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The role of genetic polymorphisms of the Renin–Angiotensin System in renal diseases: A meta-analysis

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ABSTRACT

Renal failure has a complex phenotype resulting from an underlying kidney disease as well as environmental and genetic factors. In the present study we performed a systematic review and meta-analyses to evaluate the association of the A1166C polymorphism of Angiotensin II type 1 Receptor gene (AGTR1) with Chronic Kidney Disease (CKD), End Stage Renal Disease (ESRD), IgA Nephropathy (IgAN) and Vesicoureteral Reflux (VUR) as well as the association of A1332G polymorphism of Angiotensin II type 2 Receptor (AGTR2) gene with Vesicoureteral Reflux (VUR). We found that neither AGTR1 A1166C, nor AGTR2 A1332G polymorphisms were significantly associated with any of the aforementioned renal diseases, suggesting that they cannot be used as predictive markers in either general or subgroup ethnic populations.

1. Introduction

Chronic Kidney Disease (CKD) is a global public health problem reaching high prevalence and demanding elevated health costs. It is characterized by a slow, progressive and irreversible decline of renal function; it is usually asymptomatic and thus untreated [1]. National Kidney Foundation guidelines classify the severity of Chronic Kidney Disease in five stages. Stage 5 CKD is often called End Stage Renal Disease (ESRD) and is characterized by severe illness with poor life expectancy if untreated. However, ESRD is a complex disorder with a variety of phenotypes emanating from a variety of underlying kidney disorders in conjunction with genetic and environmental factors as well as other preexisting or secondary clinical entities [2]. Treatment in ESRD is renal replacement which encounters dialysis or kidney transplantation [3]. Persons at high risk predominantly suffer from diabetes mellitus or hypertension [4]. Nevertheless, many common adult-onset kidney disorders may be due to various risk-alleles and to interactions between various genes and gene–environment interactions [5].

Immunoglobulin A Nephropathy (IgAN), where IgA deposits are found in the glomerular mesangial area, is the most common form of glomerulonephritis world-wide and leads to ESRD in about 20% of the cases [6,7]. Vesicoureteral Reflux (VUR) is a form of Congenital Anomaly of the Kidney and Urinary Tract (CACUT) [8]. It is a very common urological cause of renal insufficiency in children, culminating to ESRD in children, adolescents, and young adults, which is potentially preventable [9].

The Renin–Angiotensin System (RAS) influences sodium balance, extracellular fluid (ECF) volume, and renal and systemic vascular resistance. Thus, the RAS serves as one of the most powerful regulators of arterial blood pressure [10]. The primary effector molecule of this system is angiotensin II (ANG II) and is formed after two cleavage steps by Renin and Angiotensin Converting Enzyme (ACE). The ANG II mediates its actions via two G protein-coupled receptors, the Angiotensin II type 1 Receptor (AGTR1) and Angiotensin II type 2 Receptor (AGTR2) [10,11].

ANG II binds to AGTR1 and induces systemic vasoconstriction, a situation that leads to elevated peripheral resistance, and ultimately increases blood pressure. Arterial hypertension (HT) is frequently associated with chronic renal failure, and it is the most important risk factor for the progression of renal failure. In summary, RAS proteins convey the response of the kidneys to effective circulating volume thus regulating salt and water handling by the kidney. This fine-tuned molecular balance may be adversely influenced by a genetically mediated variability of RAS protein variants, leading to early damage of the cardiovascular or renal organ systems [10].

Although yet quite complex, there is strong evidence of genetic susceptibility in renal failure [5,10,12]. In the present study, we attempted to clarify the genetic association of polymorphisms of the angiotensin receptors with renal diseases and discus the possibility that these polymorphisms may be used as prognostic markers for renal failure.

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2. Materials and methods

2.1. Literature search

A comprehensive literature search until November 2012 was performed and 30 independent studies were retrieved that could fulfill all the eligible criteria. The keywords that were used for the search were: AGTR, AGTR1, AGTR1B, AGTR2, 'ANGIOTENSIN RECEPTOR', 'ANGIOTENSIN II RECEPTOR', GENE, VARIANT, POLYMORPHISM, MUTANT, MUTATION, ALLELE, 'CHRONIC KIDNEY DISEASE', 'KIDNEY FAILURE' 'END-STAGE KIDNEY DISEASE', 'END-STAGE RENAL DISEASE', 'END-STAGE RENAL FAILURE', DIALYSIS, 'IgA GLOMERULONEPHRITIS', 'IgA NEPHROPATHY', 'VESICOURETERAL REFLUX', VUR and combinations of them. To enrich the investigation, references of published studies were incorporated.

2.2. Data extraction

Data extraction from each study was performed by two reviewers according to the eligibility criteria. All problems of poor agreement, when they occurred, were resolved after discussion with a third investigator and the necessary data were stratified in spreadsheet. The following data were extracted from each study: Pubmed ID, first author's name, year of publication, geographical location and ethnicity of population studied, and total number of the subjects (cases and control groups). The distributions of alleles and genotypes were calculated in cases and controls for each study and are shown in Tables S1–S5. When a case–control study was designed according to a family based model, the family-trio model was encountered that distinguishes between affected offspring and non-affected parents (controls) and analysis was performed according to the transmission disequilibrium test (TDT) [13].

2.3. Statistical analysis

Odds ratio (OR) was used as the effect size of choice to test the association between the mutant alleles or genotypes (as defined in each polymorphism case), and the disease phenotypes. In case of a zero cell, a continuity correction was applied by adding 0.5 to all cells of the contingency table. Data were combined using a random-effects method [14] with inverse-variance weights, and ORs were calculated along with their 95% CIs for each genotype or allele contrast. The between study heterogeneity was evaluated using the chi-square based Cochran's Q statistic and the consistency index (I^2) [15].

