



Lessons learned from the intersection of two frequent monogenic disorders: the Marfan syndrome and autosomal dominant polycystic kidney disease

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Abstract

Aortic aneurysms, which lead to aortic dissections and ruptures if left untreated, are among the most life threatening forms of cardiovascular disease. Thoracic aortic aneurysm is a prominent clinical feature of several hereditary connective tissue disorders, including Marfan syndrome (MFS). MFS is caused by mutations in *FBNI*, which encodes fibrillin-1, an important extracellular matrix protein. Through the study of MFS mouse models and diseases related to MFS, it became clear that dysregulated TGF- β signaling contributes significantly to the pathogenesis of thoracic aortic aneurysms.

Thoracic aortic and other aneurysms do also occur in autosomal dominant polycystic kidney disease (ADPKD). Mutations in *PKD1* or *PKD2* are responsible for ADPKD. The function of the polycystins, the proteins encoded by these two genes, is not clear yet, but an upregulation of TGF- β signaling has also been suggested as a pathogenetic mechanism. Although the main manifestation of ADPKD consists of renal cysts, a clear cardiovascular involvement with aneurysm formation has been demonstrated. *Vice versa*, kidney cysts have been observed in MFS. This clinical overlap suggests a mechanistic link between ADPKD and MFS. This link provides interesting opportunities for investigations on the pathogenic mechanisms of both diseases, more in particular the mechanisms leading to formation of thoracic aortic aneurysms.

Keywords: Marfan Syndrome, autosomal dominant polycystic kidney disease, TGF- β signaling, *FBNI*, *PKD1*, *PKD2*

INTRODUCTION

Cardiovascular disease is the most prominent cause of death in Western society (1). One of the most life threatening forms consists of aortic aneurysms as these lead to aortic dissections and ruptures if left untreated (2). Thoracic aortic aneurysm is considered as a characteristic clinical feature of several hereditary connective tissue disorders, including Marfan syndrome (MFS; MIM# 154700), Ehlers-Danlos syndrome (EDS; MIM# 130000) and Loeys-Dietz syndrome (LDS; MIM# 609192) (1).

On the other hand, autosomal dominant polycystic kidney disease (ADPKD; MIM# 173900, MIM# 613095) is one of the most frequent human hereditary disorders, with a prevalence of circa 1/500-1/1000 (3). The disease mainly manifests in the kidneys (cyst formation) but is also characterized by various extra renal symptoms including cysts in the liver and spleen and a cardiovascular involvement (4). Indeed, a study on 62 deceased ADPKD patients has shown cardiovascular defects in 27% of the autopsies (5). Cardiovascular defects comprise mitral valve prolapse (up to 25% compared to 10% in healthy controls (p-value = 0,005) (6)), congestive heart failure, myocardium hypertrophy, aneurysms of the coronary or cervical cephalic arteries, cerebral aneurysms (up to 10%) and dissections of the thoracic (p-value = 0,034 (7)) and abdominal aorta. Importantly, thoracic aortic dissections are seven times more frequent in ADPKD

patients than in the general population, while in 1-10% of all ADPKD patients aortic aneurysms are found (8-11). This demonstrates a clear cardiovascular involvement in ADPKD patients. *Vice versa*, in 50-60% of the MFS patients, cysts are observed in the kidneys (12). This clinical overlap suggests a pathogenetic link between ADPKD and MFS.

CLINICAL AND GENETIC FEATURES OF MARFAN SYNDROME

Marfan syndrome (MFS) is a multisystemic connective tissue disorder affecting the skeletal, ocular and cardiovascular system. It exhibits an autosomal dominant inheritance pattern (2) and has an estimated prevalence of 1 in 5000 individuals (13, 14). The major skeletal features of MFS include skeletal overgrowth leading to disproportionate body dimensions, scoliosis, pectus deformities and long digits (arachnodactyly) (15). As a consequence of the skeletal alterations, pulmonary manifestations may arise. About 60% of the MFS patients are affected by lens dislocation which can result in retinal detachment and glaucoma. The most life threatening complication in MFS patients is progressive dilatation of the aorta leading to aneurysm formation and eventually aortic dissection and rupture. Apart from aortic dilatation, approximately 80% of the MFS patients suffer from mitral valve prolapse (16).

