G-protein signaling modulator 1 deficiency accelerates cystic disease in an orthologous mouse model of autosomal dominant polycystic kidney disease

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Polycystic kidney diseases are the most common genetic diseases that affect the kidney. There remains a paucity of information regarding mechanisms by which G proteins are regulated in the context of polycystic kidney disease to promote abnormal epithelial cell expansion and cystogenesis. In this study, we describe a functional role for the accessory protein, G-protein signaling modulator 1 (GPSM1), also known as activator of G-protein signaling 3, to act as a modulator of cyst progression in an orthologous mouse model of autosomal dominant polycystic kidney disease (ADPKD). A complete loss of Gpsm1 in the Pkd1+/−/− mouse model of ADPKD, which displays a hypomorphic phenotype of polycystin-1, demonstrated increased cyst progression and reduced renal function compared with age-matched cystic Gpsm1+/−/− and Gpsm1+/- mice. Electrophysiological studies identified a role by which GPSM1 increased heteromeric polycystin-1/polycystin-2 ion channel activity via Gβγ subunits. In summary, the present study demonstrates an important role for GPSM1 in controlling the dynamics of cyst progression in an orthologous mouse model of ADPKD and presents a therapeutic target for drug development in the treatment of this costly disease.

accessory proteins | renal epithelial cells | patch clamp | renal injury | heterotrimeric G protein

Polycystic kidney disease (PKD) is a common monogenic cause of chronic renal failure in children and adults. Positional cloning has identified the genes responsible for the etiology of PKD, with mutations in Pkd1 (1, 2) and Pkd2 (3) being associated with the dominant form of this disease. The hallmark phenotype in PKD is the manifestation of fluid-filled renal cysts, which originate from the expansion of de-differentiated and actively proliferating renal epithelial cells to directly control the function of the polycystin-2 (PC2) ion channel (7, 8), in addition to controlling the activity of specific G-protein subunits (9–13). Among its many cellular sites, PC2 localizes to the ciliary membrane and acts as a mechanosensor (14). Recently, a number of therapeutic drugs targeting GPCR to reduce cyst progression have reached the clinical trial stage, validating a central role for G proteins in cystic disease pathogenesis (5).

Over the past 15 years, our basic understanding regarding the interaction between GPCRs, G proteins, and their subsequent effector has become more diverse, largely due to the discovery of accessory proteins that regulate the G-protein activation/inactivation cycle through a GPCR-independent pathway (15, 16).

One group of accessory proteins known as activator of G-protein signaling (AGS) proteins were identified as GPCR-independent regulators of G-protein subunits (15, 16). In particular, G-protein signaling modulator 1 (GPSM1), also known as activator of G-protein signaling 3 (AGS3), was identified as an evolutionarily conserved protein with orthologs also found in fruit flies and worms (17, 18). GPSM1 contains four G-protein regulatory (GPR) motifs, also known as GoLoco motifs (18, 19), which function as a guanine nucleotide dissociation inhibitor (GDI) (18, 20).

In nonrenal mammalian cells and whole-organ systems, GPSM1 plays a critical role in regulating mitotic spindle orientation, cell polarity, and adenylyl cyclase activity (15, 21, 22). Similar biological properties have been attributed to GPSM1 orthologs in invertebrates (15, 21, 22). These same biological processes have been identified as central pathophysiological mechanisms promoting cystogenesis in PKD, but the role of GPSM1 in the kidney remains undefined. Recently, our laboratory has identified an abnormally high expression level of GPSM1 in renal epithelial cells obtained from multiple models of PKD (23) and in noncystic kidneys following renal injury (24). In the latter model, the deficiency in the expression of GPSM1 following acute kidney injury resulted in impaired recovery of the sublethally injured tubular epithelial cells (24).

This set of data suggests that GPSM1 plays a role in renal epithelial cell repair following renal injury and that the induction of this protein in PKD may be a critical modulator of the renal cystogenic process.

