Chymase-like Angiotensin II–Generating Activity in End-Stage Human Autosomal Dominant Polycystic Kidney Disease

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Abstract. Autosomal dominant polycystic kidney disease (ADPKD) is characterized by exuberant inflammation and fibrosis, a process believed to contribute to progressive loss of normal renal function. Despite early-onset hypertension and intrarenal renin/angiotensin II (AngII) activation, angiotensin-converting enzyme (ACE) inhibition does not consistently confer renal protection in ADPKD. The hypothesis was that mast cells within the inflammatory interstitium release chymase, an enzyme capable of efficient conversion of AngI to AngII, providing an ACE-independent route of AngII generation. End-stage ADPKD renal tissue extracts and cyst fluids were assayed for time-dependent, chymostatin-inhibitable conversion of 125I-AngI to 125I-AngII under conditions of ACE and aminopeptidase inhibition by means of HPLC. Thirteen of 14 ADPKD kidney extracts exhibited chymase-like AngII-generating capacity; calculated initial reaction rates averaged 3.9 ± 2.9 fmol AngII/min/µg protein with a mean maximal conversion of 55% ± 30% of added substrate. AngII-generating activity was both protein and substrate dependent. All five cyst fluid samples were negative. Chymase-like activity was detectable in only three of six non-ADPKD kidney extracts. Immunoreactive chymase protein was present in/around mast cells within the fibrotic renal interstitium in all samples. Findings demonstrate for the first time the presence of mast cells, mast cell–associated immunoreactive chymase protein, and chymase-like AngII generating capacity in ADPKD cystic kidneys. Results support the potential for ACE-independent AngII generation and for mast cell–initiated inflammatory processes in ADPKD, each with therapeutic implications for ADPKD renal progression.

Autosomal dominant polycystic kidney disease (ADPKD) affects 1 in 500 to 1 in 1000 US citizens (1). Ninety percent of ADPKD is the result of a loss of function of the PKD1 or PKD2 gene products, polycystins 1 or 2 (2,3), via random somatic mutation in the unaffected allele (1) with cyst formation by clonal expansion (3). Recent functional studies document interaction of Peys1 and Peys2 in a common signaling pathway and suggest that their physical association generates a nonselective cation channel activity (4) that is necessary for maintenance of the terminally differentiated state in normal tubular epithelial cells (1).

Clinically, ADPKD has been consistently associated with an activation of the systemic renin-angiotensin system and with early onset of hypertension (5–7). Furthermore, aberrant renin production has been noted within cyst walls (5) and in immortalized ADPKD cells of distal tubular origin (8). Surprisingly, however, ADPKD patients appear to be generally resistant to the long-term renoprotective effects of angiotensin-converting enzyme (ACE) inhibition (9,10). One of the striking pathologic findings in ADPKD, in addition to the formation and expansion of fluid-filled cysts, is an exuberant inflammatory and fibrotic process (11) believed to contribute substantially to the progressive loss of normal renal parenchyma. Mast cells are typically drawn to sites of active inflammation and produce the enzyme chymase (12–14), which in humans efficiently converts (13,14) angiotensin I (AngI) to angiotensin II (AngII) (15). A prerequisite for chymase-dependent AngII generation is renin action on its substrate, angiotensinogen, to produce AngI (14). Also, chymase must be released from mast cells to have access to AngI (14). Because chymase acts on AngI to generate AngII, this pathway bypasses ACE in the formation of AngII. Recent studies have shown that chymase produced by mast cells in the normal human heart generate a substantial proportion of cardiac AngII (12). In human and other primate vasculature, 50% to 70% of AngII generated is chymase dependent (14,16). In normal human (12,14) and dog (17) kidney, both chymase-like activity and protein have been detected. Moreover, in pathologic renal inflammation (rapidly progressive inflammatory glomerulonephritis, rejecting renal transplants, diabetic nephropathy), increased numbers of chymase-positive mast cells correlate with the degree of fibrosis, suggesting the potential for mast cell involvement in the renal inflammation associated with ADPKD.
fibroproliferative process (18–20). An analysis of PubMed revealed no prior studies examining mast cells or chymase activity/protein in ADPKD cystic renal tissue. We have therefore assayed chymase-like AngII generating activity in renal tissue, examined mast cell–associated immunoreactive chymase protein, and measured intrarenal AngII levels in endstage ADPKD kidneys.

