Kidney Androgen-Regulated Protein Transgenic Mice Show Hypertension and Renal Alterations Mediated by Oxidative Stress

O. Tornavaca, PhD*; G. Pascual, MSc*; M.L. Barreiro, PhD; M.T. Grande, MSc; A. Carretero, PhD; M. Riera, PhD; E. García-Arumi, PhD; B. Bardaji, MSc; M. González-Núñez, MSc; M.A. Montero, MD; J.M. López-Novoa, PhD; A. Meseguer, PhD

Background—Kidney androgen-regulated protein (KAP), a proximal tubule androgen-regulated gene, codes for a protein of unknown function.

Methods and Results—To investigate the consequences of KAP overexpression in kidney, we produced KAP transgenic mice and performed microarray expression analyses in kidneys of control and transgenic males. Downregulation of the androgen-sensitive Cyp4A14 monooxygenase gene in KAP transgenic mice prompted us to analyze blood pressure levels, and we observed that transgenic mice were hypertensive. Inhibition of 20-hydroxyeicosatetraenoic acid synthesis by N-hydroxy-N'-(4-n-butyl-2-methylphenyl) formamidine (HET0016) reduced the increased 20-hydroxyeicosatetraenoic acid levels in urine and normalized arterial pressure in transgenic mice, as did the NADPH oxidase inhibitor apocynin. Increased oxidative stress in transgenic mice was demonstrated by (1) enhanced excretion of urinary markers of oxidative stress, 8-iso-prostaglandin F2α, 8-hydroxydeoxyguanosine, and thiobarbituric acid–reacting substances; (2) augmented mitochondrial DNA damage and malondialdehyde levels in kidneys; and (3) diminished catalase and glutathione peroxidase activity in transgenic kidneys. Mice exhibited renal defects that included focal segmental glomerulosclerosis, proteinuria, glycosuria, and fibrosis.

Conclusions—Taken together, these results indicate that KAP expression is critical for cardiovascular-renal homeostasis maintenance and that hypertension is associated with increased oxidative stress. This is the first report showing that overexpression of an androgen-regulated, proximal tubule–specific gene induces hypertension. These observations may shed light on the molecular pathophysiology of gender differences in the prevalence and severity of hypertension and chronic renal disease. (Circulation. 2009;119:1908-1917.)

Key Words: hypertension ■ kidney ■ androgens ■ sex dimorphism ■ transgenic mice

Hypertension constitutes a major health challenge, and despite extensive research, the identification of molecular causes of hypertension remains complicated by the variety of its causes and the frequent coexistence of additional pathological conditions. Nonetheless, extensive genetic segregation and linkage analyses point to multigenic factors as being responsible for a significant component of human essential hypertension.1,2 Gender differences in the incidence and severity of hypertension have also suggested the involvement of a sex-dependent mechanism in the pathogenesis of human disease.3–6 Although the molecular mechanisms have not been elucidated completely, there is growing evidence for a role of androgens in determining sex-specific differences in the progression of hypertension and induced renal disease, both in humans and in animals.3,7 Furthermore, the importance of the kidney in blood pressure control is well known.

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The kidney androgen-regulated protein (KAP) constitutes the most abundant and specific gene expressed in proximal tubule epithelial cells.8,9 It is expressed in a tissue- and cell-specific manner and is strictly regulated by thyroid and sex steroid hormones in different segments of proximal tubules in mouse kidney.10–16 A construct that contained 1542
base pairs (bp) from the 5'-flanking region of the KAP gene, which proved to drive expression of a heterologous reporter gene in a tissue-specific, cell-specific, and androgen-regulated fashion to transgenic mice,\(^{17}\) and transient transfection assays that used several truncated and/or mutated promoter-containing constructs were used to identify the essential regulatory elements of the KAP proximal promoter that account for its complex and exquisite regulation in kidney.\(^{16,18}\)

