The *I550V* polymorphism in the renal human sodium/dicarboxylate cotransporter 1 (*hNaDC*-1) gene is associated with the risk for urolithiasis in adults from Southeastern, Mexico

Martha Medina-Escobedo[1*], Diana Franco-Bocanegra[1], Salha Villanueva-Jorge[1], Lizbeth González-Herrera[2]

[1]Departamento de Investigación en Enfermedades Renales, Hospital General O'Horán, Secretaria de Salud de Yucatán, Mérida, México
[2]Laboratorio de Genética, Centro de Investigaciones Regionales, Universidad Autónoma de Yucatán, Mérida, México
Email: *marthamedinaescobedo@hotmail.com

ABSTRACT

Urolithiasis (UL) is an endemic disease in Southeastern, Mexico. In order to evaluate the association of *I550V* polymorphism in the *hNaDC*-1 gene with risk for hypocitraturia and/or for UL; 139 adults with UL and 132 adults without UL, were included under a case-control association study. Citrate levels in 24-h urine were quantified (citraturia). The polymorphism *I550V-hNaDC*-1 was determined by PCR-RFLP. Statistical analysis was performed using the STATA10.2 software. Comparison of genotype and allele frequencies between subjects with and without UL showed significant differences for genotype bb (OR = 2.34, CI: 1.19 - 4.59, p = 0.01) and for allele b (OR = 1.62, CI: 1.15 - 2.28, p = 0.005), suggesting an association with the risk for UL. Comparison of genotype and allele frequencies between subjects with hypocitraturia and subjects with normocitraturia, did not show any significant difference (p > 0.05), suggesting that this polymorphism is not associated with the risk of hypocitraturia. Interestingly, the risk for UL was increased due to an additive effect of hypocitraturia with the genotype bb (OR = 6.6, CI: 2.38 - 18.28, p = 0.0002) or with the allele b (OR: 4.2, CI = 2.52 - 6.97, p < 0.0001) in the studied population.

*Corresponding author.

1. INTRODUCTION

The population of Yucatan, Mexico, is characterized by a high prevalence of urolithiasis (UL) [1]. Hypocitraturia, which is considered as a urine citrate excretion below 320 mg in 24 hours [2], represents the most common metabolic abnormality in adults with UL [3]. Hypocitraturia is known to be a major risk factor for the development of lithiasis [4-7], because of citrate acts as a calcium chelating agent, which reduces the formation of oxalate and calcium phosphate, thus inhibiting the generation of urinary calculus [8].

In nephrons, urine citrate is reabsorbed from the glomerular filtrate in the apical membrane of kidney proximal tubule cells by means of the transmembrane protein known as human sodium/dicarboxylate cotransporter 1 (*NaDC*-1), through a sodium-dependent mechanism [9]. In these cells, *NaDC*-1 is responsible for the reabsorption of other di- and tri-carboxylic acids most of which are citric acid cycle intermediates, such as fumarate, succinate and α-ketoglutarate, which are compounds involved in numerous important physiological processes in the body [10].

NaDC-1 is a protein with 593 amino acids, which contains 11 transmembrane domains. The gene *hNaDC*-1 belongs to the family of genes encoding the SLC13 solute carriers [11] and; it is located on locus 17p13.2. The coding portion of the *hNaDC*-1 gene contains 1953 base pairs in 12 exons [9]. Certain single nucleotide polymorphisms (SNPs) in the *hNaDC*-1 gene, have been described to produce differences in the rate of gene expres-

sion as well as, significant variation in the affinity to sodium and to various substrates in *NaDC*-1 protein; such conditions would alter the rate of tubular reabsorption of these compounds [12].

One of these SNPs, *I550V*, consists of a substitution of adenine by guanine at position 1716, in exon 12, which produces a change in amino acid 550 of *NaDC*-1 protein from isoleucine to valine. This SNP has been associated with the risk of hypocitraturia in recurrent renal stone formers [13]. However, those results have not yet been reproduced in any other population. Also, such study found no direct association between the *I550V* SNP and UL [13], nor the influence of the SNP on either the rate of gene expression or the *NaDC*-1 transport activity have been proven through *in vitro* studies [12]. Based on the above and in order to provide evidence to help determine the role performed by this SNP in the genetic risk of UL, this study focuses on evaluating the association of *I550V* SNP in *hNaDC*-1 gene with the genetic risk for develop hypocitraturia and/or urolithiasis in a population of adult subjects from Southeastern Mexico, where UL is highly prevalent and considered endemic.