Materials and Methods

ADPKD Tissue Collection

End stage kidneys from 15 patients with ADPKD and kidney samples from 10 patients without ADPKD (without fibrosis) were obtained at surgical nephrectomy via institutional review board (IRB)–approved protocols (Oregon Health and Science University IRB 6044VAV; Portland VA Medical Center IRB 00349). After nephrectomy, ADPKD kidneys were stored on ice during transport; tissue was processed immediately, with the exception of one kidney shipped overnight on ice and dry ice. Tissue to be used in chymase assays was snap-frozen in a methyl butane/dry ice slurry and stored at −80°C until use. Cyst fluid was aspirated from individual cysts, similarly snap-frozen, and stored at −80°C.

Preparation of Extract from ADPKD Tissue

The procedure for chymase extract preparation is adapted from Urata et al. (12). Frozen ADPKD tissue was thawed on ice, and visible cysts were drained. Tissue was minced with scalpels and homogenized with a PowerGen 125 homogenizer (Fisher Scientific) in 5 volumes (w/v) phosphate homogenization buffer (10 mM sodium phosphate, 0.1% nonidet P-40, 1.2 M KCl). The homogenate was incubated at 4°C for 1 h with occasional mixing, then was centrifuged at 1500 × g for 15 min at 4°C. The supernatant was centrifuged at 100,000 × g for 30 min at 4°C. The resulting supernatant was formed into aliquots and stored as a chymase extract at −80°C until assay. Bio-Rad protein assay reagent concentrate was used with visible light spectrophotometry to determine protein concentration of the extract and of cyst fluids before chymase assay.

Measurement of Chymase-like Activity in ADPKD Tissue Extracts and Cyst Fluids

Conversion of AngI to AngII by chymase-like enzymatic activity was assayed by combining renal tissue chymase extract with glycine buffer (pH 9.5) and radioiodinated AngI (125I-AngI) by a method adapted from Urata et al. (12). High pH was found to be critical to limit aminopeptidase activity and consequent angiotensin peptide product degradation during incubation because radiolabeled AngIII, AngIV, and free tyrosine were apparent at pH 8.5 but not at pH 9.5. The assay was performed on ice except for the 37°C incubation period; replicates maintained at 4°C provided negative controls. Chymase extract or cyst fluid was allowed to thaw on ice, then combined with 2× glycine buffer (200 mM glycine, 1 M KCl, 0.02% Triton X-100, 10 mM EDTA to inhibit ACE, and 0.2 mM Bacitracin). Chymostatin (100 μM), a chymase inhibitor that does not block ACE (21), or enalaprilat (1 μM), an ACE inhibitor, was added to replicate 30-min time points. 125I-AngI (20 nM final concentration) was added to start the reaction. After incubation at 37°C for the indicated times, reactions were stopped by precipitating proteins with ice-cold 10% TCA. Samples were centrifuged, and supernatants were analyzed for conversion from 125I-AngI to 125I-AngII by HPLC with a reverse-phase column (Varian) with 24% acetonitrile in triethylamine phosphate (pH 3.0) mobile phase. 125I was detected with a Beckman 170 radioisotope detector. Identity of products was based on authentic radiolabeled AngI, AngII, and free tyrosine standards. Area of peaks for 125I-AngI, 125I-AngII, and 125I-tyrosine, respectively, were analyzed by GraphPad Prism graphing and statistical software.

For AngII-generation curves, 25 μg total protein/100 μl total volume was used; samples were incubated at 37°C for 0, 10, 20, or 30 min. A small chymostatin-resistant free 125I-tyrosine peak (2% to 3% of added substrate) appeared in all incubated samples with no increase over time; otherwise, all 125I-AngI consumption was attributable to 125I-AngII formation. Initial rates were calculated by multiplying the interpolated 1-min percentage conversion of substrate by the number of picomoles of 125I-AngI added. For determination of protein dependence, 5, 10, or 25 μg extract protein per 100 μl total volume was used; samples were incubated at 37°C for 0, 2, 5, or 10 min. Rates were calculated as described above.

To determine substrate dependence, 125I-AngI (20 nM) was used as an index of rate of substrate conversion, and unlabeled AngI (0, 1, 3, 10, 30, 100, 300, and 1000 μM) was used as substrate. In this assay, 125I-AngI and AngI were added to buffer first; then extract protein was added to start the reaction. Incubation time for all samples was 10 min. It was assumed that 125I-AngI and unlabeled AngI were consumed at the same rate, and that the amount of 125I-AngI was negligible relative to the amount of unlabeled AngI. The rate of 125I-AngII generation was used to calculate the amount (fmol/min per μg protein) of unlabeled AngII generated at each substrate concentration.

