A Functional Common Polymorphism of the ABCB1 Gene Is Associated With Chronic Kidney Disease and Hypertension in Chinese

Ming Liu, Yan Li, Lorena Citterio, Qi-Fang Huang, Wei-Fang Zeng, Chang-Sheng Sheng, Fang-Fei Wei, Qian Dong, Ge-Le Li, Yuan-Yuan Kang, Lu Zhang, Ting-Yan Xu, Jing-Jing Li, Jie Song, Paolo Manunta, and Ji-Guang Wang

BACKGROUND
Permeability glycoprotein (P-gp) is encoded by the ATP-binding cassette B1 gene (ABCB1) and is an effluxer of toxic metabolites in the kidney. A functional common polymorphism (C3435T, rs1045642) in the human ABCB1 gene has been found to be associated with allograft outcome in kidney transplant patients. In this study, we investigated the association of the C3435T polymorphism with renal function and blood pressure (BP) in 2 Chinese populations.

METHODS
The discovery and replication populations were recruited from a mountainous area (Zhejiang Province) and a newly urbanized suburban area (Shanghai), respectively. We genotyped all subjects using the ABI SNapShot method. Chronic kidney disease (CKD) was defined as an estimated glomerular filtration rate <60 ml/min × 1.73 m² or 24-hour urinary albumin excretion ≥30 mg.

RESULTS
In the discovery population of 1,987 subjects, after adjustment for covariables, TT homozygosity (n = 217) was associated with a higher risk of CKD (n = 369; odds ratio [OR] = 1.73; P = 0.003) and with higher systolic BP (+3.1 mm Hg; P = 0.03) and pulse pressure (+3.4 mm Hg; P = 0.001). These associations were dependent on age (P < 0.05). In subjects aged ≥60 years (n = 374), the corresponding OR or difference was 2.40 for CKD, 15.1 mm Hg for systolic BP, and 12.4 mm Hg for pulse pressure (P < 0.001). In similar adjusted analyses in the replication population of 2,427 elderly (≥60 years) subjects, TT homozygosity was also associated with a higher risk of CKD (OR = 1.39; P = 0.02) and an enhanced association of hypertension with CKD (OR = 1.50; P = 0.04).

CONCLUSIONS
The ABCB1 C3435T polymorphism might predict CKD, especially in the elderly.

Keywords: ABCB1; albuminuria; blood pressure; chronic kidney disease; genetic polymorphism; hypertension; renal function.

doi:10.1093/ajh/hpt126

A common synonymous polymorphism (C3435T, rs1045642) in the human ABCB1 gene has been identified functional and influences the expression, activity, and function of the protein and the interindividual variation in the response for various substrates, such as digoxin and cyclosporine. Several though not all11-13 studies in renal transplant patients demonstrated that the minor T allele of this polymorphism was associated with nephrotoxicity related to calcineurin inhibitors, especially cyclosporine, and with kidney allograft outcome. Further studies suggested that this association was only apparent with the C3435T genotype of the donor but not of the recipient and in the presence of older donor age.

Correspondence: Ji-Guang Wang (jiguangwang@aim.com).

Initially submitted April 15, 2013; date of first revision June 12, 2013; accepted for publication July 5, 2013; online publication August 7, 2013.
Because P-gp in the kidney is an extruder of both endogenous and exogenous metabolites, we hypothesize that the C-to-T substitution at the ABCB1 C3435T polymorphic site might predispose to the development of chronic kidney disease (CKD) in the general population. Because CKD is a known risk factor of hypertension and one of the substrates of P-gp, ouabain, is known to cause hypertension, we further hypothesize that this polymorphism might also be associated with hypertension. In this population-based study, we therefore investigated the association of the ABCB1 C3435T polymorphism with renal function and blood pressure while accounting for age.

