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Overexpression of cytochrome P450 4F2 in mice increases 20-hydroxyeicosatetraenoic acid production and arterial blood pressure

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Cytochrome P450 4F2 (CYP4F2) activity is thought to be a factor in the pathogenesis of hypertension through its bioactive metabolite 20-hydroxyeicosatetraenoic acid (20-HETE). We previously found that a gain-in-function CYP4F2 variant in a Chinese cohort was associated with elevated urinary 20-HETE and hypertension. To further explore this association we generated a transgenic mouse model expressing CYP4F2 driven by a modified mouse kidney androgen-regulated protein promoter. This heterologous promoter regulated the expression of luciferase and his-tagged CYP4F2 in transfected HEK 293 cells. In the kidney of transgenic mice, CYP4F2 was localized to renal proximal tubule epithelia and was expressed at a higher level than in control mice, leading to increased urinary 20-HETE excretion. Assessment of CYP4F2 activity by an arachidonic acid hydroxylation assay showed that 20-HETE production was significantly higher in kidney microsomes of transgenic mice compared to control mice, as was their systolic blood pressure. There was a positive correlation of blood pressure with urinary 20-HETE levels. Our results show that increased expression of CYP4F2 in mice enhanced 20-HETE production and elevated blood pressure.

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The human cytochrome P450 4F2 (*CYP4F2*) gene (MIM no. 604426) encodes an ω-hydroxylase that catalyzes the arachidonic acid (AA) to 20-hydroxyeicosatetraenoic acid (20-HETE).[1] Previous studies have demonstrated that 20-HETE participates in the development of hypertension by regulating vascular and renal tubular functions.[2–5] On one hand, 20-HETE increases peripheral vascular resistance by potently constricting small arteries or by sensitizing the vascular smooth muscle cells to constrictor and myogenic stimuli. On the other hand, 20-HETE causes natriuresis by inhibiting sodium reabsorption. Recently, we found that a functional haplotype of *CYP4F2* with increased transcriptional activity was associated with elevated urinary 20-HETE and hypertension in a Chinese population.[6] A similar result was reported that the variant V433M in *CYP4F2* was associated with the increase of 20-HETE excretion and systolic blood pressure (SBP) in a white cohort.[7] However, a different result showed that the variant F434S in *CYP4A11*, another human 20-HETE synthase gene, with reduced catalytic activity was associated with hypertension in white populations.[8] Therefore, *CYP4F2* transgenic approach would be a pivotal and irreplaceable strategy to clarify the correlation between 20-HETE and blood pressure.

Heterologous promoters have been utilized to target transgene expression to expected tissues at controllable level in transgenic models. Native CYP4F2 is expressed at a high level in the proximal tubules of the human kidney where it accounts for the majority of renal 20-HETE production.[1] Mouse kidney androgen-regulated protein (KAP) is one of the most abundant proteins expressed in the proximal tubules in response to different hormones such as androgen and estrogen.[9] Experimental evidence from many previous transgenic models has shown that a 1542-bp fragment of the KAP promoter could successfully regulate transgene expression in the kidney.[10–14] Moreover, Soler's recent study has documented that a 224-bp truncated fragment of the KAP promoter was sufficient to drive androgen-dependent transactivation in the kidney.[15] Herein, we chose the truncated KAP promoter to highlight kidney-dominant and inducible expression of CYP4F2 in transgenic mice.

This study aims to develop a transgenic mouse model expressing CYP4F2 under the control of the heterologous KAP promoter, and to shed light on the impact of 20-HETE on blood pressure through *CYP4F2* gain-in-function.

RESULTS

KAP promoter activity in HEK293 cells

To identify the KAP promoter activity, we amplified the 224-bp fragment from 5′-flanking of the *KAP* gene to construct pKAP-*LUC* and pKAP-*CYP4F2*/his expression vectors. The expression of luciferase and his-tagged CYP4F2 was detected after transient transfection into HEK293 cells. As illustrated in Figure 1a, the luciferase activity of cells transfected with the pKAP-*LUC*, after being normalized to *Renilla* luciferase activity, was threefold over those with the pGL3-enhancer control. Western blot analysis for CYP4F2 expression in cells transfected with the pKAP-*CYP4F2*/his (Figure 1b) showed 2.6-fold of cross-reacting signal over the pGL3-enhancer control using anti-CYP4F2 antibody, wherein endogenous CYP4F2 was included. The recombinant CYP4F2 expression was further verified by anti-his antibody. These results confirmed the 224-bp KAP promoter activity in driving the expression of the heterologous *CYP4F2* gene in HEK293 cells.

