REVIEW ARTICLE

PREDISPOSITION OF GENETIC POLYMORPHISM WITH THE RISK OF UROLITHIASIS

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ABSTRACT

Urolithiasis is a relevant clinical problem with a subsequent burden for health system. The aim of this review is to provide recent progress made using genetic polymorphisms to define pathophysiology, to identify persons at risk for kidney stone disease and to predict treatment response. Population case-control studies are useful both as an alternative and an adjunct as compared to family studies. These involve either whole genome scanning or candidate gene approaches. While whole genome scanning is likely to be widely used in future, at present, candidate gene studies are more feasible. When performing candidate gene case-control studies factors such as study design, methods for recruitment of case and controls, selection of candidate genes, functional significance of polymorphisms chosen for study and statistical analysis require close attention to ensure that only genuine associations are detected. Some of the significant genes that play role in stone formation include calcitonin receptor gene (CTR), vitamin D receptor (VDR), Urokinase, Interleukin, (IL-1 β , IL-Ra), E-Cadherin, Androgen & oestrogen receptor gene, vascular endothelial growth factor (VEGF) and Arginine p21. In our case-control study we studied CTR, VDR, Urokinase, IL-1 β (-511 and +3954), IL-Ra from north India and predict that VDR, IL- β (-511) and IL-1Ra gene may be used as a possible genetic marker for earlier detection in patients who are at risk for calcium oxalate stone disease. Further, linkage disequilibrium and haplotype structure of a certain candidate gene is important for association analysis. When a certain polymorphic allele has been found to be associated with disease, it is further explained on basis of LD and haplotype structure by one or more other alleles. Once it is determined which haplotype carries the risk allele, by means of molecular biological functional analyses, the variants on that haplotype allele truly causing the effect can be determined.

KEY WORDS

Urolithiasis, Single nucleotide polymorphism, Allele, Haplotype, Candidate genes, Hypercalciurea.

INTRODUCTION

Prevalence of urinary stones is epidemic in many parts of the world. It is a major health problem, with a significant proportion of patients requiring extensive surgical procedure and a sizable minority losing a kidney. Despite intensive studies in last decades many aspects of urolithiasis still remain to be elucidated. Although the genetic causes have been studied

extensively, no chromosomal mapping have been achieved in stone patients with idiopathic hypercalciurea (1, 2). The only conclusive comment possible on the basis of genetic studies is that it is a polygenic defect and partially penetrative (3). Even though there is considerable economic and social impact in terms of lost productivity, there is no predictive marker for the disease, with most patients being diagnosed after the development of symptoms.

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Urolithiasis is a multifactorial disease for which genetic and environment are confounding factors (4). It is seen that 50-70% stone disease patients have a first relative degree with urolithiasis (5). It is still puzzling whether the increased risk is attributable to genetic factors, environmental exposure or some combination. Curhan et al showed that restricting dietary

calcium might increase the risk for stone formation in families with stone history (6). An autosomal dominant mode of inheritance among families having stone history has been supported suggesting the role of several candidate genes (7). In this review we aim to highlight the importance of genetics of a complex disease. Several genes like vitamin D receptor (VDR); vascular endothelial growth factor (VEGF), E-cadherin, p21, androgen-oestrogen receptor genes, calcitonin receptor (CTR) and cytokines have been proposed as candidates in this search for an association (8-14). The contribution of each gene is small and the major genetic determinants of urolithiasis have not yet elucidated. The contribution of subtle alterations in gene sequence to kidney stone susceptibility can only be determined once genotyped-phenotype correlations have been established. Polymorphisms that exist in these regions as variations in repeat sequences throughout the genome have served the basis for genetic linkage analysis (15).

So single nucleotide polymorphisms (SNP) has emerged as a tool for mapping the complex disease hence making it possible to search the candidate genes as cause of stone disease for calcium metabolism (16). The study of genetic polymorphisms promises to help define pathophysiological mechanisms, to identify individuals at risk for disease and to suggest novel targets for drug treatment. Based on SNPs, a population genetic approach provides a new way of identifying the genes associated with disease (17).

