

Evidence for epistatic interaction between *VDR* and *SLC13A2* genes in the pathogenesis of hypocitraturia in recurrent calcium oxalate stone formers

Domenico Rendina¹  · Gianpaolo De Filippo² · Fernando Gianfrancesco³ · Riccardo Muscariello¹ · Michele Schiano di Cola¹ · Pasquale Strazzullo¹ · Teresa Esposito^{3,4}

Received: 11 June 2016 / Accepted: 30 August 2016 / Published online: 17 September 2016
© Italian Society of Nephrology 2016

Abstract

Background Genetic factors play a key role in the pathogenesis of hypocitraturia, a common risk factor for nephrolithiasis. The Na⁺-dicarboxylate cotransporter NaDC1, encoded by the *sodium-dicarboxylate cotransporter (SLC13A2)* gene, is a major determinant of urinary citrate excretion and its biological functions are regulated also by the vitamin D/Vitamin D receptor (VDR) biological system. The aim of this case-control study was to evaluate the possible epistatic interaction between *VDR*^{rs731236} and *SLC13A2*^{rs11567842} allelic variants in the pathogenesis of hypocitraturia.

Methods Recurrent calcium-oxalate stone formers (SF) with or without hypocitraturia and healthy controls (C) were genotyped. Gene-gene interactions were estimated by the 1.0 software package of multifactor dimensionality reduction (MDR).

Results The prevalence of *VDR*^{TT} and *SLC13A2*^{GG} genotypes was higher in hypocitraturic SF compared to C (odds ratio [OR] 3.24, 95% confidence interval [CI] 1.38–7.60 for *VDR*^{TT} vs. *VDR*^{tt} and OR 4.06, 95% CI 1.75–9.42 for *SLC13A2*^{GG} vs. *SLC13A2*^{AA}). MDR analysis indicated a significant interaction between *VDR*^{TT} and *SLC13A2*^{GG} in

hypocitraturic SF compared to C [OR 3.81 (2.11–6.88)]. These data are compatible with an epistatic interaction between the *VDR*^{TT} and *SLC13A2*^{GG} genotypes with a significant impact on the magnitude of the effect (suppressive effect).

Conclusions These results point to an epistatic interaction between the *VDR* and the *SLC13A2* alleles in the pathogenesis of idiopathic hypocitraturia in calcium-oxalate SF.

Keywords Epistasis · Hypocitraturia · Nephrolithiasis · *SLC13A2* · *VDR*

Introduction

Nephrolithiasis (NL) is a complex disorder affecting 5–15% of the population worldwide and its pathogenesis is influenced by multiple metabolic, nutritional, environmental and genetic factors [1]. Similarly as for other complex disorders, the search for susceptibility loci for NL has been less successful than for simple Mendelian disorders. This is probably due to complicating factors such as a high number of contributing loci and susceptibility alleles, incomplete penetrance, and contributing environmental effects [2]. Recently, Halbritter and colleagues demonstrated that monogenic forms account for 15% of the cases of NL/nephrocalcinosis, supporting the concept that NL is genetically broadly heterogeneous [3]. At the same time, Lieske et al. demonstrated a strong heritable component for many urinary metabolic risk factors for NL [4]. Based on these considerations and observations with other complex disorders [2–5], the analysis of epistatic locus-locus interactions with regard to intermediate phenotypes may be helpful for understanding the genetic bases of NL. Low urinary citrate excretion is a well-known metabolic risk factor for calcium-oxalate

✉ Domenico Rendina
domenico.rendina@unina.it

¹ Department of Clinical Medicine and Surgery, Federico II University of Naples, via Pansini 5, 80131 Naples, Italy

² Institute of Genetics and Biophysics “Adriano Buzzati-Traverso”, Italian National Research Council, Naples, Italy

³ AP-HP, CHU Bicêtre, Service de Médecine des Adolescents, Le Kremlin-Bicêtre, France

⁴ IRCCS INM Neuromed, Pozzilli, IS, Italy

(CaOx) NL which is found in up to 85% of stone formers (SF) [6]. Hypocitraturia, generally defined as a urinary citrate excretion below 1.67 mmol/day in adults, occurs in a substantial proportion of SF, ranging from 20 to 60% in different reports, with higher prevalence in CaOx-SF [7]. Several metabolic and genetic factors are known to have an impact on renal tubular citrate handling and thus on urinary citrate excretion [7, 8]. In particular, single nucleotide polymorphisms (*snp*) of *Vitamin D receptor (VDR)* (cytogenetic location: 12q13.11; genomic coordinates [GRCh37]: 12:48,235,319–48,298,813) and of *Sodium-citrate cotransporter-1* (also called *Solute Carrier Family 13 Member 2, SLC13A2*; cytogenetic location: 17q11.2, genomic coordinates [GRCh37]: 17:26,800,663–26,824,798) genes have been associated to hypocitraturia in Caucasian and Asian SF [9–11]. The Na⁺-dicarboxylate cotransporter NaDC1, encoded by the SLC13A2 gene, is located in the apical membrane of epithelial cells of the small intestine and the renal proximal tubule, where it is involved in the absorption of citrate from the enteric juice and the tubular filtrate, respectively, via an electrogenic process, coupling 3 sodium ions to the transport of each divalent anion substrate [12]. The VDR is an intracellular hormone receptor that specifically binds the active form of vitamin D, the 1,25-dihydroxycholecalciferol (1,25[OH]₂D₃), and mediates its genomic and non-genomic pleiotropic effects [13]. The vitamin D/VDR biological system is a modulator of functional properties and expression of NaDC1 [14]. Indeed, vitamin D response elements (VDREs) have been found in the promoter region of the SLC13A2 gene and the vitamin D-VDR biological system is known to play a key role in the expression and regulation of the protein kinase pathway, which in turn regulates the function of NaDC1 [12, 15]. Against this background, we performed a case-control study to assess the possible occurrence of an epistatic interaction between *VDR* and *SLC13A2* single nucleotide polymorphisms (SNPs) relevant to the pathogenesis of hypocitraturia in recurrent CaOx SF.