MFS is a pleiotropic disease, making an accurate diagnosis sometimes

difficult. To facilitate the diagnosis of MFS patients, the Berlin nosology was formulated in 1986 (17). Because weaknesses of these criteria had emerged, they were revised and edited in 1996, giving rise to the Ghent nosology (15). The latter set of criteria are mainly based on major and minor clinical findings in the different affected tissues including the cardiovascular, skeletal, ocular, and pulmonary system in addition to dura and skin. Major manifestations comprised ectopia lentis, aortic root dilatation or dural ectasia. In 2010, the Ghent criteria have been revised, leading to straightforward diagnostic rules and putting more emphasis on the cardiovascular manifestations and molecular analysis (18). Four possible combinations can lead to a diagnosis of MFS in a proband: aortic root dilatation (Z -score >2) and ectopia lentis, aortic root dilation with an *FBNI* mutation, aortic root dilation with sufficient systemic findings (score of 7 or more on the systemic scale; for details see (18)) or finally ectopia lentis with an *FBNI* mutation that has been associated with aortic root dilation.

Since *FBNI* was identified as the MFS causing gene in 1991, more than 1000 different mutations in *FBNI* have been described (19). *FBNI* encodes the fibrillin-1 protein, an important component of the extracellular matrix (ECM). The protein contains various repetitive domains including cb EGF-like (calcium binding epidermal growth factor) domains, non-cb EGF-like domains, hybrid motifs and LTBP-like (latent transforming growth factor beta binding protein) domains characterized by an 8-cysteine domain.

The fourth LTBP-like domain contains an RGD motif (arginine-glycine-aspartic acid) responsible for binding of fibrillin-1 to integrin (Fig. 1A) (20, 21). Fibrillin-1 also interacts with many other ECM proteins such as elastin, fibulins, LTBPs and microfibril associated proteoglycans (22-24).

CLINICAL AND GENETIC FEATURES OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD)

ADPKD has an estimated prevalence of 1 in 500 to 1000 individuals, making it the most frequent hereditary renal disorder (25, 26). It is characterized by cyst formation in the kidneys and various extrarenal symptoms including cerebral, thoracic and abdominal aortic aneurysms (3, 27, 28). Cysts are not necessarily harmful and actually quite common in the general population, particularly in older people. Study of the natural history revealed that almost 12% of the population has at least one renal cyst (29). Also within the general population, renal cysts are more common in men than in women and their number and size increase with age (30). In ADPKD patients, 27% of autopsies show cardiovascular defects, including mitral valve prolapse (up to 25%), congestive heart failure, myocardium hypertrophy, aneurysms of the coronary or cervical cephalic arteries, cerebral aneurysms (up to 10%) and dissections of the thoracic and abdominal aorta (5).

ADPKD is mainly caused by *PKD1*

(85-90%) or *PKD2* (10-15%) mutations (28, 31-33). A small fraction (~ 1%) of ADPKD families cannot be linked to one of the known loci, suggesting the existence of a third disease causing gene (34-36).

PKD1 encodes the polycystin-1 (PC1) protein, an integral membrane glycoprotein (37-39). PC1 has the structure of a receptor or an adhesion molecule (see Fig. 1B) and mediates cell-cell and cell ECM interactions. It contains 16 immunoglobulin-like domains (also called PKD repeats), a receptor for egg-jelly domain and a G-protein linked receptor proteolytic site (GPS). It also has a long extracellular N-terminal region, 11

transmembrane domains, and a short intracellular C-terminal region (3, 38). This cytoplasmic tail comprises a coiled-coil domain and a G-protein domain, which plays an important role in signal transduction. PC1 is expressed in the primary cilia, cytoplasmic vesicles, the plasma membrane near focal adhesions, desmosome adherens junctions and possibly the endoplasmic reticulum (ER) and nuclei (3). It has been suggested that PC1 may regulate the mechanical adhesion strength between cells to control the formation of stable actin associated adherens junctions (40).

