Primary cilia in the pathogenesis of dentigerous cyst: a new hypothesis based on role of primary cilia in autosomal dominant polycystic kidney disease

U. R. Anoop, MDS,a Kavita Verma, BDS,b and K. Narayanan, MD, DM, DNB,c Pondicherry, India

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Autosomal dominant polycystic kidney disease (ADPKD), an inherited disease, leads to cyst formation in the kidneys. In this condition, the kidneys are grossly enlarged with multiple cysts that result in kidney failure in a majority of individuals. This condition is also associated with cysts in other organs. Recent research has focused on defects in signaling mediated by the primary cilia as the causative factor in ADPKD. Primary cilia are also present in odontogenic epithelium. Dentigerous cyst also is a developmental cyst whose pathogenesis is controversial. Recent studies have shown that loss of Ptch and Shh signaling pathways are involved in the cystogenesis of dentigerous cyst. The Shh signaling pathway is active in the primary cilia. A scanning electron microscopic study of a dentigerous cyst wall in an ADPKD patient showed structures similar to primary cilia. Based on the presentation of a dentigerous cyst in an autosomal dominant polycystic kidney patient and the demonstration of primary cilia like structures on the cyst wall by using a scanning electron microscope, a new hypothesis for the pathogenesis of dentigerous cyst is proposed. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;111:608-617)

Primary cilium is considered to be an important signaling center within the cell. It is present in almost all cells of the human body. Defects in primary cilia are seen in autosomal dominant polycystic kidney disease. Ptch (Patched) gene inactivation and a role for Shh (Sonic Hedgehog) signaling have been associated with the pathogenesis of dentigerous cyst. Because Ptch and Shh activity are seen in the primary cilia, a probable role for primary cilia in dentigerous cyst pathogenesis is discussed.

A case report of an autosomal dominant polycystic kidney disease patient with a dentigerous cyst showing primary cilia like structures under a scanning electron microscope is presented to support our new hypothesis.

PRIMARY CILIUM

The primary cilium acts as a sensory organelle to transfer information from the extracellular environment to the cell interior. Examples of specialized primary cilia include the rod receptor in the eye, the dendritic knob of the olfactory mucosa and the transient nonmotile kinocilium of the inner ear.1 It also acts as a mechanosensor through proteins Ptc1 and Ptc2.2 Receptors such as platelet-derived growth factor on the primary cilia bind to extracellular ligands and activate the Akt and Mek1/2-Erk1/2 pathways that control cell proliferation, migration, and apoptosis.3 It regulates crucial cellular processes such as cell cycle, cytoskeletal organization, and intraflagellar transport (IFT).3 Two important signaling pathways are also associated with primary cilia, namely, Wnt pathway4 and Shh pathway.5

AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

Autosomal dominant polycystic kidney disease (ADPKD) is a dominantly inherited heterogenous systemic disease that occurs in 1:300 to 1:1,000 individuals.6 Because it is an autosomal dominant disease, if 1 parent has the disease gene there is a 50% chance that a child of the parent will inherit the disease. In 10% of the cases, the condition is due to spontaneous mutation. In these cases, the parents do not have a copy of the disease gene.

ADPKD is caused by mutations in the PKD1 or PKD2 gene. PKD1 and PKD2 genes encode proteins polycystine 1 and polycystine 2, respectively. Both proteins have been recently detected on the primary cilia that are present on most of the epithelial cells in the nephron.7 Mutations in the PKD1 gene on chromosome 16 is responsible for 80% of cases. Mutations on PKD2 gene on chromosome 4 are responsible for 50%
of cases. ADPKD patients usually have germ line mutation in only 1 allele of the PKD1 or PKD2 gene. The remaining normal allele is sufficient for all developmental functions, because patients exhibit no developmental anomalies. Renal cysts in ADPKD arise after spontaneous second mutations that affect the normal allele, which results in cells devoid of a functional gene. PKD1-mutated patients are clinically indistinguishable from PKD2-mutated patients, but PKD2 patients have a less severe course of the disease.

The kidneys are grossly enlarged with multiple cysts studding the surface of the kidney. The cysts contain straw-colored fluid that may become hemorrhagic. The presence of multiple epithelial-lined kidney cysts results in gradual kidney enlargement and subsequent kidney failure in the majority of individuals. The disease penetrance is 100%. ADPKD patients also exhibit extrarenal manifestations characterized by cysts in different organs, such as liver, pancreas, and ovary.