Patients and methods

Patients

Among over 700 SF referred to the Department of Clinical Medicine and Surgery at Federico II University of Naples Medical School; Naples, Italy from 2000 to 2015, we identified for the present analysis 372 patients who declared the excretion and/or removal of two or more CaOx stones in the previous 4 years or in whom one or more calculi were observed in both kidneys. The control group (C) was made up of 88 unrelated individuals without familial or personal history of NL enrolled from among members of our research community, other hospital and clinics staff members, family

reference panels (one per family) and patients' spouses. Exclusion criteria, for both patients and controls, were: urinary tract infection, primary or secondary hyperparathyroidism [16], cystinuria >70 μmol/24 h [17], gouty diathesis [18], renal tubular acidosis [19], creatinine clearance <0.80 ml/min, chronic diarrheal states, intake of drugs such as thiazide diuretics, angiotensin converting enzyme inhibitors, glucocorticoids and estrogens. For the control group, an additional exclusion criterion was the occurrence of one or more metabolic risk factors for NL, according to previously reported diagnostic criteria [9]. There were no postmenopausal or pregnant women in the study population. Patients and controls, all of Caucasian origin, were born and resident in the Campania region. The study was approved by the Medical School Ethical Committee of Federico II University of Naples.

Methods

All participating subjects received detailed instructions and a 2.5-l container for the correct completion of a 24-h urine collection to be carried out on the day preceding their clinic visit. Relevant biochemical parameters were estimated after 7 days on a constant diet containing 1000 mg calcium, 100 mEq sodium, 900 mg phosphorus, 300 mg carbohydrates and 70 g proteins (total 2200 calories). On the examination day, at least three hours after the first morning void, a fasting urinary sample was collected and its pH was determined. Samples obtained from the 24-h urine collection were used for the analysis of calcium (uCa), magnesium (uMg), phosphate (uPO₄⁻), sodium (uNa), potassium (uK), chloride (uCl), citrate (uCit), creatinine (uCrea), oxalate (uOx), uric acid (uUr) and cystine (uCys) concentrations. On the day of the visit also fasting venous blood samples were taken for the assessment of serum ionised (sCa) and total calcium (tCa), magnesium (sMg), phosphate (sPO₄⁻), chloride (sCl), sodium (sNa), potassium (sK), parathormone (PTH), 25-hydroxycholecalciferol (25[OH]D₃), 1,25(OH)₂D₃, citrate (sCit), uric acid (sUr) and creatinine (sCrea) levels.

tCa, uCa, uMg, and sMg were measured by atomic absorption spectrophotometry. sCa was measured using an ion-selective electrode. Serum and urinary citrate levels (sCit and uCit) were assessed by the citric acid UV-method (Boehringer Mannheim, Germany). PTH serum levels were measured using an immunometric assay (Immulite intact PTH—Diagnostic Products Corporation, Los Angeles, CA, USA). 25(OH)D₃ serum levels were estimated using a modified competitive protein binding assay (Vitamin D3 screen—Buhlmann Laboratories AG; Allschwill, Switzerland). 1,25(OH)₂D₃ serum levels were estimated using a RIA Test (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Cystine urinary levels were measured by

high-performance liquid chromatography and oxalate urinary levels by ion chromatography. uNa and uK were measured by flame photometry. Tubular reabsorption of citrate (TR Cit) was calculated as $rCit = 100 \times 1 - [(sCrea) \times (uCit)] / [(uCrea) \times (sCit)]$ [20]. Other blood and urinary analyses were performed using a Hitachi 747 automatic analyzer. All parameters were measured according to the manufacturer's instructions. The stones were analyzed using Fourier-transform infrared spectroscopy and high-resolution X-ray diffraction.

Diagnostic criteria

Stones with a CaOx content >70% and a calcium apatite content <5% were considered CaOx stones [21]. Hypocitraturia was defined as a urinary citrate excretion <1.67 mmol/day [7].