Although knowledge of KAP gene regulation is extensive, little is known about the function of its encoded protein, which does not exhibit known structural or functional domains or homologies with other protein sequences in the databases, thus greatly reducing experimental approaches to elucidate KAP function. With a yeast 2-hybrid assay approach, it was first reported that KAP interacts with the cyclosporine A (CsA) binding protein, cyclophilin B (CypB), and further that a functional relationship among KAP, CypB, and CsA-mediated toxicity takes place in the kidney.\(^{19}\) We determined the effects of CsA on KAP levels in kidney and observed that although the protein diminished, the mRNA increased, which suggests the setup of a negative feedback system to recover the protein levels lost after CsA treatment. The initial hypothesis of KAP being important for kidney function was reinforced after KAP overexpression was observed to protect renal proximal tubule cells from CsA toxicity.\(^{19}\) To gain further insight into the function of this intriguing protein, we generated a KAP transgenic mouse driven by its own promoter (pKAP2-KAP) to produce androgen-inducible overexpression of KAP in proximal tubule cells in the absence of exogenous androgen administration.

**Methods**

A complete description of the methods used in the present study is provided in the online-only Data Supplement.

**Generation of pKAP2-KAP Transgenic Mice**

The mouse KAP cDNA, cloned into the pKAP2 vector,\(^{20}\) was microinjected into 1-cell–fertilized B6SJL F2 embryos.\(^{21}\) Transgenic mice were identified by Southern blot of tail genomic DNA. The study was approved by an institutional review committee.

**Reverse-Transcription Polymerase Chain Reaction Expression Analysis of RNA**

Reverse-transcription polymerase chain reactions (RT-PCRs) with RNA from different tissues were performed under linear conditions. Real-time quantitative RT-PCR was performed with the TaqMan fluorogenic PCR system.

**Western Blot Analysis**

Tissues were homogenized by \(N\) cavitation in radioimmunoprecipitation assay buffer or CHAPS-urea buffer for KAP detection. Western blot was performed by standard procedures, and quantification was performed by a Bio-Rad model DS800 calibrated densitometer (Bio-Rad Laboratories, Hercules, Calif).

**Microarray Expression Analysis**

RNA was harvested from transgenic and control littermates, 3-month-old male mice derived from 2 different founders. Eight independent microarrays, including 2 transgenic and 2 control mice for each founder, were performed with the Affymetrix mouse genome MOE 430 2.0 (Affymetrix, Santa Clara, Calif) at the Institut de Recerca Hospital Universitari Vall d’Hebron genomics facility. Raw expression values obtained directly from CEL files were preprocessed by the RMA (robust multichip average) method,\(^{22}\) a 3-step process that integrates background correction, normalization, and summarization of probe values.

Selection of differentially expressed genes was based on a linear model analysis with empirical Bayes moderation of the variance estimates according to the methodology developed by Smyth et al.\(^{23}\) Because the method proved to be too restrictive, the ranked list of genes and unadjusted probability values were used as the basis for candidate selection.

**Measurement of Blood Pressure**

Arterial pressure was measured with tail-cuff equipment adapted for mice\(^{24}\) and by radiotelemetry, as described previously.\(^{25}\)

**Assessment of Renal Oxidative Stress**

Total 8-iso-prostaglandin F\(_2\alpha\) (PGF\(_2\alpha\); isoprostane enzyme immunoassay kit [urinary] EA85, Oxford Biomedical Research, Inc, Oxford, Miss) and 8-hydroxyguanosine (8-OHdG ELISA KOG-2005/E, Japan Institute for the Control of Aging, Fukuroi, Shizuoka, Japan) were quantified in urine samples according to the supplier’s instructions. In addition, thiobarbituric acid reactive substances and mal-