Chymase Immunohistochemistry and Histology

Fresh tissue samples were snap-frozen in OCT and, 20-μm frozen cryostat sections were prepared by standard methods and stored at −80°C until use. Sections were fixed in freshly prepared 1% paraformaldehyde-PBS for 10 min at room temperature. Immunostaining was performed with a standard avidin-biotin protocol as described previously (22), except that all incubations and washes were carried out on an automated immunostainer (Dako). Mouse monoclonal antibody to human mast cell chymase (Chemicon) was used at a 1:1,500 dilution. Adjacent sections were stained with Giemsa to identify mast cells via their characteristic azurophilic granules or with nonimmune mouse immunoglobulin (2 μg/ml) as controls. Because of considerable inhomogeneity within a given ADPKD kidney for mast cell density, we did not attempt to formally quantitate this parameter. There was no systematic spatial correspondence between the tissue sample used for chymase assay and that used for immunohistochemistry.

Intrarenal Immunoreactive AngII by Radioimmunoassay

Fresh or snap-frozen ADPKD and non-ADPKD tissues were homogenized in 8 M urea with 0.1% Triton X-100 to denature proteins (3 ml 8 M urea/l g tissue) as described by Allan et al. (23). Methanol (8 ml of 80% MeOH) was added to precipitate proteins, and samples were centrifuged at 10,000 × g for 5 min at 4°C. Fifty microliters of 10% glycerol was added to the supernatant, which was then dried in a SpeedVac Drier overnight. Dried samples were reconstituted in saline and passed through activated Sep-Pak C18 cartridges, and angiotensin peptides were eluted with 80% methanol. Samples with 50 μl 10% glycerol added were dried in a SpeedVac Drier overnight. Extracts were analyzed in duplicate with an AngII radioimmunoassay kit (Phoenix Pharmaceuticals). Briefly, extracts were reconstituted in the radioimmunoassay buffer. Primary antibody (rabbit anti-AngII, Phoenix Pharmaceuticals Kit) was added, and samples incubated
overnight at 4°C. AngII peptides recognized by the primary antibody used include AngII, AngIII, and AngIV.

125I-AngII tracer solution (Peptide Radioiodination Services, Washington State University) was added (8000 to 10,000 cpm/sample), and samples were incubated overnight at 4°C. Secondary antibody (goat anti-rabbit IgG) was added, and samples incubated 90 min at room temperature. Samples were centrifuged at 1700 × g for 20 min at 4°C, and the pellet was counted with a gamma counter. The radioimmunoassay was performed three times on 16 chymase-positive ADPKD samples and 5 non-ADPKD human kidney samples. Values, expressed as immunoreactive AngII (AngII-IR), were averaged to provide a single estimate. Intra-assay variability was 6.5% ± 2.6%; interassay coefficient of variation was 9.6% ± 5.6%.

Statistical Analyses
To estimate chymase activity, initial rates (fmol AngII/min per μg protein) and maximal substrate conversion (as percentage of added 125I-AngI substrate) were derived from time-activity curves. Groups (ADPKD versus normal) were compared by nonparametric Mann-Whitney U test (SigmaStat version 1.01; SPSS) because of skewed distribution of ADPKD values for the chymase activity indices and for intrarenal AngII levels.

Results
Chymase-like AngII Generation in ADPKD Kidney Tissue
Thirteen (93%) of 14 ADPKD kidneys tested were positive for chymase-like activity, defined as time-dependent, chymostatin-sensitive 125I-AngII generation (Figure 1). Mean maximal conversion for chymase-positive samples was 55% ± 30% of added substrate (range, 17% to 108%; median, 63%); initial reaction rate for chymase-positive samples 3.9 ± 2.9 fmol AngII/min per μg protein (n = 13; Figure 1A). Representative chymase-positive and chymase-negative ADPKD samples are shown in Figure 1, B and C. Chymostatin, a chymase inhibitor that does not block ACE (21), completely blocked 125I-AngI conversion to 125I-AngII (Figure 2A). Addition of enalaprilat, an ACE inhibitor, failed to alter AngII generation, confirming that ACE was fully blocked under basal reaction conditions by 10 mM EDTA (Figure 2B).