The present study was designed to investigate the role of GPSM1 in renal epithelial cell cystogenesis using an orthologous mouse model of autosomal dominant polycystic kidney disease (ADPKD). Gpsm1 null mice were intercrossed with a mouse model of ADPKD, Pkd1+/−/−, which exhibits wild-type PC1 hypomorphism, to provide genetic insight into the role of GPSM1 in PKD biology.

Results

Expression and Localization of GPSM1 Protein in a Mouse Model of ADPKD. The expression profile and segment-specific localization of GPSM1 was examined using kidneys from an orthologous PC1 hypomorphic mouse model, Pkd1+/−/−, which previously described


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by Yu et al. (25). GPSM1 protein expression by immunoblot analysis is shown between normal and cystic mouse kidneys of various genotypes at postnatal day 11–12 (P11–12) (Fig. 1A). Quantitation of the GPSM1 band intensities revealed that cystic Gpsm1+/−;Pkd1+/− kidneys expressed significantly higher levels of GPSM1 protein by ~15-fold (P < 0.001) compared with age-matched noncystic Gpsm1+/−;Pkd1+/− mouse kidneys (Fig. 1B). Similarly, renal GPSM1 protein is readily detectable in cystic Pkd1+/− kidneys at P19, and the expression was directly correlated with the Gpsm1 genotype (Fig. S2A). Using another model of ADPKD (26), we observed that GPSM1 levels remained elevated in the cystic kidneys beyond P14 at which time normal C57BL/6 mice (i.e., Gpsm1+/−;Pkd1+/−) appear to have low-to-undetectable levels of expression (Fig. S1). The virtually undetectable expression of GPSM1 in older wild-type kidneys in our study is in agreement with previously published reports using mature kidneys from mice, rats, and humans (23, 24, 27, 28). Moreover, the temporal renal GPSM1 expression profile is similar to previous studies using brain samples (27, 29).

Immunofluorescent histochemistry of serial renal sections from Gpsm1+/−;Pkd1+/− at P12 demonstrated that GPSM1 protein expression was predominantly localized to the cystic epithelial cells of collecting duct origin [Dolichol biflorus agglutinin (DBA)-positive cells in red in Fig. 2]. In agreement with the original description of this Pkd1 model (25), renal cysts in Pkd1+/− mice are predominantly derived from collecting ducts (DBA-positive in red) with a small number of cysts in distal tubules (Calbindin 28K-positive in red) and proximal tubules [Lotus tetragonolobulus agglutinin (LTA)-positive in brown] (Fig. S3A). These results confirm the previous study by Yu et al. (25) describing this ADPKD mouse model.

**Complete Loss of Renal GPSM1 Increases Cystogenesis in Pkd1+/− Mice.** Double heterozygous Gpsm1+/−;Pkd1+/− breeder mice were produced as described in SI Materials and Methods and intercrossed to generate a panel of mice with various combinations of Gpsm1+/−;Pkd1+/− genotypes. The gross morphology of representative P11–12 whole kidneys from noncystic (Pkd1+/−;Pkd1+/−) and cystic Pkd1+/− mice with each Gpsm1 genotype (+/−;+/−; and −/−) were compared to determine genotype–phenotype correlations (Fig. 3A). Kidney-to-body weight (KW/BW) ratios provide a reliable index of renal cystogenesis in animal models of PKD. Using this parameter, the KW/BW ratios at P11–12 for Pkd1+/− mice were significantly higher (P < 0.001) regardless of the Gpsm1 genotype compared with the wild-type mice (Gpsm1+/+;Pkd1+/+) (Fig. 3B). The KW/BW ratio for Gpsm1−/−;Pkd1+/− mice was 19.4 ± 4.3% (n = 7), which was significantly higher (P < 0.05) by 85% (10.5 ± 0.6%; n = 11) in Gpsm1+/+;Pkd1+/− and by 54% (12.6 ± 2.7%; n = 8) in Gpsm1−/−;Pkd1+/− mouse kidneys. There was no difference in KW/BW ratios between all of the noncystic Pkd1+/− or Pkd1+/− mouse kidney groups regardless of the Gpsm1 genotype (Fig. 3B).