METHODS

Study populations

Our study was conducted in a general (age range = 12–87 years) and an elderly population (≥60 years) as the discovery and replication samples, respectively. The study protocol was approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiaotong University School of Medicine, China. All participants gave informed written consent. The discovery population was recruited in the framework of an ongoing, longitudinal, population-based genetic study in hypertension. From 2003 to 2008, we visited all homes in 14 villages randomly selected from JingNing County, a mountainous rural area approximately 500 km south of Shanghai. We invited all inhabitants aged ≥12 to take part. Of the 2,734 invited, 2,091 (76.5%) participated. Of these 2,091 participants, we excluded 101 subjects from this analysis because of missing information on genotype (n = 67) and phenotype (n = 37). Thus, the total number of subjects of the discovery population was 1,987.

The replication population was recruited in the framework of the Chronic Disease Detection and Management in the Elderly (≥60 years) Program supported by the municipal government of Shanghai. In a newly urbanized suburban town, 30 km from the city center, we invited all residents aged ≥60 years to take part in comprehensive examinations of cardiovascular disease and risk. A total of 2,580 subjects (participation rate = 90%) were enrolled in the period from 2006 to 2007. Of these 2,580 participants, we excluded 153 subjects because of missing information on genotype (n = 101) and phenotype (n = 52). Thus, the total number of subjects of the replication population was 2,427.

Field work

In the discovery population, 1 experienced physician measured each participant’s blood pressure 5 times consecutively by mercury sphygmomanometry after the subject had rested for at least 5 minutes in the sitting position. These 5 blood pressure readings were averaged for analysis. The same observer also administered a standardized questionnaire to collect information on medical history, smoking and drinking habits, and the use of medications. A trained technician measured body height and body weight. Venous blood samples were taken after overnight fasting for the measurement of plasma glucose and serum creatinine concentrations. Serum creatinine was measured by the Jaffe kinetic method. Estimated glomerular filtration rate (eGFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. Twenty-four-hour urine was collected in a wide-neck plastic container for the measurement of urinary albumin and creatinine, using immunoturbidimetry and enzymatic methods, respectively.

In the replication population, the field work was slightly different. Blood pressure was measured 3 times consecutively by the use of the Omron HEM-7051 device (Omron Health Care, Kyoto, Japan). Twenty-four-hour urine was not collected.

Hypertension was defined as a blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic or as the use of antihypertensive drugs. Diabetes mellitus was defined as a plasma fasting glucose of at least 7.0 mmol/L or the use of antidiabetic agents. Microalbuminuria was defined as a 24-hour urinary albumin excretion of 30–299 mg/g. CKD was defined as the presence of microalbuminuria or as an eGFR <60 ml/min × 1.73 m².

Genotyping

The ABCB1 C3435T polymorphism was genotyped using the ABI PRISM SNapShot method (Applied Biosystems, Foster, CA). Briefly, the SNapShot reaction was carried out in a 10-μl final volume containing SNapShot Multiplex Ready Mix (5 μl), primer mix (0.02–0.6 μmol/L), and templates (4 μl) consisting of the multiplex polymerase chain reaction (PCR) products, which had been purified with the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). The cycling program included 25 cycles of 94 °C for 30 seconds, 57 °C for 30 seconds, and 72 °C for 40 seconds. Extension products were purified by a 15-minute incubation with 1 U of shrimp alkaline phosphatase (Promega, Madison, WI) at 37 °C and a subsequent 15-minute incubation at 80 °C to inactivate the enzyme. The purified products (0.5 μl) were mixed with 9 μl of formamide and 0.5 μl of GeneScan-120 LIZ Size Standard (Applied Biosystems) and separated by capillary electrophoresis (ABI PRISM310 Genetic Analyzer; Applied Biosystems). The results were analyzed with GeneMapper 3.0 software (Applied Biosystems). The sense and antisense primers were 5′-CCTGTTTGTACGCGACATGG-3′ and 5′-GCAATGATCTGCGACTCTCCTT-3′, respectively. More than 97% of the total samples were successfully genotyped for all single nucleotide polymorphisms. To confirm the genotyping results, 30 samples were randomly selected and regenotyped by direct sequencing using a BigDye terminator (Applied Biosystems). All assays were 100% concordant.