CASE HISTORY

A 42-year-old male patient with history of autosomal dominant polycystic kidneys reported at the dental clinic with a swelling in the right lower jaw. He had noticed the swelling a week before and had mild pain for the past week. On inspection there was an ulcer 0.25 × 0.5 cm in the right lower canine region with a swelling in the buccal vestibule in relation to the right lower canine. Tooth 27 was missing. On palpation, there was an expansion of the buccal cortical plate from tooth 25 to tooth 30. Grade II mobility was seen in teeth 25, 26, 28, 29, and 30. As seen in Fig. 1, orthopantomography showed a radiolucent lesion in association with an impacted tooth 27 with scalloped margins. The ultrasound report of the patient was suggestive of polycystic kidney, as seen in Fig. 2. On aspiration, 1 mL blood-tinged fluid was aspirated, as seen in Fig. 3.

Incisional biopsy was done under local anesthesia from the region of tooth 27, and the histopathology, as seen in Fig. 4, was suggestive of a dentigerous cyst. Surgery was done under general anesthesia and, as shown in Figs. 5 and 6, the lesion enucleated. Gross examination of the lesion showed that the lining was attached to the neck of the canine tooth, as seen in Fig. 7. The specimen was then fixed in formalin. Excisional biopsy was suggestive of a dentigerous cyst.

A scanning electron microscopic study of the neck of the tooth and the cyst lining was done. As seen in Fig. 8, matrix-like material was seen along the cervical line of the tooth where the cyst lining was attached. A scanning electron microscopic examination of the cystic lining showed primary cilia like structures on the epithelial cells. As seen in Figs. 9-11, the primary cilia look like long hair-like projections with one end attached to the epithelial cells and the other end free facing the cystic cavity. Figure 12 shows the broad base of the primary cilium, which is attached to the epithelial cell, and a dilated tip at the free end. Figure 13 shows a long primary cilium on the epithelial surface. A smaller primary cilium like structure is also seen at the vicinity.

DISCUSSION

ADPKD, an inherited disease, leads to cyst formation in the kidneys. This condition is also associated with cysts in other organs. Dentigerous cyst is the second most common type of odontogenic cysts. It is the most common developmental cyst of the jaws and is always associated with an impacted tooth. The origin of the cyst is not yet known.

Toller suggested that the likely origin of dentigerous cysts is a breakdown of proliferating follicle cells owing to impeded eruption. Main suggested that pressure exerted by a potentially erupting tooth on an impacted follicle obstructs the venous outflow and thereby induces transudation of serum across capillary walls. Increase in hydrostatic pressure of this pooling fluid results in separation of follicle from the crown with or without decreased enamel epithelium. But all impacted teeth do not develop dentigerous cysts.

Mourshed had calculated the frequency of dentigerous cyst at 1.44 cysts for every 100 unerupted teeth. Toller estimated that 1 in every 150 impacted teeth develop dentigerous cysts. Therefore, there must be a decisive factor other than pressure that causes the cyst formation.

Primary cilia play a crucial role in mediating hedgehog signaling in vertebrates. Shh is a member of the vertebrate hedgehog signaling proteins. Ptch is the re-
ceptor for Shh ligand. In the absence of Shh, Ptch localizes to the cilium and prevents Smo (Smoothened), a transmembrane protein from moving into the cilium. When Shh binds to Ptch, a complex is formed that moves into the cytoplasm. Smo moves into the cilium and processes the transcription factor Gli (glioma-associated oncogene homolog). The transcription of \textit{SHH} target genes is regulated by the nuclear ratio of Gli activators to Gli3 repressors. Gli activators translocate to the nucleus where they activate transcription of \textit{SHH} target genes. In the absence of Shh, the cilium-mediated proteolytic processing of Gli3 generates an N-terminal fragment that functions in the nucleus as a transcriptional repressor. Recently, two cilium proteins, Pc1 and Pc2, also have been found to localize in the primary cilium. Figure 14 shows the Shh pathway in the cilium.

Studies with murine teeth have shown that primary cilia are found in tooth epithelium and mesenchyme cells at early stages of tooth development. Shh signaling is mediated by the primary cilium in odontogenic epithelium. Grafts of early mutant tooth rudiments to kidney capsules resulted in abnormal

Fig. 2. Ultrasound suggestive of polycystic kidney.

Fig. 3. Blood-tinged fluid on aspiration.

Fig. 4. Histopathology suggestive of a dentigerous cyst.