2. MATERIALS AND METHODS

2.1. Population

We performed a case-control association study. The sample consisted of 271 subjects, 95 men and 176 women, above 17 years old. The case group included 139 subjects with urolithiasis and 132 subjects without urolithiasis were used as controls. UL subjects were 51 (36.7%) men and 88 (63.3%) women, with mean age of 34 ± 10.5 years; whereas control subjects were 44 (33.3%) men and 88 (66.7%) women, with mean age of 36.2 ± 10.6 years. The presence or absence of UL in each subject was confirmed by ultrasound or X-ray. Subjects with urinary tract obstruction diseases, diabetes mellitus, hypertension, asthma, bronchitis, kidney failure, metabolic acidosis and those with prolonged medical therapy for any disease; were excluded. Likewise, the control group did not include subjects with a history of chronic disease or of diuretic drug intake. It should be noted that during the study the subjects continued their usual diet without modifications. All selected subjects were born and had lived for at least three generations in Yucatan, Mexico. We also used anthropologic and demographic parameters such as language, birth place, surnames, genealogy, and history of lifestyle, among others, to match ethnically control with case subjects belonging to the same ethnic group of Mestizos, defined as individuals born in the country having a Spanish-derived last name, and with a family history of Mexican ancestors back at least to the third generation. In addition, we previously determined the absence of substructure or population stratification within the popu-

lation of Yucatan by using 16 autosomal STR markers [14], which may represent a confounder.

Informed consent was obtained from cases and controls according to the recommendations of the Helsinki Declaration. This study was approved by the Bioethics Committee of Hospital General Agustin O'Horan de Merida, Yucatan, México.

2.2. Determination of Urine Citrate Levels (Citraturia)

To determine citrate excretion, a 24-h urine sample was collected. The concentration of citrate in urine was determined using a commercial citrate determination kit (Boehringer-Mannheim Cat # 10139076035) and citraturia was expressed in terms of mg/day measured in 24-hour urine samples. Additionally, urea and creatinine serum levels were measured to verify proper kidney function [15,16].

2.3. Genotyping

Leukocyte DNA extraction with anticoagulant (EDTA) from peripheral blood was carried out by a conventional phenol-chloroform method [17]. The polymerase chain reaction technique (PCR) was used to amplify a 520 bp fragment of exon 12 at the *hNaDC*1 gene, wherein the *I550V* SNP is located. Each 25 μl PCR reaction contained 18.3 μl of sterile H2O, 2.5 μl of 5X Green reaction buffer (PromegaCat#M5005), 1.5 mM MgCl2, 0.4 μM dNTPs, 0.3 μM of each primer; and 0.65u of *Taq* polymerase enzyme (Promega). The sequences of the primers used were 5'-ACGGGAGGACTTCCCAGAGA-3' for the forward primer and 5'-CAGGCGCACACATATC-GCA-3' for the reverse primer, previously described by Okamoto *et al*. 2007 [13]. The prepared samples were placed in an Applied Biosystems Veriti thermocycler with the following PCR conditions: initial denaturation for 1 min at 94˚C, 30 cycles of denaturation for 1 min at 94˚C, annealing for 1 min at 59˚C and extension for 1 min at 72˚C and finally 10 min final extension at 72˚C, followed by cooling for 5 min at 4˚C. The amplified DNA was incubated at 50˚C overnight in the presence of the restriction endonuclease *Bcl* I (*Bacillus caldolyticus* I) [13]. Each restriction reaction contained 10 μl of amplified DNA, 7.62 μl of sterile H₂O, 2 μl of buffer C, 0.2 BSA and 1.8u of *Bcl* I restriction enzyme Promega Cat # R4654). The *Bcl* I enzyme cuts only those fragments which have adenine (B allele) instead of guanine (b allele). When cut, the 520 bp fragment produces two fragments, one with 354 bp and one with 166 bp. After incubation, the samples were again subject to polyacrylamide gel electrophoresis, from which genotypes were identified, according to Okamoto *et al*. [13].