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**Figure 1.** Generation of KAP transgenic (Tg) mice. A complete description is provided in the Data Supplement. **A,** Schematic of the pKAP2-KAP construct. Mouse KAP cDNA was cloned into the NotI sites of the pKAP2 vector. The transgene was excised as an SpeI and Ndel fragment for microinjection. **B,** Southern blot analysis of genomic DNA extracted from mouse tail. The expected 6.5-kb band and sometimes the 0.8-kb band denoted the presence of the transgene (+) in 4 different transgenic founders (L-43, L49, L-59, and L-64). **C,** Western blot for human angiotensinogen gene. Western blot analysis of crude kidney homogenates from transgenic and control littersmates (C) with human kidney extracts (H) as positive control. Blots were hybridized with anti-human angiotensinogen antibody and reprobed with anti-\(\alpha\)-tubulin antibody for loading control.
Moxidol levels were measured in urine and kidney tissues, respectively, as described previously.26 Urinary 20-hydroxyeicosatetraenoic acid (20-HETE) levels were measured by an enzyme immunoassay (20-HETE immunoassay kit, Detroit R&D, Inc, Detroit, Mich).

Mitochondrial DNA Oxidative Damage Measurements
Total DNA was extracted with a QIamp DNA Mini Kit (Qiagen, Valencia, Calif) and quantitated spectrophotometrically. Aliquots of DNA (25 and 50 ng) then underwent PCR amplification with the GeneAmp XL PCR kit (Applied Biosystems, Foster City, Calif) according to the manufacturer’s instructions. Two fragments of mitochondrial DNA (miDNA; 8.7 kilobase [kb] and 337 bp) were amplified. An aliquot of each PCR product was resolved on a 0.8% or 2% agarose gel and electrophoresed in TBE at 90 V for 45 minutes. Density of the bands was obtained and processed with Quantity One software (Bio-Rad Laboratories).

Antioxidant Enzyme Activities
Total superoxide dismutase activity was determined according to McCord and Fridovich.27 Manganese-superoxide dismutase activity was measured in the presence of 10 mmol/L KCN. Glutathione peroxidase was measured by the method of Lawrence and Burk.28 Catalase assay was performed spectrophotometrically according to Aebi’s method.29

Histological and Pathological Assessment of Mice
Frozen kidney sections were stained with hematoxylin and eosin, periodic acid-Schiff, and Masson’s trichrome according to standard procedures. Vascular corrosion casts were performed with Mercox (Japan Vilene Co, Tokyo, Japan; supplied by Ladd Research Industries, Williston, Vt). Casts were dissected, mounted on stubs, spattered with gold, and observed in a Hitachi S-570 scanning electron microscope (Hitachi High Technologies, Pleasanton, Calif) at an accelerating voltage of 10 to 15 kV.

Immunohistochemistry and Confocal Microscopy
Paraffin-embedded kidney sections were subjected to antigen retrieval and incubated in primary anti-KAP mouse monoclonal antibody as described previously.19 Kidney sections were incubated with rabbit anti-mouse collagen IV antibody (Chemicon International, Temecula, Calif). Biotinylated anti-rabbit IgG and streptavidin Alexa Fluor 488 conjugate as fluorochrome (Molecular Probes, Carlsbad, Calif), and propidium iodide for nuclear counterstaining (Sigma-Aldrich, St Louis, Mo) were used for laser-scanning confocal analysis (TCS SP2; Leica Microsystems GmbH, Heidelberg, Germany).

Kidney Function Analysis
Urine was obtained from individual mice housed in metabolic cages for 24 hours. Blood samples (150 μL) were collected from the caudal vein. Urine and plasma creatinine concentrations were determined by a modification of Jaffé’s reaction method. Urinary electrolyte, protein, and glucose concentration were measured with a Hitachi autoanalyzer.

Statistical Analyses
The nonparametric Mann-Whitney U test was used for comparisons among the different groups when data distribution was not normal. When data were paired, they were analyzed with Student’s t test. When there were several groups or several treatments, data were analyzed by 2-way ANOVA followed by Newman-Keuls test. All data are expressed as mean ± SEM. Statistical significance was set at P<0.05. For all experiments, at least 6 different animals were used per group. Statistical analysis was performed with the Statgraphics software package (Manugistics Inc, Herndon, Va).

