Prevalence and clinical characteristics of elevated 1-alpha,25-dihydroxyvitamin D in pediatric nephrolithiasis and related disorders

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http://hdl.handle.net/2144/16260

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PREVALENCE AND CLINICAL CHARACTERISTICS OF
ELEVATED 1A,25-DIHYDROXYVITAMIN D
IN PEDIATRIC NEPHROLITHIASIS AND RELATED DISORDERS

by

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B.A., Tufts University, 2010

Submitted in partial fulfillment of the
requirements for the degree of
Master of Science
2015
I would like to dedicate this work to everyone who has supported me through several years of education and pushed me to strive for excellence. To my father Robert, my mother Joan, my brother Eric, and my sister-in-law Brooke, for their love and support throughout my life. To my fiancé, Luke Varner, whose encouragement and patience have been unending, and to whom I owe the utmost appreciation. I am truly grateful to you all.
ACKNOWLEDGMENTS

This thesis would not have been possible without the guidance and support of my two principal advisors, Dr. Michelle Baum and Dr. Ari Wassner. You have been my mentors, going above and beyond your roles as thesis advisors. You taught me not only about research but about what it means to be a dedicated clinician. I am inspired by your enthusiasm for medicine and your dedication to medicine, and I am lucky to have had two wonderful role models. I cannot thank you enough for the opportunity you gave me this year.

I would also like to thank my Boston University advisor, Dr. Vickery Trinkaus-Randall, who pushed me from the start to rise to the challenge of the MAMS program. Your guidance has been invaluable throughout the program, and I am deeply grateful for your support.
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JENNIFER DRUCKER

ABSTRACT

Introduction: The incidence of pediatric nephrolithiasis (kidney stones) has been increasing over the past several years. While environmental factors, such as poor fluid intake, high-salt diet, and obesity, can play a role, underlying metabolic factors account for at least one-third of cases of nephrolithiasis. Nephrolithiasis and related disorders, such as nephrocalcinosis and hypercalciuria, can lead to long-term kidney problems, including renal scarring, acute and chronic kidney disease, decreased renal function, or end-stage renal disease. The best treatment is prevention and is best guided by knowing the underlying cause. The majority of kidney stones are primarily comprised of calcium, and abnormal calcium metabolism and regulation can lead to nephrolithiasis, nephrocalcinosis, and hypercalciuria.

Vitamin D is an important factor in calcium regulation in the body. The physiologically active form of vitamin D is 1α,25-dihydroxyvitamin D (1,25(OH)₂D), which increases serum calcium by stimulating intestinal absorption of calcium, increasing renal calcium reabsorption, and mobilizing calcium from bone. Excess 1,25(OH)₂D has been shown to be associated with hyperabsorption of calcium in the intestine, nephrolithiasis, hypercalcemia, and hypercalciuria.
Production of $1,25(OH)_2D$ requires hydroxylation of 25-hydroxyvitamin D by the kidney enzyme $1\alpha$-hydroxylase, which is regulated in turn by serum calcium, parathyroid hormone (PTH), and by $1,25(OH)_2D$ itself. Tight control of $1,25(OH)_2D$ levels is maintained in part by the breakdown of $1,25(OH)_2D$ by the enzyme 24-hydroxylase, which is encoded by the gene $CYP24A1$. In the past few years, $CYP24A1$ mutations leading to decreased activity of 24-hydroxylase have been implicated in some cases of idiopathic infantile hypercalcemia as well as nephrolithiasis, nephrocalcinosis, and hypercalciuria.

The prevalence of 24-hydroxylase deficiency is not known, and the spectrum of its clinical manifestations is not yet fully understood. Our study aims to describe the clinical characteristics of patients with laboratory findings suggestive of 24-hydroxylase deficiency, specifically high-normal or elevated serum $1,25(OH)_2D$. We aimed to determine the prevalence of elevated $1,25(OH)_2D$ among pediatric patients with nephrolithiasis, and to compare clinical outcomes and biochemical findings in patients with normal versus elevated $1,25(OH)_2D$.