Statistical methods

We used SAS version 9.1.3 (SAS institute, Cary, NC) for database management and statistical analyses. Comparisons of means and proportions relied on the Student t test and
Table 1. Characteristics of the study populations

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Discovery (n = 1,987)</th>
<th>Replication (n = 2,427)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men, no. (%)</td>
<td>942 (47.4)</td>
<td>1,026 (42.3)</td>
</tr>
<tr>
<td>Age, y</td>
<td>45.5 ± 15.2</td>
<td>70.9 ± 7.2</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.3 ± 2.9</td>
<td>23.3 ± 3.6</td>
</tr>
<tr>
<td>Current smoking, no. (%)</td>
<td>589 (29.6)</td>
<td>551 (22.7)</td>
</tr>
<tr>
<td>Alcohol intake, no. (%)</td>
<td>862 (43.4)</td>
<td>364 (15.0)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>126.3 ± 23.7</td>
<td>138.4 ± 19.7</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>76.5 ± 12.4</td>
<td>80.7 ± 10.6</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>49.9 ± 17.9</td>
<td>57.7 ± 14.5</td>
</tr>
<tr>
<td>Hypertension, no. (%)</td>
<td>543 (27.3)</td>
<td>1,462 (60.2)</td>
</tr>
<tr>
<td>Took antihypertensive drugs, no. (%)</td>
<td>169 (8.5)</td>
<td>990 (40.8)</td>
</tr>
<tr>
<td>Plasma glucose, mmol/L</td>
<td>4.51 ± 1.02</td>
<td>5.42 ± 1.22</td>
</tr>
<tr>
<td>Diabetes mellitus, no. (%)</td>
<td>30 (1.5)</td>
<td>155 (6.4)</td>
</tr>
<tr>
<td>Serum creatinine, μmol/L</td>
<td>88.3 ± 25.5</td>
<td>97.4 ± 24.5</td>
</tr>
<tr>
<td>eGFR, ml/min × 1.73 m²</td>
<td>81.9 ± 23.9</td>
<td>60.9 ± 17.1</td>
</tr>
<tr>
<td>eGFR &lt;60 ml/min × 1.73 m², no. (%)</td>
<td>353 (17.8)</td>
<td>1,229 (50.6)</td>
</tr>
<tr>
<td>eGFR &lt;45 ml/min × 1.73 m², no. (%)</td>
<td>63 (3.2)</td>
<td>452 (18.6)</td>
</tr>
<tr>
<td>Microalbuminuria, no. (%)</td>
<td>32 (2.7)</td>
<td>—</td>
</tr>
<tr>
<td>Chronic kidney disease, no. (%)</td>
<td>369 (18.6)</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are mean ± SD or number of subjects (%). Hypertension was defined as a blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic or the use of antihypertensive drugs. Diabetes mellitus was defined as a plasma fasting glucose of at least 7.0 mmol/L or the use of antidiabetic agents. Estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration formula.\[22,23\] Urinary albumin excretion was measured in 1,184 subjects (n = 582 men) enrolled in the discovery study. Microalbuminuria was defined as a 24-hour urinary albumin excretion of 30–299 mg/g. Chronic kidney disease was defined as the presence of microalbuminuria or as an eGFR <60 ml/min × 1.73 m². “—” indicates no data for analysis.

RESULTS

Characteristics of the discovery population

The 1,987 participants (mean ±SD age = 45.5 ± 15.2 years) included 942 men (47.4%), 543 (27.3%) hypertensive patients, 353 (17.8%) patients with an eGFR <60 ml/min × 1.73 m², 32 (2.7%) patients with microalbuminuria, and 369 (18.6%) patients with CKD (Table 1).

