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Association of the P-Glycoprotein Transporter *MDR1*^{C3435T} Polymorphism with the Susceptibility to Renal Epithelial Tumors

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Abstract. Except for hereditary disease, genetic factors that contribute to the development of renal epithelial tumors are unknown. There is a possibility that the *MDR1* encoded plasma membrane transporter P-glycoprotein (PGP) influences the risk of development of renal neoplasms. PGP is known to be involved in uptake, binding, transport, and distribution of xenobiotics. There is evidence that the *MDR1*^{C3435T} polymorphism drives expression and modulates disease risk. In an explorational case-control study, constitutional genotype frequencies were established at *MDR1*^{C3435T} of 537 healthy control subjects and compared with those of 212 patients with renal epithelial tumors. There were 179 clear cell renal cell carcinoma (CCRCC) and 33 tumors collectively assigned as non-CCRCC. In a second study, genotypes of another 150 healthy control subjects and 50 patients with three non-CCRCC types (26 papillary RCC, 11 chromophobe RCC, and 13 renal oncocytic adenoma) were compared. PCR-restriction fragment length polymorphism–based analysis of constitutional DNA, and statistical analysis were applied. PGP expression was analyzed by quantitative immunohistochemistry. The explorational study showed a significant association between T allele frequency and the occurrence of tumors ($P = 0.007$). When tumors were histopathologically distinguished into frequent CCRCC and less frequent non-CCRCC, both patient groups contributed to this effect with a seemingly strong influence by the latter ($P = 0.0419$). The second study established the T allele as a risk factor especially for non-CCRCC ($P = 0.0005$) with the highest risk for homozygote TT allele carriers ($P < 0.0001$). Independently, *MDR1*^{C3435T} genotype associated variations in PGP expression were shown in normal renal parenchyma with a 1.5-fold difference of median values (TT, 1.9; CC, 2.8; $P = 0.0065$). The data provide evidence for PGP to influence the susceptibility to develop renal epithelial tumors by virtue of its *MDR1*^{C3435T} polymorphism and changes in expression. Especially T and TT carriers are at risk for developing non-CCRCC, *i.e.,* papillary and chromophobe RCC as well as oncocytic adenomas.

Renal epithelial tumors contribute approximately 3% to the overall cancer incidence and mortality. Renal cell carcinomas (RCC) compose clear cell RCC (CCRCC) in 75% to 80%, papillary (chromophilic) RCC in 10%, and chromophobe RCC in 5%. Others include granular cell carcinoma, spindle cell carcinoma, and duct Bellini and unclassified carcinomas (1). There are also benign oncocytic and papillary adenomas, which account for approximately 5% of all renal epithelial neoplasms. Although the molecular origins of these histologic subentities have been identified, *i.e.,* mutations and hypermethylations of the *VHL* and *RASSF*1A tumor suppressor genes in CCRCC (2–5), mutations of the *MET* proto-oncogene in papillary RCC (6,7), and loci for hereditary chromophobe RCC and oncocytic adenoma on chromosome 17p11.2 (8), their cause and interindividual differences in susceptibility remain elusive.

It is interesting that the incidence of renal tumors rises steadily by 2% to 4% per year in industrialized countries. This may be explained in part by exogenous carcinogens (*i.e.,* tobacco smoke), high-protein diet, and diuretic and antihypertensive drugs (9), which is in agreement with recent observa-

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tions of a possible link between carcinogen exposure, somatic mutations, and kidney cancer (10). On the constitutional level, polymorphic enzymes involved in the metabolism of xenobiotics have been discussed to modulate renal cancer risk (11,12). From this view, it may be inferred that transporter molecules involved in distribution, delivery, and penetration of environmental agents into cellular and subcellular compartments also may play a role in renal cancer risk (13,14).