The multivariate random-effects method of meta-analysis was also applied as a more advanced method for testing gene–disease associations. In this framework, the two summary log-odds ratios related to the risk allele, e.g. the log-odds ratio of heterozygotes vs. homozygotes (AB vs. AA) and the log-odds ratio of homozygotes for the risk allele vs. homozygotes for the wild type allele (BB vs. AA), are modeled simultaneously as a bivariate response. This method has several important properties, since it can infer and quantify the genetic model of inheritance directly, by estimating the ratio λ of the two log-odds ratios [16–19]. This way, we avoid multiple testing and thus the inflation of the Type I error rate. Stata 10 (StataCorp) was the statistical package that was used for all the analyses. Results with p-value <0.05 were considered statistically significant.

To estimate possible publication bias, the rank correlation method of Begg and Mazumdar [20] was used. Additionally, the fixed effects regression method of Egger was also recruited [21]. Influential meta-analysis was further performed, by removing an individual study each time, and re-calculating the effects estimates (ORs) and heterogeneity. In order to identify a possible trend of the combined estimate over years, a condition that often introduces a special kind of bias ("Proteus phenomenon"), cumulative meta-analysis was also performed. Time-trend was detected using two methods: the standard cumulative meta-analysis [22–24] approach, where we visually inspect the plot, and a more recently proposed regression-based method [25].

3. Results

A literature search was performed to identify all studies assessing the association of *Angiotensin II type 1 Receptor* (*AGTR1*) and *Angiotensin II type 2 Receptor* (*AGTR2*) gene polymorphisms with renal diseases. Meta-analyses were performed for the polymorphisms for which at least three studies were found. Polymorphisms of both genes related to disease phenotypes along with the number of studies identified and numbers of patients and controls included in each meta-analysis are shown in Table 1.

3.1. A1166C polymorphism of AGTR1 gene

In a meta-analysis to test the putative association of the A1166C (rs5186) polymorphism of the *AGTR1* gene with ESRD 109 studies were retrieved. Nevertheless, only 17 studies were included [26–42] that fulfilled the selection criteria and comprised of 2596 patients and 3866 controls. One study [32] had a family based design, (trio), and it was analyzed with the transmission disequilibrium test (TDT) according to the method presented in [13].

The characteristics of each study are shown in Table 2A, while details about alleles and genotypes are shown in Table S1. No statistical significant association was found for the per-allele contrast since OR was 1.10 with 95% CI: 0.91–1.34. Similarly, non-significant association was found when dominant and recessive models were analyzed (CC + AC vs AA: OR 1.15, 95% CI: 0.92–1.44 and CC vs AA + AC: OR 1.31, 95% CI: 0.83–2.07, Table 3). Meta-analysis in subgroups according to race did not yield any significant association (data not shown). Similarly, when meta-analysis was restricted to studies in Hardy–Weinberg Equilibrium (HWE) no significant associations were found (data not shown).

In all three meta-analyses heterogeneity was high since p-value <0.05 and I^2 > 50% (Table 3), while no publication bias was observed (p-value >0.05 for all tests). Furthermore, Proteus phenomenon was not detected in cumulative meta-analysis for the AA vs AC + CC contrast, while for the A vs C and the CC vs AA + AC contrasts a time trend was obvious (Table 4). Influential meta-analysis was also performed and showed that no individual study influenced the effect estimate (data not shown).

After that, a meta-analysis was carried out to test the association of the same polymorphism (*AGTR1* A1166C) with Chronic Kidney Disease (CKD). From the 109 studies only eight were found eligible to provide data for 812 patients and 4252 healthy subjects [36–38,40,42,44–46]. The characteristics of all studies are shown in Table 2B and numbers of alleles and genotypes in Table S2.

Table 1
Polymorphisms of *AGTR1* and *AGTR2* genes studied for their association with renal diseases.

Disease	Gene	SNP	Patients/controls	Number of studies
ESRD	AGTR1	A1166C/rs5186	2596/3866	17
ESRD	AGTR1	C521T		1
ESRD	AGTR1	A1138T		1
ESRD	AGTR1	AG214CC		1
CKD	AGTR1	A1166C/rs5186	812/4252	8
CKD	AGTR1	C573T		1
CKD	AGTR1	C521T		2
CKD	AGTR1	A1138T		1
CKD	AGTR1	AG214CC		1
CKD	AGTR1	G163A		2
CKD	AGTR2	A1332G/rs5194		1
IgAN	AGTR1	A1166C/rs5186	785/1373	5
VUR	AGTR1	A1166C/rs5186	174/216	3
VUR	AGTR2	A1332G/rs5194	352/790	3

Table 2A
Characteristics of studies included in the meta-analysis for the association of *AGTR1* A1166C polymorphism with ESRD.