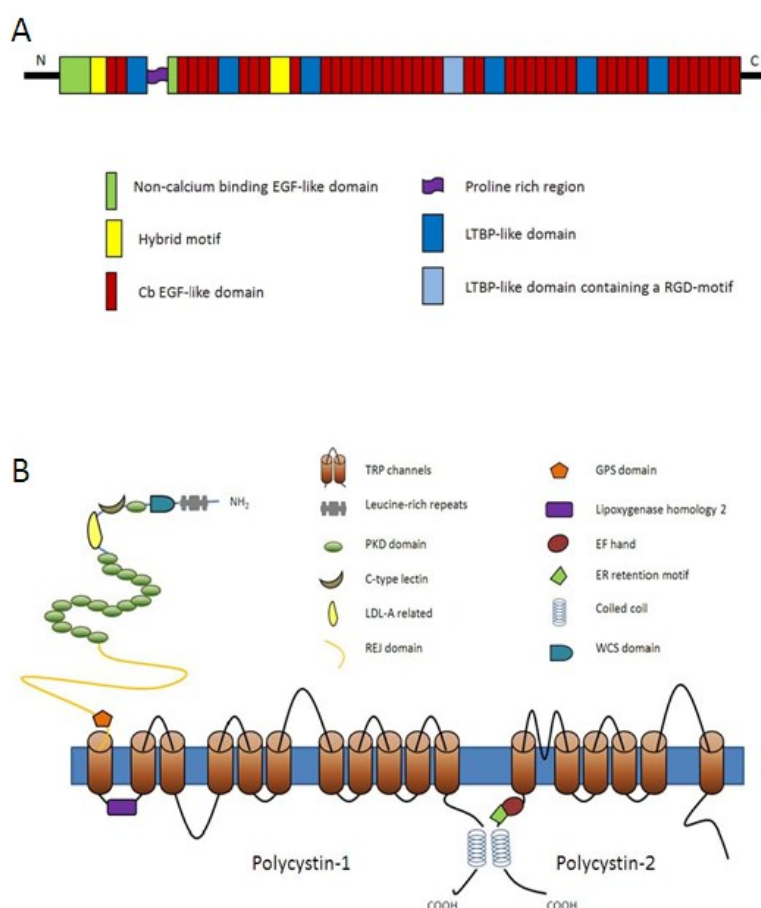


Figure 1. Fig. 1: (A) Structure of fibrillin-1, showing the different domain structures. (B) Structure of PC1 (left) and PC2 (right) and their interactions via coiled-coil domains in the C-terminus. Figures are not drawn to scale.

PKD2 encodes the polycystin-2 (PC2) protein, an integral membrane glycoprotein as well (3, 41, 42). PC2 consists of a short N-terminal cytoplasmic region with a ciliary targeting motif, six transmembrane domains and a short C-terminal tail (43). The latter has a calcium (Ca^{2+}) binding motif (EF-hand), an ER retention motif and a coiled-coil domain, responsible for various protein interactions (44, 45). PC2 is a non-selective cation channel transporting Ca^{2+} and is mainly localized in the ER, but it is also found in the primary cilia and plasma membrane of the renal tubuli (46, 47).

Both PC1 and PC2 are members of the TRPP family (Transient Receptor Potential Polycystic), a subfamily of the transient receptor potential (TRP) channels. Both proteins interact with each other via their C-termini, resulting in a complex structure formed in the primary cilium (48, 49). The interaction between PC1 and PC2 depends on the integrity of the coiled-coil domain in the C-terminus of PC1. Therefore, a first hypothesis suggests that PC1 may be functioning as a receptor controlling the cation channel activity of PC2 (42). A second hypothesis states that the polycystin complex may function as a mechanoreceptor sensing the fluid-flow in the lumen of the tubuli. This may trigger Ca^{2+} influx through the PC2 channel, hereby regulating the intracellular Ca^{2+} and cyclic AMP amounts (50). In this way, the PC1/PC2 complex can respond to flow-induced mechanosensory stimuli. Moreover, it has been shown that cultured kidney epithelial cells with mutations in *Pkd1*, do not activate flow-dependent Ca^{2+}

signaling (50). A third possibility is that PC1 and PC2 rather have a ciliary function. Both PC1 and PC2 are localized to the primary cilia of renal epithelia and it is known that cilia act as sensory organelles. Support for this hypothesis resulted from a study in which *Kif3a* deficiency, a protein of the kinesin-2 motor complex required for cilia homeostasis, resulted in cyst formation (51).

