Phenotypic and Functional Analysis of Human *SLC26A6* Variants in Patients With Familial Hyperoxaluria and Calcium Oxalate Nephrolithiasis

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Background: Urinary oxalate is a major risk factor for calcium oxalate stones. Marked hyperoxaluria arises from mutations in 2 separate loci, *AGXT* and *GRHPR*, the causes of primary hyperoxaluria (PH) types 1 (PH1) and 2 (PH2), respectively. Studies of null *Slc26a6*$^{-/-}$ mice have shown a phenotype of hyperoxaluria, hyperoxalemia, and calcium oxalate urolithiasis, leading to the hypothesis that *SLC26A6* mutations may cause or modify hyperoxaluria in humans.

Study Design: Cross-sectional case-control.

Setting & Participants: Cases were recruited from the International Primary Hyperoxaluria Registry. Control DNA samples were from a pool of adult subjects who identified themselves as being in good health.

Predictor: PH1, PH2, and non-PH1/PH2 genotypes in cases.

Outcomes & Measures: Homozygosity or compound heterozygosity for *SLC26A6* variants. Functional expression of oxalate transport in *Xenopus laevis* oocytes.

Results: 80 PH1, 6 PH2, 8 non-PH1/PH2, and 96 control samples were available for *SLC26A6* screening. A rare variant, c.487C→T (p.Pro163Ser), was detected solely in 1 non-PH1/PH2 pedigree, but this variant failed to segregate with hyperoxaluria, and functional studies of oxalate transport in *Xenopus* oocytes showed no transport defect. No other rare variant was identified specifically in non-PH1/PH2. Six additional missense variants were detected in controls and cases. Of these, c.616G→A (p.Val206Met) was most common (11%) and showed a 30% reduction in oxalate transport. To test p.Val206Met as a potential modifier of hyperoxaluria, we extended screening to PH1 and PH2. Heterozygosity for this variant did not affect plasma or urine oxalate levels in this population.

Limitations: We did not have a sufficient number of cases to determine whether homozygosity for p.Val206Met might significantly affect urine oxalate.

Conclusions: *SLC26A6* was effectively ruled out as the disease gene in this non-PH1/PH2 cohort. Taken together, our studies are the first to identify and characterize *SLC26A6* variants in patients with hyperoxaluria. Phenotypic and functional analysis excluded a significant effect of identified variants on oxalate excretion.

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INDEX WORDS: SLC26A6; hyperoxaluria; calcium oxalate urolithiasis.

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In industrialized nations, symptomatic urolithiasis has a lifetime prevalence ranging from 5.9% to 12.5% in men and 3.7% to 5% in women,[1-3] with the principal crystal component in the majority (70% to 80%) of these stones consisting of calcium oxalate monohydrate (whewellite) or dihydrate (weddellite) phases.[4] Familial aggregation patterns and, more recently, studies of twins suggested a high degree of heritability.[5-7] Of the multiple metabolic abnormalities identified in these patients, even mild increases in urinary excretion of oxalate, detected in about 20%,[8] have been shown to significantly impact on calcium oxalate supersaturation and consequently calcium oxalate crystal mass and the risk of calcium oxalate stone formation.[9]

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Marked hyperoxaluria (oxalate > 1.0 mmol/1.73 m^2/24 h) arises from mutations in 2 separate loci, *AGXT* and *GRHPR*, the causes of primary hyperoxaluria (PH) types 1 (PH1; OMIM [Online Mendelian Inheritance in Man] 259900) and 2 (OMIM 260000), respectively. To date, a third locus with large (monogenic) influence on urinary oxalate excretion is suspected, based on identification of several pedigrees with a phenotype similar to PH1 and PH2 (marked and sustained hyperoxaluria and calcium oxalate urolithiasis), in which deficiencies of the hepatic enzymes (alanine-glyoxylate aminotransferase and glyoxylate reductase/hydroxypyruvate reductase) encoded by these genes (*AGXT* and *GRHPR*) have been excluded.[10,11] Although the cause is unknown, the term non-PH1/PH2 primary hyperoxaluria has been used to describe these families.