Results

Generation of KAP Transgenic Mice
The pKAP2-KAP construct includes (noncoding) exons 2 to 5 of the human angiotensinogen (hAGT) gene,20 which proved to be necessary for androgen-dependent transgene expression in transgenic mice17 (Figure 1A). Southern blot assays show the most prominent 6.5-kb and, in some in-
stances, the 0.8-kb band size after EcoRI digestion, which were used to identify the founders and for initial screening of the offspring (Figure 1B). From 9 original founders, 2 (L-49 and L-59) were selected that were born at the expected mendelian ratios, had normal growth curves, and were fertile, regardless of their sex (Figure 1B).

KAP cDNA cloning in the NotI site downstream of the KAP promoter deletes the entire coding potential of hAGT contained in exon II, because exons 3 to 5 of hAGT are not translated into protein (Figure 1C) and only supply the distal enhancer and exon-intron splicing sites for proper expression of the KAP gene in an androgen- and cell-restricted manner. Unless otherwise indicated, experimental procedures described herein were performed in 6- to 8-month-old males from both founders and their corresponding control littermates. They yielded essentially identical results.

### Phenotypic Characterization of KAP Transgenic Mice

Transgene tissue distribution showed no expression in tissues other than kidney (Figure 2A). mRNA and protein KAP levels were approximately doubled in transgenic compared with control littermates, although the difference was not statistically significant (Figure 2A and 2B, respectively). Immunohistochemical analysis with specific anti-KAP antibodies revealed that the protein was restricted to proximal tubule cells (Figure 2C). A higher-magnification picture (40×; inset, Figure 2C) showed diffuse, intense positive staining in all areas of transgenic mice, as well as generally less intense staining and some peripheral cytoplasmic immunonegative areas in controls.

### Differential Gene-Expression Pattern in Kidneys of Control and Transgenic Mice

Differentially expressed genes, defined herein as those with $P<0.025$, were further classified into functional groups. A gene-enrichment analysis was performed with a list of 146 genes that were selected for further study. This analysis did not yield any significant Gene Ontology (GO) Database categories. To give this list a biological interpretation, we

### Table 1. Transcriptomic Analysis With Mouse Affymetrix Microarrays

<table>
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<tr>
<th>Biological Process</th>
<th>Symbol</th>
<th>Description</th>
<th>Log Fc</th>
<th>$P$</th>
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<tr>
<td>Lipid metabolism</td>
<td>Cyp4a14</td>
<td>Cytochrome P450, family 4, subfamily a, polypeptide 14</td>
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<td>Hmgcs2</td>
<td>3-Hydroxy-3-methylglutaryl-coenzyme A synthase 2</td>
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<td>A2g2p1</td>
<td>Alpha-2-glycoprotein 1, zinc</td>
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<td>0.0195</td>
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<tr>
<td></td>
<td>Cyp2d9</td>
<td>Cytochrome P450, family 2, subfamily d, polypeptide 9</td>
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<td>0.0087</td>
</tr>
<tr>
<td></td>
<td>Mtp</td>
<td>Microsomal triglyceride transfer protein</td>
<td>−1.63</td>
<td>0.0010</td>
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<td>Hao3</td>
<td>Hydroxyl acid oxidase 3</td>
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<td>Acasa</td>
<td>Acetyl-coenzyme A acyltransferase 1B</td>
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<td>0.0013</td>
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<tr>
<td></td>
<td>Lpa</td>
<td>Lysosomal acid lipase A</td>
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<td>0.0013</td>
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<tr>
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<td>Acasa2</td>
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<td>0.0030</td>
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<td>Acasa1a</td>
<td>Acetyl-coenzyme A acyltransferase 1A</td>
<td>−0.85</td>
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<td>Hsd11b1</td>
<td>Hydroxysteroid 11-beta dehydrogenase 1</td>
<td>1.89</td>
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<td>Ptgsd</td>
<td>Prostaglandin D2 synthase</td>
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<td>Arsk</td>
<td>Arylsulfatase K</td>
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<td>Idi1</td>
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<td></td>
<td>Cyp51</td>
<td>Cytochrome P450, family 51</td>
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<td>0.0149</td>
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Log Fc indicates log of fold change.