CLINICAL OVERLAP BETWEEN MFS AND ADPKD

As mentioned before, cardiovascular abnormalities are fairly common in ADPKD patients. A familial clustering of cerebral aneurysms was observed in ADPKD patients (52). Several case studies have shown an association between aortic aneurysm/dissection and ADPKD (Table 1). In 1 to 10% of ADPKD patients, aortic aneurysms are found (9-11). Moreover, dissection of the thoracic aorta is seven times more frequent in ADPKD patients (8). From the overview in Table 1, it is clear that there is no obvious association between the occurrence of aortic dissection and the gender, age, the degree of kidney dysfunction, the presence of hypertension or linkage to PKD1 or PKD2. Both type A and B dissections have been described in the presence or absence of cystic media degeneration. These data suggest that genetic modifiers confer the association of aortic aneurysms/dissection in ADPKD. On the other hand, MFS patients have more renal cysts and at an earlier age compared to healthy controls as demonstrated in a study performed on 69

Case report	Gender	Age at dissection	Hypertension	ESRD	PKD1 or 2	Aortic histology	Type of dissection (Stanford)
Biagini (95)	Female	63	No	No			
	Female	45	Mild arterial hypertension	No			
Somlo (53)	Female	60		No			
	Male	38		Yes			
	Male	31		No		Cystic medial necrosis	A
Hartman (96)	Male	72		Yes			
Paynter (97)	Female	36	Yes (200/120)	Yes		Normal	B
Osawa (98)				Yes			
Adeola (99)	Male	42	Yes				B
Lee (100)	Male	58	Yes			Cystic medial myxoid degeneration	A
						No cystic medial necrosis	
Peczowska (101)	Male	54	Yes	Creatinin 226	PKD1		A
	Male	47	Yes	Normal creatinin	PKD1		A
Keuleers (102)	Female	54	No	No	No genetic confirmation	Segmental arterial mediolysis	A
Minami (103)	Male	55		Yes			A
Ramineni (104)	Female	44	Yes				B
Gignon (105)	Male	43					A
Fukunaga (106)	Male	44	Yes			Dissection of media	B

Table 1. Case reports of thoracic aortic aneurysms and dissections in combination with ADPKD

MFS patients; in 59,4% of the MFS patients, renal cysts were present compared to 30,4% of the controls (12). Although renal cysts are seldom clinically significant in MFS, these findings suggest a molecular link between the vascular findings in MFS and cyst formation. This was further supported by the description of pedigrees in which ADPKD and connective tissue disorders (skeletal overgrowth and aortic aneurysms) appear to cosegregate. In one of these families linkage with *PKD1* was

found, while linkage with *FBNI* was excluded (53). In another family, independent segregation of the kidney phenotype with *PKD1* and of the aneurysm phenotype with *FBNI* occurred (54).

Interestingly, also in the Loeys-Dietz syndrome (LDS) there are indications of a clinical overlap with ADPKD. LDS is another thoracic aortic aneurysm syndrome, characterized by hypertelorism, a cleft palate and the development of aneurysms. It is caused by

mutations in the *TGFBR1* or 2 gene, encoding the TGF- β 1 or 2 receptors (55). Unlike MFS patients, LDS patients do not present with lens dislocations and the skeletal overgrowth is less explicit. As cysts were also found in the kidneys of LDS patients (H. Dietz, personal communication), this provides another indication of an important role for the ECM in the development of renal cysts.

LESSONS LEARNED FROM MFS AND PKD MOUSE MODELS

New insights and confirmation of hypotheses concerning the molecular and cellular mechanisms underlying the MFS

and ADPKD pathology have been gained through the generation and study of mutant mouse lines (Table 2).

MOUSE MODELS OF MFS

One of the first MFS models was the so called *mg Δ* mouse (56). Because heterozygous *mg Δ* animals did not express a MFS-like phenotype and homozygous mice deceased early in life, the *mgR* mouse was generated. This hypomorphic mouse, expressing only 15% of normal *Fbn1* levels, survived significantly longer than the *mg Δ* mice and showed a MFS-like aortic phenotype with medial calcification, intimal hyperplasia and inflammatory