**Whole-kidney sections** were obtained from the middle of the several different kidneys from Pkd1+/− mice with either wild-type Gpsm1+/+, heterozygous Gpsm1+/−, or null Gpsm1−/− (Fig. 3C). The percentage of total surface area composed of cystic space or renal parenchyma was assessed by quantitative morphometry (Fig. 3D). Cystic Pkd1+/− mice with either heterozygous (Gpsm1+/−) or wild-type (Gpsm1+/+) expression had significantly greater amounts of parenchymal surface area by 32% (36.2 ± 2.0%; P < 0.05; n = 11) and by 23% (33.5 ± 2.3%; P < 0.05; n = 7) compared to noncystic Pkd1+/− mice regardless of the Gpsm1 genotype (Fig. 3B).
with null Gpsm1-expressing Pkd1+/+ mice (Gpsm1+/−; 27.1 ± 1.7%; n = 7).

Proliferating cell nuclear antigen-positive renal cystic epithelial cells were significantly higher (P < 0.05) in Gpsm1−/−:Pkd1+/+ compared with Gpsm1−/−:Pkd1−/− mouse kidneys (Fig. S3C). Neither the extent of renal fibrosis as determined by Trichrome staining (Fig. S3B) nor the level of α-smooth muscle actin staining in the kidney was markedly different between cystic kidneys with or without the expression of Gpsm1 (Fig. S3D). Macrophage infiltration into the kidney was absent as determined by a lack of F4/80-positive cells in the cystic kidneys (data not shown). At P19, there was a similar trend as observed at P11–12 where the KW/BW ratio (Fig. S2B) and morphometric tissue-to-tubular space analysis (Fig. S2C) were elevated in the absence of Gpsm1 compared with the mice expressing Gpsm1 (+/+ or +/−) in the Pkd1−/− mice.

Reduction of Renal Function in Pkd1−/− Mice with a Genetic Loss in Gpsm1. To assess the level of renal function in the normal and cystic mice, blood urea nitrogen (BUN) (Fig. 4A) and plasma creatinine (Fig. 4B) were measured at P11-12, and BUN was measured at P19 (Fig. S2F). In Fig. 4A, the BUN was not different between Pkd1−/+ mice with or without a functional Gpsm1 gene. BUN was significantly higher (P < 0.001 to P < 0.05) in all of the Pkd1−/− mice regardless of Gpsm1 genotype compared with the Pkd1+/+ mice. However, the BUN levels increased with a complete loss of the Gpsm1 genes, such that BUN was significantly higher (P < 0.001) in the Gpsm1−/−:Pkd1−/− mice (n = 7) compared with Gpsm1+/−:Pkd1−/− (P < 0.01; n = 9) and Gpsm1+/−:Pkd1−/− (P < 0.05; n = 5) mice. Subsequently, plasma creatinine showed a consistent range of 0.10–0.13 mg creatinine/dL (average = 0.11 ± 0.01 mg/dL; n = 8) in noncystic Gpsm1−/−:Pkd1−/− mice (Fig. 4B). Consistent with the BUN results, the trend of the plasma creatinine levels was highest in the mice with a complete loss of the Gpsm1 gene. Plasma creatinine was 0.194 ± 0.022 mg/dL (n = 5) in the Gpsm1−/−:Pkd1+/+ mice, which was 49% (P < 0.01; n = 11) and 24% (n = 5) higher than the levels detected in the Gpsm1+/−:Pkd1+/+ (0.13 ± 0.01 mg/dL) and Gpsm1+/−:Pkd1+/+(0.156 ± 0.01 mg/dL) mice, respectively. The reduced renal function in each of the mouse groups was consistent with the increased KW/BW ratios (Fig. 3B) and the decreased tissue surface area (Fig. 3D).