Genotyping

Genomic DNA was extracted from the participants' venous blood samples as described previously [9]. Polymerase chain reactions (PCRs) were also performed as previously described [10]. For the human *SLC13A2* gene, located on chromosome 17, spanning a genomic region of 24.136 bases and split into 13 exons, two SNPs were selected consulting the dbSNP database (<http://www.hapmap.org/index.html.en>): the coding SNP rs11567842 [c.1648A>G; p.I550V] in exon 12, already described by Okamoto and co-workers [10], and the SNP rs3764866 [c.-527G>A] located in the promoter region. Primers used for genotyping of rs11567842 (forward: 5-ACGGGAGGACTTCCCAGAGA-3; reverse: 5-CAGGCGCACACATATGCGCA-3) amplified a 520-bp fragment which was then digested with 15 U of Bcl-I endonuclease (Roche, Mannheim, Germany) at 50°C and size-fractionated on a 1.5% agarose gel containing 0.6 ml/ml ethidium bromide. The promoter SNP rs3764866 was genotyped using the forward primer 5-AGTTGTCTAATTTGAGCCTC-3 combined with the reverse primer 5-CTGAGCACTTTATGTGACAG-3, which amplified a 263-bp fragment which was then digested with 5 U of Alu-I endonuclease (Roche, Mannheim, Germany) at 37°C and size-fractionated on a 2% agarose gel containing 0.6 ml/ml ethidium bromide. In a preliminary analysis on a smaller sample of patients and controls (50 cases vs. 50 controls), only the coding SNP rs11567842 showed a trend to a different allelic distribution between cases and controls; thus it was subsequently analyzed in the whole study population. Genotyping of the TaqI *VDR* polymorphism (rs731236) was performed as previously described [9]. The genotypes were defined as "T" (in the absence of the restriction site) or "t" (in the presence of the restriction site).

Statistical analysis

Quantitative and qualitative data are expressed as mean \pm standard deviation or as absolute (percentage) values, respectively. Statistical analysis was performed with a SPSS statistics package, version 18.0 (SPSS, Chicago, IL, USA). Contingency table χ^2 and ANOVA (with Bonferroni correction when required) were used to compare qualitative and quantitative data, respectively. Genotypes and allele frequencies for the *VDR* and *SLC13A2* variants were calculated from the observed genotype counts. As a statistical control for systematic genotyping error and population stratification, the expected genotype proportions according to the Hardy–Weinberg law were calculated and compared with the observed genotypes using the deFinetti program (<http://ihg2.helmholtzmuemchen.de/cgi-bin/hw/hwa1.pl>). To estimate haplotype frequencies and the occurrence of linkage disequilibrium between *VDR* and *SLC13A2* variants, the computer program Estimated Haplotype (EH) (Linkage Program version 5.1) was used (<http://www.jurgott.org/linkage/eh.htm>). Gene–gene interactions were estimated by the 1.0 software package of multifactor dimensionality reduction (MDR) [22]. All statistical tests were two-sided. Statistical significance was defined as $p < 0.05$.

Results

Of the 372 SF patients, 190 were found to be hypocitraturic. The main clinical and biochemical features of hypocitraturic and normocitraturic CaOx-SF and C are shown in Table 1. Hypocitraturic CaOx SF showed significantly higher TR Cit compared to the other two study groups and both groups of CaOx SF showed higher uCa compared to C. The other clinical and biochemical parameters examined were not significantly different among the groups.

Table 2 shows the prevalence of *VDR*^{rs731236} and *SLC13A2*^{rs11567842} alleles and genotypes in the three study groups. *VDR*^{rs731236} and *SLC13A2*^{rs11567842} genotype frequencies were in Hardy–Weinberg equilibrium in C ($p=0.68$ and $p=0.16$, for *VDR*^{rs731236} and *SLC13A2*^{rs11567842} genotypes, respectively). As reported in Table 2, the prevalence of *VDR*^T and *SLC13A2*^G alleles was significantly higher in hypocitraturic CaOx SF than C. Also the prevalence of *VDR*^{TT} and *SLC13A2*^{GG} genotypes was significantly higher in hypocitraturic CaOx SF compared to C (odds ratio [OR] 3.24, 95% confidence interval [CI] 1.38–7.60 for *VDR*^{TT} vs. *VDR*^{tt}; OR 4.06 [1.75–9.42] for *SLC13A2*^{GG} vs. *SLC13A2*^{AA}). These differences in the prevalence of *VDR*^{rs731236} and *SLC13A2*^{rs11567842} alleles and genotypes were not observed between normocitraturic CaOx SF and C and between normocitraturic and hypocitraturic CaOx SF (Table 3). Gene–gene interactions among

Table 1 Clinical characteristics of calcium-oxalate stone formers with or without hypocitraturia and control subjects