As an example, P-glycoprotein (PGP) is a member of the ABC family of transporters that extrudes various hydrophobic drugs and peptides from the inside to the outside of the plasma membrane. This ATP-driven efflux transport mechanism involves binding to drugs (Vinca alkaloids, anthracyclines, and epipodophyllotoxins) (15). Although the physiologic role of PGP is not fully understood, it is conceivable that PGP may prevent intracellular accumulation of potentially toxic substances and metabolites (16). PGP is highly expressed in the apical membranes of organs with excretory function, such as liver, small intestine, and kidney (17,18), where it mediates transport for excretion of xenobiotics via the canalicular membrane of hepatocytes into the bile, via the luminal brush border membrane of enterocytes into the gut lumen, and via the luminal brush border membrane of proximal tubule cells into the urine (13). PGP is also abundant in many important epithelial barriers, including blood-brain, blood-nerve, blood-testis, and maternal-fetal, formed by placental trophoblasts (13,19,20).

PGP is encoded by the *MDR1* gene, which contains at least 15 polymorphic sequence changes. Recently, the wobble base *MDR1*^{C3435T} polymorphism in exon 26 has attracted attention as a possible modulator of health and disease. Although no functional consequence may be predicted, individuals homozygous for the T allele showed lower intestinal PGP expression and a higher intestinal uptake of the orally administered PGP substrate digoxin (21). There is a 24% to 29% prevalence of this genotype and phenotype in Caucasian individuals (21–23). In kidney cancer, high *MDR1* expression causes cancer cells to become refractory to treatment with chemotherapeutic agents, a phenomenon known as multidrug resistance (24,25). Furthermore, in patients who are infected with human immunodeficiency virus-1 (HIV-1), there is considerable variability in the response to antiretroviral therapy as a result of the *MDR1*^{C3435T} polymorphism (26). Because the level of *MDR1* expression limits drug penetration into pharmacologic sanctuaries, it is plausible to hypothesize that genetically driven low constitutive *MDR1* expression in the kidney may limit local detoxification of carcinogens. Thus, we sought to document a relationship between *MDR1*^{C3435T} genotype and expression and the risk of development of renal epithelial tumors.

Materials and Methods

Patients and Control Subjects

We established and analyzed data sets of two patient populations (Table 1). A first explorational case-control study included 212 unrelated patients who were treated for kidney tumors at German University hospitals Mannheim, Heidelberg, Mainz, and Munich. There were 212 Caucasian patients with renal epithelial tumors: 179 CCRCC and 33 non-CCRCC referred to us as granular (n = 9), papillary (chromophilic; n = 4), chromophobe (n = 6), and unclassified RCC (n = 4); oncocytic adenoma (n = 2); duct Bellini (n = 1); and others (n = 7). There were 121 CCRCC that had been previously analyzed for somatic *VHL* mutations (4). There were 69 women and 143 men. Age ranged from 28 to 89 yr (median, 62 yr).

Table 1. Samples analyzed in *MDR1*^{C3435T} genotyping

	First Study	Second Study
Caucasian control subjects	537	150
Total renal epithelial tumors	212	50
Total CCRCC	179	
Total non-CCRCC	33	50
granular	9	
papillary	4	26
chromophobe	6	11
unclassified	4	
duct Bellini carcinoma	1	
others	7	
oncocytic adenoma	2	13

For further investigation of non-CCRCC, a second case-control study included 50 unrelated Caucasian patients with non-CCRCC–type renal epithelial tumors with a confirmed histopathologic diagnosis of papillary (chromophilic) RCC (n = 26), chromophobe RCC (n = 11), and oncocytic adenoma (n = 13). There were 32 men, 9 women, and 9 patients without known gender. Age ranged from 28 to 80 yr (median, 60 yr).

Genotypes of unrelated, seemingly healthy subjects of two control groups were randomly collected as described previously and used for comparison with patients (23). For avoiding confounding by mixed ethnicity, only individuals of Caucasian origin were included. First, 537 individuals were used for the initial explorational study in which patients and control subjects were matched 1:2.5 (Table 1). Age ranged from 17 to 62 yr (median, 25 yr). Second, an additional 150 individuals were used for the second case-control study, in which patients and control subjects were matched 1:3.0 (Table 1). Age ranged from 19 to 60 yr (median, 25 yr).