Genetic markers of calcium oxalate stone disease could be clinically useful for identifying subjects at risk of stone disease and for preventing its occurrence. Genes that regulate calcium and oxalate metabolism are believed as a genetic marker of urolithiasis that influences the formation of stone. Some of the significant genes which play role in stone formation include: calcitonin receptor gene (CTR), vitamin D receptor (VDR), Urokinase, Interleukin, (IL-1 β , IL-Ra), E-Cadherin, Androgen & oestrogen receptor gene, VEGF (vascular endothelial growth factor) and Arginine p21. In this article we review the above-mentioned genetic polymorphisms that may have an etiological role in stone disease. Moreover, Linkage disequilibrium (LD) and haplotype analysis has been performed in VDR and IL-1 gene as in both the genes, the polymorphic sites studied were in strong linkage disequilibrium. We include associations identified in molecular epidemiological studies and the consistency of findings reported till date.

A search of the English literature using the National Library of Medicine MEDLINE and essential search terms for the years 1985-2006 was undertaken to identify all published articles or abstracts in which the frequency distribution of genotypes for

each gene was determined for kidney stone by PCR method. Articles selected for meta-analysis were case-control in design, published in primary literature and had no obvious overlap of subjects with other studies (Table 1). In the Table 1 we have also provided our published data of case control study from North Indian population. Haplotype frequencies for all SNPs within a gene were estimated using the expectation-maximization (EM) algorithm. Pair-wise linkage disequilibrium (LD) between each pair of VDR and IL-1 loci was calculated using D^x implemented in SNPAnalyzer software (18).

Calcitonin Receptor Gene

Calcitonin (CT) is a polypeptide hormone produced and secreted by parafollicular cells of thyroid gland. CT inhibits osteoclastic bone reabsorption and stimulates urinary calcium excretion. The Calcitonin Receptor gene (CTR) is a 7 pass transmembrane G-protein coupled receptor located on chromosome 7q21.3 that reacts in response to the calcium metabolism, related hormone calcitonin (19). The frequent use of SNP's site for detection is located at the 1377th nucleotide polymorphism. This implies a C/T base polymorphism altering the encoded amino acid from proline to leucine. This polymorphism is involved in signal transduction coupled with adenylate cyclase and phospholipase C pathway regulating many intracellular events including calcium regulation and phospholipid metabolism, which increases lipid peroxidation. Therefore, CTR gene polymorphism has been proposed as a candidate gene in association with calcium oxalate urolithiasis. Though Chen et al reported the association of calcitonin receptor (CTR) gene polymorphism with calcium oxalate stone patients (20), we did not find significant association between CTR gene polymorphism and calcium oxalate urolithiasis in north Indian stone formers (21).

Vitamin-D receptor (VDR) gene

Vitamin-D is a member of the steroid receptor family and mediates the effects of active metabolite 1, 25(OH)₂ Vit D₃ by regulating transcription of number of different cellular genes (22). Effect of vitamin-D on target cells is mediated by the interaction between its active metabolite, calcitriol, and its cellular receptor (VDR). The VDR gene, located on chromosome 12, is 5.6 kb in size. In response to hormone binding, the VDR regulates the transcriptional activity of 1, 25(OH)₂ D₃-responsive genes complexed with a vitamin-D response element located in promoter region of target genes. Several polymorphisms have been identified in the VDR gene (figure-1), and their functional significance and potential effects on disease susceptibility have been investigated (23).

One of the known DNA sequence variants is a thymine/cytosine (T/C) polymorphism in first of two potential start (ATG) codons separated by 3 codons. This polymorphism results in two alleles that can be distinguished by RFLP using the endonuclease Fok-I (24). Other polymorphisms found in the 3' region of the VDR gene, in the intron between exons 8 and 9, are identified using endonuclease BsmI and Apal; and at the 3' noncoding sequences in exon 9 is TaqI site.