Non-ADPKD control human kidney samples from eight patients were tested (five from individual kidneys plus a sixth sample pooled from three kidneys). Three of these six samples were negative (no AngII increase over time; maximal conversion averaged 6% ± 1% (Figure 1C). Three non-ADPKD samples were positive for chymase-like activity: maximal percentage conversion was 13% ± 5%; initial rate averaged 0.59 ± 0.09 fmol AngII/min per μg protein. By ANOVA on ranks,
both indices of chymase-like activity were significantly increased in ADPKD samples over non-ADPKD control kidneys (each \( P < 0.001 \)).

Protein dependence was assessed in three ADPKD kidney samples with 5, 10, or 25 \( \mu \)g total extract protein per reaction. Mean initial reaction rates, calculated by interpolating the 1-min point on the time-activity curve (0-, 2-, 5-, and 10-min time points) were 94 ± 28, 135 ± 15, and 173 ± 18 fmol \(^{125}\)I-AngII generated per minute for 5, 10, and 25 \( \mu \)g extract protein, respectively (Figure 3; \( r^2 = 0.999 \)). Mean maximal conversion of substrate at each protein concentration was, respectively, 49% ± 23%, 56% ± 19%, and 103% ± 43% of added substrate. However, although increasing conversion of substrate with increasing protein concentration was apparent, the initial reaction rates per microgram of protein in fact demonstrated a decreasing conversion rate with increasing protein concentration: for 5, 10, and 25 \( \mu \)g protein per tube, reaction rates per microgram of protein were, respectively, 12.5 ± 3.5, 8.0 ± 2.5, and 4.9 ± 1.2 fm/min per \( \mu \)g protein.

To determine substrate dependence of the chymase-like activity, increasing concentrations (0, 1, 3, 10, 30, 100, 300, and 1000 \( \mu \)M) of unlabeled AngI (cold substrate) together with 20 nM \(^{125}\)I-AngI (labeled substrate) were incubated for 10 min in the presence of ADPKD kidney extract (25 \( \mu \)g protein per reaction). We assumed that unlabeled AngI was converted at the same rate as \(^{125}\)I-AngI and that the amount of \(^{125}\)I-AngI was negligible relative to the amount of unlabeled substrate. On the basis of the percentage conversion of labeled substrate, the amount of unlabeled AngI converted at each time point was calculated. The results indicate that AngII generation by ADPKD kidney extracts in this assay is substrate-dependent in the pattern expected for an enzymatic reaction (Figure 4).

**Effect of Protein Concentration on Initial Reaction Rate**

*Figure 3.* Protein dependence of reaction rate. Initial reaction rate (1-min interpolation) increased in a nonlinear pattern with increasing protein concentration. However, when expressed per \( \mu \)g extract protein, initial reaction rate decreased with increasing tissue extract protein (inset).

**Substrate Dependence of AngII Generation**

*Figure 4.* Substrate dependence of angiotensin II (AngII) generation. Tissue homogenate was incubated under AngII converting enzyme (ACE)-inhibited conditions for 10 min with 20 nM \(^{125}\)I-angiotensin I (AngI) and increasing concentrations of unlabeled AngI. The graph shows that AngII generation is dependent on substrate concentration.

**Chymase-like Activity in ADPKD Cyst Fluid**

Of five cyst fluid samples from five ADPKD kidneys, none exhibited detectable chymase-like AngII generating activity (data not shown).

**Immunoreactive Chymase Protein in ADPKD Renal Tissue**

In ADPKD tissue, chymase immunohistochemistry identified multiple discrete sites of immunoreactivity corresponding to mast cells (Figure 5). Immunoreactive chymase protein was present both within and around mast cells in the intercystic fibrotic interstitium (Figure 5, A and B). In some cases, mast cells clustered along extended segments of cyst wall in a subepithelial location (data not shown). Adjacent Giemsa-stained sections confirmed the presence of abundant mast cells with typical azurophilic granules (Figure 5C). Control slides exposed to nonimmune mouse globulin were consistently negative for chymase immunoreactivity (data not shown). Because of the marked variability of mast cell density within and among ADPKD kidneys, and because the tissue used for chymase activity assay was not spatially related to the immunostained samples, we did not attempt formal quantitation of mast cell density.