DNA Isolation and Genotyping Analysis

Constitutional DNA was isolated from blood samples according to standard procedures. In cases for which blood could not be obtained, constitutional DNA was isolated from normal kidney parenchyma of surgical specimens. Genotyping of the C3435T polymorphism was carried out by PCR-restriction fragment length polymorphism and denaturing high-performance liquid chromatography (DHPLC) analyses as described previously (27).

PGP Expression Analyses

Quantitative immunohistochemistry with mouse monoclonal antibody JSB-1 (Roche, Mannheim, Germany) was performed on 2.5-μm tissue sections of noncancerous renal tissues of 33 patients homozygous for the *MDR1*^{3435} C allele and of 52 patients homozygous for the T allele (21). Microscopic inspection showed normal morphology and no indication of malignancy. Antibody dilution was 1:10 with a final concentration of 5 μg/ml. Slides were stained with diaminobenzidine (Vectastain ABC complex) and counterstained with hematoxylin and eosin (Merck, Darmstadt, Germany). For quantification of stained

slides, an image analysis workstation (Histoanalyzer, Institute of Physical Electronics, University of Stuttgart, Stuttgart, Germany) was used, and ratios of specific signals of tubuli to background were calculated as described previously (28–30). Tissues were subjected to blinded analysis by an independent investigator. Snap-frozen tissue for RNA isolation and Western blot analysis was not available.

Statistical Analyses

An association between *MDR1* genotypes and kidney tumor risk was calculated from contingency tables using χ^2 statistics. When appropriate, Fisher's exact test was applied. Odds ratios (OR) appeared with 95% confidence intervals (CI) and two-sided *P*. For all calculations, GraphPad Instat software (version 3.0; GraphPad Software, San Diego, CA) was used. $P < 0.05$ was considered statistically significant. In cases of testing of an association between control subjects and patient subgroups, hierarchical testing corrected according to Bonferroni was applied using the software SSPS (version 10.1; SPSS Inc., Chicago, IL). For PGP expression analysis, median values of two groups (TT and CC) were compared using the Mann-Whitney *U* test.

Results

Frequencies of MDR1^{C3435T} *Genotypes in Caucasian Control Subjects*

MDR1^{C3435T} allelotype and genotype frequencies were established for control groups of 537 and 150 Caucasian individuals. In the first group, the number of T alleles was 540 and the number of C alleles was 534. Frequencies of genotypes were 25.9% for CC, 47.7% for CT, and 26.4% for TT (Table 2). In the second group, the number of T alleles was 147 and the number of C alleles was 153. Frequencies of genotypes were 26% for CC, 50% for CT, and 24% for TT. All frequencies ranged within calculated CI and matched those previously reported for the Caucasian population (21–23).

Frequencies of MDR1^{C3435T} *Genotypes in Renal Epithelial Tumors*

We tested the constitutional DNA of 212 patients with renal epithelial tumors for *MDR1*^{C3435T} genotypes. This group of patients showed a moderate but significant increase of the frequency of the T allele (OR, 1.3; $P = 0.007$; CI, 1.1 to 1.7) and for the TT genotype (OR, 1.8; $P = 0.0098$; CI, 1.2 to 2.9; Table 2). When the data were stratified according to histopathologic tumor type considering the two major classes (CCRCC and others collectively assigned to non-CCRCC), frequencies for T were increased by an OR of 1.3 ($P = 0.028$; CI, 1.0 to 1.7) and for TT by an OR of 1.7 ($P = 0.0299$; CI, 1.1 to 2.8) in patients with CCRCC. For non-CCRCC, frequencies for T were increased by an OR of 1.7 ($P = 0.0419$; CI, 1.0 to 2.9) and for TT by an OR of 2.5 ($P = 0.073$; CI, 0.9 to 6.5), indicating a stronger influence to the overall result.