Fig 1. Schematic representation of VDR gene demonstrating various restriction sites

The vitamin D receptor (VDR) is considered a possible candidate gene for calcium stone disease because of the fact that allelic variation may be affecting the activity of receptor and subsequent downstream vitamin-D mediated effects such as calcium absorption and excretion. An increase in VDR activity has been reported to be involved in stone formation in genetically hypercalciuric rats (25). Absorptive hypercalciuria is a common cause of kidney stones. The involvement of VDR in calcium kidney stones has been suspected because half of calcium stone forming patients have idiopathic hypercalciuria, most of them with increase intestinal calcium absorption and low BMD(26). The cause of hypercalciuria may be an excessive intestinal absorption due to increase vitamin-D synthesis or alteration in the VDR. The involvement of the VDR gene in nephrolithiasis was proposed by Scott et al, (1999) who showed a linkage between stone formation and the VDR gene locus (12q12-14), using specific microsatellite and endonucleases Fok-I and Tru9I (27). Chen et al (2001) reported significant association of VDR (Fok-I) polymorphism with calcium oxalate urolithiasis (28). VDR (Taq-I) polymorphism has a significant association in case of familial calcium stone disease (29). Gunes et al (2006) did not find any significant association of VDR Apal, BsmI, and TaqI polymorphisms with urolithiasis except with Apal polymorphism and family history (30). In another study by Relan et al (2004) no association was observed for Bsm I and Fok I (31). We

found a significant association of VDR (Fok-1) polymorphism and the FF genotype was associated with 3.4 fold-increased risks for renal stone compared with the Ff/ff (32).

Interleukin-1 gene cluster

Interleukin-1 (IL-1) is one of the most potent proinflammatory cytokines involved in physiological response as well as development of various immunopathological disorders. It has a central role in joint inflammation and tissue destruction. In addition, it induces bone resorption and formation of osteoclast (33). Previous studies have shown a correlation between decreased bone mass and increased production of IL-1 by mononuclear cells in postmenopausal osteoporosis and hypercalciuria, suggesting a role of interleukin-1 in the pathogenesis of bone resorption and thus an increased bone turnover may account for the hypercalciuria in the patients (34).

The genes encoding members of IL-1 family are clustered on the long arm of chromosome 2 and located on 2q14-21, figure 2, (35).

Fig 2. Schematic representation of IL-1 gene cluster

Different polymorphisms have been described in the IL-1 β gene, and at least 2 of them influence the protein production: one is located within the promoter region, the other in exon-5 (36). Five alleles of the IL-1Ra gene have been reported, corresponding to 2,3,4,5 and 6 copies of an 86-base pair sequence repeats located in intron 2 (37). Because 3 potential protein-binding sites are located in this 86-bp sequence, the number of repeats may influence gene transcription and protein production.

IL-1 receptor antagonist (IL-1Ra), one of the most powerful endogenous anti-inflammatory agents known, is a 22-25kd protein that is related structurally to IL-1 α and IL-1 β . It

competes with these molecules for occupancy of cell surface receptors, but does not stimulate signal transduction, thus acting as an inhibitor of IL-1 action (38). IL-1 β is produced by wide range of cells, particularly of monocyte-macrophase lineage. IL-1 β is important in response to tissue damage and infection, but is not required for normal development and homeostasis.

IL-1Ra is a hormone receptor coupled with a G protein, a single change in the intracellular domain may occasionally cause disease. It is considered possible that allelic variants of the IL-1Ra gene may have an influence over the variation of intracellular signaling pathways and may be associated with stone disease (39). However, because the relationship between IL-1 and stone disease is not only involved in an inflammatory pathway, the linkage disequilibrium of the genes (IL-1Ra, IL-1 β promoter & exon) during recombination may be one of the causes (40). Cytokines are present in urine and several researches have reported their association with stone disease. An excessive amount of IL-1 mRNA transcription was noted in hypercalciuric patients, indicating that IL-1 may be a candidate for the SNPs study of calcium oxalate stone disease (41). We observed a significant difference in genotype frequencies between stone patients and control group for IL-1 β (promoter region) and IL-1Ra gene polymorphism ($p < 0.001$ and $p = 0.039$, respectively) was observed whereas, IL-1 β gene (exon-5) polymorphism remained unchanged. ($p = 0.408$). Frequency of TT (IL-1 β promoter) and I/II (410/240; IL-1Ra) genotype was higher in patients as compared to controls (50/130 vs. 16/150 and 55/130 vs. 38/150, respectively) (42).