Study	Year	Country	Race	Cases	Diagnostic criteria	Controls	Diagnostic criteria
Zsom M	2011	Hungary	Caucasian	134	ESRD with primary glomerulonephritis interstitial nephritis, hypertension related CKD	200	Healthy and age-matched controls
Elshamaa MF	2011	Egypt	Other	44	Pediatric patients with ESRD based on e GFR on MHD	70	Healthy control subjects with no clinical signs of vascular or renal disease and no family history
Huang HD	2010	China	Asian	47	ESRD patients a) mainly on MHD, b) transplant recipients c) IgA Nephropathy	120	Healthy subjects
Ayed Kh	2006	Tunisia	African	131	Renal transplant recipients	50	Normotensive healthy subjects with clear yearly examinations and negative hypertension history
Tabel Y	2005	Turkey	Other	13	Children with end-stage renal insufficiency	287	Healthy adult subjects
Buraczynska M	2006	Poland	Caucasians	745	Hemodialysis (n = 687) and peritoneal dialysis (n = 58) patients	520	Healthy control subjects with no clinical signs of vascular or renal disease and no family history of renal disease
Lau YK	2004	Singapore	Asian	32	Biopsy-proven primary IgAN-ESRD on MHD	94	Healthy subjects
Liu KP	2004	Taiwan	Asian	16	Children with VUR progressing to ESRD	117	Unrelated healthy adults without renal disease
Lee KB	2003	Korea	Asian	24	ADPKD-ESRD patients	105	Normotensive controls
Coll E	2003	Spain	Caucasian	104	Dialysis patients	131	Healthy subjects with absence of nephropathy, renal failure, diabetes mellitus, or cardiovascular diseases
Papp F	2003	Hungary	Caucasian	70	ESRD patients (20 pediatric, 50 adult)	150	Normotensive healthy subjects (130 adults, 20 children)
Losito A	2002	Italy	Caucasian	160	Hemodialysis patients	169	Healthy blood donors and hospital staff
Buraczynska M	2002	Poland	Caucasian	430	Hemodialysis (n = 407) and peritoneal dialysis (n = 23) patients	260	Healthy control subjects, with no clinical signs of vascular or renal disease and no family history of renal disease
Basset el-EA	2002	France	Caucasian	294	Transplant recipients	181	Gender matched normal local subjects
Filler G	2001	Germany	Caucasian	100	Pediatric transplant recipients	100	Healthy consecutive newborns
Frimat L	2000	France	Caucasian	76	IgA-ESRD patients	960	Healthy Caucasian men in the Stanislas cohort
Gumprecht J	2000	Poland	Caucasian	176	ESRD patients	352	Not reported

MHD: hemodialysis, ADPKD: Autosomal dominant polycystic kidney disease.

Association of CKD and A1166C polymorphism of *AGTR1* gene could not be found neither with per allele contrast nor with genotype contrasts. The ORs were 1.16 (95% CI: 0.83–1.64) for the per allele contrast (C vs A), 1.06 (95% CI: 0.50–2.25) for the CC vs AA + AC contrast and 1.16 (95% CI: 0.82–1.63) for the CC + AC vs AA contrast. Excluding one study of which the population was not in HWE did not grant significance to the association (data not shown). Heterogeneity was rather low in all cases with p-values >0.05 and I^2 < 50% (Table 3), with no publication bias (p-value >0.05 for all tests, data not shown). No time trend was observed in any of the contrasts (Table 4). No individual study was found to influence the effect estimate of the remaining of the studies at an influential meta-analysis (data not shown).

Afterwards, IgA Nephropathy was investigated for its association with the A1166C polymorphism of the *AGTR1*. 36 studies were found from the literature search, however, only five fulfilled all the appropriate criteria and were used in the meta-analysis [31,37,38,47,48]. In total, they contained 785 patients and 1373 controls (Tables 2C and S3) and all populations were in HWE. Meta-analysis for the allele contrast (C vs A) produced an OR of 1.00 (95% CI: 0.84–1.17) indicating the absence of any association of the A1166C polymorphism with IgA Nephropathy. Likewise, no association was found in the other two genotype contrasts (CC vs AA + AC and CC + AC vs AA) as shown in Table 3. Heterogeneity was very low in all contrasts with p-values >0.05 and I^2 < 50% (Table 3) and no publication bias (p-value >0.05 for all tests, data not shown) was observed. Proteus phenomenon was observed in the C vs A and the CC + AC vs AA contrast while in the CC vs AA + AC contrast no time trend was observed (Table 4). According to the influential meta-analysis performed, there was no study to influence the ORs of the remaining studies (data not shown).

Subsequently, we wished to analyze the association of A1166C polymorphism of *AGTR1* gene with Vesicoureteral Reflux (VUR). From the literature search 14 studies were initially retrieved, but only three could be used in the meta-analyses [40,49–51]. Altogether they included 174 patients and 216 healthy controls (Tables 2D and S4). However, the

Table 2B
Characteristics of studies included in the meta-analysis for the association of *AGTR1* A1166C polymorphism with CKD.

Study	Year	Country	Race	Cases	Diagnostic criteria	Controls	Diagnostic criteria
Su SL	2012	Taiwan	Asian	135	Patients with stages 3–5 CKD according to US National Kidney Foundation [1], modified	270	Healthy subjects age- and sex-matched
Zsom M	2011	Hungary	Caucasian	61	CKD patients with primary glomerulonephritis, interstitial nephritis, Hypertension related CKD	200	Healthy and age-matched controls
Elshamaa MF	2011	Egypt	Egyptian	32	Pediatric patients with advanced CKD (stage 4) based on e GFR under CT	70	Healthy subjects with no clinical signs of vascular or renal disease and no family history
Huang HD	2010	China	Asian	83	IgAN non-ESRD patients	120	Healthy subjects
Hsu CC	2006	US	African Americans	307	CKD progression defined as a) increase in SCr ≥35 μmol, b) hospitalization discharge, c) death coded for chronic renal disease [ICD-9] codes 581 to 583 or 585 to 588	3331	Not reported
Peruzzi L	2005	Italy	Caucasian	50	Patients with renal hypodysplasia	50	Healthy subjects matched for sex, age and origin
Lau YK	2004	Singapore	Asian	86	Biopsy-proven primary IgAN-non-ESRD	94	Healthy subjects
Liu KP	2004	Taiwan	Asian	58	VUR patients diagnosed by voiding cystourethroradiography and graded as I–V	117	Unrelated healthy adult volunteers without renal disease

e GFR: estimated glomerular filtration rate, CT: conservative treatment, ICD-9: international classification of diseases, ninth revision, SCr: serum creatinine.