To assess whether there may have been a prerenal effect, such as volume status and dehydration, in the Pkd1−/− mice, we measured the plasma Na+ and K+ levels in each of the mouse groups at P19 (Fig. S2 D and E). No difference was measured between the noncystic and cystic mice. Cyst fluid analysis similarly did not detect differences in the Na+ and K+ levels nor in osmolality regardless of the Gpsm1 genotype (Fig. S2 F–H).

Electrophysiological Studies Demonstrating Increased PC1/PC2 Channel Activity in the Presence of Gpsm1. The expression of PC1 and PC2 leads to a physical interaction between PC1 and PC2 (7, 8, 30–33) to form a Ca2+-permeable nonselective cation channel (10, 34, 35). Fig. 5 demonstrates that Gpsm1 modulates PC1/PC2 channel activity. PC1/PC2 channel activity was measured.

![Fig. 3](https://example.com/fig3.png)  
**Fig. 3.** Biological analysis of the renal cystic index in various Gpsm1:Pkd1 genotyped mice. Mouse kidneys were harvested between postnatal days 11 and 12 from noncystic Pkd1+/+ and Pkd1−/− and cystic Pkd1−/− mice with various Gpsm1 genotypes (i.e., Gpsm1+/−, Gpsm1−/−, or Gpsm1−/−). (A) Representative whole kidneys were photographed to demonstrate the differences in gross morphology among the various genotyped mice. (B) KW/BW ratios were calculated and graphed as a percentage. Numbers analyzed for each group with a distinct genotype are provided in the graph. *P < 0.05: significant difference between Gpsm1−/−:Pkd1−/− and Gpsm1−/−:Pkd1−/− versus Gpsm1+/−:Pkd1−/−. **P < 0.01: significant difference between the Pkd1−/− and Pkd1−/− mouse kidneys. (C) Kidneys obtained from mice at postnatal days 11–12 as shown in A were sectioned and stained with H&E. Four representative whole-kidney sections were shown from the three different Gpsm1 genotyped mutant strains on the Pkd1−/− background. (Scale bar: 2.98 mm.) Kidney sections were morphometrically analyzed as described in Materials and Methods and graphed in D to determine the surface area of the remaining kidney tissue relative to the tubular (cyst) space. *P < 0.05: significant difference between Gpsm1+/−:Pkd1−/− and Gpsm1−/−:Pkd1−/− versus Gpsm1−/−:Pkd1−/− mouse kidneys.

![Fig. 4](https://example.com/fig4.png)  
**Fig. 4.** Decreased renal function in Pkd1−/− mice with Gpsm1 gene deficiency. Plasma was obtained from mice between P11 and P12 to measure the levels of either BUN (A) or creatinine (B) as a measure of renal function. (A) *P < 0.05 to P < 0.01: significant difference between Gpsm1+/−:Pkd1−/− and the other two Pkd1−/− mouse groups. **P < 0.05 to P < 0.001: higher significant difference between all Pkd1−/− groups and both Pkd1+/− groups. (B) *P < 0.05: significant difference between noncystic Gpsm1+/−:Pkd1−/− mice and the Pkd1−/− groups. **P < 0.01: significant difference between Gpsm1−/−:Pkd1−/− and Gpsm1−/−:Pkd1−/− mice. The numbers for each distinct genotype are shown in the bars of each graph. ND, not determined.
as the La^{3+}-sensitive inward Na^+ current at −100 mV under voltage-clamp conditions. La^{3+} is a nonselective cation channel inhibitor and has been shown to inhibit PC2 channel activity (34, 35). As shown in Fig. 5A, CHO cells did not exhibit endogenous La^{3+}-sensitive currents. Macroscopic currents from representative whole-cell experiments elicited by a family of test pulses stepping from a holding potential of 0 to 100 to −100 mV are shown in the absence and presence of GPSM1 (Fig. 5B). Fig. 5A shows currents before (left) and following (right) treatment with LaCl₃ (1 mM). The current-voltage (I-V) relation for macroscopic PC1/PC2 currents in the presence of GPSM1 is shown in Fig. 5B.