Urokinase gene

Urokinase gene is yet another piece of puzzle to be explored in the causation of urolithiasis. Urokinase is a proteolytic enzyme that belongs to a serine Proteinase superfamily and is involved in fibrinolytic pathways. This Proteinase activity breaks down the matrix protein within the stone and further prevents stone growth. According to matrix theory changes in protein can influence crystal growth, hence, it is hypothesized that urokinase is involved in the formation of urinary stones (43). The urokinase gene is expressed in kidney cells and its protein is present in urine. This gene is located at chromosome 10q24 (44). The polymorphic site is located at 3' untranslated region of the urokinase gene, which is associated with calcium oxalate stone disease (45).

Hence this gene was explored for the involvement of urokinase gene polymorphism in kidney stone disease in our Indian population of calcium oxalate stone formers. A significant

difference ($p = 0.035$) was found in the distribution of the urokinase gene 3' untranslated region (UTR) C/T polymorphism between patients with stones and controls in our population, however no risk was observed (OR=1.006, 95% CI 0.63-1.62). The T allele of 3' UTR of the urokinase gene may not be associated with a higher risk of stone formation (46). An observation by Tsai et al reported a significant association between urokinase gene polymorphism and calcium stone disease (45).

Stone formation may be assumed to be caused by initiation of uromucoid decomposition in optimal conditions (43) and the concentration of uromucoid decomposition in turn is regulated by urokinase and sialidase. Therefore urokinase may be speculated to play a role in the complex process of stone formation. Marked decrease in urinary urokinase concentration in stone patients have also been reported, which further supports the theory of stone formation supporting Van Aswegan et al hypothesis (43). Lower urinary urokinase activity can increase the uromucoid levels in favor of conversion of urinary mucoid to mineralize the matrix. It is also believed that activity of urokinase is inhibited by some stone-promoting factors, i.e. urease-producing bacteria, calcium, and magnesium. Therefore, urokinase is assumed to play a role in the complex process of stone formation.

E-Cadherin gene

Urolithiasis is a multifactorial disease and there is evidence that crystals, which interact with the tubular epithelium, may lead to retention and accumulation of crystalline material in the kidney, which eventually leads to renal stone formation. Cadherin is one of the families of intracellular junction proteins. It mediates calcium-independent cell adhesion and transmits signals from the extra cellular matrix to the cytoplasm and is expressed in almost all epithelial cells, including renal tubule cells (48). E-cadherin (CDH-1) is an epithelial cellular junction protein that plays an important role in the maintenance of epithelial development, organization and cell integrity. Therefore, destruction of cell integrity in conjunction with cellular injury may be responsible for stone formation. The CDH-1 gene is located at 16q22.1 and a polymorphism involving a single base change from C to T at position 3'-UTR +54 after stop codon of the gene is known to exist (49). CDH-1 has been implicated as a contributor to the formation of urolithiasis, as a 'signpost' or physical landmark on the chromosome as a possible genetic marker (50). Tsai et al reported a significant association of E-cadherin gene 3'-UTR C/T polymorphism with calcium oxalate stone disease (51).

Vascular endothelial growth factor (VEGF) gene

Growth factor related genes regulate cell growth, differentiation, and apoptosis in kidney in response to cellular injury. It has been reported that an unknown sequence of events caused by cellular injury is involved in stone formation (52). One of the most important growth and survival factors for endothelium is vascular endothelial growth factor (VEGF) (53). VEGF induces angiogenesis and endothelial cell proliferation and plays an important role in regulating vasculogenesis. VEGF causes vasodilatation; partly through stimulation of nitric oxide synthase in endothelial cells and stimulate cell migration and inhibit apoptosis (54).