Mouse	Genetic engineering	Heterozygous mice	Homozygous mice	Conclusion	Reference
Marfan					
<i>mgΔ</i>	Deletion of <i>Fbn1</i> exons 19 to 24	Normal phenotype	Perinatal death because of vascular complications	Difficult to study because of prenatal lethality	57
<i>mgR</i>	Hypomorphic <i>Fbn1</i> allele (expresses 15% of normal levels)	Normal phenotype	Survive longer as <i>mgΔ</i> mice Medial calcification Intimal hyperplasia Inflammatory responses	Threshold hypothesis <i>Fbn1</i> functions in homeostasis of elastic tissues	58
<i>Fbn1</i> ^{C1039G/+}	<i>Fbn1</i> c.1039C>G	From 2 months of age: Elastic fiber fragmentation Thickening of aortic wall No intimal hyperplasia No Inflammation No aortic dissection	Not applicable	Loss of function contributes to disease mechanism Currently preferred MFS animal model	61
<i>Tsk</i>	Naturally occurring duplication in <i>Fbn1</i> resulting in a larger protein	Thickened skin Bone overgrowth Lung emphysema No vascular abnormalities	Not applicable	Threshold hypothesis Naturally occurring SSS animal model	60

ADPKD					
<i>Pkd1^{del34}</i>	Deletion of <i>Pkd1</i> exon 34	Normal phenotype Few kidney cysts at older age	Perinatal death because of: Enlarged kidneys Pancreatic cysts Pulmonary hypoplasia No vascular abnormalities	Difficult to study because of lethality	66, 67
<i>Pkd1^L</i>	Premature stop codon in <i>Pkd1</i> exon 43	Normal phenotype	Embryonic lethal with: Edema Focal vascular leaks Hemorrhage	PC1 functions in tissue integrity	68
<i>Pkd1^{del17-21βgeo}</i>	Deletion of <i>Pkd1</i> exons 17 to 21	Renal cysts Occasionally liver cysts	Embryonic lethal with: Disorganized myocardium Abnormal atrio-ventricular septation	First ADPKD animal model	69
<i>Pkd1⁻</i>	Deletion of <i>Pkd1</i> exons 2 to 6	Subtle endothelial dysfunction Defective NOx production	Embryonic lethal with: Hemorrhage Progressive renal cystogenesis	Difficult to study	70
<i>Pkd1^{nl}</i>	Hypomorphic <i>Pkd1</i> allele (expresses 20% of normal levels)	Not applicable	Viable Renal, liver, pancreatic cysts Cardiovascular abnormalities	Good ADPKD animal model	71
<i>Pkd2^{WS25}</i>	Disrupted exon 1 in tandem with WT exon 1	Renal cysts	Renal cysts (more severe)	Loss of capacity to express PC2 leads to cyst formation	72
<i>Pkd2⁻</i>	Deletion of <i>Pkd2</i> exon 1	Renal cysts Early death	Embryonic lethal with: Structural cardiac defects Renal and pancreatic cysts	Haplo-insufficiency is mechanism	73

Table 2: Existing mouse models for MFS and ADPKD

responses (fibroproliferation and elastolysis) (57). Because heterozygous mgR mutant animals expressed a normal phenotype throughout life, a threshold hypothesis was suggested in which the relative amount of functionally normal fibrillin-1 is decisive for MFS severity.

Detailed study of the elastic vessels from both mgR mice and MFS patients revealed loss of cell attachments at the surface of elastic laminae, causing morphological changes in neighboring cells (58). This suggested an important role for fibrillin-1 in the homeostasis of elastic tissues.

Further support for the threshold hypothesis came from the *Tight skin (Tsk)* mouse, characterized by thickened skin, bone overgrowth and lung emphysema (59). Heterozygous *Tsk* mice express a decreased amount of functional microfibrils, hereby exceeding the threshold levels for bone overgrowth and lung emphysema, but not for vascular abnormalities. Because *mgΔ* and *mgR* mice both rely on homozygosity of the mutant allele for expression of a phenotype, a heterozygous MFS mouse model was generated: the *Fbn1*^{C1039G/+} mouse (60). These mice had a normal life span, while elastic fiber fragmentation and thickening of the aortic wall was significant starting at 2 months of age. Intimal hyperplasia and aortic wall inflammation were not observed. Unfortunately, also aortic dissections did not occur. As transgenic addition of the wild type fibrillin-1 allele to the *Fbn1*^{C1039G/+} mice resulted in rescue of the MFS phenotype, loss-of-function was confirmed as the disease mechanism in MFS pathogenesis.