**Endogenous Intrarenal AngII-IR Levels**

Intrarenal AngII-IR concentration in ADPKD kidney samples was significantly higher than that of non-ADPKD kidney samples (\( P = 0.001 \); Mann-Whitney) with no overlap between groups (Figure 6). Mean ADPKD AngII-IR was 1736 ± 670 pg AngII/g tissue versus 543 ± 41 pg/g tissue in non-ADPKD control renal tissue.
Discussion

Unlike many forms of chronic renal disease, ADPKD is generally resistant to the renoprotective effects of ACE inhibitor therapy (9,10). Because AngII has been implicated in progressive inflammatory/fibrotic renal disease models (24), we hypothesized that an alternative pathway of AngII formation could be operative in ADPKD. With the recent discovery of the AngII-generating capacity of human chymase in heart and vascular tissue (12,14,16,17), we further proposed that mast cell–derived chymase mediates an ACE-independent pathway of AngII generation in ADPKD.

To demonstrate the feasibility of this pathway, we have provided three supporting lines of evidence. First, we showed the presence of immunoreactive chymase protein located in and around azurophilic mast cells within the fibrotic interstitium, documenting a previously unrecognized source of chymase in ADPKD kidney tissue. Second, chymase-like AngII-generating activity—defined here as chymostatin-inhibitable/ACE-resistant AngII generation that increases with time—was present in 13 of 14 ADPKD kidneys studied as compared with three of six non-ADPKD kidneys. Furthermore, the level of activity—measured both by maximal percentage of substrate conversion and by the estimated initial reaction rate—was significantly higher in ADPKD than in non-ADPKD kidneys. Because the AngII-generating capacity was assayed in the presence of 10 mM EDTA, a highly effective ACE inhibitor in vitro, and AngII generation was not changed by the further addition of enalaprilat, it is unlikely that AngII generation measured here represents ACE activity. Chymase-like AngII-generating activity exhibited both protein- and substrate-dependence typical of a classic enzymatic reaction. Third, we have shown that endogenous intrarenal AngII levels are significantly higher in ADPKD than in non-ADPKD kidney tissue, supporting the functional activity and clinical relevance of AngII-generating systems in ADPKD in vivo. Taken together, these results support the presence of mast cell–associated, enzymatically active chymase as an alternative AngII-generating pathway in ADPKD.

Figure 5. Immunoreactive mast cell–associated chymase protein in autosomal dominant polycystic kidney disease (ADPKD) cystic kidney. Discrete foci of immunoreactive chymase protein correspond to mast cells scattered throughout the fibrotic interstitium. Staining is apparent both within and surrounding individual mast cells. (A) Low-power view (original magnification, ×20). (B) Higher-power view (original magnification, ×100); arrowheads indicate chymase-positive mast cells. (C) Giemsa-stained mast cells from an adjacent section can be identified by their typical azurophilic granules (arrowheads).

Figure 6. Intrarenal immunoreactive angiotensin II (AngII) levels in autosomal dominant polycystic kidney disease (ADPKD). ADPKD samples showed significantly higher intrarenal immunoreactive AngII (AngII-IR) than non-ADPKD samples, \( P = 0.001 \) (Mann-Whitney \( U \) test).
Incomplete Substrate Conversion and Tissue Linearity in Time-Activity Curves

Classic time-activity curves assessing enzymatic activity approach 100% substrate conversion with time, although reaction rate slows with substrate depletion. However, in ADPKD renal tissue homogenates, the majority failed to approach 100% conversion. Our studies cannot determine whether this finding reflects high endogenous AngI in the homogenates with subsequent competition for chymase, high AngII levels exerting product inhibition of the reaction, endogenous non-AngI chymase substrates competing for chymase binding, and/or chymase inhibitors.

The lack of tissue linearity (decreasing initial reaction rate with increasing microgram of tissue protein; Figure 3, inset) in fact suggests that endogenous inhibitors or competitors present in tissue homogenates may variably modulate the rate of reaction. Preliminary measurements of extracted endogenous tissue immunoreactive AngI show concentrations that, even if fully protected in the chymase-assay samples, are unlikely to exert significant substrate inhibition. The high endogenous AngII levels (Figure 6), however, could potentially exert an inhibitory effect. We cannot exclude non-AngI chymase substrates or other tissue-derived chymase inhibitors as contributors to the loss of tissue linearity.

