Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic form of kidney failure worldwide. The disorder is characterized by the formation and enlargement of renal cysts that distort renal architecture, resulting in massively enlarged kidneys and end-stage kidney disease (ESKD). The disorder is also associated with numerous extrarenal complications, including polycystic liver disease and intracranial aneurysms. The most common form of ADPKD is caused by mutations in 2 genes: ~85% of affected individuals have mutations in the PKD1 gene, while the rest have mutations in PKD2. Mutations in these genes result in similar phenotypes, but PKD1-associated disease is typically more severe, with patients reaching ESKD about 20 years earlier than patients with PKD2 mutations. At the cellular level, ADPKD is recessive and the process of cystogenesis is thought to originate when a somatic mutation occurs in the PKD gene inherited from the unaffected parent.

Since PKD1 and PKD2 were identified more than 20 years ago, we have learned much about their protein products. PKD1 encodes a large polypeptide, polycystin 1 (PC1; Fig 1A) of 4,302 amino acids, with 11 transmembrane helices, a short intracellular carboxy-terminal (C-terminal) tail, and a large extracellular fragment that is thought to be involved in cell-cell or cell-matrix interactions. PC1 undergoes regulated cleavage, leaving the extracellular fragment noncovalently tethered to the rest of the protein. The product of the PKD2 gene, polycystin 2 (PC2; Fig 1A), is homologous to the transient receptor potential (TRP) family of cation channels, and like many TRP channels, early studies suggested that PC2 was primarily a voltage-gated ion channel fold. The CTD of PC1 fits with the homologous portions of 3 PC2 molecules to form a voltage-gated ion channel complex that regulates Ca^{2+}-dependent signal transduction pathways. Interestingly, the last 6 transmembrane helices of PC1 have homology to PC2, but whether PC1 forms part of the channel was unknown. Despite years of intense investigation, polycystin’s ligands (molecules that bind this complex, activating it and triggering downstream signaling pathways) have remained elusive. Now, in a report from Su et al, exciting new methods have been applied to the structure of the polycystin complex to gain more robust insights into its physiologic function.

What Does This Important Study Show?
Solving the protein crystal structure for transmembrane proteins has historically been extremely difficult, resulting in few mammalian transporter and channel structures. However, recent advances in cryogenic electron microscopic (cryo-EM) structural visualization methods have led to a rapidly expanding catalogue of mammalian channel structures, including a recent structure for PC2. In this new study, Su et al report a high-resolution cryo-EM structure of the PC1/PC2 complex for the first time. This structure provides unanticipated details of the PC1-PC2 interaction that have the potential to yield critical insights into the function of the polycystin complex.

The first innovation of Su et al was to engineer versions of PC1 and PC2 that allowed the isolation of sufficient quantities of purified protein for structural analysis. This had proved to be a major stumbling block to similar efforts in the past. After screening several alternatives, they found that versions of PC1 and PC2 lacking the flexible amino and carboxy terminal regions were the most successful. When these shortened proteins were expressed together, the authors found that they combined to form a structure consisting of 3 PC2 molecules and 1 PC1 molecule, a 1+3 assembly (Fig 1B). In many respects, the new mixed PC1 and PC2 assembly resembles previously reported structures containing 4 PC2 molecules or other TRP channel complexes, but with the much larger PC1 molecule intercalating with the PC2 molecules. The successful assembly of the PC1/PC2 complex reported here is an unexpected given that previous work had described the carboxy termini of PC1 and PC2 to be required for the interaction of the 2 proteins.

The Su et al structure also confirmed the homology of the final 6 transmembrane helices of PC1 (the carboxy-terminal domain [CTD]) to the corresponding helices of PC2. Both proteins have a large extracellular loop, the “TOP” domain, between the first and second transmembrane helices, along with a voltage-gated ion channel fold. The CTD of PC1 fits with the homologous portions of 3 PC2 molecules to form a structure with a pore and selectivity filter (S5-S6; Fig 1A and B), which is similar to other TRP channels and voltage-gated ion channels. In the previously obtained structures formed entirely from PC2 (see below), the TOP domain proved to be critical in the assembly of the channel complex. Although PC1 also possesses a TOP domain, it differs in key aspects, which is predicted to disrupt the symmetry of the 1+3 assembly. The TOP and CTD of PC1 are inserted at an angle compared with the other subunits, suggesting a potential instability in the complex. Su et al
surmise that this deformation may be what limits the number of PC1 subunits in the complex to 1; any more and the complex might not hold together.

The cryo-EM structure of the PC1-PC2 channel also reveals several unusual features, which suggest that the channel may not be functional. First, the channel pore is disrupted by the inclusion of PC1. Unlike PC2, the S6 (the last transmembrane domain of the CTD) of PC1 possesses a bend that may disrupt not only the pore itself, but also the selectivity of the pore for Ca\(^{2+}\). A second highly unusual aspect of the PC1 S6 is the presence of many positively charged amino acids (the arginines at positions 4,100 and 4,107 and the histidine at 4,111) that face the pore, thereby discouraging the penetration of positively charged ions such as Ca\(^{2+}\) (Fig 1C). In contrast, the same region of PC2 has an accumulation of negatively charged residues that would promote permeation by cations. Theoretically the PC1/PC2 channel would only be permeable to cations if the broken S6 segment of PC1 were to rotate significantly, shifting the positively charged amino acids away from the pore. The authors note that at least one of the positive residues, the arginine at 4,100, is highly conserved in PC1 molecules across vertebrate species, supporting the importance of this unique amino acid arrangement.