Given the low number of non-CCRCC in this study and their histopathologic diversity (Table 1), the data of an association between *MDR1* genotype and the risk for non-CCRCC needed to be explored further. For this purpose, we extended *MDR1* genotyping to a second group of 50 patients who had been previously established for a collection of papillary (chromophilic) and chromophobe RCC as well as oncocytic adenoma (4). For avoiding multiple testing, allelotypes and genotypes of this patient group were compared with a second control group of seemingly healthy subjects. Frequency and statistical data of these analyses are given in Table 3. The

Table 2. Case-control study on frequencies of *MDR1*^{C3435T} allelotypes and genotypes of patients with renal epithelial tumors

Control Subjects and Patients	Total	Genotypes and Frequencies[b]			Comparison of Frequencies of Allelotypes or Genotypes			
					T *versus* C		TT *versus* CC	
		CC	CT	TT	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Caucasian	537	139	256	142				
		25.9%	47.7%	26.4%				
		22.2 to 29.8	43.4 to 52.0	22.8 to 30.4				
Renal epithelial tumors[a]	212	39	100	73	1.3	0.007	1.8	0.0098
		18.4%	47.2%	34.4%				
		13.4 to 24.3	40.3 to 54.1	28.1 to 41.3	1.1 to 1.7		1.2 to 2.9	
CCRCC	179	33	88	58	1.3	0.028	1.7	0.0299
		18.4%	49.2%	32.4%				
		13.0 to 24.9	41.6 to 56.7	25.6 to 39.8	1.0 to 1.7		1.1 to 2.8	
non-CCRCC	33	6	12	15	1.7	0.0419	2.5	0.073
		18.2%	36.4%	45.4%				
		7.0 to 35.5	20.4 to 54.9	28.1 to 63.7	1.0 to 2.9		0.9 to 6.5	

[a] Renal epithelial tumors were composed of frequent CCRCC and less frequent tumors collectively assigned as non-CCRCC.
[b] Genotypes and frequencies are given in total numbers, in percentages, and with binomial 95% CI.

Table 3. Second case-control study on frequencies of *MDR1*^{C3435T} allelotypes and genotypes in patients with histopathologically defined rare renal epithelial tumors

Control Subjects and Patients	Total	Genotypes and Frequencies[b]			Comparison of Frequencies of Allotypes or Genotypes			
					T *versus* C		TT *versus* CC	
		CC	CT	TT	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Caucasian control subjects	150	39	75	36				
		26%	50%	24%				
		19.2 to 33.8	41.7 to 58.3	17.4 to 31.7				
Non-CCRCC patients[a]	50	1	29	20	2.3	0.0005	21.7	<0.0001
		2%	58%	40%				
			43.2 to 71.8	26.4 to 54.8	1.4 to 3.8		2.8 to 169.9	
I. RCC	37	0	23	14	2.3	0.0026	31.4	0.0002
		0%	62.2%	37.8%	1.3 to 4.0		1.8 to 545.6	
Ia: Papillary RCC	26	0	15	11	2.6	0.0040	24.9	0.0008
			57.7%	42.3%	1.4 to 4.9		1.4 to 438.0	
Ib: Chromophobe RCC	11	0	8	3	1.8	0.2817	7.6	0.2403
			72.7%	27%	0.7 to 4.5		0.4 to 134.0	
II. Oncocytic adenomas	13	1	6	6	2.2	0.0725	6.5	0.1096
		7.7%	46.1%	46.1%	1.0 to 5.2		0.8 to 56.7	

[a] Non-CCRCC are distinguished into RCC (I) and oncocytic adenomas (II). RCC are further subdivided into papillary (chromophilic) RCC (Ia) and chromophobe RCC (Ib).