Fig. 5. Cyst at surgery.
development of tooth germs, small-sized teeth or formation of keratinized cysts depending on the stage at which the teeth were isolated.\textsuperscript{18}

Shh stimulation leads to increased ciliary localization of Smo. Mobilization of Smo to cilia is blocked if the protein is truncated even in the presence of Shh, leading to loss of signaling. Cyclopamine also decreased Smo in mouse nodal cilia. These prove that Smo localization to cilia is important for signaling.\textsuperscript{3}

Smo-mutant ameloblasts failed to assume the morphologic features of differentiating cells, and stratum intermedium remained squamous. Smo-mutant ameloblasts failed to grow in size, did not polarize, and had a paucity of organelles.\textsuperscript{19}

Intraflagellar transport proteins are highly conserved in all ciliated eukaryotic cells. Mutation of the proteins that are associated with the IFT process result in defects in cilia formation in all organisms studied to date.\textsuperscript{17} Homozygous Tg737orpk mice exhibit kidney and pancreatic cysts, preaxial polydactyly, and supernumerary teeth.\textsuperscript{17} Tg737orpk mice also showed stunted primary cilia in the renal epithelium with an abrogated calcium response.\textsuperscript{20}

Studies from IFT mutants indicate that IFT is required for Gli activation and for the proteolytic processing of Gli3 into Gli3 repressors but is not required for trafficking of Gli proteins to the nucleus. In IFT mutants with aberrant or absent cilia, the formation of both Gli activators and Gli repressors is impaired, which can lead to Shh loss- or gain-of-function phenotypes in different tissues.\textsuperscript{7} Gli2 mutant mice showed abnormal development of maxillary incisors. Gli3 mutants had no major tooth abnormalities. Gli2/Gli3 double homozygous mutants did not develop any normal teeth and did not survive beyond the embryonic period.\textsuperscript{21} The above discussion shows the importance of cilium-mediated Shh signaling in odontogenic epithelium.

Ptc, which is a member of the Shh pathway, appears to be inactivated in dentigerous cysts. Levanat et al. had stated that Ptc alterations may be a necessary, and perhaps the initiating, event in the formation and growth of various noninflammatory cysts.\textsuperscript{22} Pavelić et al. also suggested that a Ptc inactivation can occur at any age and can lead to a monoclonal proliferation from a progenitor cell from the decreased enamel epithelium.\textsuperscript{14,22}

Loss of heterozygosity (LOH) for the \textit{PTCH} gene has been identified in dentigerous cysts. It is also seen in basal cell carcinomas and keratocysts.\textsuperscript{23} But the clinical presentation in all these lesions is different. Ptc is a member of the Shh pathway. Shh signaling has been found to be tissue and gradient specific.\textsuperscript{24} Therefore, defects in the signaling pathway may produce different effects in the skin and odontogenic tissue. Also, Shh-induced lesions appear to lack the aggressiveness of other malignancies characterized by multiple genetic alterations.\textsuperscript{25} Levanat et al. also stated that the local \textit{PTCH} inactivation can, under favorable circumstances, lead to persistent though not by itself truly aggressive cell proliferation.\textsuperscript{22} Therefore, we can surmise that \textit{PTCH} inactivation may lead to low-grade cell proliferation, and further clinical manifestations such as cystic change or aggressiveness may depend on the accompanying genetic alterations and the type of tissue involved.

The Shh receptor Ptc1, its activator Smo, and the transcriptional effector Gli1 are all found to localize in primary cilia.\textsuperscript{17} Primary cilia are found in odontogenic epithelium. Odontoblasts in vitro were found to express ciliary components such as tubulin, inversine, rootletin, Odf1, Bbs4, Bbs6, Alms1, Ki63a, Pc1, and Pc2. Calcium channels were also concentrated in the vicinity of the basal body.\textsuperscript{26} Mutations in Pc1 and Pc2 are seen in
ADPKD. The present ADPKD patient presented with a dentigerous cyst. Primary cilia were detected on the lining epithelial cells of the cyst under a scanning electron microscope. Therefore, a similar pattern of cystogenesis as in renal cysts can be hypothesized for the dentigerous cyst in this case.

As seen in Fig. 15, in patients prone to cyst formation, the decreased enamel epithelium must be harboring a mutated gene. This could be the PTCH gene. But because it is not specific for the dentigerous cyst, there must be some accompanying mutations that characterize this manifestation. The involvement of the Shh pathway and the occurrence of the dentigerous cyst in a patient prone to Pc1 and Pc2 mutations suggest that accompanying alterations of proteins in the primary cilium can be expected. The normal alleles
maintain the cell as long as the normal genes are functional. A second hit in the cell results in loss of heterozygosity. This leads to cell proliferation from the progenitor cell. This is supported by the clonality of the cysts and the loss of heterozygosity in the lining cells.