Transcriptomic analyses with mouse Affymetrix microarrays were performed in kidneys of male control ($n=4$) and transgenic ($n=4$) mice at 3 months of age. The results from 8 independent experiments are shown. Genes selected by statistical criteria and included in the lipid metabolism function category according to the Gene Ontology Database and bibliographic references are listed in Table 2. $P$ values ($<0.025$) and log of fold change are shown for selected genes.

Figure 3. Real-time quantitative RT-PCR analysis. Differentially expressed genes from Table 1, including Cyp4a14 and Cyp2d9, and nonselected genes by statistical criteria (absent in Table 1) that included Cyp4a10 and Cyp4a12 were analyzed by real-time RT-PCR assays with genus-specific TaqMan probes. Results correspond to mean values of controls ($n=10$) and transgenic mice (Tg; $n=10$). Mean and SD are represented. $^{*}$Significant differences ($P=0.05$) between groups.
searched each gene manually in PubMed and assigned to each gene a category that we considered accurately described its biological function.

The main categories corresponded to lipid metabolism (14.8%), cell proliferation (15.8%), cell adhesion and migration (19.8%), renal tubular transport (8.9%), immune responses (11.8%), protein metabolism (10.8%), response to stress (5.9%), and genes of unknown function (11.8%; supplemental Figure I). Because genes included in the lipid metabolism category are relevant to the results reported here,

Figure 4. Hemodynamic analysis of KAP transgenic (Tg) mice. A, Tail-cuff measurements of systolic (SAP) and diastolic (DAP) arterial pressure in L-49 transgenic mice and control male littermates. Results for this and the following figures correspond to mean values of controls (n=4) and transgenic mice (n=8) at 6 to 8 months of age. B, Radiotelemetric measurements of SAP, DAP, and mean arterial pressure (MAP) of control and L-49 transgenic mice. C, Tail-cuff measurements of SAP and DAP in control (n=7) and L-59 transgenic (n=7) mice. D, Tail-cuff measurements of SAP and DAP in control (n=4 and 7) and transgenic mice (n=8 and 7) from L-49 and L-59, respectively, recorded from 2 up to 10 months of age. Mean and SEM are represented. *Significant difference (P<0.05, 2-way ANOVA) between groups.

Figure 5. Urinary excretion of 20-HETE and effects of HET0016 administration on blood pressure. A, Urinary excretion of 20-HETE in transgenic (Tg; n=6) and control (n=6) littermates between 6 and 8 months of age and effect of specific 20-HETE synthesis inhibitor HET0016 in same animals. Data are shown as mean±SEM. B, Effects of 20-HETE synthesis inhibitor HET0016 administration on systolic (SAP) and diastolic (DAP) arterial pressure levels in control (n=6) and transgenic (n=6) mice from Figure 5A. Data are shown as mean±SEM. *Significant difference between transgenic and control mice; #significant difference between HET0016- and vehicle-treated animals (P<0.05, 2-way ANOVA).
they are shown in Table 1, in which the log of fold change for each gene is also indicated. Among these, Cyp4A14 and Cyp2D9 were selected for further confirmation of differences by real-time RT-PCR assays in the general population, which included animals from both transgenic founders (Figure 3A and 3B, respectively). Two more genes, Cyp4A10 (Figure 3C) and Cyp4A12 (Figure 3D), which also fall into this category but are not shown in Table 1, were used as negative controls. Figure 3 shows the bona fide results of the microarrays and the statistical analysis performed because all genes selected proved to be expressed as predicted when analyzed by RT-PCR.