Genotype frequencies of the discovery population

Genotype frequencies of the ABCB1 C3435T polymorphism did not deviate from the Hardy–Weinberg equilibrium (CC = 42.7%, CT = 46.4%, and TT = 10.9%; \(P = 0.15\)) (Table 2). Genotype frequencies differed according to the presence and absence of eGFR <60 ml/min × 1.73 m² (\(P = 0.01\)), microalbuminuria (\(P = 0.02\)), and CKD (\(P = 0.004\)). Although the difference in genotype frequencies between hypertensive and normotensive subjects did not reach statistical significance (\(P = 0.06\)), the frequency of the
Table 2. Genotype frequencies

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Discovery</th>
<th>Replication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC 707 (43.3)</td>
<td>473 (39.5)</td>
</tr>
<tr>
<td></td>
<td>CT 765 (46.8)</td>
<td>588 (49.1)</td>
</tr>
<tr>
<td></td>
<td>TT 162 (9.9)</td>
<td>137 (11.4)</td>
</tr>
</tbody>
</table>

Values are number of subjects with percentage of the column total in the parentheses. Estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration formula. Microalbuminuria was defined as a 24-hour urinary albumin excretion of 30–299 mg/g. Chronic kidney disease was defined as the presence of microalbuminuria or as an eGFR <60 ml/min x 1.73 m². Hypertension was defined as a blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic or the use of antihypertensive drugs.

3435T allele was significantly higher in hypertensive subjects than normotensive subjects (36.9% vs. 33.1%; P = 0.02).

ABCB1 polymorphism and renal function in the discovery population

After adjustment for age, sex, body mass index, current smoking, alcohol intake, diabetes mellitus, and the use of antihypertensive drugs, TT homozygotes, compared with C allele carriers, had significantly higher serum creatinine (96.2 vs. 87.3 µmol/L; P < 0.001), lower eGFR (77.7 vs. 82.4 ml/min x 1.73 m²; P = 0.003) (Table 3), and higher risk of eGFR <60 ml/min x 1.73 m² (OR = 1.62; 95% CI = 1.11–2.37; P = 0.01) (Figure 1) or 45 ml/min x 1.73 m² (OR = 7.19; 95% CI = 3.74–13.85; P < 0.001), microalbuminuria (OR = 2.83; 95% CI = 1.24–6.45; P = 0.01) and CKD (OR = 1.73; 95% CI = 1.20–2.50; P = 0.003).

These genetic associations were dependent on age (P values for the C3435T x age interaction ≤ 0.05). eGFR declined with age significantly faster in TT homozygotes than the CC and CT subjects (Figure 2). In the 374 subjects aged ≥60 years, eGFR was 12.9 ml/min x 1.73 m² (95% CI = 6.8–19.1; P < 0.001) lower in TT homozygotes than C allele carriers. The corresponding OR in TT homozygotes vs. C allele carriers was 2.16 (95% CI = 1.09–4.28; P < 0.03) for eGFR <60 ml/min x 1.73 m²; 5.18 (95% CI = 2.45–10.93; P < 0.001) for eGFR <45 ml/min x 1.73 m²; 5.08 (95% CI = 1.37–18.92; P=0.02) for microalbuminuria; and 2.41 (95% CI = 1.21–4.82; P = 0.01) for CKD. In this group of elderly people, the PAR of CKD to the TT genotype was 14%.

ABCB1 Polymorphism and blood pressure in the discovery population

With similar adjustments applied as above, TT homozygotes, compared with C allele carriers, had significantly higher systolic blood pressure (129.1 ± 1.3 vs. 126.0 ± 0.5 mmHg, P = 0.03) and pulse pressure (52.9 ± 1.0 vs. 49.5 ± 0.3 mm Hg; P = 0.001) (Table 3) and slightly and nonsignificantly higher risk of hypertension (OR = 1.21; 95% CI = 0.85–1.71; P = 0.29).