VEGF, a homodimeric glycoprotein with a relative molecular mass of 45 kDa, plays an important role in neovascularization and is a potent inducer of endothelial cell growth (55). Hypoxia, androgen, interleukin-1 and tumor necrosis factor are reported to regulate or at least correlate with VEGF in the enhancement of neovascularization indicating that it is activated by upstream signaling (56). Therefore the pathogenesis of stone disease occurs via a VEGF-related signal transduction pathway. Chen et al reported significant association of vascular endothelial growth factor gene polymorphism with stone disease (57).

Table1 : Molecular epidemiology studies of different gene polymorphism and risk of urolithiasis

Gene	Age Range/Years	Authors, Year	Alleles	No. Cases/ Controls	Ethnicity	P value/ Odds ratio
Calcitonin receptor gene	23-76	Chen et al, 2001	C/T at the 1377th nucleotide	102/105	Taiwan	0.0002/ 5.634
	Mean-30.0	Mittal et al, 2004	C/T at the 1377th nucleotide	100/105	India	0.185
Vitamin-D receptor	42-73	Chen et al, 2001	Start codon Fok-I	146/90	Taiwan	0.032/1.672
	21-72	Bid et al, 2005	Start codon Fok-I	138/166	India	<0.001/1.654
	18-65	Relan et al, 2004	Bsm I and Fok I	150/100	India	>0.05; >0.05
	22-68	Ruggiero M, et al, 1998	Bsm I	27/150	Italy	0.004
		Jackman SV et al, 1999	Taq I	37/19	USA	
	23-74	Chen et al, 2001	Bsm-I	124/90	Taiwan	0.89/ 1.162
Interleukin	23-76	Chen et al, 2001	IL-1Ra IL-1 β -511 IL-1 β +3954	105/152	Taiwan	0.005/6.041 0.627 0.403
	40.0 $\pm$ 11.5	Mittal et al, 2007	IL-1Ra IL-1 β -511 IL-1 β +3954	130/150	India	0.039/2.8 <0.001/18.7 0.408/1.2
	24-73	Tsai et al, 2001	TNF- α	103/114	Taiwan	0.76
Urokinase	24-73	Tsai et. al., 2001	3'UTR	153/105	Taiwan	0.025
	21-62	Mittal et al, 2006	3' UTR +4065	130/150	India	0.035/1.006
VEGF	26-78	Chen et al, 2003	Bst U1 C/T -470; CYP17 5' UTR; EGFR 2073; IGF-2 exon-9	230/230	Taiwan	<0.001/1.30; 0.263; 0.584; 0.180;
Arginine p21	24-73	Chen et al, 2001	Codon 31	95/119	Taiwan	0.037/0.719
E-Cadherin	24-73	Tsai et al, 2003	C/T, 3'-UTR + 54	148/103	Taiwan	0.001/2.0
Androgen-oestrogen receptor (AR& ER)	41-70	Chen et al, 2001	CAG repeats in AR & TA repeats in ER gene	149/102	Taiwan	0.828 & 0.001

Androgen and Esterogen receptor gene

The androgen receptor (AR), a member of steroid receptor superfamily, is involved in various biological processes such as sexual differentiation, maturation and spermatogenesis (58). The gene is located in the chromosome Xq11-q12 region and has a polymorphic cytosine, adenine and guanine (CAG) microsatellite on exon-1, which codes for variable length glutamine repeats in the N-terminal domain of the AR protein (59). The oestrogen receptor (ER) gene is located at chromosome 6q25.1 and has a thymine-adenine (TA) dinucleotide repeat polymorphism 1174 bp upstream from exon-1 (60). It is unclear why men have higher mean urinary oxalate concentration and incidence of calcium oxalate stones than women. Chen et al reported the association of androgen and estrogen-receptor gene polymorphisms with urolithiasis (61).