[b] Genotypes and frequencies are given in total numbers, in percentages, and with binomial 95% CI.

frequency of the T allele increased greater than twofold considering all 50 patients (OR, 2.3; $P = 0.0005$; CI, 1.4 to 3.8), for the subgroup of combined papillary and chromophobe RCC (OR, 2.3; $P = 0.0026$; CI, 1.3 to 4.0), and for the subgroup of papillary RCC (OR, 2.6; $P = 0.004$; CI, 1.4 to 4.9). OR dramatically increased and were highly significant when frequencies of homozygous T carriers were compared with those of homozygous C carriers. This applied to the group of all 50 non-CCRCC (OR, 21.7; $P < 0.0001$; CI, 2.8 to 169.9), the combined group of papillary and chromophobe RCC (OR, 31.4; $P = 0.0002$; CI, 1.8 to 545.6), and the subgroup of papillary RCC (OR, 24.9; $P = 0.0008$; CI, 1.4 to 438.0). Likewise, homozygote C carriers had a 10-fold reduced risk of developing any rare renal epithelial tumor and an even 25-fold reduced risk of developing papillary or chromophobe RCC (not shown).

The numbers for chromophobe RCC and oncocytic adenoma were low, and OR did not reach statistical significance (Table 3). However, it is noteworthy that among patients with papillary and chromophobe RCC, no single CC homozygote carrier was identified. Similarly, among patients with oncocytic adenoma, there was only one CC carrier, indicating a clear underrepresentation of this genotype.

In the group of CCRCC with moderate but significant increase of T allelotype and genotype, we further stratified the data according to the *VHL* mutation status of the patients' tumors. There were 45 patients with and 76 patients without somatic *VHL* mutations (4). Patients without somatic *VHL* mutations showed a T allele frequency by an OR of 1.4 ($P = 0.0459$; CI, 1.0 to 2.0) and patients with somatic *VHL* mutations by an OR of 1.6 ($P = 0.0615$; CI, 1.0 to 2.4). When genotypes were compared, CCRCC patients whose tumors did not carry a *VHL* mutation showed a significant increase in TT genotype frequency by an OR of 2.5 ($P = 0.025$; CI, 1.1 to 5.56; not shown).

PGP Expression

Quantitative immunohistochemistry was performed on noncancerous renal tissues of individuals homozygous for *MDR1*^{3435} C and T alleles. Immunohistochemical staining of renal parenchyma (Figure 1A) of individuals homozygous for *MDR1*^{3435} C showed markedly higher PGP expression than those for *MDR1*^{3435} T homozygotes. Calculated expression values varied from 0.9 to 6.2 (Figure 1B). The difference in PGP expression levels of 52 TT and 33 CC individuals was 1.5-fold (TT, 1.9; CC, 2.8) and statistically significant ($P = 0.0065$), indicating lower PGP expression in TT homozygous individuals.

Discussion

As excretory organs, the kidneys are committed to the detoxification and excretion of water-soluble metabolites and carcinogens. During the process of plasma concentration, tubular cells may prevent carcinogens and metabolites of the glomerular filtrate from back-diffusion into the plasma volume by active transport mechanisms. Similar to its protective role at many biologic barriers, PGP as a plasma membrane efflux pump may be involved in the clearance of carcinogens via the