2.4. Statistical Analysis

Mean levels of citrate excretion between cases and controls, as well as among genotypes were compared with Student t test. A value of p < 0.05 was considered significant. Hardy-Weinberg equilibrium deviations were determined in the studied population by means of Chi square test. Genotype and allele frequencies of *I550V SNP* were also evaluated and compared between cases and controls in order to determine significant associations. To determine whether the studied genotypes or alleles conferred a significant increase to the risk of hypocitraturia and/or UL, we calculated the odds ratio (OR) with a confidence interval (CI) of 95%, using the Woolf approximation. Significance was considered when p < 0.05. All statistical tests were performed using the STATA version 10.2 software.

3. RESULTS

Genotype and allele frequencies of the *I550V* polymerphism in *hNaDC* gene in 139 subjects with urolithiasis and 132 subjects without urolithiasis are shown in **Table 1**. Genotype frequencies for the *I550V* polymorphism in *hNaDC*-1 gene were distributed according to Hardy-Weinberg equilibrium expectations (p > 0.05). The three *I550V-hNaDC*-1 SNP genotypes were observed in the studied population, being the heterozygous genotype Bb the most frequent in control subjects, whereas the homozygous bb genotype was the most frequent in subjects with UL. The frequency of the allele b in the control population was 47.73%. Comparison of genotype and allele frequencies between subjects with and without UL, showed significant differences for the homozygous genotype bb (p = 0.017); and for the allele b (p = 0.005); suggesting that the *I550V* polymorphism in *hNaDC* gene is associated with the genetic risk for UL in the studied population.

Mean levels of citrate excretion obtained in subjects with UL were 309 ± 223 mg/24h, which were significantly lower than mean levels of citrate excretion in control subjects of 408 ± 187 mg/24h (p = 0.0001). This finding remarked the hipocitraturic condition en cases of UL. Moreover, hypocitraturia (<320 mg/24h) was significantly more frequent in cases of UL (61.9%, N = 86), than in control subjects (38.1%, N = 53), suggesting that hypocitraturia might be an associated risk factor for UL in the studied population (OR = 2.5, CI: 1.49 - 4.20, p < 0.001).

According to the levels of citrate excretion, studied subjects were stratified in hypocitraturia and normocitraturia in order to evaluate its association with the *I550V* polymorphism in *hNaDC*-1 gene, independently of the UL. We identified an overall of 138 subjects with hypocitraturia and 132 subjects with normocitraturia in our population. Genotype and allele frequencies of the *I550V* polymorphism in *hNaDC*-1 gene in subjects with hypocitraturia and subjects with normocitraturia, as well as the comparison of frequencies on an association analysis are listed in **Table 2**. Genotype frequencies for the *I550V* polymorphism in *hNaDC*-1 gene was distributed according to Hardy-Weinberg equilibrium expectations (p > 0.05). The heterozygous genotype Bb was the most frequent in both subjects with hypocitraturia (50.72%) and normocitraturia (40.60%). The comparison of genotype and allele frequencies of *I550V* polymorphism in *hNaDC*-1 gene between subjects with hypocitraturia and subjects with a normal level of citrate excretion (normocitraturia) did not show significant difference for any genotype or allele (p > 0.05), suggesting that the *I550V* polymerphism in *hNaDC*-1 gene is not associated with the risk for low levels of citrate excretion or with the risk for hypocitraturia within the studied population.

Mean levels of citrate excretion according to genotype for *I550V-NaDC*-1 polymorphism, are shown in **Table 3**. Mean levels of citrate excretion for each genotype were compared within genotypes, showing no significant dif-

Table 1. Genotype and allele frequencies of the *I550V* polymorphism in *hNaDC*-1 gene in subjects with urolithiasis (UL) and subjects without lithiasis, Hardy-Weinberg expectations; and association analysis (OR, IC, p), N (%).

Genotypes and Alleles of the SNP *I550V* in *hNaDC*-1 gene	Subjects with urolithiasis	Subjects without urolithiasis	Cases vs. control		
			OR	95% CI	p
	N = 139	N = 132			
	2N = 278	2N = 264			
BB	29 (20.86)	34 (25.76)		Reference	
Bb	54 (38.85)	70 (53.03)	0.90	0.49 - 1.66	0.757
bb	56 (40.29)	28 (21.21)	2.34	1.19 - 4.59	0.017[**]
Allele B	112 (40.29)	138 (52.27)		Reference	
Allele b	166 (59.71)	126 (47.73)	1.62	1.15 - 2.28	0.005[**]
Hardy-Weinberg (p)	(0.291)	(0.905)			

[**]significant.