INVOLVEMENT OF DYSREGULATED TGF- β SIGNALING IN MFS PATHOGENESIS

For a long time it was assumed that MFS was caused by pure structural deficiency of the microfibrils. This provided a plausible explanation for some manifestations of MFS, such as lens dislocation and aortic aneurysm, but others, including skeletal overgrowth, could not be explained by loss of structural

tissue integrity. Studies on the *Fbn1*^{C1039G/+} mouse model demonstrated that increased TGF- β signaling played an important role in the aorta pathology (61, 62) and that fibrillin-1 is thus not only a structural component of the ECM but also a key regulator of TGF- β activation (21, 63) (see Fig. 2). The key experiment involved rescue of the mutant phenotype in *Fbn1* deficient mice by the administration of TGF- β -neutralizing antibodies (62). This mutant phenotype included impaired distal airspace septation in the respiratory tract (62), and elastic fiber fragmentation, aneurysms (64) and mitral valve prolapse (61) in the cardiovascular system.

MOUSE MODELS OF ADPKD

Pkd1^{del34} was the first *Pkd1* mouse (65). Heterozygous *Pkd1*^{del34/+} mice have a normal phenotype although a few kidney cysts developed at older age (66), while homozygous animals died early in the perinatal period. No vascular abnormalities were observed. In a second *Pkd1* mouse model, homozygous mice (*Pkd1*^{L/L}) died *in utero* between E14.5 and E15.5 (67). The animals exhibited edema, focal vascular leaks and hemorrhage, indicating that PC1 has an important role in maintenance of vascular tissue integrity. Homozygosity for a third mutant *Pkd1* allele (*Pkd1*^{del17-21 β geo}) also turned out to be embryonic lethal, but both homozygous and heterozygous mutant mice developed renal cysts (68). Homozygous mutant mice of the fourth *Pkd1* mouse model (*Pkd1*^{-/-}) died from E14.5 onwards (69). Surviving *Pkd1*^{-/-} mice developed renal cysts starting from E15.5,

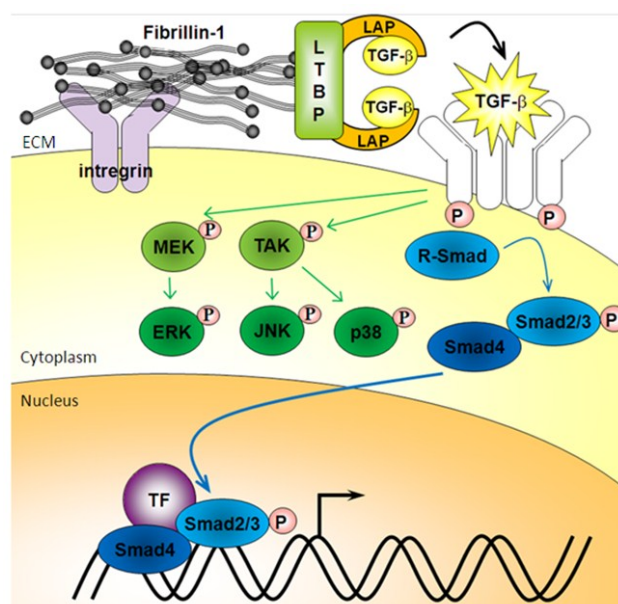


Figure 2: Overview of the TGF- β pathways involved in the pathogenesis of MFS. TGF- β is the prototype of a family of secreted polypeptide growth factors essential in development, cell growth, differentiation, migration, apoptosis and ECM production (74, 75). TGF- β is secreted as part of a latent complex, consisting of TGF- β , LAP- β (latency associated protein) and LTBP (latent TGF- β binding protein), and binds to the ECM. Once activated, TGF- β binds to the cell surface receptors, T β RI and T β RII (76). Smads (combination of the *C. elegans* Sma protein and the *Drosophila* Mad (Mothers against decapentaplegic) protein) are transcription factors shuttling between the cytoplasm and nucleus which regulate the intracellular responses with regard to TGF- β (77-80). TGF- β can activate two pathways: the canonical (indicated in blue) and the non-canonical (indicated in green). In the canonical signaling pathway, Smad2 and/or Smad3 are phosphorylated by T β RI/II, followed by binding of Smad4. The Smad2/3-Smad4 complex translocates to the nucleus where transcription of Smad dependent genes occurs (81). The non-canonical (alternative) TGF- β pathways include the RhoA and MAPK cascades (ERK, JNK and p38) (82-84).