Table 2C
Characteristics of studies included in the meta-analysis for the association of *AGTR1* A1166C polymorphism with IgA Nephropathy.

Study	Year	Country	Race	Cases	Diagnostic criteria	Controls	Diagnostic criteria
Huang HD	2010	China	Asian	130	IgAN by renal biopsy	120	Healthy subjects
Lau YK	2004	Singapore	Asian	118	IgAN patients	94	Not reported
Maruyama K	2001	Japan	Asian	95	IgAN patients	99	Healthy adult volunteers with no history of renal disease or abnormal urinary findings
Frimat L	2000	France	Caucasian	274	IgAN defined as glomerulo-nephritis with predominantly IgA deposits in the mesangium of all glomeruli.	960	Healthy subjects in the Stanislas cohort
Pei Y	1997	Canada	Caucasian	168	IgA by renal biopsy	100	Healthy subjects with no history of renal disease or hypertension

study of Liu and coworkers [40] could be used only for allele contrasts since no genotype data was presented. As shown in Table 3, meta-analysis under the C vs A allele contrast illustrated an OR 1.07 (95% CI: 0.68–1.67) suggesting no statistical significant association. Likewise, the genotype contrasts did not give any evidence for a significant association of *AGTR1* A1166C polymorphism with VUR (Table 3).

Between study heterogeneity was very low in all contrasts since p-values >0.05 and $I^2 < 50\%$ (Table 3). No publication bias was observed with p-value >0.05 for all tests (data not shown). Time trend was observed (Proteus phenomenon) for the C vs A contrast (Table 4), while for the other two contrasts calculations could not be performed since only two studies were included. In an influential meta-analysis no individual study was found to influence the ORs of the rest (data not shown).

3.2. A1332G polymorphism of AGTR2 gene

Finally, another polymorphism, A1332G of the *AGTR2* gene was also analyzed for its association with VUR. Initially 14 studies were retrieved from the literature search, yet, only three abided with the selection criteria and were used in the meta-analysis [50,52,53]. Altogether, they comprised 352 patients, 790 controls. All studies included Caucasian populations (Tables 2E and S5). Because *AGTR2* gene is on X chromosome, one study [53] gave data for males and females separately, and thus two cohorts were included in the initial meta-analysis. One study [50] presented data only for males, and data from [52] was on mixed population. Meta-analysis for the allele contrast on mixed populations revealed no association of *AGTR2* A1332G polymorphism since OR was 1.13 with 96% CI 0.66–1.92. No publication bias was observed (p-value >0.05 for all tests, data not shown) and significant heterogeneity appeared (p-value 0.041 and $I^2 = 63.8\%$, Table 3). Time trend (Proteus phenomenon) was also observed (Table 4). Meta-analysis for male populations was additionally carried out, but did not present any significant association (Table 3). Thus, association of *AGTR2* A1332G polymorphism in males with VUR could not be proven (Table 3).

3.3. AGTR1 A1166C and hypertension in ESRD patients

From the 17 studies we recruited in the meta-analysis for the association of *AGTR1* A1166C with ESRD, three of them were found to test the influence of the variant on hypertension development in patients with ESRD [28,39,42]. Data was given only for the AA vs CC + AC contrast and thus we were able to perform a meta-analysis concerning this contrast. The OR was found equal to 0.98 with 95% CI: 0.68, 1.42 suggesting no association of A1166C polymorphism with hypertension in ESRD patients. According to Begg and Egger tests there was no publication bias and heterogeneity was very low (data not shown).

3.4. Multivariate meta-analyses for the association of AGTR1 A1166G polymorphism with renal disease phenotypes

To validate the above results, multivariate meta-analyses were performed. The analysis that was performed revealed no evidence for the association of AGTR1 A1166C polymorphism with ESRD, since the AC vs AA yields a p-value = 0.181 and OR: 1.14 (95% CI: 0.94, 1.37) and the CC vs AA, an OR: 1.29 (95% CI: 0.78, 2.15) and p-value = 0.319 (Table 5). Likewise, multivariate meta-analysis did not detect any significance for the association of this polymorphism with CKD. No association of AGTR1 A1166C polymorphism with IgA Nephropathy could be proven since the ORs were 1.01 (95% CI: 0.81, 1.25) and 0.95 (95% CI: 0.61, 1.47) for the AC vs AA and CC vs AA contrasts respectively. Similarly, multivariate meta-analysis suggested no significant association with VUR for either dominant AC vs AA [OR: 1.29 (95% CI: 0.73, 2.29)] or the recessive contrast [CC vs AA [OR: 0.16 (95% CI: 0.02, 1.39)]] (Table 5). Nevertheless, these findings were expected since the majority of the univariate tests were unable to show an association. Multivariate meta-analysis could help in avoiding an inflation of the Type I error rate (i.e. reduce false positive findings), but it does not offer greater statistical power.

4. Discussion

The Renin–Angiotensin System plays a pivotal role in the physiology of the kidneys. In non-dialyzed CKD patients, ACE inhibitors and AGTR blockers are used as the treatment of choice since they grant greater survival [10,54]. It has been recently shown [55] that *AGT* M235T gene polymorphism is associated with ESRD susceptibility in Caucasians. In addition, a meta-analysis [56] demonstrated genetic association of *ACE* I/D polymorphism with ESRD risk which actually correlates well with findings that increased circulating ACE levels in plasma are related with *ACE* I/D polymorphism [57].