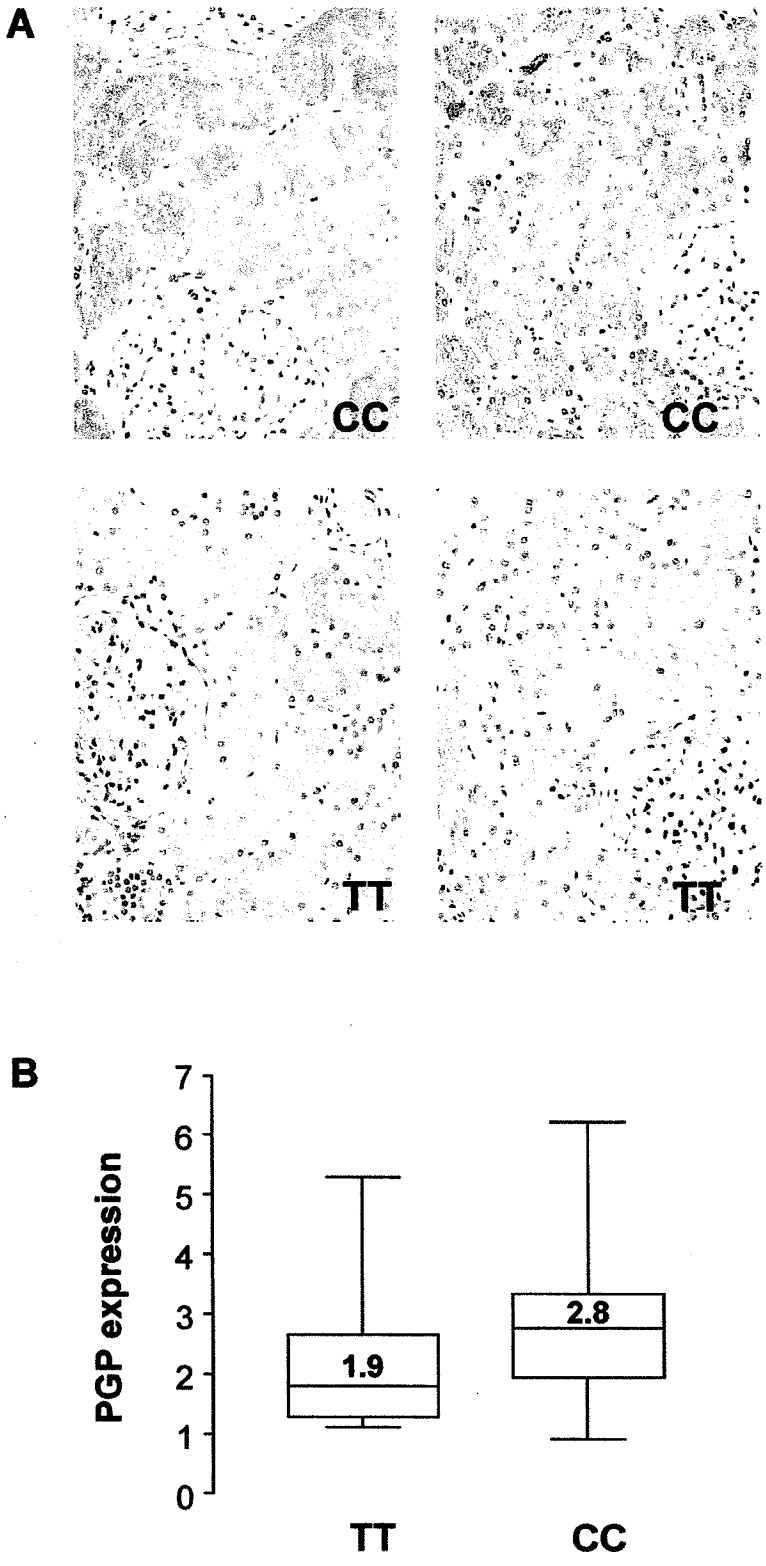

Figure 1. Variable P-glycoprotein (PGP) expression in kidney parenchyma. (A) Immunohistochemically stained tissue sections with anti-PGP antibody JSB-1. (Top row) Individuals with a *MDR1*^{3435} CC genotype. (Bottom row) Individuals with *MDR1*^{3435} TT genotype. PGP tubular expression varies between carriers of CC and TT genotypes with markedly stronger signals in tubuli of CC carriers. (B) Levels of PGP expression are blotted for 52 individuals with TT and 33 individuals with CC genotypes and ranged between 0.9 and 6.2. The boxes represent the distribution of the 25th to 75th percentiles, and the bars represent the 5th to 95th percentiles. Mean values are given at respective positions within the boxes. The difference of median expression values between TT (1.9) and CC (2.8) carriers is 1.5-fold ($P = 0.0065$). Magnification, ×250.

brush border of the tubular lumen and be critical in the processes of reabsorption and secretion.