	HSF	NSF	C	p values		
				HSF vs. NSF	HSF vs. C	NSF vs. C
Number	190	182	88			
M:F ratio	101\89	107\75	50\38	0.30	0.61	0.79
Age (years)	41.1±14.9	41.2±13.3	40.8±14.1	0.76	0.57	0.58
BMI (kg/m ²)	26.4±3.9	26.5±4.6	26.4±4.1	0.78	0.83	0.86
Age at onset NL (years)	33.3±11.3	30.3±10.8	–	0.23	–	–
Serum parameters						
Calcium (mmol/l)	2.37±0.11	2.36±0.12	2.37±0.11	0.77	0.76	0.81
Magnesium (mmol/l)	1.08±0.10	1.09±0.11	1.07±0.10	0.64	0.63	0.66
Phosphate (nmol/l)	1.11±0.19	1.13±0.20	1.14±0.19	0.43	0.41	0.75
Chloride (mmol/l)	106.2±2.6	106.1±3.0	106.1±2.5	0.69	0.71	0.79
Sodium (mmol/l)	139.7±2.1	139.8±2.2	139.8±2.3	0.75	0.76	0.74
Potassium (mmol/l)	4.29±0.39	4.30±0.40	4.26±0.35	0.67	0.34	0.35
PTH (pmol/l)	4.18±1.85	4.21±1.84	4.05±1.93	0.48	0.29	0.28
25(OH)D ₃ (nmol/l)	74.7±19.6	74.9±19.8	78.4±19.7	0.67	0.31	0.29
1,25(OH) ₂ D ₃ (pmol/l)	89.3±14.7	89.8±21.4	97.8±13.2	0.58	0.29	0.28
Citrate (mmol/l)	0.29±0.17	0.29±0.14	0.29±0.16	0.76	0.75	0.76
Urate (mmol/l)	0.27±0.06	0.27±0.07	0.28±0.07	0.76	0.43	0.43
Urinary parameters						
Calcium (mmol/24 h)	6.61±2.51	6.18±1.64	4.25±1.67	0.22	<0.01	<0.01
Magnesium (mmol/24 h)	3.78±1.93	3.76±1.28	3.80±1.41	0.56	0.42	0.41
Phosphate (mmol/24 h)	21.8±9.3	24.1±8.1	23.9±8.5	0.33	0.39	0.56
Citrate (mmol/24 h)	1.14±0.22	3.02±0.95	2.97±0.98	<0.01	<0.01	0.37
TR Cit (%)	95.3±2.9	90.8±3.9	90.1±4.3	<0.01	<0.01	0.78
Oxalate (μmol/24 h)	336.4±219.9	327.6±192.9	268.1±95.4	0.52	0.17	0.18
Urate (mmol/24 h)	2.93±1.08	2.89±0.98	2.41±1.02	0.53	0.22	0.24

Data are expressed as mean±standard deviation and absolute values for quantitative and qualitative parameters, respectively. Hypocitraturia was defined as urinary citrate excretion lower than 1.67 mmol/24 h

Contingency table χ^2 and ANOVA (with Bonferroni correction when required) were used to compare qualitative and quantitative data, respectively

HSF hypocitraturic calcium-oxalate stone formers, NSF normocitraturic calcium-oxalate stone formers, C healthy controls, M male, F female, BMI body mass index, TR Cit tubular reabsorption of citrate

VDR and *SLC13A2* were analyzed by MDR software and summarized in Fig. 1. The MDR analysis indicated a significant interaction among *VDR*^{TT} and *SLC13A2*^{GG} in hypocitraturic CaOx SF compared to C [training balance accuracy=0.65; testing balance accuracy=0.63; cross-validation consistency=10/10; $\chi^2=21.05$; $p<0.001$; OR 3.81 (2.11–6.88)] and to hypocitraturic CaOx SF [training balance accuracy=0.59; testing balance accuracy=0.58; cross-validation consistency=10/10; $\chi^2=13.51$; $p<0.001$; OR 2.20 (1.44–3.37)]. This interaction was not observed between normocitraturic CaOx SF and C [training balance accuracy=0.56; testing balance accuracy=0.45; cross-validation consistency=10/10; $\chi^2=3.29$; $p=0.07$; OR 1.62 (0.96–2.73)] (Fig. 1). Data from MDR analysis support the occurrence of an epistatic interaction between

VDR^{rs731236} and *SLC13A2*^{rs11567842} genotypes in hypocitraturic CaOx SF. Compared to C, the ORs for hypocitraturia in hypocitraturic CaOx SF were 3.24 for *VDR*^{TT} vs. *VDR*^{tt} and 4.06 for *SLC13A2*^{GG} vs. *SLC13A2*^{AA}, whereas cumulative OR generated by MDR analysis for the same genotypes was 3.81. This is compatible with an epistatic interaction between *VDR*^{rs731236} and *SLC13A2*^{rs11567842} genotypes with a significant change in the magnitude of effect (suppressive effect). Moreover, hypocitraturic CaOx SF with at least one copy of both risk alleles (*VDR*^T and *SLC13A2*^G) showed a mean age at onset of NL significantly lower compared to the remaining CaOx SF (27.9±8.1 vs. 34.3±12.8, years, $p=0.02$). This difference remained significant after correction for age, body mass index (BMI) and gender.