Hemodynamic Analyses of KAP Transgenic Mice

Of the genes shown in Table 1, we were interested in the downregulation of the androgen-sensitive Cyp4a monoxygenase gene, because knockout mice for this cytochrome display a hypertensive phenotype. Tail-cuff measurements of systolic and diastolic arterial pressure in KAP transgenic mice of line 49 showed these animals to be hypertensive (Figure 4A). Data were confirmed in this founder line by telemetric measurements (Figure 4B), and results were further reproduced in animals from the L-59 founder (Figure 4C) with the tail-cuff technique. Sequential measurements of systolic and diastolic arterial pressure in transgenic and control mice of each founder line revealed that hypertension appeared in transgenic mice at 4 months of age compared with controls. Systolic and diastolic arterial pressure continued to increase from 4 to 6 months, stabilizing between 7 and 10 months of age, in both transgenic and control mice (Figure 4D). The present data demonstrated that mice from 2 independent transgenic founders became hypertensive in a time-related manner.

Because the role of the Cyp4A cytochrome family in the pathophysiology of hypertension is mediated in part by the production of vasoconstrictor metabolites such as 20-HETE through the \( \gamma \)-HSL hydroxylation of arachidonic acid, we determined 20-HETE levels in urine of transgenic and control mice and demonstrated that urinary excretion of 20-HETE was significantly higher in transgenic mice (Figure 5A). Moreover, treatment of the animals with N-hydroxy-N-(4-n-butyl-2-methylphenyl) formamidine (HET0016), a potent and selective inhibitor of 20-HETE synthesis, in renal microsomes resulted in almost complete elimination of this metabolite in urine (Figure 5A). HET0016-treated mice also revealed a significant decrease in systolic and diastolic arterial pressure in transgenic mice (Figure 5B), which indicates that KAP overexpression is associated with increased 20-HETE production and thus with increments in mean arterial pressure.

Oxidative Stress

We measured the urinary excretion of 8-iso-PGF\(_{2\alpha}\), thiobarbituric acid reactive substances, and 8-hydroxyguanosine
together with mtDNA damage and malondialdehyde production in kidney tissues of control and transgenic mice. Urinary excretion of 8-iso-PGF$_2\alpha$, 8-hydroxyguanosine, and thiobarbituric acid reactive substances was increased significantly in transgenic mice (Figure 6A) and correlated with greater oxidative damage in kidneys of transgenic mice compared to control mice, as assessed by mtDNA damage (Figure 6B, left) and malondialdehyde levels (Figure 6B, right). mtDNA is more sensitive to oxidative stress than nuclear DNA and serves as a useful biomarker for reactive oxygen species–associated disturbances in DNA. These results support the concept that KAP overexpression-induced hypertension is associated with increased oxidative stress, which is caused, at least in part, by the kidney. Treatment of transgenic and control mice with a specific inhibitor of NADPH oxidase, apocynin, lowered both systolic and diastolic arterial pressure in transgenic mice (Figure 6C), which indicates that reactive oxygen species might be produced from NADPH oxidase in this model of hypertension.

Antioxidant Enzyme Activities

The results of antioxidant enzyme activity in kidneys of transgenic and control littermates shown in Figure 7 indicate that although activities of total, mitochondrial, or cytoplasmic superoxide dismutase were similar between controls and transgenic mice (Figure 7A), catalase (Figure 7B), total glutathione peroxidase, and selenium-dependent glutathione peroxidase (Figure 7C) activities were significantly reduced in transgenic mice.

Figure 7. Antioxidant enzyme activity. Activity of total superoxide dismutase (SOD T), manganese-superoxide dismutase (Mn-SOD), and CuZn superoxide dismutase (SOD CuZn; A), catalase (CAT; B), and total (GSH-Px) and selenium-dependent glutathione peroxidase (SeGSH-Px; C) in kidneys of male transgenic and control mice. All samples were analyzed in duplicate, and results of 8 animals per group are depicted. Mean and SD are represented. *Significant difference between transgenic and control mice (P < 0.05, Mann–Whitney U test).