As summarized in Fig. 5C, electrophysiological analysis of CHO cells transfected with rGpsm1- or hPKD1-expressing plasmids alone or in combination produced minimal La^{3+}-sensitive currents, which represent basal activity of the endogenous channels in CHO cells. CHO cells transfected with mPkd2-expressing plasmid generated ion channel activity, but the overexpression of GPSM1 did not significantly alter the magnitude of the activity. Coexpression of hPKD1 and mPkd2 did not significantly alter the ion channel activity compared with the CHO cells expressing mPkd2 alone. However, coexpression of GPSM1 with PC1/PC2 significantly increased (P < 0.001) La^{3+}-sensitive current by 2.5-fold from 23.0 ± 3.3 pA/pF (n = 25) to 58.3 ± 7.7 pA/pF (n = 22). Blockade of Gβγ function with GRK2ct, a selective Gβγ scavenger, prevented the GPSM1-mediated increase in PC1/PC2 activity (21.7 ± 3.0 pA/pF; n = 18) compared with the basal PC1/PC2 channel activity in the presence of GRK2ct (34.1 ± 5.6 pA/pF; n = 17).

In pertussis toxin (PTX)-treated CHO cells transfected with hPKD1/mPkd2 (Fig. 8A), the La^{3+}-sensitive ion channel current density was 14.7 ± 3.3 pA/pF (n = 7). Upon overexpression of GPSM1, the PCI/PC2 channel activity significantly increased (P < 0.05) by 2.4-fold to 33.3 ± 3.4 pA/pF (n = 12). As pertussis toxin pretreatment does not inhibit the GPSM1–Gai interaction (36), these data would further suggest that PCI/PC2 channel activity is regulated by unbound Gβγ dimers liberated by GPSM1.

Discussion
The association between extrinsic cues, including hormones and mechanical forces, with cell-surface GPCRs has long been studied to understand the activation/inactivation states of heterotrimeric G proteins to modulate cell-signaling pathways (5). With the identification of accessory proteins, however, the control of G-protein mediated signaling has become more diverse (16, 37, 38). Accessory proteins are postulated to possess a multitude of intracellular functions, including modulating signal intensity and/or duration, orienting signaling components to specific subcellular locations, and contributing to the formation of functional signaling complexes that regulate the directionality of signaling pathways. One example of such an accessory protein is the group II AGS protein, GPSM1 (16, 37).

At present, there remains a paucity of information regarding the role of accessory proteins, including GPSM1, in the kidney. Our recently published data (23, 24) in combination with the results of our present study are consistent with GPSM1 playing an integral role in modulating mechanisms involved in epithelial cell repair and cyst formation. The morphogenetic changes that cystic epithelial cells follow are similar to those observed during regeneration and repair processes in noncystic renal epithelial cells following an acute insult, such as ischemic injury (39, 40). Following injury, kidneys undergo highly organized and coordinated repair in which epithelial cells undergo partial dedifferentiation, cell migration, and proliferation to reform functional nephrons (41, 42). The role of the polycystins play in this repair process is not fully known, but the expression of Pkd1 and Pkd2 mRNA and proteins is increased following ischemia-reperfusion injury (IRI) (43, 44). Moreover, the genetic loss of either one of the Pkd1 or Pkd2 genes leads to an impairment in the recovery of the epithelial cell and promotes focal cyst formation following renal injury (45–47). It is entirely possible that kidneys with mutations in either ADPKD gene would have areas of localized ischemia and cell damage due to cystic growth. This would initiate a repair process very similar to the process that occurs following ischemia-reperfusion injury (IRI). As the cysts
expand, the repair process continues to be activated and renal epithelial cells with cystic gene mutations remain in a continual state of repair, leading to cyst expansion and abnormal tissue architecture (39, 40). Because we have shown that mice deficient for full-length GPSM1 expression have a markedly reduced capacity to promote renal epithelial cell recovery following IRI compared with wild-type (GPSM1+/+ mice) (24) and that there is an association with GPSM1 and polycystin ion channel activity (see below), we believe that there may be a close association between GPSM1, polycystins, and pathways involved in epithelial cell repair to modulate cystogenesis in animal models of PKD.