Table 2 Prevalence of *VDR*^{rs731236} and *SLC13A2*^{rs11567842} alleles and genotypes in the three study groups

Subjects	<i>SLC13A2</i> ^{rs11567842}									
	<i>VDR</i> ^{rs731236}					Alleles				
	Alleles		Genotypes			Alleles		Genotypes		
	<i>T</i>	<i>t</i>	<i>tt</i>	<i>Tt</i>	<i>TT</i>	<i>A</i>	<i>G</i>	<i>AA</i>	<i>AG</i>	<i>GG</i>
HSF	96 (25.3)	284 (74.7)	14 (7.4)	68 (35.8)	108 (56.8)	178 (46.8)	202 (53.2)	34 (17.9)	110 (57.9)	46 (24.2)
NSF	118 (32.4)	246 (67.6)	14 (7.7)	90 (49.5)	78 (42.9)	202 (55.5)	162 (45.5)	50 (27.5)	102 (56.0)	30 (16.5)
C	70 (39.8)	106 (60.2)	13 (14.8)	44 (50.0)	31 (35.2)	108 (61.4)	68 (38.6)	30 (34.1)	48 (54.5)	10 (11.4)

Data are expressed as absolute (percentage) values

The statistical significant data (p < 0.05) after multivariate analysis are shown in bold

HSF hypocitratric calcium-oxalate stone formers, *NSF* normocitratric calcium-oxalate stone formers, *C* healthy controls

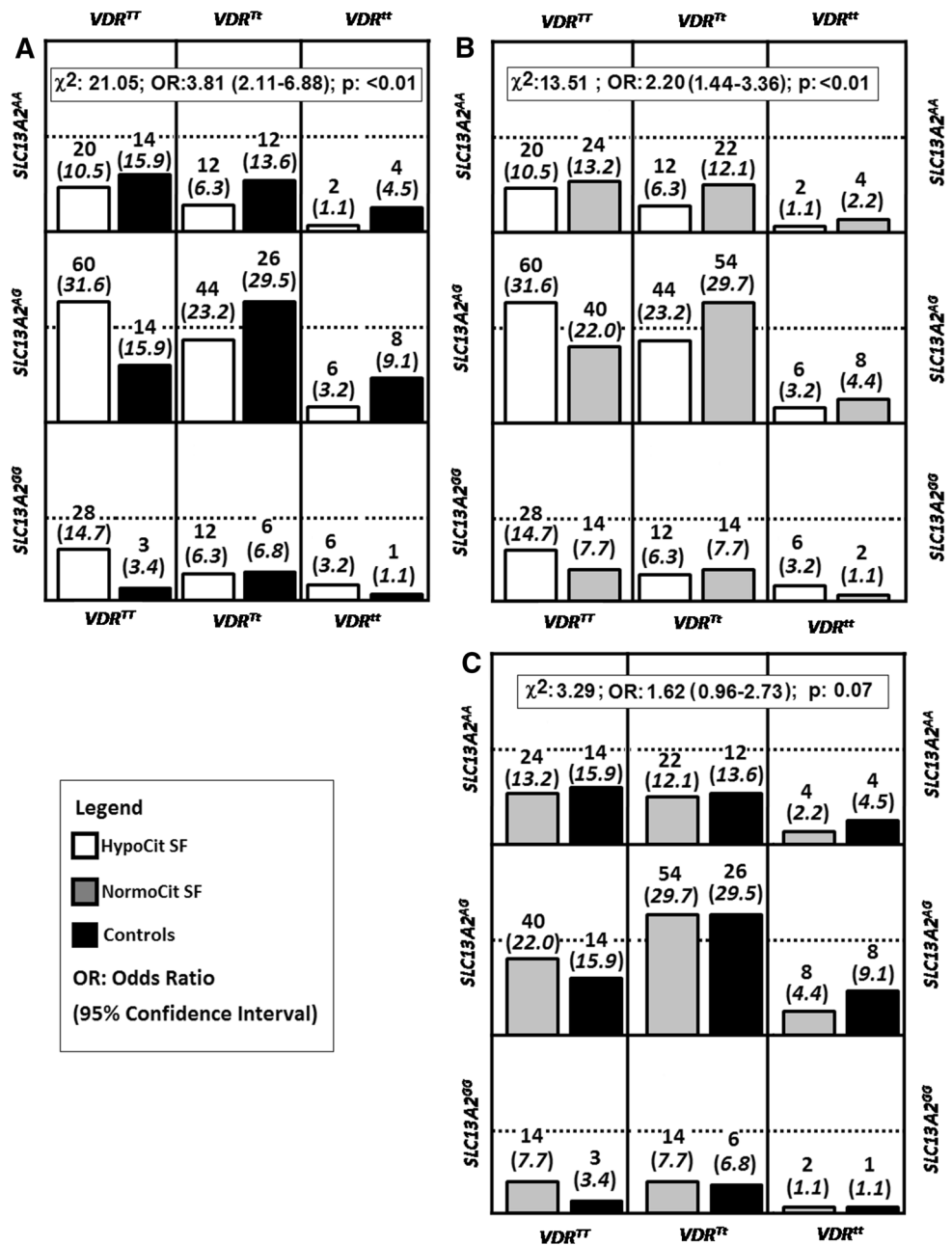
Table 3 Comparison of prevalence of *VDR*^{rs731236} and *SLC13A2*^{rs11567842} alleles and genotypes in the three study groups