There was also significant interaction between the C3435T polymorphism and age in relation to systolic blood pressure (P < 0.001) (Figure 3), pulse pressure (P < 0.001), and the risk of hypertension (P = 0.05). In the 374 subjects aged ≥60 years, systolic blood pressure and pulse pressure were 15.1 mm Hg (95% CI = 6.6–23.5 mmHg; P < 0.001) and 12.4 mm Hg (95% CI = 5.9–18.8 mm Hg; P < 0.001) higher in TT homozygotes than C allele carriers. The corresponding OR for hypertension in TT homozygotes vs. C allele carriers was 2.17 (95% CI = 1.00–4.76; P = 0.05).

GEE analyses in the discovery population

The results of GEE analyses accounting for covariables as well as family structure were confirmatory. Briefly, in 1,802
subjects, TT homozygotes, compared with C allele carriers, had significantly higher serum creatinine concentration (94.1 vs. 85.5 μmol/L; \( P < 0.001 \)), lower eGFR (79.1 vs. 83.3 ml/min × 1.73 m\(^2\); \( P = 0.003 \)), and higher pulse pressure (51.9 ± 1.1 vs. 49.1 ± 0.4 mm Hg; \( P = 0.02 \)), and tended to have high systolic blood pressure (128.2 ± 1.3 vs. 125.6 ± 0.5 mm Hg; \( P = 0.07 \)).

**Replication population**

We performed a replication study in 2,427 elderly Chinese (Table 1). Frequencies of the TT genotype tended to be higher in the presence of reduced eGFR, especially when eGFR was <45 ml/min × 1.73 m\(^2\) (\( P = 0.05 \) vs. eGFR

**Table 3.** Renal function and blood pressure in relation to the \textit{ABCB1} C3435T polymorphism

<table>
<thead>
<tr>
<th></th>
<th>Discovery</th>
<th>Replication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC (n = 848)</td>
<td>CT (n = 922)</td>
</tr>
<tr>
<td>Serum creatinine, μmol/L</td>
<td>87.3 ± 0.8</td>
<td>87.3 ± 0.8</td>
</tr>
<tr>
<td>eGFR, ml/min × 1.73 m(^2)</td>
<td>82.4 ± 0.7</td>
<td>82.4 ± 0.7</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>126.0 ± 0.7</td>
<td>126.0 ± 0.6</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>76.4 ± 0.4</td>
<td>76.6 ± 0.4</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>49.6 ± 0.5</td>
<td>49.4 ± 0.5</td>
</tr>
</tbody>
</table>

Values are mean ± SE. The analysis was adjusted for age, sex, body mass index, current smoking, alcohol intake, diabetes mellitus, and the use of antihypertensive drugs. Estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration formula.\(^{22,23}\)
Figure 2. Association between estimated glomerular filtration rate (eGFR) and age according to the ABCB1 C3435T genotype in the discovery population. eGFR was calculated according to the Chronic Kidney Disease Epidemiology Collaboration formula.\(^{22,23}\) Symbols (○ CC, △ CT, and □ TT) and vertical lines denote mean value and SE per age subgroup, respectively. The analysis was adjusted for age, sex, body mass index, current smoking, alcohol intake, diabetes mellitus, and the use of antihypertensive drugs. The $P$ value for interaction ($P_{int}$) between the C3435T polymorphism and age is given. For each genotype, the number of participants is given alongside the symbols.

Figure 3. Association between systolic blood pressure and age according to the ABCB1 C3435T polymorphism in the discovery population. Symbols (○ CC, △ CT, and □ TT) and vertical lines denote mean value and SE per age subgroup, respectively. The analysis was adjusted for age, sex, body mass index, current smoking, alcohol intake, diabetes mellitus, and the use of antihypertensive drugs. The $P$ value for interaction ($P_{int}$) between the C3435T polymorphism and age is given. For each genotype, the number of participants is given alongside the symbols.
>60 ml/min × 1.73 m²), but did not differ between hypertensive and normotensive subjects (P = 0.91) (Table 2).