Table 2. Genotype and allele frequencies of the *I550V* polymorphism in *hNaDC*-1 gene in subjects with hypocitraturia and subjects with normocitraturia, Hardy-Weinberg expectations; and association analysis (OR, IC, p), N (%).

Genotypes and Alleles of the SNP *I550V* in *hNaDC*-1 gene	Subjects with hipocitraturia	Subjects with normocitraturia	Cases vs. control		
			OR	95% CI	p
	N = 138	N = 133			
	2N = 276	2N = 266			
BB	28 (20.29)	35 (26.32)		Reference	
Bb	70 (50.72)	54 (40.60)	1.62	0.87 - 2.98	0.125
bb	40 (28.99)	44 (33.08)	1.13	0.58 - 2.18	0.740
Allele B	126 (45.65)	124 (46.62)		Reference	
Allele b	150 (54.35)	142 (53.38)	1.03	0.74 - 1.45	0.444
Hardy-Weinberg (p)	(0.998)	(0.328)			

Table 3. Comparison of mean citrate excretion (mg/24hr) according genotypes for the SNP *I550V-hNaDC*-1 in the studied overall population.

Genotype	*Mean of citrate excretion* (mg/24hrs.)	Standard deviation	Significance (p) versus:		
			BB	Bb	bb
BB N = 63	374.71	216.22	---	0.296	0.912
Bb N = 124	340.09	207.80	0.296	----	0.307
bb N = 84	370.77	214.65	0.912	0.307	----

ferences ($p > 0.05$), which leads towards the point that citrate excretion itself is not associated with any allele or genotype for the *I550V* polymorphism in gene.

Case and control subjects were also compared independently between two groups according hypocitraturia or normocitraturia; and within these groups into the genotype and allele frequencies for the *I550V* polymorphism in the *hNaDC*-1 gene (intra-group analysis). Significant differences were found in the group with normocitraturia for both the bb genotype (OR = 2.52, CI: 1.00 - 6.31, p = 0.038), and b allele (OR = 1.82, CI: 1.10 - 3.00, p = 0.023), whereas subjects with hypocitraturia did not show any significant difference for any genotype or allele (**Table 4**). This finding supports that the *I550V* polymorphism in the *hNaDC*-1 gene might be associated with UL itself, rather than hypocitraturia.

Given that the bb genotype and hypocitraturia were independently found to be risk factors associated with the presence of UL; the additive effect of these factors was assessed, in order to determine if the risk is increased when both factors occur concurrently. The genotype frequencies of the SNP *I550V-hNaDC*-1 of subjects with UL and hypocitraturia were compared with frequencies for the reference conditions of control subjects with normocitraturia and BB or Bb genotype (**Table 5**). Significant associations were obtained for these comparisons (p < 0.05); except for subjects with UL, hypocitraturia and genotype Bb (p = 0.06). The highest risk was associated with subjects with UL, hypocitraturia and bb genotype, OR = 6.6, CI: 2.38 - 18.28, p = 0.0002, when compare

with homozygous BB; and OR = 8.18, CI: 3.18 - 21.03, p = 0.000; when compare with heterozygous Bb. These findings suggest that the presence of bb genotype added to the presence of hypocitraturia, noticeably increases the risk for UL.

Comparison of mean urine citrate levels (mg/24h.) stratified according to genotypes for the SNP *I550V-hNaDC*-1; showed significant differences between subjects with UL and control subjects for the heterozygous genotype Bb (0.002) and for the homozygous genotype bb (0.004) (**Table 6**); suggesting that these genotypes might be implicated in the lower urine citrate excretion in presence of urolithiasis.

4. DISCUSSION

The results of this study confirmed that hypocitraturia was present with a significantly higher frequency in subjects with UL (61.9%) than in controls (38.1%), representing an identifiable risk factor for UL in the study population ($p < 0.05$). This observation is consistent with results of previous studies which evaluated the same association in other populations [4-7] and; with the results obtained in a previous study conducted in the Yucatan population, in which hypocitraturia was found in 63.6% of patients with UL [3]. Given that we did not find any association between *I550V-hNaDC*-1 genotype and hypocitraturia itself, the genetic contribution of this SNP towards hypocitraturia was not demonstrated in this Mexican population; so the possible genetic basis should

Table 4. Genotype and allele frequencies of the *I550V* polymorphism in *hNaDC*-1 gene in subjects with urolithiasis and subjects without urolithiasis, according to hipocitraria or normocitraturia under an intragroup analysis; (OR, IC, p), N (%).