**Patients and Methods:** This study was a retrospective chart review. To determine the prevalence of high-normal (56-75 pg/mL) and high (>75 pg/mL) serum $1,25(OH)_2D$, we reviewed electronic medical records of patients seen in the Boston Children’s Hospital Stone Clinic. We identified 346 patients who were evaluated for nephrolithiasis, were under 18 years of age at the time of presentation, and had at least one measurement of
1,25(OH)$_2$D. Patients were classified based on their highest measured level of 1,25(OH)$_2$D.

To determine the clinical characteristics of patients with elevated 1,25(OH)$_2$D, we reviewed clinical records and laboratory data of patients at Boston Children’s Hospital with a diagnosis of nephrolithiasis, nephrocalcinosis, or hypercalciuria. We identified 83 patients who met our inclusion criteria: age of onset <18 years, at least one measurement of 1,25(OH)$_2$D, and a pre-treatment urine solute analysis. Data collected included demographic information, diagnoses, family history of kidney disease, treatments, laboratory data, and urine solute analyses. We compared findings in patients with normal 1,25(OH)$_2$D (≤55 pg/mL) versus elevated 1,25(OH)$_2$D (>55 pg/mL).

**Results:** Of 346 children with nephrolithiasis in whom 1,25(OH)$_2$D was measured, 100 (28.9%) had high 1,25(OH)$_2$D, and an additional 120 (34.7%) had high-normal 1,25(OH)$_2$D. To determine the clinical characteristics of elevated 1,25(OH)$_2$D, we analyzed the data of 40 patients with normal 1,25(OH)$_2$D and 43 patients with elevated 1,25(OH)$_2$D who had a history of nephrolithiasis, nephrocalcinosis, or hypercalciuria. Seventy-five children had nephrolithiasis, and 25/37 (67.6%) of children with elevated 1,25(OH)$_2$D had a recurrence of nephrolithiasis, compared to only 9/38 (23.7%) of children with normal 1,25(OH)$_2$D (p < .001). Urine calcium/creatinine ratio did not differ between the two groups. However, linear regression analysis showed an association between 1,25(OH)$_2$D levels and urine calcium/creatinine ratio. Important secondary
findings included a younger age of onset, higher serum 25-hydroxyvitamin D, and lower parathyroid hormone levels in patients with elevated 1,25(OH)₂D.

**Conclusions:** Important clinical findings of this study were the increased rate of recurrence and the younger age of onset in patients with elevated 1,25(OH)₂D. While we recognize that mutations in *CYP24A1* do not account for the majority of cases of elevated 1,25(OH)₂D, we do advocate for special consideration for these patients. In the absence of a commercially-available assay for 24-hydroxylase activity, children with nephrolithiasis, nephrocalcinosis, or hypercalciuria and elevated 1,25(OH)₂D should be closely monitored for recurrence or worsening of symptoms. Furthermore, we advise caution in the use of vitamin D repletion in at-risk patients.
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LIST OF ABBREVIATIONS

1,25(OH)₂D ......................................................................................... 1α,25-dihydroxyvitamin D
25(OH)D ................................................................................................. 25-hydroxyvitamin D
APRTD .............................................................. Adenine phosphoribosyltransferase deficiency
BCH ....................................................................................... Boston Children’s Hospital
Ca/Cr ............................................................................................. Calcium/Creatinine ratio
CKD ................................................................................................. Chronic kidney disease
DBP ................................................................................................. Vitamin D binding protein
ESRD ............................................................................................. End-stage renal disease
ESWL ........................................................................ Extracorporeal shock wave lithotripsy
GFR ............................................................................................... Glomerular filtration rate
IIH ................................................................................... Idiopathic infantile hypercalcemia
IOM ...................................................................................................... Institute of Medicine
IU .............................................................................................................. International Unit
NHANES ............................................. National Health and Nutrition Examination Survey
NLRP3 ........................ NOD-like receptor, leucine-rich repeat, pyrin-domain containing 3
PCNL .................................................................................... Percutaneous nephrolithotomy
PTH ...................................................................................................... Parathyroid hormone
RDA ...................................................................................................... Recommended dietary allowance
INTRODUCTION