For understanding a possible role of PGP in the maintenance of renal physiology, it is important to reconsider the key findings of *MDR1* genotype/phenotype relationship in human intestine. In duodenal mucosa, the degree of *MDR1* expression and activity varied significantly among individuals with different constitutional genotypes of the $MDR1^{C3435T}$ polymorphism (21). Individuals with a CC genotype had significantly higher levels of functional PGP than TT homozygous individuals or CT heterozygotes. Recently, $MDR1^{C3435T}$-associated variable PGP expression and function was also shown for peripheral blood mononuclear cells (26,27). Unlike the gut and lymphocytes, the normal kidney is not accessible to *ex vivo* studies of *MDR1* expression; therefore, any prediction or conclusion on functional variations giving rise to renal epithelial tumors may be assessed only from constitutional *MDR1* genotypes and expression studies with archived tissue.

We established $MDR1^{C3435T}$ genotypes of patients with renal epithelial tumors and compared the frequencies of allelotypes and genotypes to those of apparently healthy control subjects. When these frequencies were compared with those of the patient group, we observed a significant disequilibrium with respect to a higher T prevalence in patients. Histopathologically, their tumors were represented by the six major subtypes of renal epithelial tumors. When the data were stratified according to tumor type, it became clear that both main groups, *i.e.,* frequent CCRCC and also infrequent tumors collectively assigned to non-CCRCC, contributed to this significant result. Although the latter represent a heterogeneous group of renal epithelial neoplasms, they were grouped together for the purpose of statistical analysis.

The first study group included only a few non-CCRCC because of their rare occurrence. Because these also represented a heterogeneous group of rare benign and malignant tumors, it became necessary to conduct a second study with a larger number of patients with histopathologically confirmed diagnosis of papillary (chromophilic) RCC, chromophobe RCC, or renal oncocytic adenoma. Genotype analysis of 50 patients confirmed the highly significant prevalence of the T allele in non-CCRCC renal epithelial tumors, which now led us to conclude that the T allele was associated with an increased risk for these tumors. Homozygote T carriers significantly showed a 22-fold increased overall risk, a 31-fold increased risk for carcinomas, which was 25-fold for papillary RCC. For the subentities of chromophobe RCC and oncocytic adenoma, the numbers failed to reach statistical significance probably as a result of overall low numbers. From this association between T allele and risk, we may likewise predict a protective effect of the C allele, which was confirmed to be 25 times reduced for C allele carriers with respect to papillary or chromophobe RCC.

Our data, although with more moderate results, further suggest a 1.7-fold increased risk for CCRCC in homozygote T carriers. CCRCC is the major malignant renal neoplasm and is frequently associated with somatic alterations in the *VHL* tumor suppressor gene. *VHL* affected tissues suffer from the disruption of a regulatory pathway for controlled protein degradation, a molecular defect that initiates approximately 50% of CCRCC (4,31,32). It is interesting that a significant association between CCRCC and homozygous $MDR1^{3435}$ T allele carriership was limited to the group of patients without somatic *VHL* alterations. Although there is evidence that these carcinomas may develop as a result of other somatic changes, such as silencing of the *RASSF1A* tumor suppressor (5), our data point to a role of the constitutional *MDR1* genotype in CCRCC susceptibility. Accordingly, non-*VHL*–driven CCRCC may preferably develop on the basis of an $MDR1^{3435}$ TT genotype, a hypothesis that is also in agreement with different environmental carcinogens potentially involved in *VHL*-associated and non-*VHL*–associated CCRCC (11,12).

With respect to these association studies, it is important to note that our control subjects were not age and gender matched. However, we feel that these data are sound for the following reasons. Sample sizes were sufficient and patients and control subjects were matched for ethnicity. We deliberately avoided age matching because evidence for age dependence of drug metabolizing enzyme polymorphisms is missing and control subjects should be free of disease, which is difficult to realize in the age groups of sporadic kidney cancer. We consider these control subjects adequate especially because our $MDR1^{C3435T}$ genotype frequency data matched those of the Caucasian population established in independent large-scale frequency studies (21–23).