Arginine p53 gene

The formation of urinary stones is associated with cell death in response to various injuries. p21 is down stream protein of p53 and can arrest the cell cycle at G1/S resulting in cell death. It is a cyclin dependent kinase inhibitor that regulates the cell cycle (62). Its ability to inhibit cyclin dependent CDK complexes resulting in G1 cell cycle arrest is regulated by wild type tumor suppressor protein p53. Alterations in p21 could interrupt the p53-mediated pathway of cell cycle arrest and influence the progression of cell death in response to cell injury. Human p21 gene is localized to the chromosome 6p21.2 and not to a sex chromosome that's why there is no significant difference when considering gender differences. The most frequently seen gene polymorphism is codon 31 with a base change from AGC to AGA and amino acid change from serine to arginine. Individuals possessing arginine form of p21 codon 31 may have marginally less risk of developing calcium oxalate stone disease (62).

Calcium-sensing receptor gene

Calcium-sensing receptor (CaSR) is a G-protein-coupled membrane receptor. It allows parathyroid and kidney tubular cells to regulate parathyroid hormone (PTH) secretion and tubular calcium re-absorption according to the plasma calcium concentration (63). The CaSR molecule comprises a large extracellular domain, a transmembrane domain, and an intracellular tail. Binding of calcium to the extracellular domain stimulates the interaction of transmembrane and intracellular domains with G-proteins and activates a complex network of intracellular messengers, leading to phospholipase C-

dependent inositol 1,4,5-triphosphate production which results in release of calcium from intracellular stores (64).

Three SNPs of CaSR gene (Ala986Ser, base change 2956G4T; Gln1011Glu, base change 3031C4G and Arg990Gly, base change s2968A4G) have been mapped on the portion of exon 7 coding for the CaSR intracellular tail. R990G, base change 2968A4G has been associated with primary hypercalciuria, the 990G variant allele was more frequent in hypercalciuric calcium stone-forming patients, compared with normocalciuric stone formers or healthy individuals (65). Vezzoli et al further reported that R990G polymorphism resulted in a gain-of-function of the calcium-sensing receptor and increased susceptibility to primary hypercalciuria (66).

Thus, identification of numerous polymorphisms has dictated the need to evaluate the disease risk susceptibility associated with progression of more than one risk factor. Indeed, assessment of the contribution of a single polymorphism to disease risk is no longer rational. The polygenic analyses present several challenges, so the generated data must be interpreted with caution. Due to the low frequency of most variant alleles, analyses are often compromised by the small number of samples and the resulting lack of statistical power. Furthermore, it is well established that the frequency of specific polymorphism varies depending on the ethnicity and geographic location of the sample population.

Haplotypes and Linkage disequilibrium (LD)

Linkage disequilibrium is the nonrandom association of alleles at linked loci. LD analysis is crucial to assess genes predisposing to disease and provide valuable information for disease association. It remains the fundamental paradigm by which genetic epidemiologists map loci contributing to genetic disorders. The variation of the recombination rate along chromosomal DNA is one of the important determinants of the patterns of linkage disequilibrium. Characterization of the linkage disequilibrium (LD) structure of candidate genes is

Table 2 : Distribution of haplotypes of the VDR gene (Fok-I and Taq-I) in patients and healthy controls

Haplotype	Patient (%)	Control (%)	p value
F-T	60 (46)	73(49)	
f-T	30 (23)	36 (24)	P= 0.002
f-t	23 (18)	7 (4)	$\chi^2=14.66$
F-t	17 (13)	34(23)	df= 7

(F-f and T-t represent alleles of Fok-I and Taq-I respectively)

the basis for an effective association study of complex diseases like urolithiasis.