Kidney stones, or nephrolithiasis, are a disease of the urinary tract in which crystals aggregate to form mineral deposits within the kidney. There are several metabolic causes for nephrolithiasis as well as contributing environmental factors, including low fluid intake, high salt intake, and obesity. Calcium nephrolithiasis may be seen in conjunction with increased urine calcium crystals in the kidney. Over the past several years, there has been an increase in the incidence of nephrolithiasis in industrialized countries (Milliner, 2009), with a current lifetime prevalence of nephrolithiasis estimated between estimated between 10-15%, dependent upon age, sex, race, and geographic location (Nesterova et al., 2013). In addition to the acute, severe pain described by many stone patients, the formation of crystals and stones, passage of stones, and treatment can cause long-term damage, including chronic kidney disease (CKD) and renal scarring. Moreover, the overall recurrence rate of kidney stones is estimated to be 20-40% and is especially high in children with a metabolic basis for stone formation (Milliner, 2009).

Incidence and Epidemiology

In a report from the 2007-2010 National Health and Nutrition Examination Survey (NHANES), Scales et al. found an 8.4% population-adjusted prevalence of nephrolithiasis in adults in the United States, a significant increase from the NHANES estimated prevalence of 5.2% in 1994 (Scales, Smith, Hanley, Saigal, & Urologic Diseases in America Project, 2012). The incidence of nephrolithiasis increases with age
(Milliner, 2009; Sas, Hulsey, Shata t, & Orak, 2010; Scales et al., 2012). Adults ages 60-69 are at the highest risk with a prevalence of 14.3% (Sas et al., 2010).

In the United States, pediatric nephrolithiasis accounts for 0.1-1.3% of hospitalizations of children and adolescents, a number that is less than 2% of the reported adult rate (Milliner, 2009). Like adult nephrolithiasis, pediatric nephrolithiasis appears to be increasing in incidence. VanDervoort et al. conducted a retrospective chart review of a single center over two time periods, 1994-1996 and 2003-2005 (VanDervoort et al., 2007). Adjusting for the number of new visits, they report a 4-fold increase in the number of new cases of nephrolithiasis in children between the two periods. Sas et al. (2010) conducted a study of the incidence of pediatric nephrolithiasis in South Carolina emergency departments between 1996 and 2007 and found an overall incidence of 12.0 per 100,000 children in the 12-year period. There were 8.1 diagnoses of nephrolithiasis per 100,000 visits to the emergency department in 1996. This number rose steadily to 13.9 per 100,000 in 2007.

The higher rate of diagnosis of pediatric nephrolithiasis may be in part due to increased use of radiographic techniques, such as ultrasound and CT, resulting in more incidental findings of nephrolithiasis (Milliner, 2009; VanDervoort et al., 2007). It may also be attributed in small part to the longer survival of patients with childhood diseases that are associated with nephrolithiasis. In addition, increased awareness and consideration of the diagnosis in a child with colicky abdominal pain may also play a role.
There are many factors that contribute to lifetime risk of stone development. Historically, men have had a higher prevalence of stone disease than women. In the 2007-2010 NHANES, the estimated prevalence was higher for men than women in a 1.5:1 ratio (Scales et al., 2012). However, this does not appear to hold true for pediatric nephrolithiasis. Sas et al. (2010) found a higher prevalence of nephrolithiasis among girls than boys, especially in adolescents. Furthermore, while girls account for only 46% of pediatric hospitalizations overall, they accounted for 56% of hospitalizations for nephrolithiasis and 61% of readmissions between 2002 and 2007 (Bush et al., 2010; Sas, 2011). The only adult age group for whom prevalence was higher in women was 20 to 29 years (Sas, 2011). The reasons for this discrepancy are not yet understood, although frequent urinary tract infections (UTIs) are implicated as a contributing factor.