After adjustment for covariates, TT homozygotes, compared with C allele carriers, had a significantly higher serum creatinine concentration (mean difference = +6.3 μmol/L; 95% CI = 3.5–9.1 μmol/L; P < 0.001), lower eGFR (mean difference = −2.9 ml/min × 1.73 m²; 95% CI = −4.5 to −1.2 ml/min × 1.73 m²; P < 0.001) (Table 3), and higher risk of eGFR <60 ml/min × 1.73 m² (OR = 1.39; 95% CI = 1.06–1.91; P = 0.02), but had similar risk of hypertension (OR = 0.96; 95% CI = 0.75–1.24; P = 0.77).

Nonetheless, the prevalence of hypertension in patients with a reduced eGFR tended to be higher in TT homozygotes than in CC homozygotes, especially when eGFR was <45 ml/min × 1.73 m² (OR = 1.38; 95% CI = 1.00–1.91; P = 0.05) (Figure 4). The adjusted relative contribution of eGFR <45 ml/min × 1.73 m² to hypertension was significantly greater in TT homozygotes than in C allele carriers (OR = 1.50; 95% CI = 1.02–2.22; P = 0.04).

**DISCUSSION**

To the best of our knowledge, our study is the first that has demonstrated significant association of the ABCB1 C3435T polymorphism with CKD and hypertension at the population level. In our discovery population, TT homozygotes, compared with C allele carriers, had a 73% and 141% increase in the risk of CKD in all and elderly subjects, respectively. The corresponding genetic penetrance rate of CKD was 25.3% and 64.4%, respectively. In elderly subjects, TT homozygotes, compared with C allele carriers, also had 15.1 mm Hg higher systolic blood pressure, 12.4 mm Hg higher pulse pressure, and 117% higher risk of hypertension. In our replication population, which is much larger, more urban, and solely elderly, the results are confirmatory, but the size of the association is shrunk. Nonetheless, TT homozygotes, compared with C allele carriers, had a 39% increase in the risk of CKD and a 50% higher risk of hypertension that could be attributable to CKD. Our finding, if confirmed by other population studies, may have immediate clinical implications. Genetic determination of the ABCB1 gene 3435 C/T polymorphism in young age might predict late-onset CKD and hypertension and become an efficient reminder for the prevention of these serious chronic diseases.

Although there is solid and clear evidence that this synonymous variant is functional, and the results of previous studies in renal transplant patients are indicative of renal consequence of this genetic variation, our renal observations should be cautiously interpreted and require experimental and pathophysiological evidence. The risk of cyclosporine-related nephrotoxicity associated with this polymorphism suggests that exogenously ingested drugs that require active eflux by P-gp can cause renal damage in persons with a reduced extruding capacity from the kidney. In our population-based study, none of the study subjects took cyclosporine or other potentially harmful calcineurin inhibitors. We searched from the drugs commonly used in our study populations, such as pain killers and analgesics, and looked for compounds whose metabolism requires P-gp. We found that there is indeed a compound, acetaminophen, that...
which is a sole component or one of the components of many over-the-counter analgesics in China, and requires P-gp for metabolism. Acetaminophen has previously been found to be associated with renal damage,\textsuperscript{27–29} such as papillary necrosis.\textsuperscript{28} These drugs are inexpensive and hence more often used by rural Chinese who did not have health insurance until recently. The use of acetaminophen might partially explain the enhanced association between CKD and ABCB1 variant in our rural discovery population. In addition, our finding is in line with the major results of a previous Chinese study in end-stage renal disease.\textsuperscript{30} In 244 patients with a serum creatinine concentration of at least 442 μmol/L, Zhang et al. found that 37 TT homozygotes, compared with 92 CC homozygotes, had significantly higher serum creatinine (987.0 ± 512.0 vs. 753.8 ± 276.0 μmol/L; \( P < 0.05 \)).\textsuperscript{30}