Both A1166C of *AGTR1* and A1332G of *AGTR2* are within the 3′ untranslated regions of the genes. Though these polymorphisms do not lead to amino acid substitutions, these 3′ untranslated regions may play a pivotal role in the genomic context of the genes and may influence their expression levels, since they could result in defects in

Table 2D
Characteristics of studies included in the meta-analysis for the association of *AGTR1* A1166C polymorphism with VUR.

Study	Year	Country	Race	Cases	Diagnostic criteria	Controls	Diagnostic criteria
Liu KP	2004	Taiwan	Asian	74	VUR diagnosed by VCUG and graded as I–V	117	Unrelated healthy adult volunteers without renal disease
Haszon I	2002	Hungary	Caucasian	77	VUR graded as I–V	80	Healthy blood donors
Hohenfellner K	1999	Germany	Caucasian	23	VUR diagnosed by radiological investigations including VCUG and graded as I–V	19	boys with absence of any disorder of the urinary tract

VCUG: voiding cysto-urethrography.

Table 2E
Characteristics of studies included in the meta-analysis for the association of *AGTR2* A1332G polymorphism with VUR.

Study	Year	Country	Race	Cases	Diagnostic criteria	Controls	Diagnostic criteria
Rigoli L	2004	Italy	Caucasian	27	Primary VUR according to the International Reflux Classification [43]	92	Children with no renal disease
Yoneda A	2002	Ireland	Caucasian	302	Male and female VUR patients from 88 families	679	Healthy controls, and non-affected family members
Hohenfellner K	1999	Germany	Caucasian	23	Male VUR patients (grades I–V, according to the International Reflux Classification)	19	Healthy boys with absence of any disorder of the urinary tract

Table 3
Univariate meta-analysis for all contrasts performed for both *AGTR1* (A1166C) and *AGTR2* (A1332G) polymorphisms for its association with diseases as indicated.

SNP	Contrast	Disease	Number of studies	Odds ratio (random effects)	95% confidence interval	Cochran's Q	p-value for heterogeneity	I^2 (%)	Between studies variance (τ^2)
A1166C/*AGTR1*	A vs C	ESRD	16	1.10	0.91 1.34	53.06	0.000	71.7%	0.097
		CKD	7	1.16	0.83 1.64	10.41	0.109	42.3%	0.087
		IgAN	5	0.99	0.84 1.17	2.31	0.678	0.0%	0.000
		VUR	3	1.07	0.68 1.67	2.29	0.318	12.8%	0.022
	CC vs AA + AC	ESRD	14	1.31	0.83 2.07	30.80	0.004	57.8%	0.370
		CKD	6	1.06	0.50 2.25	3.16	0.675	0.0%	0.000
		IgAN	5	0.94	0.62 1.45	0.51	0.973	0.0%	0.000
		VUR	2	0.14	0.02 1.22	0.10	0.749	0.0%	0.000
	CC + AC vs AA	ESRD	15	1.15	0.92 1.44	38.42	0.000	63.6%	0.108
		CKD	7	1.16	0.82 1.63	11.03	0.087	45.6%	0.091
		IgAN	5	1.00	0.81 1.23	3.05	0.550	0.0%	0.000
		VUR	2	1.15	0.66 2.01	0.36	0.551	0.0%	0.000
A1332G/*AGTR2*	A vs C	VUR	4 (mixed)	1.13	0.66 1.92	8.28	0.041	63.8%	0.649
			2 (males)	0.67	0.41 1.10	0.61	0.433	0.0%	0.000

messenger RNA (mRNA) processing, mRNA half-life, or affect the function of regulatory elements such enhancers and insulators [2,58]. The deletion/insertion polymorphism in intron 16 of the ACE gene is an example of such non-coding sequence polymorphisms that influence gene function. Moreover, Sethupathy et al. [59] have shown that there is a miRNA from chromosome 21, namely miR155, that downregulates the expression of the 1166A allele but not of the 1166C. They hypothesize that the 1166C allele is associated with hypertension just because miR155 cannot negatively control the expression levels of AGTR1.

In view of the above data, considering the fact that AGT, ACE and AGTRs perform in the same biochemical pathways, and taken the number of case–control studies investigating relationship of *AGT receptors* gene variants with kidney diseases, in the present meta-analysis we set out to explore putative genetic associations of *AGTR1* and *AGTR2* gene polymorphisms with renal diseases. We investigated these associations for sub-group renal diseases such as ESRD, CKD, IgA Nephropathy and VUR.

Though very promising, and despite the inclusion of 2596 patients and 3866 healthy controls from 17 studies, no statistical significant association of *AGTR1* A1166C polymorphism with ESRD was found either in the allele contrasts or in the dominant and recessive models. Subgroup analysis by ethnicity or by studies in HWE did not change this pattern of results. The main analysis revealed significant heterogeneity under the allele contrast, the recessive and dominant models, implying that these results may not change even in a future meta-analysis that would contain additional studies. This is further corroborated by our findings showing that contrasts for the allele, recessive and dominant models needed two to three times more subjects to obtain significant results (data not shown) according to calculations based on the Barrowman et al. method [60]. Multivariate meta-analysis did not change the significance of the results. Thus no significant association of *AGTR1* A1166C polymorphism with ESRD was proven.

Similarly, meta-analyses revealed lack of association of *AGTR1* A1166C polymorphism with CKD and non-significant heterogeneity under the allele contrast, the recessive and dominant models. This pattern did not change under subgroup analysis by race and by HWE, neither did it change when multivariate meta-analysis was performed. Time–trend related bias (Proteus phenomenon) was not detected reinforcing the absence of association of *AGTR1* A1166C with CKD. Besides, due to low heterogeneity and according to our calculation (needing four to 1500 times more subjects to reach significance) based on the Barrowman et al. method [60], we believe that the absence of association of *AGTR1* A1166C polymorphism with CKD is rather factual.