Genotypes and Alleles of the SNP *I550V* in *hNaDC*-1 gene	Subjects with urolithiasis	Subjects without urolithiasis	Cases vs. control		
			OR	95% CI	p
Normocitraturia	N = 53	N = 80			
BB	12 (22.64)	23 (28.75)		Reference	
Bb	16 (30.19)	38 (47.50)	0.80	0.34 - 2.00	0.648
bb	25 (47.17)	19 (23.75)	2.52	1.00 - 6.31	0.038[**]
Allele B	40 (37.74)	84 (52.50)		Reference	
Allele b	66 (62.26)	76 (47.50)	1.82	1.10 - 3.00	0.023[**]
Hipocitraturia	N = 86	N = 52			
BB	17 (19.77)	11 (21.15)		Reference	
Bb	38 (44.19)	32 (61.54)	0.76	0.31 - 1.87	0.654
bb	31 (36.04)	9 (17.31)	2.22	0.77 - 6.44	0.178
Allele B	72 (41.86)	54 (51.92)		Reference	
Allele b	100 (58.14)	50 (48.08)	1.50	0.91 - 2.44	0.107

[**]significant.

Table 5. Genotype frequencies of the *I550V* polymorphism in *hNaDC*-1 gene in subjects with urolithiasis and hipocitraturia versus the reference condition of subjects without urolithiasis, normocitraturia and BB or Bb (across group analysis; OR, IC, p), N (%).

Genotypes and Alleles of the SNP *I550V* in *hNaDC*-1 gene	Subjects with urolithiasis	Subjects without urolithiasis	Cases vs. control		
			OR	95% CI	p
Normocitraturia and homozygous BB	2 (22.64)	23 (28.75)		Reference	
versus					
Hipocitraturia and					
BB	17 (19.77)	11 (21.15)	2.96	1.05 - 8.30	0.045[**]
Bb	38 (44.19)	32 (61.54)	2.27	0.98 - 5.28	0.063
bb	31 (36.04)	9 (17.31)	6.06	2.38 - 18.28	0.0002[**]
Normocitraturia and Heterozygous Bb	16 (30.19)	38 (47.50)		Reference	
versus					
Hipocitraturia and					
BB	17 (19.77)	11 (21.15)	3.67	1.40 - 9.56	0.009[**]
Bb	38 (44.19)	32 (61.54)	2.82	1.33 - 5.96	0.006[**]
bb	31 (36.04)	9 (17.31)	8.18	3.18 - 21.03	0.000[**]

[**]significant.

be in relation to different implicated genes or different SNPs. However, we were able to demonstrate that the polymorphism *I550V-hNaDC*-1 might contribute to the genetic susceptibility to develop urolithiasis; showing a higher risk for subjects with hypocitraturia and the bb genotype. Implication of the SNP *I550V-hNaDC*-1 in the mechanism of stone formation might be due to the isoleucine-to-valine alteration at position 550 in h*NaDC*-1 is located in the last trans membrane domain and the C-terminal half, which contains amino acid residues that determine substrate recognition and cation affinity; therefore, altering the function for transferring citrate. It is also possible that the rate of transcription or stability, of *hNaDC*-1 is modified by the SNP [13].

It is worth to note that the intestinal absorption of citrate, is the main source of plasma citrate [18]. The absorbed citrate is utilized mainly in the liver, and also fulfills the function of promoting the sodium and water absorption in the jejunum [19]. In the intestine, the absorption of citrate is carried out by the same transporter, *NaDC*-1 as in the renal proximal tubules. The presence of this protein in the intestine was documented by

Table 6. Comparison of mean citrate excretion (mg/24h.) according to genotypes for the SNP *I550V-hNaDC*-1, between cases and control.

Genotype	Subjects with Urolithiasis N = 139	Subjects without Urolithiasis N = 132	Significance p
BB	339.89 ± 216.22 N = 29	395.75 ± 164.11 N = 34	0.259
Bb	277.54 ± 222.19 N = 54	391.63 ± 182.82 N = 70	0.002[**]
bb	324.87 ± 202.11 N = 56	470.62 ± 212.53 N = 28	0.004[**]

[**]significant.