In our study, the difference in blood pressure across the C3435T genotype became prominent in the late years of life, slightly behind the appearance of difference in renal function. We therefore hypothesize that high blood pressure might be a consequence of renal damage earlier in life. If this is the case, hypertension can be defined as a kind of CKD-associated hypertension that may be preventable by treating CKD. Nonetheless, other mechanisms cannot be excluded. For instance, ouabain, regardless of whether endogenously synthesized or exogenously ingested, has to be extruded by P-gp.\textsuperscript{31,32} Ouabain is known to cause hypertension in animal experiments\textsuperscript{17} and is associated with various cardiovascular disorders.\textsuperscript{33} We recently found that the ABCB1 C3435T polymorphism was associated with plasma levels of ouabain,\textsuperscript{34} and higher plasma levels of endogenous ouabain were associated with an increased risk of acute kidney injury after cardiac surgery.\textsuperscript{35}

In spite of consistent and convincing observations on the association of the C3435T polymorphism with renal function and blood pressure, it is still a question how retaining of a toxic metabolite due to reduced P-gp function would cause renal damage in the glomerulus. The results of a recent study are suggestive.\textsuperscript{36} In an elegant study of 290 Africans from 62 pedigrees, the 3435T allele was associated with significantly higher effective renal plasma flow (28.1 ml/min; \( P = 0.007 \)) and lower renal resistance (0.011 mm Hg/ml/min; \( P = 0.004 \)), indicating a phenomenon of hyperfiltration.\textsuperscript{36} This could be a biofeedback effect of reduced extruding of toxic metabolites in the renal tubules. Hyperfiltration in the long run will promote or accelerate the decay of renal function with aging. Nonetheless, it is also possible that retained toxic metabolites directly damage both glomeruli and renal tubules. Acute papillary necrosis in relation to the overdose of acetaminophen provided indirect evidence on this hypothesis.\textsuperscript{27} We observed similar genetic associations with reduced eGFR and albuminuria.

Our study should be interpreted within the context of its strengths and limitations. A major strength was that our findings were generally consistent between 2 different study populations. However, our study was cross-sectional, and hence does not allow causal inference. Our study included >4,000 subjects. However, according to the current standard, the sample size could not be considered sufficiently large. In addition, renal function was based on a single measurement of serum creatinine or urinary albumin excretion. Finally, a genetic association study can always be confounded by population stratification.

In conclusion, the ABCB1 C3435T polymorphism is associated with renal function and blood pressure, especially in the elderly. If confirmed in other study populations and prospective studies, genetic determination of the C3435T polymorphism might be useful in the prediction of CKD and in the definition of a form of CKD-associated hypertension. Future studies should investigate the role of several known substrates or inhibitors of P-gp, such as verapamil and acetaminophen, in the development of renal damage associated with the 3435TT genotype.

ACKNOWLEDGMENTS

We gratefully acknowledge the voluntary participation of all study subjects, the support of Jingning Bureau of Health (Jingning County, Zhejiang Province), and the technical assistance of the physicians and nurses of Zhaoxiang Community Health Centre (Qingpu District, Shanghai). We also appreciate the expert assistance of Jing-Ling Han, Bi-Hua Liu, Jie Wang, Li Zeng, and Yi Zhou (Shanghai Institute of Hypertension, China). This study was financially supported by grants from the National Natural Science Foundation of China (grants 30871360, 30871081, 81170245, and 81270373), the Ministry of Science and Technology (a grant for China-Europe Union collaborations (1012)), and the Ministry of Education (NCET-09-0544), Beijing China, the Shanghai Commissions of Science and Technology (grant 07JC14047, the “Rising Star” program 06QA14043 and 11QH1402000) and Education (grant 07ZZ32 and the “Dawn” project 08SG20), the Shanghai Bureau of Health (grants XBR2011004, Shanghai Jiaotong University School of Medicine (a grant of Distinguished Young Investigators to Yan Li), and the European Union (grants LSHM-CT-2006–037093 and HEALTH-F4-2007–201550).

DISCLOSURE

The authors declared no conflict of interest.

REFERENCES