Similarly, no association was found between *AGTR1* A1166C polymorphism and IgA Nephropathy and VUR, under all contrasts (allele and genotypes) tested. While for the association with IgAN the absence of association was pretty clear, the ORs for the association with VUR were fluctuating between various contrasts, due to the very small number of studies (three for allele contrast and two for the genotypes contrasts). Further evaluations suggested very low heterogeneity of the studies and no publication bias. Time–trend related bias (Proteus phenomenon) detected in these meta-analyses denote that more studies will improve the significance of our results. Additional multivariate meta-analyses that we performed confirmed the lack of association of *AGTR1* A1166C polymorphism with IgAN and VUR.

Furthermore, we attempted to investigate the involvement of the A1332G polymorphism of the *AGTR2* gene, located in the X chromosome, in the pathogenesis of VUR. Meta-analysis of the available data

Table 4
Time trend results for all univariate meta-analyses.

SNP	Disease		p-value cumulative	Proteus phenomenon
A1166C/*AGTR1*	CKD	C vs A	0.789	NO
		CC vs AA + AC	0.501	NO
		AA vs CC + AC	0.432	NO
	ESRD	C vs A	0.017	YES
		CC vs AA + AC	0.000	YES
		AA vs CC + AC	0.783	NO
	IgA Nephropathy	C vs A	0.008	YES
		CC vs AA + AC	0.488	NO
		AA vs CC + AC	0.000	YES
	VUR	C vs A	0.000	YES
A1332G/*AGTR2*	VUR	G vs A	0.000	YES

Table 5
Multivariate meta-analysis for all contrasts performed for *AGTR1* (A1166C) polymorphism.

Disease	Number of studies	Contrast	OR	95% confidence interval	
ESRD	14	AC vs AA	1.14	0.94	1.37
		CC vs AA	1.29	0.78	2.15
CKD	6	AC vs AA	1.17	0.73	1.88
		CC vs AA	1.08	0.46	2.52
IgA Nephropathy	5	AC vs AA	1.01	0.81	1.25
		CC vs AA	0.95	0.61	1.47
VUR	2	AC vs AA	1.29	0.73	2.29
		CC vs AA	0.16	0.02	1.39

from three studies including both male and female populations showed no association under the allele contrast.

It should be mentioned that the absence of any association of the two aforementioned polymorphisms was relatively unexpected, considering the fact that polymorphisms of the other two RAS proteins genes (*AGT* and *ACE*) do associate with renal diseases. Nevertheless, no Genome Wide Association Study (GWAS) revealed *AGTR*s polymorphisms as putative markers for renal disease progression. However, these findings did not discourage our study because GWASs do have some limitations since they often explain only a few percent of the variance of the phenotype, and it is difficult to assign mechanistically the loss of function of a specific gene to one polymorphism [5,61]. In support of our first idea of *AGTR* association with renal diseases is the fact that the *AGT* M235T and *AGTR1* A1166C polymorphisms have been associated with diabetic nephropathy in a meta-analysis [62], but not with diabetes (to the best of our knowledge). On the other hand, and in support of our results, a publication came during the preparation of the present manuscript showing lack of association of *AGTR1* A1166C polymorphism with the risk for ESRD [63], though including only eight studies as compared to 17 that we included in the present study. Taken together our data suggest that neither *AGTR1* A1166C nor *AGTR2* A1332G polymorphisms can be used as reliable markers to predict the risk for CKD, ESRD, IgAN or VUR.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.csbj.2014.05.006.