For interpreting our results further, it is important to consider the functional consequences of the $MDR1^{C3435T}$ polymorphism. As a result of its wobble base location, there shall be no expected change in PGP function (21). However, expression experiments first conducted in the gut mucosa and lymphocytes (21,26,27) and now established for renal epithelial tissue showed reduced PGP expression for $MDR1^{3435}$ TT carriers. Although there is a possibility that variations in PGP expression may not directly be caused by $MDR1^{C3435T}$ but rather be linked to some other polymorphism within *MDR1* or elsewhere in the genome, and despite evidence from the *MDR1* knockout mouse that other renal transporters may compensate for PGP deficiency (33), our data suggest that $MDR1^{C3435T}$ genotypes will be useful to predict the relative degree of renal PGP expression and tumor risk. Functional changes as a result of *MDR1* variations were first observed in tumors in which increased expression was associated with multidrug resistance and poor prognosis (24,25). Although the phenomenon of multidrug resistance is not fully understood, a common feature of multidrug-resistant tumor cells is the expression of PGP. Thus, it is believed that multidrug resistance is conveyed by PGP-mediated increased drug efflux that removes drugs from the cell before they have a chance to exert their cytotoxic effects (34). PGP displays a broad substrate specificity that includes chemotherapeutics and other lipophilic compounds. There is experimental evidence that the major determinant of the ability of a given substance to be transported by PGP may be its relative hydrophobicity (35). Thus, although little is known about renal tissue–specific carcinogens, it seems plausible that individuals with a lower PGP efflux pump function in

normal cells are less protected from potential carcinogenic effects of intracellular accumulated lipophilic substances with PGP sensible characteristics.

To explain the relative high risk for the development of non-CCRCC in *MDR1*^{3435} TT carriers, we may further view this genotype/phenotype relationship with respect to the linear organization of the renal tubular system. It is important to note that CCRCC and papillary RCC develop from the proximal tubule responsible for the bulk reabsorption of isosmotic fluid, and chromophobe RCC and oncocytic adenoma are said to develop from the cortical portion of the collecting duct (36). PGP expression so far has been described only for proximal tubular cells but not for the collecting duct epithelia (18). Our genetic and expression data support the notion that any normal decrease in PGP expression along the renal tubular system may in addition be subject to genetically driven modulation. Individuals with an *MDR1*^{3435} TT genotype may be compromised in renal PGP expression and protective mechanisms against toxic agents in the various tubular segments. Given any critical carcinogen challenge, a physiologically low and in addition genetically determined below-low expression of PGP at the brush border of proximal tubular cells may explain an increased susceptibility to papillary RCC and some CCRCC, respectively. This effect would be most severe at the distal tubular segment and the collecting duct with the physiologically lowest PGP expression and may explain the associated high risk for chromophobe RCC and oncocytic adenoma. Although our study provided evidence for an association between the *MDR1* TT genotype and the relative risk of development of renal epithelial neoplasms, there is a possibility that this might also be explained by a defect in an *MDR1*-linked renal cancer–causing gene. However, it is noteworthy that our view of *MDR1*^{C3435T} modulating the risk of development of renal epithelial tumors is in agreement with the known low incidence of RCC in the sub-Saharan African population and the observed low frequencies of 0% to 6% of *MDR1*^{3435} TT genotypes (23,37,38).

In summary, we provided evidence for PGP to influence the natural history of especially rare renal epithelial tumors by virtue of its *MDR1*^{C3435T} polymorphism and changes in expression. In light of recent findings that PGP is part of a protective barrier against both bacteria and viral particles, that it protects CD4$^+$ cells from HIV-1 infection, and that there is a significant benefit for *MDR1*^{3435} TT HIV1 patients in response to antiretroviral therapy (13,26,39), our study underscores the role of *MDR1* as a critical modulator of health and disease and adds significantly to its role in renal tumor susceptibility.

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