Northern blot analysis, in which, mRNA of *NaDC*-1 was detected in intestinal tissue [9]. The fact that the same protein regulates citrate absorption in both the intestine as in the renal proximal tubules is interesting in the sense that one SNP affecting the recognition of the *NaDC*-1 substrate would result in a poor citrate absorption at the intestine level, which could be reflected with the onset of hypocitraturia [20]. Poor intestinal citrate absorption should not be dismissed as a possible cause for the high frequency of hypocitraturia in the Yucatan population.

Compared with other UL associated SNPs, *I550V* SNP has been scarcely studied. The only study regarding this SNP, analyzed in a human population before the present work was carried on, was in Japan [13]. The results obtained in the present study markedly contrast those reported in the Japan study [13], in which, when comparing the genotype and allele frequencies of this SNP between subjects with hypocitraturia and subjects with normocitraturia, significant differences were revealed for both genotype (p = 0.0007), and allele (p = 0.00005), exhibiting a higher frequency of the BB genotype and B allele in subjects with hypocitraturia, pointing to these factors as associated to the presence of hypocitraturia. Those data contrast with findings in our study in which no significant differences neither in genotype nor in allele frequencies between subjects with hypocitraturia and normocitraturia, were shown.

Okamoto *et al.*, 2007 [13] explain the results suggesting that the b allele of the *I550V* SNP causes a change in the structure of the transporter, which would result in a decrease of its affinity to the substrate. With this, the rate of reabsorption of the filtered citrate would be reduced and, therefore, citrate excretion increased, whereby the b allele would be conferring a protection factor against hypocitraturia, and hence against UL. Nevertheless, these results have not been reproduced in any other population in the world, and the reported association between genotype *I550V* and citraturia has not been confirmed through *in vitro* studies. A site-directed mutagenesis technique study in COS-7 cells has reported no significant differ-

ences in the rate of gene expression or in the transport activity of *NaDC*-1 among the different genotypes of *I550V* SNP [12].

Lack of association between *I550V* SNP and citraturia obtained in this study, might be explained because of the *NaDC*-1 is capable of carrying other di- and tri-carboxylic acids, by having greater affinity with these than with citrate, as are fumarate and succinate as well as methyl-, dimethyl succinate, which can be present in the luminal liquid [21]. It has been reported from *in vitro* studies that fumarate and succinate significantly inhibit citrate uptake by *NaDC*-1 in brush border membrane vesicles through a competitive inhibition mechanism [19]. Likewise, it is known that sulfate may inhibit citrate transport; this inhibition, unlike that of succinate and fumarate is noncompetitive, since sulfate hinders the binding of other substrates to the transporter, but without being carried in to the tubular cell interior [22]. It is possible that the transporter saturation by these luminal components causes an inhibition of citrate transport, which may explain the observed lack of association among *I550V* genotype and citraturia in this study. Studies of the urinary profile, taking into account the determination of the concentration of succinate and fumarate in plasma and urine, are needed to shed some light on this point.

Another explanation is that other SNPs, either by themselves, or through an interaction with *I550V* SNP; might have effects on the amount of transporters existing in the membrane, which could influence substrate concentration at which the transporter reaches saturation.

In the Mexican population analyzed for this study, significant differences were found with respect to *I550V-hNaDC*-1 genotype and allele frequencies between cases and controls, suggesting an association between the b allele and the presence of UL, opposite to what was obtained in the Japanese population studied by Okamoto *et al.*, 2007, [13] who did not evidence such association. Given that in the present study, we did not find any association of the bb genotype with citraturia, whereas a possitive association was found with the presence of UL, the outcome suggests the existence of another mechanism, unrelated to urinary citrate excretion, whereby the transporter is involved in the lithogenic process.

As mentioned above, in addition to *I550V*, other polymorphisms that have implications on the activity and functioning of *hNaDC*-1 have been identified [12]. It is possible that some alleles of other polymorphisms are associated with the b allele in *I550V* SNP through linkage disequilibrium [23] and; that this would explain the association found between b allele, bb genotype and UL. The association among these polymorphisms and the presence of UL has not been evaluated whatsoever.

The complete lack of association between genotype and citraturia was confirmed once again by dividing the

study population into subjects with hypocitraturia and normocitraturia and comparing the genotype and allele frequencies between cases and controls, within each group. In this case, as mentioned before, frequency differences were obtained, pointing at the bb genotype and the b allele as risk factors for UL, only in the group of subjects with normocitraturia. The above confirms that the citraturia is not associated with genotype in this population, since the association between genotype and UL was present in subjects with normal levels of urinary citrate.