which increased in number and size up to E18.5. Next, a hypomorphic *Pkd1* mutant model (*Pkd1^{nl}*), expressing only 20% of normal PC1, was generated (70). In contrast to the previous *Pkd1* mutant mouse models, *Pkd1^{nl}* mice were viable and presented renal, liver and pancreatic cysts. They also showed cardiovascular abnormalities, in line with the human ADPKD phenotype. These findings demonstrate that a decreased *Pkd1* expression level is sufficient to cause polycystic kidneys and vascular abnormalities.

In the first *Pkd2* mouse model

(*Pkd2^{WS25}*), about half of the homozygous and heterozygous mutant animals developed renal cysts, with cyst formation being more severe in the homozygous mice. In a minority, liver cysts were observed as well (71). PC2 immunohistochemistry on kidneys of *Pkd2^{+/WS25}* mice with a non-cystic phenotype was comparable to that of WT mice. In contrast, in kidneys of *Pkd2^{+/WS25}* mice with a cystic phenotype, a complete absence of PC2 immunoreactivity was observed in the renal cysts and in cells lining the cysts, while the surrounding noncystic regions did show PC2

expression. This suggested that tubular epithelial cells which lose the complete capacity to express PC2 may give rise to cysts. The second *Pkd2* mouse model carried a true null mutation (*Pkd2*^{-/-}) (72). Homozygous mice died at E13.5. Kidney cysts development in heterozygous mice (*Pkd2*^{+/-}) resulted in renal failure and early death. Heterozygous mice without kidney cysts had an intermediate survival. These findings suggested that haploinsufficiency was the disease causing mechanism. Further evidence for haploinsufficiency as the disease causing mechanism came from the evaluation of two rat models expressing truncated PC2 (73).

OPPORTUNITIES FOR THE STUDY OF ANEURYSM PATHOGENESIS FROM THE INTERSECTION OF MFS AND ADPKD

ROLE OF TGF- β IN MFS AND ADPKD

As described above, dysregulated TGF- β signaling is an important contributor to MFS pathogenesis. Likewise, experiments on ADPKD epithelial cells show an increased TGF- β activation (85), while also in *Pkd1*^{nl} mice an important role for TGF- β signaling was demonstrated in the more advanced stages of disease, including cyst progression and fibrogenesis (86). This suggests a clear contribution of dysregulated TGF- β signaling to ADPKD progression. For the time being, it is not clear exactly how the polycystins fit within this pathogenic mechanism, although it is conceivable that the polycystins play a role in the ECM.

The latter hypothesis is built on several arguments. First, polycystin 1 has several extracellular motifs that may function in possible cell-cell and cell-matrix interactions (87). Second, an altered expression of matrix proteins, such as collagen and fibronectin, occurs in polycystic kidneys (88, 89). Finally, in *Pkd1* and *Pkd2* deficient zebrafish, a persisting expression of multiple collagen mRNAs transcripts and low levels of collagen-crosslinking inhibitors were found, implicating an involvement of the polycystins in the modulation of collagen expression (90).

ROLE OF PC1/PC2 IN VASCULAR REMODELING

Polycystin 1 and 2 are expressed in the smooth muscle cells of the arterial wall and in the dense plaques, where they anchor the intracellular contractile filaments to the extracellular elastic lamellae (91, 92). Based on the hypomorphic *Pkd1*^{nl} mouse model, Hassane et al. examined vascular remodeling in ADPKD (93). These studies led to the formulation of a model for aneurysm formation in which matrix components accumulate between the elastic lamellae, followed by an increase of smooth muscle cells leading to weakening of the vessel wall. Subsequently, endothelial cells detached from the elastic lamellae in the intima. This leads, in combination with the weakened aortic media, to a rupture in the intima, giving rise to intramural bleeding. Therefore, polycystins may play a role in the smooth

muscle cell-adhesion complex and the maintenance of the structural integrity of the vasculature, and thus seem to function in vascular homeostasis rather than in vascular development (93).