The cysts are characteristically associated with an impacted tooth. In normal individuals, the pressure from the tooth may be sensed by the primary cilium in reduced enamel epithelium, leading to an influx of calcium, which decreases cyclic adenosine monophosphate (cAMP) in the cells and maintains the cells in quiescence. An increase in cAMP leads to proliferation in cells prone to cyst formation. Therefore, abnormal calcium response in cells prone to cystogenesis can play a role in formation of dentigerous cyst. This is
supported by the fact that loss of function of Polaris has been found to affect IFT in both renal and odontogenic cells. IFT mutants have a stunted primary cilium and an abnormal calcium response in renal cells. Further dentigerous cysts have also been reported in patients taking cyclosporine and calcium channel blockers. The drugs also interact with the calcium signaling pathway. Calcium signaling is also associated with cell proliferation. In mutated individuals, an abnormal calcium response due to a defective primary cilium when induced by drugs also may lead to increased cAMP in the cells, resulting in cellular proliferation.

The primary epithelial band forms by a programmed change in the orientation of the mitotic spindle and plane of cleavage of dividing epithelial cells. The Shh pathway plays a role in tooth initiation. There is a
Fig. 14. The Shh pathway in the primary cilium.

Fig. 15. Cyst formation due to mutation in reduced enamel epithelium resulting in monoclonal proliferation with abnormal basement formation.
possibility that an alteration in Shh signaling owing to defective primary cilia can also induce a change in orientation of the spindles in the decreased enamel epithelium, leading to abnormal proliferation of cells.

The mutated cells may also secrete an abnormal extracellular matrix that may form a basement membrane as in kidney cysts. The basement membrane may attach to the cementoenamel junction as well as to the surface of enamel. Therefore, the cyst can enclose the tooth as well as line the surface of the enamel. Electron microscopic study of the tooth associated with the dentigerous cyst in the patient also showed the presence of matrix-like material at the cementoenamel junction.

Toller and Main had discussed the issue of reduced enamel epithelium being attached to the enamel in some dentigerous cysts.\textsuperscript{12,14,33} The reduced enamel epithelial cells seen by Toller attached to the enamel may be mononclonal cells derived from a mutated progenitor cell of reduced enamel epithelium lining the enamel surface of the cystic cavity. The lining cells on the enamel surface may also be lost as the cyst expands, probably because the cells are pressed against a hard surface such as enamel or because of a weak adhesion between the lining cells and the surface of enamel, giving the appearance of the tooth communicating with the cyst cavity directly.

Toller, Main, and Browne have found that the osmolality of cystic fluid in noninfected dentigerous cyst was almost similar to serum.\textsuperscript{12,14,34,35} Therefore, we propose that a transcellular or paracellular movement of fluid from the surrounding vasculature may result in formation of the cystic fluid similar to that seen in kidney cysts. The monoclonal proliferation of cells from the mutated progenitor cell may line the formed cystic cavity.

CONCLUSIONS
We premise the following observations on the basis of our novel hypothesis of the role of primary cilia in the pathogenesis of the dentigerous cyst:

1. Extrarenal manifestations are common in autosomal dominant kidney disease. The dentigerous cyst, which is also developmental in origin, may be an odontogenic manifestation of the condition.

2. Primary cilium may play a role in pathogenesis of dentigerous cysts.

3. Ptc1 inactivation has been reported in other developmental cysts as well.\textsuperscript{22,36} Abnormal Shh signaling is implicated in malignancies also. Further studies downstream of the pathway may help us to identify the defects specific for the cysts.

4. If primary cilium and ciliary proteins are involved in the development of cysts, then drugs can be used to reduce the size of big cysts or to prevent expansion of cysts, as in kidney cysts.\textsuperscript{37} This can prevent major resections when the cysts involve inaccessible areas of the maxilla and mandible.

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Reprint requests:
Dr. U. R. Anoop
K. K. Uthaman Dental Care and Implant Centre
Pondicherry Medical Mission
1, Sabthagiri Gardens
Solai Nagar Main Road
Muthialpet, Pondicherry
Pondicherry 605003
India
anoopkuthaman@rediffmail.com