

## What Is the Role of Somatic Mutation in Autosomal Dominant Polycystic Kidney Disease?

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A 50% dosage reduction of gene product, known as haploinsufficiency, as found in heterozygotes with an inactivating mutation, is not usually associated with a detectable physiologic effect. Consequently, either loci associated with dominant disease are those rare ones that are sensitive to haploinsufficiency, or an additional mechanism plays a role to enhance the effect of the single mutant allele. In the case of autosomal dominant polycystic kidney disease (ADPKD), adult-onset disease is characterized by focal cyst development and expansion as a result of heterozygous mutation in *PKD1* or *PKD2*.

In mice, homozygous inactivating mutations to either *Pkd1* or *Pkd2* results in embryonic lethality with the development of renal and pancreatic cysts from embryonic day 13.5.<sup>1,2</sup> Humans with two fully penetrant mutations are also thought to have a nonviable phenotype. The wide range of human mutations causing ADPKD, approximately 70% of which are clearly inactivating, and the lack of clear genotype–phenotype correlations suggest that a dominant negative mechanism, whereby the mutant product adversely affects the function of the normal ADPKD allele product, is unlikely to be important in the human disease.<sup>3</sup> Rather, a widely accepted view is that ADPKD is recessive at the cellular level and that cysts develop clonally from a tubular cell only once the cell has acquired a second, somatic mutation to inactivate the remaining normal allele.

There are considerable data to support this two-hit hypothesis for cystogenesis in ADPKD. This includes the demonstration of deletion (loss of heterozygosity) or inactivating point mutations to the remaining normal *PKD1* or *PKD2* allele in epithelial cyst linings isolated from single cysts, in either the kidney or the liver.<sup>4–8</sup> In addition, there is evidence for the clonality of cysts.<sup>7</sup> Further data come from a *Pkd2* mouse model (*Pkd2*<sup>WS25</sup>) that is prone to a high level of somatic rearrangement, generating a null allele. These animals as homozygotes or compound heterozygotes with a null allele (*Pkd2*<sup>WS25/-</sup>) develop progressive cystic disease similar to human ADPKD,<sup>9</sup> and the cysts that form are negative for the PKD2 protein, polycystin 2.

Likewise, conditional *Pkd1* knockout mice (*Pkd1*<sup>flox/-</sup>) that are induced to inactivate the floxed allele in the kidney before postnatal day 13 rapidly develop cysts<sup>10–12</sup>; however, mouse

studies also indicate that the timing of polycystin 1 inactivation has profound effects on the phenotype, with inactivation after postnatal day 13 resulting in more slowly progressive disease that manifests in adulthood.<sup>10–12</sup> The switch between rapid and slow cystogenesis upon loss of polycystin 1 may correspond to completion of kidney development that continues approximately 2 weeks postnatally in mice.<sup>11</sup> The slow development of cysts when all polycystin 1 is lost at later stages suggests that other events are required for cysts to develop in the adult; renal injury is one such event shown to hasten cystogenesis.<sup>10,12–14</sup>

Although the two-hit mechanism is attractive, increasingly, phenotypes have been associated with haploinsufficiency of *PKD1* or *PKD2*. Some manifestations of disease, such as an increased level of intracranial aneurysms, are unlikely due to secondary somatic events. Rather, altered properties and reactivity of vascular smooth muscle combined with reduced levels of polycystin 1 or 2 may be critical.<sup>15–17</sup> Further evidence that haploinsufficiency is significant in ADPKD comes from *Pkd2*<sup>+/-</sup> mice, which have a shorter lifespan that is not due to renal failure because they develop only a few liver and kidney cysts.<sup>2</sup> Proliferation also seems higher in noncystic tubules in human ADPKD and *Pkd2*<sup>+/-</sup> and *Pkd1*<sup>+/-</sup> animals.<sup>13</sup> Both *Pkd1* and *Pkd2* heterozygotes are especially sensitive to renal injury, resulting in increased inflammation, apoptosis, and fibrosis<sup>18</sup> and hastening the development of microcysts.<sup>19,20</sup> In addition, altered water balance (antidiuresis), with high urine and low plasma osmolality, has been noted in *Pkd1*<sup>+/-</sup> mice, whereas cells from patients with PKD2 have altered Ca<sup>2+</sup> homeostasis.<sup>21</sup> Together, these data indicate that polycystin 1 and 2 are proteins that are sensitive to heterozygous dosage reduction.