VDR (Fok-I and Taq-I)

Haplotype frequencies between the two sites of VDR gene (Fok-I and Taq-I) in patients and controls are shown in Table 2. All the four possible haplotypes were common in normals and patients and a significant difference in haplotype frequency distribution was observed ($p=0.002$). Frequency of haplotype f-t was higher in patients as compared to controls (18% vs. 4%, $p=0.001$) and it represents four-time risk in patients $OR=4.391(1.817-10.612)$. Linkage disequilibrium was found significant between Fok-I and Taq-I sites in VDR gene both in the patients as well as controls ($D^* = 0.28$; $p=0.000$ & $D^* = 0.40$; $p=0.001$ respectively). Genetically, we need to understand which alleles are linked to each other. Our results showing a significant p-value for LD suggest that the two sites (Fok-I and Taq-I) on VDR gene are strongly linked and the haplotype f-t represents four times higher risk. Strong LD at the 3' end of the gene has been observed early on for the Bsm-I-, Apa-I-, EcoRV-, and Taq-I-RFLPs (67).

IL-1 gene cluster (IL-1Ra, Promoter region and Exon-5)

Haplotype frequencies among the three IL-1 gene polymorphisms in patients and controls are shown in Table 3. All the eight possible haplotypes were common in normal and patients and no significant difference was observed ($\chi^2=6.484$, $df=7$; $p=0.485$). Linkage disequilibrium was found to be significant between IL-1Ra and IL-1 β (promoter region and exon-5) polymorphism in the controls as well as patients indicating strong association among the variants ($D^* = 0.37$,

Promising markers in Urolithiasis from north India

$p<0.0001$ & 0.35 , $p=0.000$). The importance of IL-1 haplotypes may reflect differential regulation of IL-1Ra expression by IL-1 β and coordinated effects of polymorphisms that regulate IL-1 bioactivity in vivo have been previously reported (68). However, in our study no significant difference in IL-1 β and IL-1Ra haplotype distribution between patient and controls was observed. The significant value of linkage disequilibrium shows a strong association among the variants. Functional studies suggest that it is the combination of alleles, which is important in the regulation of IL-1 gene expression.

Other risk factors of kidney stones

Besides genetic factors, it is important to understand epidemiological factors associated with kidney stones. A few of them are listed in this section.

A family history of kidney stones substantially increases the risk of stone formation. A higher percentage of stone formation is seen in patients with a positive family history (5). Lavan et al showed that 16.8% of men and 22.7% of women form kidney stones with a family history as compared to 6% of controls (69). Age and sex are also risk factors for kidney stones. Baker et al found that the peak age for development of calcium oxalate stones was between 50 and 60 years. Further, uric acid stones occur more frequently in older population (60–65 years) while infection stones occurred in younger people (20 and 55 years) and most commonly in women (70). Greater risk is seen in men for developing kidney stones with incidence and prevalence rates between two and four times that of women (71). Racial differences may also be the risk factor for developing kidney stones. Soucie et al showed the prevalence of kidney stones was highest among White people and lowest in Black people. Hispanic and Asian people had an intermediate prevalence (72). The association between diet and kidney stones has long been suspected. It is seen that

Table 3 : Distribution of haplotypes constructed for the IL-1 β (promoter-region), IL-1 β (exon-5) and IL-1Ra polymorphism in stone patients and healthy controls

Haplotype	Patient (%)	Control (%)	P-value	^a OR at 95% CI
C-E2-I	10 (4)	18 (6)	0.459	1.0 (Ref)
C-E1-I	57 (22)	64 (21)	0.277	1.603 (0.68-3.7)
T-E1-I	120 (46)	114 (38)	0.124	1.895 (0.84-4.3)
T-E2-I	13 (5)	21 (7)	0.838	1.114 (0.39-3.14)
C-E1-II	10 (4)	18 (6)	1.000	1.000 (0.33-2.98)
T-E1-II	39 (15)	51 (17)	0.476	1.376 (0.57-3.3)
C-E2-II	8 (3)	12 (4)	0.762	1.200 (0.37-3.9)
T-E2-II	3 (1)	2 (1)	0.318	2.700 (0.38-18.9)

(C-T, E1-E2 and I-II represent the alleles of IL-1 β promoter, exon-5 and IL-1Ra respectively)

high dietary calcium intake reduces the risk of kidney stones, possibly by reducing gut absorption of oxalate (73). High fluid intake is associated with a lower risk of developing kidney stones (74). It is also reported that an increased risk for kidney stones is seen in individuals with the absence of intestinal oxalate degrading bacteria (75).