Limitations of This Study

Although our results demonstrate the potential for participation of chymase in ADPKD, findings do not address whether chymase in fact plays a significant role in vivo in progression of ADPKD renal disease. One limitation is the nonphysiologic conditions of the in vitro assay, which was designed to optimize chymase activity and limit angiotensin peptide degradation by aminopeptidases and may not reflect in vivo enzymatic activity. A second limitation is the requirement for chymase release from the mast cell to interact with AngI in the extracellular compartment: the presence of immunoreactive chymase surrounding mast cells in the fibrotic ADPKD interstitium could be an artifact of tissue processing. Third, we do not know whether ADPKD patients were receiving ACE inhibitors and/or AngII receptor blockers at the time of nephrectomy; these agents could potentially upregulate chymase activity in vivo. A potentially similar phenomenon was observed in ACE knockout mice, where intrarenal chymase activity was increased 14-fold in the absence of ACE activity (25). This was especially notable given the fact that normal rodents—in contrast to human and other primate species—express little or no renal chymase-dependent AngII formation (25,26). Finally, a novel smooth muscle–associated chymase has been recently described (27); although not detected by the antibody used in our studies, this could be present and contributing to chymase-like activity.

Despite these limitations, a number of observations support the plausibility of in vivo chymase activity and specifically chymase-like AngII generation in ADPKD kidneys. Hollenberg et al. (14) demonstrated, in the renal vasculature of normal humans, that around two thirds of AngI conversion to AngII was independent of ACE. In the monkey, 50% to 70% of vascular AngI conversion to AngII was shown to be chymase dependent (14,16). In normal kidney tissue, both chymase-like activity and protein have been detected in vitro (12,14,17). Of special note, vascular chymase has been shown to be upregulated in pathologic states such as balloon injury (28), myocardial infarction (29), and atheromatous plaque erosion (30,31).

Mast cell infiltration has been described in inflammatory renal diseases such as rapidly progressive glomerulonephritis, where increased numbers of chymase-positive mast cells were correlated with the degree of fibrosis and with the presence of activated myofibroblasts, suggesting the potential for mast cell participation in the fibroproliferative process (18). Similarly, in rejecting renal transplants, increased expression of chymase in mast cells correlated with severity of interstitial fibrosis (19). Finally, in diabetic nephropathy, by using a monoclonal anti-chymase antibody that detects chymase in mesangial cells and subintimal myofibroblasts, Huang et al. (20) report marked upregulation of both glomerular and arterial-wall chymase protein.

Regardless of the enzymatic source of AngII generation, elevated levels of endogenous intrarenal AngII in ADPKD—whether acting locally to augment vascular resistance and/or sodium retention or escaping to elevate circulating AngII—would be expected to contribute substantially to the early hypertension typical of ADPKD (6,7). In addition to these hypertensive actions, AngII has also been shown to have independent proinflammatory and profibrotic effects (24,32) in many forms of inflammatory renal disease, actions that typically involve activation of TGF-β (24,32), release of monocyte chemotactic protein-1 (MCP-1) (33), and stimulation of collagen synthesis (34,35). Thus, the consistently increased intrarenal AngII reported here in ADPKD cystic kidneys could additionally mediate or augment inflammatory cascades.

Importantly, potential roles for chymase in ADPKD disease progression are not limited to AngII generation. Chymase also has widespread proinflammatory effects independent of AngII (36), including chemotraction of circulating mast cells and mast cell precursors, neutrophils, eosinophils, and macrophages (37,38), activation of TGF-β (39), and angiogenesis (40). Furthermore, the role of the mast cell as a crucial factor in initiation and amplification of the inflammatory cascade has been recently documented in genetic models of autoimmune diseases (41). Thus, mice lacking mast cells (W/Wv) exhibit reduced susceptibility to autoimmune inflammatory encephalitis and to rheumatoid arthritis (41); susceptibility is restored by reconstitution of mast cells. Accordingly, not only chymase but also other mast cell products (tryptase, TNF-α, interleukins) and mast cell functions could provide potentially useful therapeutic targets in an integrated approach to the prevention of ADPKD disease progression.

In summary, the study presented here shows for the first time the presence of chymase-like AngII generating capacity in end-stage ADPKD renal tissue. The presence of AngII-forming activity in the solid tissue (but not in cyst fluid), together with our finding of abundant chymase-containing mast cells, points to a previously unrecognized source of AngII generation from
AngII within the fibrotic interstitium of ADPKD kidneys. Additionally, this is the first report of elevated intrarenal AngII concentration in human ADPKD, indicating that the increased intrarenal renin previously demonstrated in ADPKD cystic epithelium (5) is functionally active and may potentially contribute to both intrarenal AngII and circulating AngII levels. If chymase-like AngII generation is relevant in vivo, both the hypertensive and the inflammatory components of ADPKD disease progression may benefit from long-term AngII receptor blockade as well as from anti-inflammatory interventions that target mast cell–initiated pathways.

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