Hypomorphic *Pkd1* or *Pkd2* alleles (alleles that have reduced levels of gene activity; *Pkd1*<sup>nl</sup>, *Pkd1*<sup>L3</sup>, or *Pkd2*<sup>rf3</sup>) allow

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the phenotypic consequences of very low levels of polycystin 1 or 2 to be explored.<sup>22–24</sup> These mice are viable as homozygotes but express just 13 to 33% of normally spliced product and develop progressive cystic disease in the kidney, liver, and pancreas, despite the presence of some polycystin1. They also exhibit intramural bleeding of the aorta and growth retardation.<sup>25</sup> Recently, viable humans who were homozygous or compound heterozygous for hypomorphic alleles and developed typical to severe polycystic disease, including a renal phenotype similar to the infantile-onset autosomal recessive polycystic kidney disease, were described.<sup>26,27</sup> The presence of a hypomorphic allele inherited in *trans* with an inactivating *PKD1* allele (on the other chromosome homolog) can also result in severe, early-onset disease. Often the renal appearance is one of multiple, small, uniformly sized cysts rather than the cystic heterogeneity typical of ADPKD, further suggesting cyst development without the necessity of a somatic mutation. Additional evidence that the level of polycystin 1 and 2 is significant in preventing cystogenesis comes from transgenic studies in which overexpression or aberrant expression of functional *PKD1* or *Pkd1* could result in the development of kidney and liver cysts.<sup>28–30</sup> Transgenic overexpression of *Pkd2* results in a renal tubulopathy, whereas expression of a truncated *Pkd2* causes cyst development and retinal degeneration, suggesting a dominant negative mechanism may sometimes be in play.<sup>31,32</sup>

It seems that cyst development can occur when the level of the polycystin protein is lower or higher than a critical window required to maintain normal tubulogenesis. Although cyst development in these cases can be focal, it seems unlikely that somatic mutation of the normal allele is required for cyst initiation but rather that a threshold effect, perhaps associated with stochastic factors such as renal damage, is sufficient to initiate cystogenesis.

It remains uncertain in typical human ADPKD whether haploinsufficiency alone is sufficient for cyst initiation or somatic inactivation of the remaining normal allele is always necessary. Could the observed somatic mutations be giving the cyst a survival or growth advantage analogous to the additional genetic events associated with tumor development, rather than being required for initiation? Indeed, comparative genomic hybridization and loss-of-heterozygosity analysis of individual cyst linings identified a number of regions that were deleted or duplicated (several found in multiple cysts), suggesting a succession of genetic changes.<sup>33</sup> Consistent with this hypothesis, a phenotypic transition to a more dedifferentiated state was seen as cysts enlarged in the *Pkd2*<sup>WS25/–</sup> model.<sup>34</sup> Additional genetic events have been noted in ADPKD cysts; for instance, *PKD1* mutations have been found in PKD2 kidney cysts and *vice versa*,<sup>35,36</sup> purporting to show that haploinsufficiency at both loci is sufficient to trigger cyst development. Mice or humans who are transheterozygous for a *Pkd1/PKD1* and *Pkd2/PKD2* mutation also have significantly more severe disease than those with a mutation in either gene alone, although they are fully viable.<sup>37,38</sup> These data argue that the mutation to the second gene modifies the phenotype and at the level of the cyst may help to

promote cyst initiation (in combination with other factors) or be an additional step that favors growth or survival of the cyst.

Another event that can significantly modify the *PKD1* phenotype is contiguous deletion of *PKD1* and the adjacent tuberous sclerosis gene, *TSC2*, which results in early-onset polycystic disease.<sup>39</sup> In this case, haploinsufficiency of both polycystin 1 and tuberin (the *TSC2* protein) enhances the severity of disease, either by accumulative disruption to the same pathway or synergistic effects in separate pathways that are important for cyst initiation and growth.<sup>40,41</sup> It seems reasonable to propose that an array of similar genetic events may occur somatically in a developing cyst, influencing their rate of growth. The genetic background of the host may also influence the rate of cyst growth and even susceptibility to somatic mutations.

The emerging model in ADPKD is of loci that are dosage sensitive, which likely alone explains some disease phenotypes. Sufficient dosage reduction can cause cyst initiation, suggesting that a *PKD1/PKD2* threshold effect in combination with stochastic and/or environmental factors may be sufficient for cyst development even in typical ADPKD. In addition, cyst initiation can occur by complete loss of polycystin as a result of somatic second hits; the relative importance of these two mechanisms in cystogenesis in human ADPKD is not fully resolved. Regardless of the importance of somatic events to cyst initiation, it seems likely that a variety of genetic events in the developing cyst play important roles in dictating the rate of expansion and its ultimate contribution to the clinical burden of disease.

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## DISCLOSURES

None.

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See related article, "Incompletely Penetrant *PKD1* Alleles Mimic the Renal Manifestations of ARPKD," on pages 1097–1102.