At this time, the mechanism of action for GPSM1 in the renal epithelial cells remains elusive, although several modes of action have been proposed. First, GPSM1 is known to function as a GDP-Gi by selectively binding to Gaαi subunits in the GDP state (20, 28, 48). Because Gaαi subunits inhibit adenylyl cyclase activity and elevated levels of cAMP is a classic phenotype observed in cystic renal epithelial cells (5), the relationship between GPSM1, Gaαi, and adenylyl cyclase was initially of particular interest. Numerous clinical trials based on somatostatin receptor agonists, which activate Gaαi subunits, are currently being investigated as an anticystic agent in PKD (5). Because a single molecule of GPSM1 can simultaneously bind up to four Gaαi subunits (49), it is conceivable that GPSM1 could short-circuit the ability of Gaαi subunits to inhibit adenylyl cyclase. Fan et al. (50) demonstrated this phenomenon using cultured neuronal cells by measuring increased activation of cAMP production in the presence of elevated GPSM1 levels. To date, however, our laboratory and others have been unable to show any measurable difference in cAMP production in cultured renal epithelial cells and other nonrenal-derived cells with or without GPSM1 expression (23, 24, 51).

For this reason, we believe that the role of GPSM1 and Gaαi-mediated signaling is substantially more diverse than previously anticipated (36). The GPR motifs in the C-terminal region of GPSM1 function as a GDI for Gaαi/o-mediated signaling is substantially more diverse than previously anticipated (36). The GPR motifs in the C-terminal region of GPSM1 function as a GDI for Gaαi/o complexes (61). This would conduce to dissociate Gaαi/o-GDP complexes and limit the extent of GPCR-mediated signaling (51, 52). Because of the diversity of function by GPSM1, there may be a context-dependent role for this protein in epithelial cell repair. In the absence of other genetic defects, biological injury to the renal epithelial cells would induce the expression of GPSM1 to promote accelerated epithelial cell recovery through Gaαi/o-mediated signal transduction events. In cystic epithelial cells, the increased levels of GPSM1 may act to partially compensate for reduced PC1/PC2 function, such that the cystic disease progression is blunted. Thus, the genetic ablation of GPSM1 leads to increased epithelial cell proliferation and accelerated cystic disease.

In conclusion, our genetic evidence sheds light on the mechanistic importance of accessory proteins and their associated heterotrimeric G proteins to control the dynamics of cystic disease progression through the modulation of polycystin function in renal epithelial cells. These data open the door for the development of drug therapies targeting GPSM1 and its associated downstream pathways to modulate cystic disease progression in PKD.

Materials and Methods

Mouse Strains and Breeding. Details on the animal breeding and genotyping are available in SI Material and Methods (25, 63).

Biological Measurements and Tissue Morphometry. Plasma, cyst fluid, and kidneys were isolated from mice at postnatal days 11-12 and 19, and these biological samples were processed as described in SI Material and Methods.

GPSM1 Immunoblot Analysis. The right kidneys were removed and immediately frozen on dry ice to measure the GPSM1 expression by immunoblot analysis as previously described by our laboratory (23, 24).

Renal Immunohistochemistry. Serial sections (4-μm thick) were stained with tubelet segment-specific markers, Trichrome, or primary antibodies as described in SI Material and Methods.

Electrophysiological Measurements of PC1/PC2 Ion Channels. Reconstituted PC1/PC2 channel activity in the presence or absence of GPSM1 expression was measured in CHO cells as described in SI Material and Methods (34, 35).

Statistical Analysis. Data are expressed as mean ± SEM. All statistical analyses were performed using Prism 5.0 software (SAS Institute Inc.) as described in SI Material and Methods.

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