Notably, among recent findings in 2 separate lines of null *Slc26a6*$^{-/-}$ mice are hyperoxaluria[12,13] with additional observations of hyperoxalemia and calcium oxalate urolithiasis in 1 of these null lines.[12] A defect in intestinal oxalate secretion by chloride/oxalate exchange mediated by Slc26a6 has been shown to account for the hyperoxaluria. In humans, the product of the *SLC26A6* gene, solute carrier family 26, member 6, is widely expressed in many tissues, with abundant expression in kidney, pancreas, and colon.[14-16] Although functional expression studies have shown that SLC26A6 can function in multiple modes of anion exchange involving chloride, formate, bicarbonate, sulfate, hydroxyl, and oxalate as substrates,[17-21] direct assessment of SLC26A6 transporter function in vivo through generation of the null *Slc26a6*$^{-/-}$ mice has clearly emphasized a key role in oxalate homeostasis. These studies led to the hypothesis that *SLC26A6* mutations may cause or modify hyperoxaluria in humans.[12]

To explore the possibility that sequence alterations in *SLC26A6* may impart large (monogenic) or small (polygenic) phenotypic effects, we screened families with non-PH1/PH2, PH1, and PH2 for *SLC26A6* variants. Because of the significant intrafamilial and interfamilial variability observed in patients with PH1 and PH2 and the still undefined genetic basis of non-PH1/PH2 primary hyperoxaluria, we deemed *SLC26A6* to represent an especially relevant and logical biological modifier or candidate gene in these phenotypes.

METHODS

Study Design and Population

We recruited cases from the International Primary Hyperoxaluria Registry (IPHR) for participation. The IPHR is a secure web-based international disease registry for patients with PH (types 1, 2, and non-PH1/PH2). All IPHR patients from the Mayo Clinic who consented to molecular research testing were included and provided informed consent or assent after approval by our Institutional Review Board. Control DNA samples were from a pool of adult subjects who identified themselves as being in good health. Because this was not a prospective study, specific clinical characteristics of the control group other than healthy status were not available. Both case and control populations were predominantly white. Genomic DNA was extracted from peripheral-blood leukocytes by using standard methods.

A total of 80 PH1, 6 PH2, 8 non-PH1/PH2, and 96 control samples were available for *SLC26A6* screening. A diagnosis of PH (AGT deficiency, PH1; GRHPR deficiency, PH2) was confirmed by means of hepatic enzyme analysis in the proband or an affected sib (n = 73 PH1, n = 2 PH2) or by demonstration of 2 known causative mutations and supportive biochemical data (n = 7 PH1, n = 4 PH2). In the 8 patients with non-PH1/PH2, normal hepatic activities of AGT and GRHPR were documented in the proband or affected sib, and secondary causes of marked hyperoxaluria, including hyperabsorption of oxalate from the gastrointestinal tract, were excluded. Clinical features of 6 of these 8 patients with non-PH1/PH2 have been published previously.[10] Urine and plasma oxalate were measured by using the oxalate oxidase method.[22]

Genotyping

The entire coding sequence (21 exons) of *SLC26A6*, including exon-intron boundaries, was amplified and sequenced by using the primer sequences and polymerase chain reaction conditions established by Waldegger et al[15] using 96-well plates. All samples were sequenced in both directions using an ABI Prism 3700 DNA Analyzer (Applied Biosystems, Foster City, CA) and chromatograms were analyzed using both Sequencher v4.5 software (Gene Codes Corp, Ann Arbor, MI) and Mutation Surveyor v2.41 software from SoftGenetics LLC (State College, PA).

SLC26A6 and *Slc26a6* denote the human and mouse orthologs, respectively, in keeping with the recommendations of the Human Genome Organization for genetic nomenclature. Nucleotide numbering refers to the complementary DNA (cDNA) sequence of human *SLC26A6* splice isoform 1 (NM_022911.2), except that the +1 position corresponds to the "A" of the first ATG.

Statistical Analysis

The χ^2 or Fisher exact test was used to test the association of genotype (homozygous wild, heterozygous, and homozygous variant) with PH subtype (PH1, PH2, and non-PH1/