Subjects	SNPs	Alleles		Allele 1 vs. 2		Genotype 11 vs. 22		Genotype 11 vs. 12+22		Genotype 11+12 vs. 22	
		1	2	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
		Alleles		Allele 1 vs. 2		Genotype 11 vs. 22		Genotype 11 vs. 12+22		Genotype 11+12 vs. 22	
NSF vs. HSF	<i>VDR</i> ^{rs731236}	<i>t</i>	<i>T</i>	1.42 (1.03–1.95)	0.03	1.38 (0.62–3.06)	0.42	1.05 (0.48–2.26)	0.90	0.57 (0.38–0.86)	<0.01
C vs. HSF	<i>VDR</i> ^{rs731236}	<i>t</i>	<i>T</i>	1.95 (1.34–2.86)	<0.01	3.24 (1.38–7.60)	<0.01	2.18 (0.97–4.86)	0.05	0.413 (0.24–0.70)	<0.01
C vs. NSF	<i>VDR</i> ^{rs731236}	<i>t</i>	<i>T</i>	1.37 (0.95–2.01)	0.09	2.34 (0.98–5.53)	0.05	2.08 (0.93–4.64)	0.07	0.72 (0.43–1.23)	0.23
NSF vs. HSF	<i>SLC13A2</i> ^{rs11567842}	<i>A</i>	<i>G</i>	1.41 (1.06–1.88)	0.01	2.25 (1.20–4.25)	0.01	1.74 (1.06–2.85)	0.03	0.62 (0.37–1.03)	0.06
C vs. HSF	<i>SLC13A2</i> ^{rs11567842}	<i>A</i>	<i>G</i>	1.80 (1.25–2.59)	<0.01	4.06 (1.75–9.42)	<0.01	2.37 (1.33–4.22)	<0.01	0.40 (0.19–0.84)	0.01
C vs. NSF	<i>SLC13A2</i> ^{rs11567842}	<i>A</i>	<i>G</i>	1.28 (0.88–1.84)	0.20	1.80 (0.77–4.19)	0.17	1.37 (0.79–2.36)	0.26	0.65 (0.30–1.4)	0.27

The statistical significant data after multivariate analysis are shown in bold

HSF hypocitratric calcium-oxalate stone formers, *NSF* normocitratric calcium-oxalate stone formers, *C* healthy controls, *OR* odds ratio, *CI* confidence interval

Fig. 1 Gene–gene interaction between $VDR^{rs731236}$ and $SLC13A2^{rs11567842}$ allelic variants in hypocitraturic and normocitraturic calcium-oxalate stone formers and healthy control subjects. **a** $VDR^{rs731236}$ and $SLC13A2^{rs11567842}$ genotype interaction between hypocitraturic calcium-oxalate stone formers (*HypoCit SF*) and healthy control subjects (*Controls*). **b** $VDR^{rs731236}$ and $SLC13A2^{rs11567842}$ genotype interaction between *HypoCit SF* and normocitraturic calcium-oxalate stone formers (*NormoCit*). **c** $VDR^{rs731236}$ and $SLC13A2^{rs11567842}$ genotype interaction between *NormoCit* and *Controls*. Gene–gene interactions among VDR and $SLC13A2$ were analyzed using the 1.0 software package of multifactor dimensionality reduction (MDR). In each window, data are expressed as absolute (percentage) prevalence and the dotted line represents 25%. Hypocitraturia was defined as urinary citrate excretion lower than 1.67 mmol/24 h



Discussion

Genetic factors play a key role in the pathogenesis of NL and their metabolic risk factors: twin studies demonstrated that the heritability of the risk for NL was higher than 50% [3, 4, 23–25]. The most novel finding of our study is the demonstration of an epistatic interaction between two SNPs of the VDR and $SLC13A2$ genes, $VDR^{rs731236}$ and $SLC13A2^{rs11567842}$, in the pathogenesis of hypocitraturia. Hypocitraturia is a common and well recognized metabolic risk factor for CaOx NL. Citrate directly inhibits the spontaneous nucleation of calcium oxalate salts as well as the growth, agglomeration and aggregation of calcium oxalate

crystals in urine [7, 8]. Urinary citrate excretion is a function of filtration, reabsorption, peritubular transport and synthesis by the renal tubular cells [26]. Since tubular citrate secretion is usually negligible, urinary citrate excretion is essentially due to the amount of filtered citrate which is not reabsorbed at the tubular level [7, 8, 14, 26]. Based on these elements, the NaDC1 activity is considered a major determinant of urinary citrate excretion and, in turn, $SLC13A2$ is seen as a candidate locus for NL [12, 27]. Pajor and Sun have evaluated the effects of known missense mutations of the $SLC13A2$ gene, including the $SLC13A2^{rs11567842}$ SNP, on functional properties and expression of hNaDC1 [27]. According to Pajor and Sun, the $SLC13A2^{rs11567842}$