The fact that the same association was not found in the group of subjects with hypocitraturia may be due to citraturia itself representing a heavier risk factor than the genotype (which is consistent with the higher value of OR obtained for hypocitraturia than that for genotype), which implies that subjects with hypocitraturia develop UL more often, even in the absence of the risk genotype.

Another important aspect to consider, especially when dealing with multicausal origin conditions is the fact that gene expression is a mechanism capable of changing according to outer environmental stimuli, performing what is known as gene-environment interactions. UL is known to be a multifactorial pathology resulting from the interaction between environmental influences and genetic as well as hormonal factors [24,25]. The differences between the results obtained in the Japanese population [13] and; those reported in this paper can also owe to the many variations that exist in the environmental conditions to which the subjects in both populations are exposed, same which, through repression or induction mechanisms could produce differences in *hNaDC*-1 gene expression, conversely, the combination of a certain genotype with diverse environmental variables could produce a difference in the effect of this genotype on various phenotypic features

With regard to the increased risk for UL due to the interaction of both risk factors observed in this study: hypocitraturia and the bb genotype or b allele, might be explained because UL is a disorder with a multifactorial etiology, the presence of only one of the risk factors is not sufficient to develop the disease, however, these risk factors show additive effect [26], in this study we showed that the presence of two risk factors significantly increases the risk of acquiring the disease compared with the presence of each of these factors independently.

In order to find the answer to complete the new questions arisen from this study, the dynamics of di- and tricarboxylic acid transport must be analyzed further; as well as the determination of plasma citrate; and other intermediates of the citric acid cycle in urine as part of the UL patient's metabolic profile.

In conclusion, our study demonstrated that the polymorphism *I*550*V* in the *hNaDC*-1 gene is associated with the risk for urolithiasis. Moreover, the risk for UL might increase due to an additive effect of hypocitraturia with the genotype bb or with the allele b in the population of Southeastern, Mexico.

5. ACKNOWLEDGEMENTS

REFERENCES

[1] Medina-Escobedo, M.M., Zaidi, M., Real-de-León, E. and Orozco-Rivadeneyra, S. (2002) Prevalencia y factores de riesgo en Yucatán, México, para litiasis urinaria. *Salud Pública de México*, **44**, 541-545. doi:10.1590/S0036-36342002000600006

[2] Arrabal, M., Fernández, A., Arrabal, M.A., Ruiz, M.J. and Zuluaga, A. (2006) Estudio de factores físico-químicos en pacientes con litiasis renal. *Archivos Españoles de Urología*, **59**, 583-594.

[3] Villanueva-Jorge, S., Medina-Escobedo, M.M. and Arcos, A. (2007) Excreción de oxalatos y citratos en adultos con litiasis urinaria. *Bioquimia*, **32**, 134-140.

[4] Akçay, T., Konukoğlu, D. and Celik, C. (1996) Hypocitraturia in patients with urolithiasis. *Archives of Disease in Childhood*, **74**, 350-351. doi:10.1136/adc.74.4.350

[5] Gambaro, G., Fabris, A., Puliatta, D. and Lupo, A. (2005) Lithiasis in cystic kidney disease and malformations of the urinary tract. *Urological Research*, **34**, 102-107. doi:10.1007/s00240-005-0019-z

[6] Spivacow, F.R., Valle, E.E. and Zanchetta, J.R. (2006) Litiasis renal. Modificaciones bioquímicas durante el seguimiento. *Medicina*, **66**, 201-205.

[7] Deshmukh, S.R. and Kahn, Z.H. (2006) Evaluation of urinary abnormalities in nephrolithiasis patients of Marathwada region. *Indian Journal of Clinical Biochemistry*, **21**, 177-180. doi:10.1007/BF02913091

[8] Fan, J., Schwille, P.O., Schmiedl, A., Fink, E. and Manoharan, M. (2001) Calcium oxalate crystallization in undilluted postprandial urine of healthy male volunteers as influenced by citrate. *Arzneimittel-Forschung*, **51**, 848-857.

[9] Pajor, A.M. (1996) Molecular cloning and functional expression of a sodium-dicarboxylate cotransporter from human kidney. *American Journal of Physiology*, **270**, F642-F648.

[10] Pajor, A.M. and Sun, N.N. (2000) Molecular cloning, chromosomal organization and functional characterization of a sodium-dicarboxylatecotransporter from mouse kidney. *Renal Physiology*: *American Journal of Physiology*, **279**, F482-F490.