### Table 2. Renal Function Analyses of Male Transgenic and Control Mice at 6 to 8 Months of Age

<table>
<thead>
<tr>
<th></th>
<th>Control (n=5)</th>
<th>KAP Tg (n=7)</th>
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<tbody>
<tr>
<td>Creatinine clearance, mL/24 h</td>
<td>0.63±0.17</td>
<td>0.56±0.12</td>
</tr>
<tr>
<td>Urinary glucose, mg/24 h</td>
<td>0.072±0.012</td>
<td>0.114±0.017*</td>
</tr>
<tr>
<td>Urinary protein, mg/24 h</td>
<td>9.36±1.66</td>
<td>14.01±2.35*</td>
</tr>
<tr>
<td>BUN, mg/dL</td>
<td>41.63±4.05</td>
<td>45.36±3.37</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>0.41±0.01</td>
<td>0.43±0.01</td>
</tr>
<tr>
<td>Blood potassium, mmol/L</td>
<td>9.49±1.06</td>
<td>10.31±0.35</td>
</tr>
</tbody>
</table>

Tg indicates transgenic; BUN, blood urea nitrogen.

The results of antioxidant enzyme activity in kidneys of transgenic and control mice, as assessed by mtDNA damage (Figure 6B, left) and malondialdehyde levels (Figure 6B, right), support the concept that reactive oxygen species–associated disturbances in DNA. These results also indicate that reactive oxygen species might be produced from NADPH oxidase in this model of hypertension.

### Discussion

KAP transgenic mice represent a unique model to observe the effects of KAP overexpression in the absence of androgen manipulations, thereby avoiding a generalized activation of the androgenic response that would mask KAP-specific effects. In this regard, a major finding of the present study is that KAP overexpression mimics the effects of androgens on transcriptional downregulation of cytochromes Cyp4A14 and Cyp2D9 (16 α-OH), which are associated with the hypertensive phenotype. These data suggest that KAP might constitute a downstream mediator of some of the effects attributed to androgens in arterial pressure.

A role for cytochrome P450 (Cyp) 4A arachidonic acid ω/ω-1 hydroxylases in the pathophysiology of hypertensive rats was first suggested by McGiff in the spontaneously hypertensive rat (SHR)/Wistar-Kyoto rat model. More recently, Holla et al uncovered a link between Cyp4A expression, 20-HETE synthesis, and the development of androgen-induced hypertension. Additional studies in rats
treated with 5α-dihydrotestosterone confirmed this link and provided additional evidence for a causal relationship between Cyp4A expression, 20-HETE synthesis, and the development of androgen-induced endothelial dysfunction and hypertension. Moreover, 5α-dihydrotestosterone had minimal effects on the activity of liver microsomal arachidonic acid ω/ω-1 hydroxylase or epoxygenase, which indicates that the androgen effects on arachidonic acid metabolism are kidney specific.33

Treatment of KAP transgenic mice with the highly specific 20-HETE synthesis inhibitor HET0016 reduces urinary 20-HETE levels and normalizes mean arterial pressure levels, which indicates that 20-HETE plays a major role in the hypertensive phenotype shown by these animals. Singh et al34 reported that administration of HET0016 prevented 5α-dihydrotestosterone–induced hypertension in male Sprague-Dawley rats, thereby strongly implicating 20-HETE production in the development of androgen-induced hypertension.

Increased 20-HETE production has been related to increased oxidative stress. The present data demonstrated a relationship between increased oxidative stress, hypertension, and 20-HETE production in KAP transgenic mice. Although increased generation of superoxide and augmented expression of NADPH oxidase in rats overexpressing Cyp4A had been reported previously,35 a direct demonstration of reactive oxygen species production by the Cyp4A-derived vasoconstrictor 20-HETE itself has only been described recently in bovine pulmonary arterial endothelial cells.36 Those authors

