study⁵³ so it appears that the negative impact of PREP inhibition on autophagy is related to lithium-induced changes but not to PREP inhibition alone. It is possible that when lithium decreases IP₃ levels, PREP inhibition does not have its normal impact on Bcl2 and Beclin1 phosphorylation, but this would require more detailed studies. The major shortcoming of the current study was that we could not verify the effects of PREP modifications on IP₃ levels in our models. Widely used radioligand assay for IP₃ levels has been discontinued, and available enzyme-linked immunosorbent assays for IP₃ were not reliable in our hands.

PP2A dysfunction has been widely studied in the context of cancer (for review, see Wlodarchak and Xing⁶¹) and in Alzheimer’s disease (for review, see Clark and Ohlmeyer⁶²) but not in the mental disorders. There are studies reporting that the mutations in the B-subunit of PP2A are involved with the intellectual disability⁶³,⁶⁴ and with schizophrenia.⁶⁵ Moreover, SNPs and risk haplotypes for bipolar disorder were found in PPP2R2C gene that encodes the brain-specific PP2A-ß regulatory subunit.⁶⁶ Mutation in the PP2A-ß altered the function of...
KCNQ2 potassium channel, leading to decreased lithium response in cells and to reduced neuronal excitability. Lithium has been shown to inhibit PP2A in several pre-clinical studies, promoting the role of PP2A on intracellular signalling cascades that are regulated by lithium treatment.\(^{67,68}\) Additionally, taking in the account that wide range of PP2A target kinases is related, for example, to pathophysiology of bipolar disorder, such as GSK3\(\beta\) and Akt,\(^{12}\) the role of PP2A in mental disorders should be studied in more detail. It is also interesting that lowered PREP activity has been connected with bipolar disorder\(^{22}\) and major depressive disorder,\(^{24}\) and in both diseases, lithium is used as a therapy. PREP deletion or inhibition elevates PP2A activity\(^{31}\) that then again has an effect on GSK3\(\beta\) and pAkt as shown in the current study. Based on this, it would be interesting to investigate if lowered PREP activity in the mood disorders contributes to elevated PP2A activity and altered intracellular signalling.

Although the elevated PP2A activation in PREPko cells and after PREP inhibition explains the lack of effects by lithium on GSK3\(\beta\) and pAkt, studies by Williams et al\(^{18,19}\) have shown that the PREP deletion and inhibition increases IP\(_3\) levels in \textit{dictyostelium}, and Schulz et al showed the same in U343 cells after PREP silencing and inhibition.\(^{20}\) Interestingly, none of these studies report the changes in the intracellular Ca\(^{2+}\) levels although the release of Ca\(^{2+}\) from endoplasmic reticulum is the main effect of IP\(_3\) signalling.\(^{69}\) In the study by Rostami et al, PREP inhibition on intracellular Ca\(^{2+}\) levels was studied,\(^{57}\) but the Ca\(^{2+}\) levels were measured after 48-h PREP inhibition that is too long timeframe for rapid Ca\(^{2+}\) alterations caused by IP\(_3\). PP2A inhibition decreases the IP\(_3\) levels in the rabbit platelets\(^{70}\) and human erythrocytes,\(^{71}\) and PP2A has a negative regulatory effect on IP\(_3\) signalling receptor complex.\(^{72,73}\) Therefore, it is possible that the effects of PREP modifications on IP\(_3\) levels are mediated by PP2A activity, but this should be studied in more detail. Therefore, it is possible that part of the PREP-related effects on lithium come also via IP\(_3\) pathway where PP2A may have a role and in part via PP2A-dependent GSK3\(\beta\) and Akt pathway.

**ACKNOWLEDGEMENTS**

This study was supported by grants from Academy of Finland (grant 318327), Jane and Aatos Erkko Foundation and Sigrid Juselius Foundation to TTM. Funding bodies did not participate in the study design nor in the collection, analysis and interpretation of data nor in writing of the report.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.
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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.