References

[1] Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, et al. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann Intern Med 2003;139:137–47.
[2] Balakrishnan VS, Rao M, Jaber BL, DialGene C. Genomic medicine, gene polymorphisms, and human biological diversity. Semin Dial 2005;18:37–40.
[3] McDonald SP, Tong B. Morbidity burden of end-stage kidney disease in Australia: hospital separation rates among people receiving kidney replacement therapy. Nephrology 2011;16:758–66.
[4] Luke RG. Can we prevent end-stage renal disease due to hypertension or to diabetes mellitus? JAMA 1992;268:3119–20.
[5] Hildebrandt F. Genetic kidney diseases. Lancet 2010;375:1287–95.
[6] D'Amico G, Minetti L, Ponticelli C, Fellin G, Ferrario F, Barbiano di Belgioioso G, et al. Prognostic indicators in idiopathic IgA mesangial nephropathy. Q J Med 1986;59:363–78.
[7] Gong R, Liu Z, Chen Z, Li L. Genetic variation of mannose-binding protein associated with glomerular immune deposition in IgA nephropathy. Chin Med J (Engl) 2002;115:192–6.
[8] Woolf AS, Winyard PJ. Advances in the cell biology and genetics of human kidney malformations. J Am Soc Nephrol 1998;9:1114–25.
[9] Arant Jr BS. Vesicoureteric reflux and renal injury. Am J Kidney Dis 1991;17:491–511.
[10] Harrison-Bernard LM. The renal renin–angiotensin system. Adv Physiol Educ 2009;33:270–4.
[11] Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. Am J Physiol Cell Physiol 2007;292:C82–97.
[12] McKnight AJ, Currie D, Maxwell AP. Unravelling the genetic basis of renal diseases; from single gene to multifactorial disorders. J Pathol 2010;220:198–216.
[13] Bagos Pantelis G, Dimou Niki L, Liakopoulos Theodore D, Nikolopoulos Georgios K. Meta-analysis of family-based and case-control genetic association studies that use the same cases. Stat Appl Genet Mol Biol 2011:1.
[14] DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–88.
[15] Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557–60.
[16] Bagos PG. A unification of multivariate methods for meta-analysis of genetic association studies. Stat Appl Genet Mol Biol 2008;7 [Article31].
[17] Bagos PG, Nikolopoulos GK. A method for meta-analysis of case-control genetic association studies using logistic regression. Stat Appl Genet Mol Biol 2007;6 [Article17].
[18] Minelli C, Thompson JR, Abrams KR, Thakkinstian A, Attia J. The choice of a genetic model in the meta-analysis of molecular association studies. Int J Epidemiol 2005;34:1319–28.
[19] Minelli C, Thompson JR, Abrams KR, Lambert PC. Bayesian implementation of a genetic model-free approach to the meta-analysis of genetic association studies. Stat Med 2005;24:3845–61.
[20] Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics 1994;50:1088–101.
[21] Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629–34.
[22] Ioannidis JP, Trikalinos TA. Early extreme contradictory estimates may appear in published research: the Proteus phenomenon in molecular genetics research and randomized trials. J Clin Epidemiol 2005;58:543–9.
[23] Lau J, Antman EM, Jimenez-Silva J, Kupelnick B, Mosteller F, Chalmers TC. Cumulative meta-analysis of therapeutic trials for myocardial infarction. N Engl J Med 1992;327:248–54.
[24] Lau J, Schmid CH, Chalmers TC. Cumulative meta-analysis of clinical trials builds evidence for exemplary medical care. J Clin Epidemiol 1995;48:45–57 [discussion 9–60].
[25] Bagos PG, Nikolopoulos GK. Generalized least squares for assessing trends in cumulative meta-analysis with applications in genetic epidemiology. J Clin Epidemiol 2009;62:1037–44.
[26] Basset el EA, Berthoux P, Cecillon S, Deprle C, Thibaudin D, De Filippis JP, et al. Hypertension after renal transplantation and polymorphism of genes involved in essential hypertension: ACE, AGT, AT1 R and ecNOS. Clin Nephrol 2002;57:192–200.
[27] Buraczynska M, Ksiazek P, Drop A, Zaluska W, Spasiewicz D, Ksiazek A. Genetic polymorphisms of the renin–angiotensin system in end-stage renal disease. Nephrol Dial Transplant 2006;21:979–83.
[28] Buraczynska M, Ksiazek P, Zaluska W, Spasiewicz D, Nowicka T, Ksiazek A. Angiotensin II type 1 receptor gene polymorphism in end-stage renal disease. Nephron 2002;92:51–5.
[29] Coll E, Campos B, Gonzalez-Nunez D, Botey A, Poch E. Association between the A1166C polymorphism of the angiotensin II receptor type 1 and progression of chronic renal insufficiency. J Nephrol 2003;16:357–64.
[30] Filler G, Yang F, Martin A, Stolpe J, Neumayer HH, Hocher B. Renin angiotensin system gene polymorphisms in pediatric renal transplant recipients. Pediatr Transplant 2001;5:166–73.
[31] Frimat L, Philippe C, Maghakian MN, Jonveaux P, Hurault de Ligny B, Guillemin F, et al. Polymorphism of angiotensin converting enzyme, angiotensinogen, and angiotensin II type 1 receptor genes and end-stage renal failure in IgA nephropathy: IGARAS—a study of 274 Men. J Am Soc Nephrol 2000;11:2062–7.
[32] Gumprecht J, Zychma MJ, Grzeszczak W, Zukowska-Szczechowska E. Angiotensin I-converting enzyme gene insertion/deletion and angiotensinogen M235T polymorphisms: risk of chronic renal failure. End-Stage Renal Disease Study Group. Kidney Int 2000;58:513–9.
[33] Losito A, Kalidas K, Santoni S, Ceccarelli L, Jeffery S. Polymorphism of renin–angiotensin system genes in dialysis patients—association with cerebrovascular disease. Nephrol Dial Transplant 2002;17:2184–8.
[34] Papp F, Friedman AL, Bereczki C, Haszon I, Kiss E, Endreffy E, et al. Renin–angiotensin gene polymorphism in children with uremia and essential hypertension. Pediatr Nephrol 2003;18:150–4.
[35] Tabel Y, Berdeli A, Mir S, Serdaroglu E, Yilmaz E. Effects of genetic polymorphisms of the renin–angiotensin system in children with nephrotic syndrome. J Renin Angiotensin Aldosterone Syst 2005;6:138–44.
[36] Zsom M, Fulop T, Zsom L, Barath A, Maroti Z, Endreffy E. Genetic polymorphisms and the risk of progressive renal failure in elderly Hungarian patients. Hemodial Int 2011;15:501–8.
[37] Huang HD, Lin FJ, Li XJ, Wang LR, Jiang GR. Genetic polymorphisms of the renin–angiotensin–aldosterone system in Chinese patients with end-stage renal disease secondary to IgA nephropathy. Chin Med J (Engl) 2010;123:3238–42.
[38] Lau YK, Woo KT, Choong HL, Zhao Y, Tan HB, Chong SM, et al. Renin–angiotensin system gene polymorphisms: its impact on IgAN and its progression to end-stage renal failure among Chinese in Singapore. Nephron Physiol 2004;97:p1–8.
[39] Lee KB, Kim UK. Angiotensinogen and angiotensin II type 1 receptor gene polymorphism in patients with autosomal dominant polycystic kidney disease: effect on hypertension and ESRD. Yonsei Med J 2003;44:641–7.
[40] Liu KP, Lin CY, Chen HJ, Wei CF, Lee-Chen GJ. Renin–angiotensin system polymorphisms in Taiwanese primary vesicoureteral reflux. Pediatr Nephrol 2004;19:594–601.

