In Silico Screening and Molecular Dynamic Study of Nonsynonymous Single Nucleotide Polymorphisms Associated with Kidney Stones in the SLC26A6 Gene

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Abbreviations and Acronyms

3D = 3-dimensional

CFTR = cystic fibrosis transmembrane conductance regulator

MD = molecular dynamic

NCBI = National Center for Biotechnology Information

PCR = polymerase chain reaction

SLC26A6 = solute carrier family 26 member 6

SNP = single nucleotide polymorphism

STAS = sulphate transporter and anti-sigma factor antagonist

Purpose: SLC26A6 is a multifunctional anion transporter with a critical physiological role in the transport of oxalate anions. Recognizing a genetic variant of *SLC26A6* would advance our understanding of oxalate transport in the formation of calcium oxalate stones.

Materials and Methods: All nsSNPs (nonsynonymous single nucleotide polymorphisms) reported in human *SLC26A6* were investigated using 4 in silico tools, including SIFT (Sorting Intolerant From Tolerant), PROVEAN (Protein Variation Effect Analyzer), PhD-SNP (Predictor of human Deleterious Single Nucleotide Polymorphisms) and MutPred. A total of 426 subjects, including 225 with kidney stones and 201 healthy controls, were included in study to genotype the candidate disease associated nsSNP using allele specific polymerase chain reaction. Furthermore, the structural consequences due to the mutation were assessed using homology modeling and molecular dynamics simulation methods.

Results: The nsSNP rs184187143 was identified as a more probable disease associated variant in the *SLC26A6* gene by in silico screening. The C allele carrier showed a 6.1-fold increased kidney stone risk compared with G allele carriers in the nsSNP (OR 6.1, 95% CI 1.36–27.38, $p = 0.007$). We found that the mutation from arginine to glycine leads to the loss of 2 hydrogen bonds and to an unstable structure in the STAS domain of SLC26A6.

Conclusions: Our results indicate that the variant G539R in the *SLC26A6* gene is associated with kidney stone risk, providing a clear clue to further achieve insight into oxalate transport in kidney stone formation.

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KIDNEY stones are a complex multifactorial disease resulting from interaction among environmental, dietary and genetic factors. There may be as high as a 5% to 10% lifetime risk of kidney stone disease.[1] Approximately 80% of kidney stones are composed of calcium oxalate. Oxalate is a well-known risk factor for kidney stones, which is toxic to renal tubular cells. Hyperoxaluria leads to urinary calcium oxalate supersaturation, resulting in the formation and retention of crystals in renal tissue. It is found in major stone formers. Recognizing a genetic disorder of oxalate transport would advance our understanding of the formative mechanisms of calcium oxalate stones.

The SLC26 transporter family consists of 11 members with significantly different anion specificities. Mutations in the domains of SLC26 transporters have been linked to a number of human diseases, such as congenital chloride diarrhea for SLC26A3,[2] Pendred syndrome deafness for SLC26A4[3] and dysplasia for SLC26A2.[4] Recently, the anion exchanger SLC26A6, a member of the Slc26 family, was reported to be an important oxalate transporter.[5] In that study *Slc26a6* null mice showed high urinary oxalate excretion, an increased plasma oxalate concentration and a defect in intestinal oxalate secretion. These findings clearly demonstrate that SLC26A6 has a critically important role in oxalate transfer in the intestine and kidney, which is thought to be associated with kidney stone disease.

To our knowledge the details of SLC26A6 topology are as yet unknown. Monico et al previously analyzed a Val206Met polymorphic variant of the *SLC26A6* gene and found a 30% reduction in oxalate transport.[6] However, Corbetta et al were unable to find this polymorphic variant associated with kidney stone development.[7] To date hundreds of variants of the *SLC26A6* gene have been identified in the NCBI database but *SLC26A6* gene variant relationships with kidney stone disease in humans are still unclear. Determining disease associated variants and an in-depth understanding of the effect of these variants may provide insight into kidney stone disease pathogenesis.

A nsSNP is a single base change in the coding region of a gene which may result in an amino acid substitution in the corresponding protein product. nsSNPs may affect protein structure and are thought to have more potential to alter protein function and lead to pathogenic phenotypes. About 50 million human SNPs have been identified in the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/snp/). However, most of them remain unclear in terms of an association with disease. Bioinformatics application to analyze probable diseases associated nsSNPs and evaluate the molecular mechanisms has become a well-known methodology. A number of studies have proved the effectiveness of using computational algorithms for a precise prediction of disease associated nsSNPs.[8,9]

In this study to gain insight into the *SLC26A6* gene we focused on pathogenic nsSNPs in the *SLC26A6* gene and their structural consequences at the molecular level. We used the SIFT (http://sift.jcvi.org/),[10] PROVEAN (http://provean.jcvi.org/index.php),[11] PhD-SNP (http://snps.biofold.org/phd-snp/)[12] and MutPred (http://mutpred.mutdb.org/)[13] tools to predict deleterious disease associated nsSNPs from available SNP data sets obtained from the NCBI dbSNP database. We then performed homology modeling and MD simulation of the native and most deleterious variant SLC26A6 protein. We used g_hbond gromacs built-in tools (http://www.gromacs.org) for atomic structure analysis. Our results showed that SNPrs184187143 (G539R) is a novel kidney stone associated variant that leads to loss of the hydrogen bond and to unstable structure in the STAS domain of SLC26A6.

MATERIALS AND METHODS

Data Set Collection

We obtained nsSNP information about the *SLC26A6* gene from the NCBI dbSNP database. Human SLC26A6 (Accession ID: NP_075062.2) protein sequence data were collected from NCBI protein sequence database. Template structures (PDB: 3LL0) were obtained from the Brookhaven Protein Data Bank (RCSB [Research Collaboratory for Structural Bioinformatics] Protein Data Bank, http://www.rcsb.org/pdb/home/home.do) to model the topological structure of the STAS domain of SLC26A6 protein.

Disease Associated nsSNP Prediction

We used the SIFT, PROVEAN, PhD-SNP and MutPred tools to examine the disease associated nsSNP occurring in the SLC26A6 protein coding region. SIFT predicts disease associated nsSNPs based on the degree of conservation of amino acid residues in sequence alignments. The prediction accuracy of SIFT is 76.99% in the human data set. A prediction score of 0.05 or less is considered damaging. PROVEAN predicts whether an amino acid substitution has an effect on the biological function of a protein. The prediction accuracy of PROVEAN is 79.19% in the human data set. The variant is considered