Pkd2^{+/-} mice were used to examine intracellular Ca^{2+} homeostasis (94). Hypertension was surgically induced in WT and *Pkd2*^{+/-} mice. Since the majority of *Pkd2*^{+/-} mice developed cerebral arterial lesions compared to only one WT animal, loss of PC2 was associated with an increased risk of vascular complications. Furthermore, *Pkd2*^{+/-} mice had decreased sarcoplasmic reticulum Ca^{2+} storage caused by reduced store-operate calcium activity. These data support the hypothesis that the PC1/PC2 complex might play a role in flow-dependent Ca^{2+} regulation. Most probably, abnormal intracellular Ca^{2+} regulation is contributing to the vascular phenotype observed in ADPKD. This may warrant experiments to deduce the

intracellular Ca^{2+} levels in MFS mouse models as well.

In conclusion, although the pathogenic mechanisms leading to aneurysm formation in MFS and ADPKD remain largely undeciphered, it is clear that an interesting overlap exists (Fig.3) and that this overlap may provide interesting opportunities for investigations on the pathogenic mechanisms of both diseases.

FUTURE PERSPECTIVES

Although mutations in PKD1 and PKD2 explain the large majority of the ADPKD families, further genetic heterogeneity has been suggested based on the fact that some ADPKD families are not linked to either PKD1 or PKD2. But, so far the suggested third locus has not been identified. The availability of ADPKD families with known vascular involvement and in whom linkage to the PKD1, PKD2,

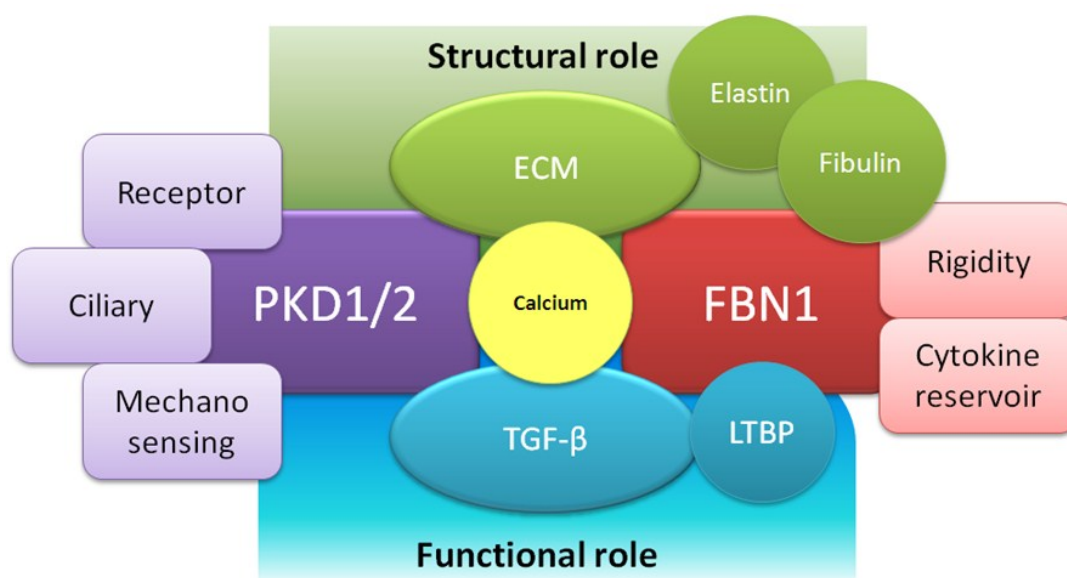


Figure 3. Overlap between the different proteins involved in MFS and ADPKD and their function.

FBN1 and TGFBR1/2 loci has been excluded, can offer a solution. If these families are large enough linkage analysis can be performed, possibly resulting in the identification of new loci. Additionally, if tissue samples from these families are available immunohistochemistry can be performed, enabling the investigation of the contribution of TGF- β in ADPKD pathogenesis. If human tissues cannot be obtained, experiments on aortic wall tissue from different Pkd mouse models can be informative. The results of these experiments will lead to a better understanding of the different pathways

that are involved in the pathogenesis of aneurysm formation in MFS and ADPKD and will provide an explanation for the intersection of both diseases.

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