Environmental factors likely play a role in the risk for stone formation. National data show an increased prevalence of kidney stones in the southeastern region of the United States in what is known as the “Stone Belt” (Milliner, 2009; Sas et al., 2010). This regional difference may be due to higher temperatures leading to dehydration, which is supported by the higher incidence of nephrolithiasis in the summer months, or due to dietary factors such as a high-salt diet (Sas et al., 2010). However, the full cause is not understood. Additionally, socioeconomic standing seems to play a role, with the highest prevalence of stone disease in individuals with the lowest household income (Scales et al., 2012). This may again be related to dietary factors, such as a high-salt diet, or to obesity.
**Signs and Symptoms**

Although some patients may present with gross hematuria, the most common symptom associated with nephrolithiasis is extreme pain (renal colic). However, renal colic as the primary symptom of nephrolithiasis is highly age-dependent. While 94% of adolescents complain of flank or abdominal pain at presentation, the number drops to 72% in school-age children and only 56% in children ages zero to five (Milliner, 2009). Younger children may report abdominal pain rather than renal colic, or they may be unable to clearly articulate what is bothering them (VanDervoort et al., 2007). Approximately half of patients with nephrolithiasis under five years of age are diagnosed during evaluation for urinary tract infection or via an incidental radiographic finding (Milliner, 2009). This means that without screening, pediatric nephrolithiasis may remain undiagnosed until later in life, leading to potential injury to the kidneys and urinary tract before a diagnosis is made.

**Pathophysiology**

The majority of urinary tract stones originate in the kidney, and 60-78% of stones are still present in the kidney at the time of diagnosis (Milliner, 2009). The majority of stones found in the ureters and bladder at the time of diagnosis originate in the kidneys. Approximately 10% of stones originate in the bladder and are believed to be primarily caused by dietary factors. Most stones are chemically comprised of calcium. Forty to sixty percent are calcium oxalate, 15-25% are calcium phosphate, and 10-25% are mixed calcium phosphate and calcium oxalate.
There are many causes of nephrolithiasis. Metabolic causes and structural abnormalities of the urinary tract are the most common, causing 33% and 32% of stones, respectively (Milliner, 2009). However, the proportion of metabolic causes may be underrepresented due to incomplete metabolic workup in some patients. In one study cohort in whom a full metabolic evaluation was performed, a metabolic basis for stones was found in 53% of patients (Noe, Stapleton, Jerkins, & Roy, 1983). There is also strong evidence for a genetic basis of abnormal calcium metabolism among stone formers. Forty to forty-five percent of patients with idiopathic hypercalciuria have at least one family member with nephrolithiasis (Dinour et al., 2013), and approximately 40% of stone formers report a family history of nephrolithiasis (Nesterova et al., 2013). Although the precise genetic factors associated with calcium-based nephrolithiasis are mostly unknown, they are believed to be associated with increased intestinal calcium absorption, renal reabsorption, and bone resorption (Dinour et al., 2013).

Related Disorders

Unsurprisingly, nephrolithiasis is often related to other urinary calcium disorders, including hypercalciuria and nephrocalcinosis. Pediatric hypercalciuria is defined as a urine excretion greater than 4 mg/kg/day or a urinary calcium/creatinine (Ca/Cr) ratio greater than 0.21 mg/mg in patients over 2 years of age (Milliner, 2009; Stapleton, 1990). In children under 2 years of age, urine calcium excretion is greater, and a urine Ca/Cr ratio up to 0.8 mg/g may be normal. A prospective study of 184 children with microscopic hematuria found that 60 children had hypercalciuria, of whom eight (13%)
developed nephrolithiasis during a 1- to 4-year follow-up period (Stapleton, 1990). An earlier retrospective study of 68 children with hypercalciuria found that 49 children (72%) developed nephrolithiasis during a follow-up period of 2 to 15 years (Turi et al., 1989). Approximately 50-60% of stone formers are found to have hypercalciuria (Dinour et al., 2013; Nesterova et al., 2013), in contrast to 5-10% of healthy children (Milliner, 2009).