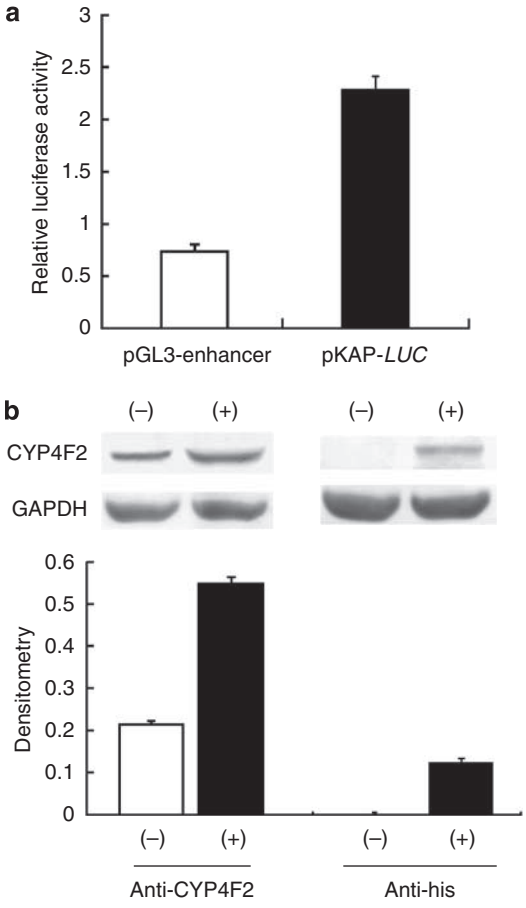

Figure 1 | KAP promoter activity in transfected HEK293 cells. (**a**) Relative luciferase activity assay. Using the Dual Luciferase Reporter assay system, the relative luciferase activity is presented as the ratio of luciferase activity of firefly to that of *Renilla* as internal control. (**b**) Western blot analysis of CYP4F2 expression. CYP4F2 level was determined by anti-CYP4F2 antibody and anti-his antibody in cells transfected with the pKAP-*CYP4F2*/his (+) and the pGL3-enhancer (–). The 53-kD band represented CYP4F2 expression, and the 36-kD GAPDH was shown as internal control. Data are presented as mean ± s.d. All experiments were performed three times independently.

Generation of *CYP4F2* transgenic mice

The linearized pKAP-*CYP4F2*/his (Figure 2a) was microinjected into FVB/N mouse to generate transgenic mice. We routinely screened the mice by PCR (data not shown) for the integration of *CYP4F2* using specific primers, and further evaluated the presence of the transgene by Southern blot analysis (Figure 2b). Three transgenic founders, F0-6 (female), F0-16 (female), and F0-56 (male), were successfully bred to establish independent transgenic mouse lines. There was no difference in the number of offsprings between male and female, indicating the *CYP4F2* gene may insert into the autosomes of the three founders. Line F0-16 seemed to be less reproducible and thereby the number of positive transgenic pups per brood was fewer than the others.

CYP4F2 expression profile in transgenic mice by Western blot analysis

CYP4F2 was expressed in all kidney samples of the three transgenic lines, among which line F0-16 exhibited the highest expression level (Figure 3a). Detailed expression profile of female and male transgenic offspring in line F0-16 was illustrated in Figure 3b and c, respectively, demonstrating a wide expression spectrum of CYP4F2. Densitometry scanning (Figure 3d) revealed that the highest expression level was in the kidney of male mice, about 2.5-fold over female littermates. In addition, the extra-renal expression of CYP4F2 was prominent in reproductive organs. These results

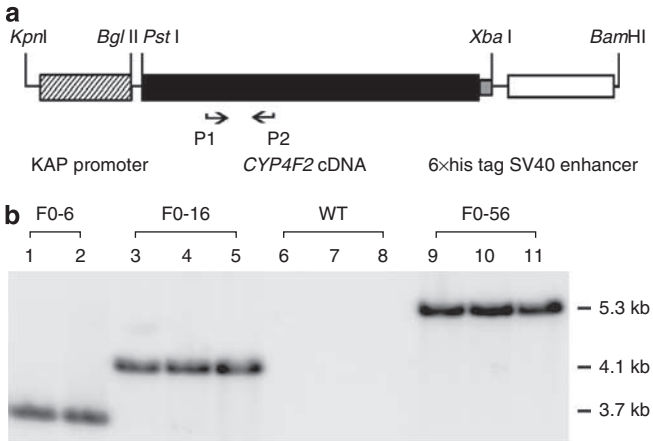

Figure 2 | Schematic map of the linear pKAP-CYP4F2/his and Southern blot analysis of CYP4F2 in transgenic mice. (**a**) The fragment for microinjection contained the 224-bp KAP promoter, *CYP4F2* cDNA, 6 × his tag, and SV40 enhancer. Arrow-indicated P1 and P2 are the primers specific for *CYP4F2* to screen positive mice by PCR. (**b**) Southern blot analysis. The hybridization bands of tail DNA with specific *CYP4F2* probe confirmed the integration of the transgene in the three transgenic lines. WT: wild-type.