Figure 8. Histological and pathological assessment of KAP transgenic kidneys. A, Histological examination. Frozen kidney sections from male control and transgenic (Tg) mice were stained with Masson’s trichrome, hematoxylin-eosin (H-E), and periodic acid-Schiff (PAS) reaction at 6 to 8 months of age. Transgenic mice showed afferent arteriole dilatation and focal glomerulosclerosis compared with control mice (left). B–D, Glomerulosclerosis assessment. B, Laser-scanning confocal analysis showing collagen IV expression in representative kidney sections of male control and transgenic mice at 6 to 8 months of age; C, Western blots of crude kidney homogenates from male transgenic and control littermates were hybridized with anti-collagen I and anti-collagen IV, fibronectin, and anti-α-smooth muscle actin. Blots were reprobed with anti-α-tubulin for loading control. Ratios are expressed as arbitrary units. D, Scanning electron microscopy of vascular corrosion casts showing representative affected glomeruli of male control and transgenic mice at 6 to 8 months of age. For all experiments, at least 6 different animals were used per group.
claimed that 20-HETE stimulates reactive oxygen species production, at least in part, through activation of NADPH oxidase. This multimolecular enzyme represents the major source of superoxide anion that leads to increased oxidative stress in cardiovascular tissues.37 This is compatible with our finding that inhibition of NADPH oxidase with apocynin normalizes blood pressure in KAP transgenic mice.

There is much evidence supporting the role of increased oxidative stress in the pathophysiology of arterial hypertension. It has been reported that renal production of anion superoxide is higher in SHR than in normotensive rats38 and that increasing oxidative stress in male SHR rats with molsidomine further increases blood pressure.39 The same effects have been described in normotensive animals when superoxide dismutase is inhibited.40 Moreover, it has been reported that oxidative stress and the hypertensive response to superoxide scavengers is higher in male than in female SHR rats. The causative role for renal NADPH oxidase–dependent oxidative stress in the development of hypertension in male SHR rats37 has been demonstrated to be mediated by androgens.41 KAP transgenic mice show indirect evidence of increased oxidative stress, such as increased urinary excretion of oxidative stress markers and a marked hypertensive effect of the NADPH oxidase inhibitor apocynin. We found it very interesting that mechanisms underlying KAP-induced hypertension are reminiscent of those attributed to androgens.

The increased oxidative stress found in transgenic mice was not only related to a putative increased activity of NADPH oxidase, through increased 20-HETE and superoxide anion production, but also to the observed significant deficiencies in antioxidant glutathione peroxidase and catalase activity in kidney. KAP transgenic mice also showed glomerulosclerosis and proteinuria, which are frequently associated with hypertension.

In conclusion, these data demonstrate that specific KAP overexpression in proximal tubule cells is associated with hypertension, Cyp4A regulation, increased 20-HETE synthesis, and increased reactive oxygen species production, likely because of augmented NADPH oxidase activity and diminished antioxidant enzyme activity in kidney of transgenic mice. Hypertension appears to be caused by increased oxidative stress and increased 20-HETE production. Because activation of these pathways and their final outcome have been classically attributed to androgens, we postulate that KAP itself is, at least in part, a mediator of the androgen-dependent effects on the cardiovascular system. We report for the first time that a slight overexpression of a kidney-specific and androgen-regulated protein is associated with hypertension and renal disease.

Acknowledgments

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

These data demonstrate for the first time a key role of the kidney androgen-regulated protein (KAP) in the regulation of responses outside the kidney, and they link the function of KAP with hypertension. The present study provides one of the first mechanistic insights to explain the higher prevalence of hypertension in a sexually dimorphic manner on the basis of a molecule expressed exclusively in kidney that is also able to affect the cardiovascular system. It also connects KAP with oxidative stress, another key mechanism of cardiovascular disease and hypertension. In summary, these data may shed some light on the gender-related molecular mechanisms underlying essential hypertension, an increasingly prevalent disease in highly developed countries that remains elusive at the pathophysiological level, and they suggest KAP as a possible target for therapeutic interventions.
Kidney Androgen-Regulated Protein Transgenic Mice Show Hypertension and Renal Alterations Mediated by Oxidative Stress


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