Nephrocalcinosis is a diffuse deposition of calcium salts in the renal parenchyma, most commonly in the medullary pyramids. Nephrocalcinosis has many of the same risk factors and physiological mechanisms as nephrolithiasis (Milliner, 2009). Because mild nephrocalcinosis is often asymptomatic, the overall prevalence is not well known (Nesterova et al., 2013), but it occurs more often in infants and young children (Milliner, 2009). When not accompanied by nephrolithiasis, nephrocalcinosis often occurs with hypercalciuria or hyperoxaluria and is more likely to be associated with loss of renal function.

**Prognosis**

In general, stones under 5 mm in diameter are often passed in the urine, but passage of a stone may lead to scarring in the kidney, ureters, and urethra (Milliner, 2009). Stones that do not pass may grow in size and lead to obstruction of the urinary tract. If the obstruction is not relieved, it can lead to dilatation of the urinary collecting system due to obstruction (hydronephrosis), thinning of the renal cortex, and impaired renal function. Large stones may need intervention, such as extracorporeal shock wave
therapy (ESWL), ureteroscopic lithotripsy and/or removal, percutaneous stone removal (PCNL), or, in rare cases, open lithotomy. Each intervention entails a risk of damaging renal tissues. The risk of renal insufficiency among patients with idiopathic calcium oxalate nephrolithiasis is 1.7% (Milliner, 2009).

The long-term effects of nephrocalcinosis vary widely. In some cases, it remains asymptomatic and causes no problems with renal function. In others, it can lead to acute and chronic kidney injury, decreased renal growth, CKD, and, in some cases, end-stage renal disease (ESRD) (Milliner, 2009).

Hypercalciuria has been targeted as a cause for renal scarring in patients with nephrolithiasis and hypercalciuria due to both direct effects of urinary crystals and resulting inflammatory responses. Animal models have shown that inflammatory markers such as the NOD-like receptor, leucine-rich repeat, pyrin-domain containing 3 (NLRP3) may explain the inflammatory mechanism of crystal-mediated nephropathy (Knauf et al., 2013; Lorenz, Darisipudi, & Anders, 2014). In other crystallopathies such as gout and atherosclerosis, inflammation involved the secretion of IL-1β via the NLRP3/apoptosis-associated speck-like protein containing CARD/caspase-1 axis (Mulay et al., 2013). Calcium oxalate crystals activate the same pathway by acting as damage-associated molecular patterns (DAMPs), resulting in damage to the tubules and renal parenchyma in hypercalciuria as well as nephrocalcinosis (Lorenz et al., 2014; Mulay et al., 2013).

The cause of nephrolithiasis may affect the prognosis. Some diseases, such as Dent’s disease (which is associated with hypercalciuria), primary hyperoxaluria, and adenine phosphoribosyltransferase deficiency (APRTD), put patients at an increased risk
for significant nephrolithiasis or nephrocalcinosis (Milliner, 2009). Because of the renal damage caused by urinary calcium disorders, the best treatment is prevention, through both dietary and pharmacological interventions.

**Calcium Homeostasis**

Because calcium is important for many physiological functions- including muscle contraction, blood coagulation, nerve conduction, and bone mineralization- calcium homeostasis must be tightly regulated. Low serum calcium (hypocalcemia) or high serum calcium (hypercalcemia) can have severe negative effects including seizures, cardiac arrhythmias, or death.

Most calcium in the body is stored in bone as hydroxy-apatite crystals (Portale & Perwad, 2009; Portale & Perwad, 2009). A small amount exists in a rapidly exchanging pool between bone and plasma and contributes to the physiological effects of calcium. Healthy adults maintain a zero balance of calcium, that is, calcium intake is equivalent to calcium excretion. In contrast, in growing children a net daily retention of calcium of 150-200 mg supports skeletal growth. Calcium homeostasis is maintained by actions primarily within the intestine, kidneys, and bone. Healthy adults absorb 20-25% of ingested calcium. Intestinal calcium absorption occurs primarily in the duodenum and proximal jejunum through both passive diffusion and by a saturable active transport mechanism stimulated by vitamin D. Ionized and complexed calcium account for 60% of plasma calcium and are freely filtered by the glomerulus. About 98-99% of this is reabsorbed, or approximately 8 gm/day, primarily in the proximal tubule. There does not
appear to be a maximum tubular reabsorptive rate ($T_m$) for calcium within the normal physiologic range (Portale & Perwad, 2009).