[41] Ayed K, Ayed-Jendoubi S, Ben Abdallah T, Bardi R, Gorgi Y, Sfar I, et al. Polymorphism of the renin–angiotensin–aldosterone system in patients with chronic allograft dysfunction. Transpl Immunol 2006;15:303–9.
[42] Elshamaa MF, Sabry SM, Bazaraa HM, Koura HM, Elghoroury EA, Kantoush NA, et al. Genetic polymorphism of ACE and the angiotensin II type 1 receptor genes in children with chronic kidney disease. J Inflamm 2011;8:20.
[43] Medical versus surgical treatment of primary vesicoureteral reflux: report of the International Reflux Study Committee. Pediatrics 1981;67:392–400.
[44] Peruzzi L, Lombardo F, Amore A, Merlini E, Restagno G, Silvestro L, et al. Low renin–angiotensin system activity gene polymorphism and dysplasia associated with posterior urethral valves. J Urol 2005;174:713–7.
[45] Su SL, Lu KC, Lin YF, Hsu YJ, Lee PY, Yang HY, et al. Gene polymorphisms of angiotensin-converting enzyme and angiotensin II type 1 receptor among chronic kidney disease patients in a Chinese population. J Renin Angiotensin Aldosterone Syst 2012;13:148–54.
[46] Hsu CC, Bray MS, Kao WH, Pankow JS, Boerwinkle E, Coresh J. Genetic variation of the renin–angiotensin system and chronic kidney disease progression in black individuals in the atherosclerosis risk in communities study. J Am Soc Nephrol 2006;17:504–12.
[47] Maruyama K, Yoshida M, Nishio H, Shirakawa T, Kawamura T, Tanaka R, et al. Polymorphisms of renin–angiotensin system genes in childhood IgA nephropathy. Pediatr Nephrol 2001;16:350–5.
[48] Pei Y, Scholey J, Thai K, Suzuki M, Cattran D. Association of angiotensinogen gene T235 variant with progression of immunoglobin A nephropathy in Caucasian patients. J Clin Invest 1997;100:814–20.
[49] Haszon I, Friedman AL, Papp F, Bereczki C, Baji S, Bodrogi T, et al. ACE gene polymorphism and renal scarring in primary vesicoureteric reflux. Pediatr Nephrol 2002; 17:1027–31.
[50] Hohenfellner K, Hunley TE, Yerkes E, Habermehl P, Hohenfellner R, Kon V. Angiotensin II, type 2 receptor in the development of vesico-ureteric reflux. BJU Int 1999;83:318–22.
[51] Hohenfellner K, Fogo A, Kon V. Renin–angiotensin genes in renal development and the occurrence and progression of renal diseases. Semin Nephrol 1999;19:148–54.
[52] Rigoli L, Chimenz R, di Bella C, Cavallaro E, Caruso R, Briuglia S, et al. Angiotensin-converting enzyme and angiotensin type 2 receptor gene genotype distributions in Italian children with congenital uropathies. Pediatr Res 2004;56:988–93.
[53] Yoneda A, Cascio S, Green A, Barton D, Puri P. Angiotensin II type 2 receptor gene is not responsible for familial vesicoureteral reflux. J Urol 2002;168:1138–41.
[54] Molnar MZ, Kalantar-Zadeh K, Lott EH, Lu JL, Malakauskas SM, Ma JZ, et al. ACE inhibitor and angiotensin receptor blocker use and mortality in patients with chronic kidney disease. J Am Coll Cardiol 2014;63:650–8.
[55] Zhou TB, Yin SS, Qin YH. Association of angiotensinogen M235T gene polymorphism with end-stage renal disease risk: a meta-analysis. Mol Biol Rep 2013;40:765–72.
[56] Zhou TB, Yin SS, Qin YH. Association between angiotensin-converting enzyme insertion/deletion gene polymorphism and end-stage renal disease susceptibility. J Renin Angiotensin Aldosterone Syst 2014;15:22–31.
[57] Lee JM, Lo AC, Yang SY, Tsau HS, Chen RJ, Lee YC. Association of angiotensin-converting enzyme insertion/deletion polymorphism with serum level and development of pulmonary complications following esophagectomy. Ann Surg 2005;241:659–65.
[58] Atkinson TJ, Halfon MS. Regulation of gene expression in the genomic context. Comput Struct Biotechnol J 2014;9:e201401001.
[59] Sethupathy P, Borel C, Gagnebin M, Grant GR, Deutsch S, Elton TS, et al. Human microRNA-155 on chromosome 21 differentially interacts with its polymorphic target in the AGTR1 3′ untranslated region: a mechanism for functional single-nucleotide polymorphisms related to phenotypes. Am J Hum Genet 2007;81:405–13.
[60] Barrowman NJ, Fang M, Sampson M, Moher D. Identifying null meta-analyses that are ripe for updating. BMC Med Res Methodol 2003;3:13.
[61] Altshuler D, Daly MJ, Lander ES. Genetic mapping in human disease. Science 2008;322:881–8.
[62] Ding W, Wang F, Fang Q, Zhang M, Chen J, Gu Y. Association between two genetic polymorphisms of the renin–angiotensin–aldosterone system and diabetic nephropathy: a meta-analysis. Mol Biol Rep 2012;39:1293–303.
[63] Mao S, Huang S. Lack of association of angiotensin II type 1 receptor A1166C gene polymorphism with the risk of end-stage renal disease. Ren Fail 2013;35:1295–301.