[11] Pajor, A.M. (2006) Molecular properties of the SLC13 family of dicarboxylate and sulfate transporters. *Pflügers Archiv*, **451**, 597-605. doi:10.1007/s00424-005-1487-2

[12] Pajor, A.M. and Sun, N.N. (2010) Single nucleotide polymorphisms in the human Na+-dicarboxylate co-transporter affect transport activity and protein expression.

Renal Physiology: *American Journal of Physiology*, **299**, F704-F711. doi:10.1152/ajprenal.00213.2010

[13] Okamoto, N., Aruga, S., Matsuzaki, S., Takahashi, S., Matsushita, K. and Kitamura, T. (2007) Associations between renal sodium-citrate cotransporter (*hNaDC*-1*)* gene polymorphism and urinary citrate excretion in recurrent renal calcium stone formers and normal controls. *International Journal of Urology*, **14**, 344-349. doi:10.1111/j.1442-2042.2007.01554.x

[14] González-Herrera, L., Vega-Navarrete, L., Roche-Canto, C., Canto-Herrera, J., Virgen-Ponce, D., Moscoso-Caloca, G., *et al.* (2010) Forensic parameters and genetic variation of 15 autosomal STR Loci in Mexican Mestizo populations from the States of Yucatan and Nayarit. *Open Forensic Science Journal*, **3**, 57-63.

[15] Gotsman, I., Zwas, D., Planer, D., Admon, D., Lotan, C. and Keren, A. (2010) The significance of serum urea and renal function in patients with heart failure. *Medicine*, **89**, 197-203. doi:10.1097/MD.0b013e3181e893ee

[16] Perrone, R.D., Madias, N.E. and Levey, A.S. (1992) Serum creatinine as an index of renal function: New insights into old concepts. *Clinical Chemistry*, **38**, 1933-1953.

[17] Blin, N. and Stafford, D.W. (1976) A general method for isolation of high molecular weight DNA. *Nucleic Acids Research*, **3**, 2303-2308. doi:10.1093/nar/3.9.2303

[18] Hamm, L.L. (1990) Renal handling of citrate. *Kidney International*, **38**, 728-735. doi:10.1038/ki.1990.265

[19] Rolston, D.D., Moriarty, K.J., Farthing, M.J., Kelly, M.J., Clark, M.L. and Dawspm, A.M. (1986) Acetate and citrate stimulate water and sodium absorption in the human jejunum. *Digestion*, **34**, 101-104. doi:10.1159/000199317

[20] Rudman, D., Dedonis, J.L., Fountain, M.T., Chandler, J.B., Gerron, G.G., Fleming, G.A., *et al.* (1980) Hypocitraturia in patients with gastrointestinal malabsorption. *New England Journal of Medicine*, **303**, 657-661. doi:10.1056/NEJM198009183031201

[21] Yao, X. and Pajor, A.M. (2000) The transport properties of the human renal Na+-dicarboxylate cotransporter under voltage-clamp conditions. *Renal Physiology*: *American Journal of Physiology*, **279**, 54-64.

[22] Aruga, S., Pajor, A.M., Nakamura, K., Liu, L., Moe, O.W., Preisig, P.A., *et al.* (2004) OKP cells express the Na-dicarboxylate cotransporter *NaDC*-1. *Cell Physiology*: *American Journal of Physiology*, **287**, C64-C72. doi:10.1152/ajpcell.00061.2003

[23] Reich, D.E., Cargill, M., Bolk, S., Ireland, J., Sabeti, P.C., Richter, D.J., *et al.* (2001) Linkage disequilibrium in the human genome. *Nature*, **411**, 199-204. doi:10.1038/35075590

[24] van Vliet, J., Oates, N.A. and Whitelaw, E. (2007) Epigenetic mechanisms in the context of complex diseases. *Cellular and Molecular Life Sciences*, **64**, 1531-1538. doi:10.1007/s00018-007-6526-z

[25] Healy, K.A. and Ogan, K. (2005) Nonsurgical management of urolithiasis: An overview of expulsive therapy. *Journal of Endourology*, **19**, 759-767. doi:10.1089/end.2005.19.759

[26] Clayton, D. and McKeigue, P.M, (2001) Epidemiological methods for studying genes and environmental factors in complex diseases. *The Lancet*, **358**, 1356-1360. doi:10.1016/S0140-6736(01)06418-2