Calcium homeostasis is regulated primarily by parathyroid hormone (PTH) and vitamin D. Both are stimulated by low plasma calcium and act to increase the absorption and resorption of calcium. PTH stimulates osteoclast activity to release calcium from bone. It is also the primary determinant of renal calcium reabsorption both by stimulating renal reabsorption directly and by partially reducing excretion by decreasing the glomerular filtration rate (GFR). Vitamin D plays a secondary role by promoting renal reabsorption at the distal tubule. Vitamin D also stimulates intestinal calcium absorption via a saturable active transport mechanism.

Disorders of either PTH or vitamin D metabolism may cause nephrolithiasis, nephrocalcinosis, and hypercalciuria. Hyperparathyroidism, which results in excess PTH, is known to mobilize too much calcium from bone and increase renal reabsorption, leading to hypercalcemia, hypercalciuria, and nephrolithiasis. Vitamin D has long been implicated in hyperabsorptive hypercalciuria and nephrolithiasis. The direct relationship between vitamin D and intestinal absorption of calcium is exaggerated in hyperabsorptive nephrolithiasis (Alvarez-Arroyo, Traba, Rapado, de la Piedra, & Torralbo, 1992). It is this hyperabsorptive calcium excess on which our study is focused.

**Vitamin D**

The clinical benefits of vitamin D have been well-established in particular for the treatment of vitamin D-deficient rickets (Samuel, 1964; Schlingmann et al., 2011). It is
also useful for the prevention of osteoporosis. While beneficial effects of vitamin D for cardiovascular health and the prevention of neoplastic disease have been suggested (Leaf et al., 2012), the association has yet to be proven and is highly debated (Manson & Bassuk, 2015).

*Metabolism, Regulation, and Action*

Vitamin D is produced primarily in human skin cells by the action of UVB radiation on 7-dehydrocholesterol (Institute of Medicine, 2011; Mugg et al., 2015; Portale & Perwad, 2009). Vitamin D$_3$ is an inactive pro-hormone that is activated by consecutive hydroxylation by multiple cytochrome P450 enzymes. Vitamin D is first hydroxylated in the liver by CYP2R1 (and possibly CYP27A1) to form 24-hydroxyvitamin D (25(OH)D) (Institute of Medicine, 2011; Mugg et al., 2015; Portale & Perwad, 2009; Wolf et al., 2014). This step is not tightly regulated, and has little, if any feedback regulation. 25(OH)D levels are therefore highly dependent on sunlight exposure and dietary intake.

25(OH)D is converted to the physiologically active form, 1α,25-dihydroxyvitamin D (1,25(OH)$_2$D) by the enzyme 25-hydroxyvitamin-D-1α-hydroxylase (1α-hydroxylase, CYP27B1), primarily in the kidneys. There is evidence to suggest that CYP27B1 also has function in other tissues including keratinocytes, lungs, macrophages, brain, testes, and osteoblasts (Mugg et al., 2015; Prosser & Jones, 2004).

Both 25(OH)D and 1,25(OH)$_2$D are inactivated by the action of the enzyme 24-hydroxyvitamin-D-24-hydroxylase (24-hydroxylase, CYP24A1) (Portale & Perwad, 2009; Prosser & Jones, 2004; St-Arnaud, 1999). CYP24A1 is expressed in most, if not
all, vitamin D target tissues, though the main sites of vitamin D inactivation are the kidney and liver (Portale & Perwad, 2009). CYP24A1 catalyzes the breakdown of 1,25(OH)$_2$D to calcitriol and of 25(OH)$_2$D to 24,25-dihydroxyvitamin D (24,25(OH)$_2$D), both of which are inactive.