variant had only a modest effect on the transporter function and was associated with a 20% reduction in plasma membrane protein abundance and a corresponding decrease in the transport activity. The authors then concluded that the hypocitraturia associated with kidney stone formation could not be directly attributable to the missense mutation rs11567842 in the *SLC13A2* gene [27]. Experimental and clinical data indicated that the *VDR* is a candidate locus for NL because its allelic variation affects the activity of the receptor and the subsequent downstream vitamin D-mediated effects [13, 24]. The SNP of the *VDR* gene examined in our study, *VDR*^{rs731236}, is a T/C nucleotide substitution (ATT–ATC) leading to a synonymous change at codon 352 (isoleucine) in exon 9. This change could be responsible for differences in translational efficiency or in messenger RNA stability, resulting in changes in *VDR* expression [28]. Moreover, subtle variation in the expression and/or function of *VDR* may contribute to major differences in the regulation of other target genes. Indeed, mutations in the *VDR* gene selectively and specifically alter *VDR* subcellular distribution [29]. The epistatic interaction between the SNPs *VDR*^{rs731236} and *SLC13A2*^{rs11567842} demonstrated in our study suggest a possible pathogenic mechanism of hypocitraturia in recurrent CaOx SF. One can argue that the suppressive epistatic interaction among *VDR*^{rs731236} and *SLC13A2*^{rs11567842} may influence the expression of NaDC1 in the apical membrane of the epithelial cells of the renal proximal tubule significantly increasing the risk of hypocitraturia in recurrent CaOx SF and anticipating the mean age at onset of NL by more than 6 years. Epistasis is a form of gene–gene interaction whereby one gene interferes with the phenotypic expression of another non-allelic gene such that the phenotype is determined effectively by the former and not by the latter, even when both genes occur together in an individual [30, 31]. Epistatic interactions for quantitative traits fall into two categories: a change of the magnitude of effects, in which the phenotype of one locus is enhanced or suppressed by genotypes at the other locus; or a change of direction of effects [32]. In this context and according to Fisher's definition [33], additive variations represent the cumulative effect of individual loci (OR locus 1 + OR locus 2 = cumulative OR locus 1 + 2), whereas epistasis refers to a deviation from additivity in the effect of alleles at different loci with respect to their contribution to a quantitative phenotype (OR locus 1 + OR locus 2 ≠ cumulative OR locus 1 + 2) [30]. Our results are in agreement with a suppressive influence of *VDR*^{rs731236} SNP on the cellular expression of NaDC1^{I550V} variant, confirming recent pioneering *in vitro* observations [34]. A similar model of epistatic interaction was observed in asthmatic patients from the Chinese Han population by Liu et al. [35]. Further experimental studies are nevertheless necessary to confirm our findings.

A limitation of our study is its cross-sectional design, which prevents both the prospective evaluation of the associations observed and the influence of the detected interaction on the pharmacological response to citrate salts assumption, which is considered a milestone in the treatment of NL [6, 7]. Furthermore, validation of our findings in a second independent sample, also of different ethnicities, is currently lacking. This notwithstanding, the ethnic and geographic homogeneity of our cohort may be a good starting point to evaluate such gene interaction in other populations, with the objective to detect additional environmental interaction, if any. Considering the significant impact of NL on worldwide health status [1, 6], an improved knowledge of genetic susceptibility factors could be useful to better identify at-risk populations.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

- Romero V, Akpınar H, Assimos DG (2010) Kidney stones: a global picture of prevalence, incidence, and associated risk factors. *Rev Urol* 12:e86–e96
- Civelek M, Lusic AJ (2014) Systems genetics approaches to understand complex traits. *Nat Rev Genet* 15:34–48. doi:10.1038/nrg3575
- Halbritter J, Baum M, Hynes AM et al (2015) Fourteen monogenic genes account for 15 % of nephrolithiasis/nephrocalcinosis. *J Am Soc Nephrol* 26:543–551. doi:10.1681/ASN.2014040388
- Lieske JC, Turner ST, Edeh SN et al (2014) Heritability of urinary traits that contribute to nephrolithiasis. *Clin J Am Soc Nephrol* 9:943–950. doi:10.2215/CJN.08210813
- Wei WH, Hermani G, Haley CS (2014) Detecting epistasis in human complex traits. *Nat Rev Genet* 15:722–733. doi:10.1038/nrg3747
- Worcester EM, Coe FL (2010) Clinical practice. Calcium kidney stones. *N Engl J Med* 363:954–963. doi:10.1056/NEJMc1001011
- Zuckerman JM, Assimos DG (2009) Hypocitraturia: pathophysiology and medical management. *Rev Urol* 11:134–144
- Zacchia M, Preisig P (2010) Low urinary citrate: an overview. *J Nephrol* 23(Suppl 16):S49–S56
- Mossetti G, Vuotto P, Rendina D et al (2003) Association between vitamin D receptor gene polymorphisms and tubular citrate handling in calcium nephrolithiasis. *J Intern Med* 253:194–200
- Okamoto N, Aruga S, Matsuzaki S et al (2007) Associations between renal sodium-citrate cotransporter (hNaDC-1) gene