Therefore, what is the function of the first 5 PC1 transmembrane domains? Interestingly, these helices, along with the cytoplasmic “PLAT” domain (together termed the amino-terminal domain)\(^{18}\) interface with the pore-forming CTD of PC1 directly through a string of hydrophobic residues on the helix designated “TM1” in Figure 1A. These structural elements raise the possibility that binding of a ligand to the extracellular amino terminus of PC1 could result in deformation of the amino-terminal domain that might in turn influence the conformation or activation of the PC1/PC2 channel. Because the protein

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**Figure 1.** The polycystin 1 (PC1)/PC2 complex. (A) Topology of the polycystin proteins. PC1/poly cystic kidney disease 1 (PKD1) in red with the amino-terminal domain (NTD) and carboxy-terminal domain/voltage gated ion channel fold (CTD/VGIC) labeled. The missing amino- and carboxy-termini of PC1 and PC2 are indicated in pink. (B, C) Visualization of the Su et al\(^{13}\) cryo-EM structure of the PC1/PC2 heteromeric channel complex.\(^{25}\) (B) Top down view of the 1+3 stoichiometry of PC1+PC2 assembly, with the NTD S1-S6 of PC1 interdigitating with the homologous VGIC folds of PC2. (C) Bottom up view of the PC1/PC2 complex pore and activation gate with the acidic negatively charged residues of the PC2 trimer highlighted in orange and the incongruous positively charged residues found in the PC1 S6 highlighted in yellow.
fragments used in the study did not include the large extracellular amino-terminal fragment of PC1, this hypothesis awaits experimental confirmation.

Su et al. went on to map known ADPKD disease missense mutations onto the PC1/PC2 structure. Their efforts suggest an enrichment of mutations in specific functional areas and domains. In PC1, they report that the TOP, amino-terminal, and PLAT domains have many known causal mutations, supporting the importance of each of these domains in assembly, channel activation, and trafficking, respectively. They also noted a dearth of causal mutants in the pore-forming regions of PC1, suggesting that the pathogenic mechanisms of PKD1 mutations may be unrelated to a role for PC1 in ion conductance.

**How Does This Study Compare With Prior Studies?**

During the past several years, advancements in cryo-EM have produced new structures for several ion channels, including PC2 and a PC2 homolog called PKD2L1. Collectively, these studies demonstrated that 4 PC2 proteins can organize as a channel forming a recognizable voltage-gated ion channel pore with a distinct voltage sensor and secondary activation gate. However, overexpression of PC2 in multiple cell types failed to yield a discernible current in electrophysiologic experiments. Because PKD2L1 spontaneously conducts cations, with significant permeability to Ca2+, a 2016 study examined a chimeric molecule that had the pore of PKD2L1, but the selectivity filter of PC2. This channel conducted cations, but with much higher permeability for potassium and sodium ions versus Ca2+. The low Ca2+ permeability of PC2-containing channel complexes has been described by multiple investigators, raising a question about which cation is pertinent in vivo.

The new Su et al. PC1/PC2 structure reveals a 1+3 assembly of PC1 and PC2. This was previously demonstrated biochemically using an elegant fluorescence microscopy–based technique. Interestingly, the key portions of the proteins necessary for complex assembly in the older study were the respective carboxy-terminal tails. The carboxy termini of PC1 and PC2 were missing from the constructs used by Su et al, making it difficult to anticipate how the cytosolic interactions they promote might affect the structure of the PC1/PC2 channel complex. Su et al concluded that the PC1/PC2 complex may not primarily function as an ion channel, based on the position of PC1 S6. Inclusion of these protein elements, which were previously shown to be important for assembly, and to possess a number of missense mutations, could alter the conclusions of Su et al. Additional studies will be necessary to determine whether PC1/PC2 functions as an ion channel under physiologic conditions.

**What Are the Implications for Nephrologists?**

The work of Su et al. represents an important advance in our understanding of the intricacies of the polycystin complex, which could also have significant clinical impact. Mutation testing for PKD1 and PKD2 has been available for some time. Indications for gene testing include to screen young kidney donors from ADPKD families, perform preimplantation genetic diagnosis, and establish an ADPKD diagnosis when the clinical picture is not clear. However, the large number of amino acid variants of unknown significance, particularly in PKD1, has hampered the utility of gene testing. It is sometimes difficult to predict what change, if any, an amino acid substitution will have on the protein’s function. With this new PC1/PC2 structure, we should have a better template on which to map variants, which will allow more accurate prediction of their pathogenicity. Ideally the structure will also result in a functional assay that could be used to confirm predictions about the functionality of DNA variants.

A second potential consequence of this work is to allow for the rational design of therapeutic agents to treat and prevent cyst formation. Although the first vasopressin receptor antagonist was approved this year for the treatment of ADPKD, these agents may target pathways that are several steps along in the pathogenesis, resulting in a more modest therapeutic effect. A mechanistic understanding of the PC1/PC2 channel structure could allow one to screen for small molecules that might open the channel if disease-causing mutations are found to render it inactive. One can also envision molecules or correctors that might be specific for certain classes of disease-causing mutations in the pore region or selectivity filter or regions required for complex assembly. Arguably, malfunction of the channel proper is the event most proximate to ADPKD pathogenesis.

However, there are several open questions that need to be addressed before one can use the Su et al structure and possibly that of the previously published PC2-only structure to screen for therapeutic agents. A more precise definition of what constitutes the active channel complex, whether it is 4 subunits of PC2 or the PC1/PC2 1+3 assembly, is key. Can the latter conduct ions if activated by a ligand and if so, is Ca2+ conductance physiologically relevant? It is certainly possible that this will vary depending on the subcellular location. In summary, establishing the structure of the PC1/PC2 complex is a significant breakthrough that opens a new conversation about the role and functions of the polycystins and the pathogenesis of ADPKD.

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