1α-hydroxylase activity is stimulated by PTH and by phosphorus deficiency to increase phosphorus absorption in the intestine (Portale & Perwad, 2009). Conversely, enzyme activity is suppressed by high serum levels of calcium, by fibroblast growth factor 23 (FGF-23), and by 1,25(OH)$_2$D itself via a negative regulatory vitamin D response element (VDRE) upstream of the CYP27B1 promotor region (Prosser & Jones, 2004). Increased levels of 1,25(OH)$_2$D also induce expression of CYP24A1, leading to vitamin D catabolism (Portale & Perwad, 2009). In the kidney, PTH suppresses CYP24A1 expression, thus increasing renal reabsorption of calcium in the distal tubule via high levels of 1,25(OH)$_2$D. However, in osteoblasts, PTH enhances 1,25(OH)$_2$D-mediated induction of CYP24A1 in order to prevent sudden spikes in 1,25(OH)$_2$D and resultant bone formation abnormalities (Jones, Prosser, & Kaufmann, 2012).

One of the most important actions of vitamin D is to maintain calcium balance. 1,25(OH)$_2$D increases serum calcium and phosphate primarily by increasing intestinal absorption. It plays a minor role in mobilizing calcium from bone and renal reabsorption of calcium and phosphate. 1,25(OH)$_2$D is the most important stimulus for intestinal calcium absorption, which it achieves by stimulating synthesis of the epithelial calcium channel TRPV6, calbindin 9, and the calcium adenosine triphosphatase pump (Nesterova
et al., 2013). By similar mechanisms, 1,25(OH)$_2$D stimulates reabsorption of calcium in the kidney.

Clinical Uses of Vitamin D

Vitamin D supplementation is routinely advocated, especially among pediatric and geriatric patient populations, to promote bone health, although there continues to be debate about the recommended dietary allowance (RDA). Currently, the Institute of Medicine (IOM) recommends 400 IU per day for infants, 600 IU per day for people ages 1 to 70 years and 800 IU per day for people over 70 years of age, which can be achieved through the consumption of fortified foods or over-the-counter supplements (Institute of Medicine, 2011; Manson & Bassuk, 2015).

A benefit of dietary supplementation of vitamin D is its wide therapeutic window. However, even in healthy patients, excess vitamin D can lead to toxicity due to severe hypercalcemia. Symptoms of vitamin D toxicity (hypervitaminosis D) include thirst, itchiness, diarrhea, general malaise, polyuria, and diminished appetite (Blank, Scanlon, Sinks, Lett, & Falk, 1995; Deluca, Prahl, & Plum, 2011). In severe cases, calcifications may be found in the kidneys, aorta, heart, lung, and subcutaneous tissues, and renal failure or death can result. Two early reports of infant vitamin D fatalities were linked to extreme vitamin D dosages, with a daily administration of 30,000-40,000 IU over a course of 8-12 months (Ketha, Wadams, Lteif, & Singh, 2015). Between 1988 and 1991, eight patients in the greater Boston area were diagnosed with vitamin D toxicity due to overfortification of milk from a local dairy (Blank et al., 1995). It was found that the
samples from the dairy contained between 70 to 600 times the state limit of 500 IU per quart.

The precise mechanism of vitamin D toxicity is unclear. Hypotheses include increased production of 1,25(OH)$_2$D or overwhelming of the binding capacity of vitamin D binding protein (DBP), thus releasing excess 1,25(OH)$_2$D to enter target cells (Ketha et al., 2015). However, both of these hypotheses were called into question by studies of Cyp27b1$^{-/-}$ knockout mice (Deluca et al., 2011). Cyp27b1$^{-/-}$ and wild type mice both had similar reactions to high doses of 25(OH)D. Because the Cyp27b1$^{-/-}$ mice cannot produce 1,25(OH)$_2$D, this implicates 25(OH)D as the primary metabolite responsible for vitamin D toxicity in otherwise healthy patients. An alternate hypothesis for the mechanism of vitamin D toxicity is that high doses over 25(OH)D overwhelm the DBP binding capacity, allowing for excess 25(OH)D in the plasma to exert a direct effect on target cells at extremely high serum concentrations (Ketha et al., 2015).