CONCLUSION

The accumulating information of genome science will help understand the pathogenesis of urolithiasis and find the useful ways for the prevention and the treatment of this disease. We have conducted a study in which polymorphisms of many genes have been examined. Our case-control study from north India may predict that VDR, IL-B (-511) and IL-1Ra gene may be used as a possible genetic marker for earlier detection in patients who are at risk for calcium oxalate stone disease (fig 3). However, calcitonin, IL-1B +3954 and urokinase gene polymorphism did not show any significant association with the stone disease in our population. Knowledge of specific gene association with stone disease could provide additional risk identification, improved preventive medicine and the option of unique treatment for stone disease. Although this list of genes likely to be involved in urolithiasis is by no means exhaustive, we consider it an important first step toward evaluating and summarize the role of multiple genes in the pathogenesis and development of urolithiasis. We have mentioned nine different genes whose genotype correlate with urolithiasis susceptibility and/or progression and could have potential diagnostic or therapeutic value by pointing to possible new disease mechanisms. The study has illustrated the diversity and complexity of the genetics of urolithiasis and highlights the difficulties of picking candidates precedence on the basis of physiologic function.

Genetic factors have been postulated to play an important role in the risk of urolithiasis, as demonstrated by the evidence that positive family history is a well know risk factor for urolithiasis (1, 76). Genetic studies so far demonstrated that urolithiasis might be the result of a polygenic defect with partial penetrance (77).

It follows that the LD and haplotype structure of a certain candidate gene is important for association analyses to understand how polymorphic variation in such a gene can contribute to risk of disease and population variance of certain phenotypes of interest. When a certain allele of a polymorphism has been found to be associated with a disease, in question it is further explained on the basis of LD and the haplotype structure by one or more other alleles, which happen

to be linked to this allele within the haplotype. Once it is determined which haplotype carries the risk allele, by means of cell biological and molecular biological functional analyses, the variants on that haplotype allele truly causing the effect can be determined (78).

These studies indicate that multiple genes with differing effects are involved in kidney stone susceptibility and progression and combinations of genes might prove more useful than any individual polymorphism for detecting large effects on the risk of developing urolithiasis. Recently polymorphisms in several genes like the gene encoding for calcium-sensing receptor (CaSR), NPT2a gene, a sodium-phosphate co-transporter, transporter associated with antigen-processing protein (TAP) related to immune response and inflammation, osteopontin gene have been studied to explore the association with urolithiasis (79-82). These studies raise the hope of the emergence of a genetic diagnostic technique to predict urolithiasis susceptibility and progression based on multiple SNPs. It also illustrates the complexity and diversity of disease mechanisms involved in urolithiasis.

Future Prospects

Rapid advances in high-throughput gene analysis by DNA chip technology are likely to spread the identification of new mutations in predisposing disease gene. The main task, however, will be to characterize the functional significance of these genes variants in humans. Such efforts are underway, e.g. the Human Genome Project by Human Genome Organization (83).

To accomplish this prospect, studies involving larger control and case populations, precisely and uniformly defined clinical classification of urolithiasis and better exposure histories will undoubtedly lead to a more thorough understanding the role of various genes in the development of urolithiasis. Future studies that examine associations among several genetic polymorphisms should take into account risk factors for urolithiasis, such as diet and other environmental exposures, as well as possible biological pathways. Of particular interest are studies of gene-environment interactions and gene-gene-environment interactions. To date, molecular epidemiology studies of urolithiasis have rarely looked at a variety of potential gene-environment interactions or explored associations and interaction

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