- polymorphism and urinary citrate excretion in recurrent renal calcium stone formers and normal controls. *Int J Urol* 14:344–349
11. Zhu C, Ye Z, Chen Z et al (2010) Association between vitamin D receptor gene polymorphisms and idiopathic hypocitraturia in the Chinese population. *Urol Int* 85:100–105
 12. Pajor AM (1996) Molecular cloning and functional expression of sodium-dicarboxylate cotransporter from human kidney. *Am J Physiol* 270:F642–648.
 13. Haussler MR, Jurutka PW, Mizwicki M et al (2011) Vitamin D receptor (VDR)-mediated actions of $1\alpha,25(\text{OH})_2$ vitamin D 3: genomic and non-genomic mechanisms. *Best Pract Res Clin Endocrinol Metab* 25:543–559
 14. Hamm LL, Hering-Smith KS (2002) Pathophysiology of hypocitraturic nephrolithiasis. *Endocrinol Metab Clin North Am* 31:885–893
 15. Pajor AM, Sun N (1999) Protein kinase C-mediated regulation of the renal Na^+ /dicarboxylate cotransporter, NaDC-1. *Biochim Biophys Acta* 1420:223–230
 16. Eastell R, Brandi ML, Costa AG et al (2014) Diagnosis of asymptomatic primary hyperparathyroidism: proceedings of the fourth international workshop. *J Clin Endocrinol Metab* 99:3570–3579. doi:10.1210/jc.2014-1414
 17. Lindberg JS, Sprague SM (2001) Nephrolithiasis. In: DeGroot LJ, Jameson JL (eds) *Endocrinology*. W.B. Saunders Company, Philadelphia, pp 1169–1180
 18. Levy FL, Adams-Huet B, Pak CY (1995) Ambulatory evaluation of nephrolithiasis: an update of a 1980 protocol. *Am J Med* 98:50–59
 19. Smulders MY, Frissen PHJ, Slaats EH et al (1996) Renal tubular acidosis. Pathophysiology and diagnosis. *Arch Intern Med* 156:1629–1636
 20. Rudman D, Dedonis JL, Fountain MT et al (1980) Hypocitraturia in patients with gastrointestinal malabsorption. *N Engl J Med* 303:657–661
 21. Pak CY, Poindexter JR, Adams-Huet B et al (2003) Predictive value of kidney stone composition in the detection of metabolic abnormalities. *Am J Med* 115:26–32
 22. Hahn LW, Ritchie MD, Moore JH (2003) Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions. *Bioinformatics* 19:376–382
 23. Goldfarb DS, Fischer ME, Keich Y et al (2005) A twin study of genetic and dietary influences on nephrolithiasis: a report from the Vietnam Era Twin (VET) Registry. *Kidney Int* 67:1053–1061
 24. Arcidiacono T, Mingione A, Macrina L et al (2014) Idiopathic calcium nephrolithiasis: a review of pathogenic mechanisms in the light of genetic studies. *Am J Nephrol* 40:499–506. doi:10.1159/000369833
 25. Vezzoli G, Terranegra A, Arcidiacono T et al (2011) Genetics and calcium nephrolithiasis. *Kidney Int* 80:587–593. doi:10.1038/ki.2010.430
 26. Lee Hamm L (1990) Renal handling of citrate. *Kidney Int* 38:728–735
 27. Pajor AM, Sun N (2010) Single nucleotide polymorphisms in the human Na^+ -dicarboxylate cotransporter affect transport activity and protein expression. *Am J Physiol Renal Physiol* 299:F704–F711. doi:10.1152/ajprenal.00213.2010
 28. Uitterlinden AG, Fang Y, Van Meurs JB et al (2004) Genetics and biology of vitamin D receptor polymorphisms. *Gene* 338:143–156
 29. Barsony J, Renyi I, McKoy W (1997) Subcellular distribution of normal and mutant vitamin D receptors in living cells. *J Biol Chem* 272:5774–5782
 30. Cordell HJ (2002) Epistasis: what it means, what it doesn't mean, and statistical methods to detect it in humans. *Hum Mol Genet* 11:2463–2468
 31. Burga A, Lehner B (2013) Predicting phenotypic variation from genotypes, phenotypes and a combination of the two. *Curr Opin Biotechnol* 24:803–809. doi:10.1016/j.copbio.2013.03.004
 32. Mackay TF (2014) Epistasis and quantitative traits: using model organisms to study gene-gene interactions. *Nat Rev Genet* 15:22–33. doi:10.1038/nrg3627
 33. Fisher RA (1918) The correlation between relatives on the supposition of Mendelian inheritance. *Trans R Soc Edin* 52:399–433
 34. Li K, Mo Y, Shen J et al (2016) Expression of VDR and NaDC1 in HK-2 cells and their significance in hypocitraturia. *Kunming yi ke da xue xue bao* 37:35–39
 35. Liu Y, Saccucci P, Qi H et al (2006) ADA polymorphisms and asthma: a study in the Chinese Han population. *J Asthma* 43:203–206