While 25(OH)D appears to be a culprit for vitamin D toxicity in otherwise healthy patients, patients with certain lymphomas and granulomatous diseases demonstrate symptoms of vitamin D toxicity that has been shown to be associated with elevated 1,25(OH)$_2$D. In recent years, elevated serum 1,25(OH)$_2$D has been implicated in several cases of idiopathic infantile hypercalcemia, nephrolithiasis, and nephrocalcinosis (Castanet, Mallet, & Kottler, 2013; Dauber et al., 2012; Dinour et al., 2013; Dowen, Sayers, Hynes, & Sayer, 2014; Fencl, Blahova, Schlingmann, Konrad, & Seeman, 2013; Jacobs et al., 2014; Meusburger et al., 2013; Nesterova et al., 2013; Schlingmann et al., 2011; Skalova et al., 2013; Streeten, Zarbalian, & Damcott, 2011; Wolf et al., 2014).
Vitamin D Supplementation and Idiopathic Infantile Hypercalcemia

In 1943, the British Paediatric Associated recommended vitamin D supplementation for the general population to help prevent rickets (Samuel, 1964). Nationwide efforts led to the fortification of milk, infant cereals, and cod-liver oil compounds to aid in providing children with the recommended dose of up to 4,000 IU of vitamin D daily (Schlingmann et al., 2011). In the early 1950s, Great Britain saw a spike in cases of infantile hypercalcemia with nephrocalcinosis with no known origin, first described by R. Lightwood as idiopathic infantile hypercalcemia (IIH). Between January 1953 and June 1955, there were 216 reported cases of IIH, or 7.2 per month. Meanwhile, in the United States, where vitamin D supplementation was 10-25% of the dosage recommended in Great Britain, there were only 10 reported cases of IIH (Schlingmann et al., 2011). In 1957, the Ministry of Health suggested a reduction by half of the amount of supplemental vitamin D given to infants (Samuel, 1964). Between January 1960 and May 1961, the reported number of cases of IIH dropped to 50, or 3.0 per month. While a causative link was not established, a correlation was strongly implied. Importantly, only some patients manifested with IIH, despite national vitamin D fortification, suggesting a hypersensitivity to vitamin D in certain individuals. While some cases of IIH could later be explained by other disorders, such as Williams-Beuren syndrome, thyroid or adrenal disorders, disordered bone metabolism, or subcutaneous fat necrosis, others still remained unexplained (Castanet et al., 2013; Nguyen et al., 2010).
It was later found that some patients with IIH may have had increased 1,25(OH)\(_2\)D and suppressed PTH, suggesting a problem with 1,25(OH)\(_2\)D regulation (Dauber et al., 2012; Schlingmann et al., 2011; Scott et al., 1998). Activating mutations in \(\text{CYP27BI}\) were postulated, but Scott et al. (1998) were unable to find evidence of these. However, the fact that ketoconazole, an inhibitor of cytochrome P450 enzymes, was useful in treating IIH served as strong indication that IIH might be caused by problems with 1,25(OH)\(_2\)D metabolism. Schlingmann et al. (2011) studied six patients from four families with IIH and four additional patients who suffered vitamin D intoxication and hypercalcemia after prophylactic vitamin D pulse therapy. All 10 patients were found to have either homozygous or compound heterozygous mutations of \(\text{CYP24A1}\), the gene encoding 24-hydroxylase. Furthermore, while healthy patients maintain stable levels of 1,25(OH)\(_2\)D after a bolus of 25(OH)D due to tight regulation, patients with IIH were found to have spikes in 1,25(OH)\(_2\)D, indicating impaired catabolism. A concurrent study by Dauber et al. (2012) confirmed that hypercalcemia is due to calcium hyperabsorption in the gut via calcium isotope studies, suggesting the underlying mechanism. However, they looked specifically at a cohort of patients with IIH and found no other \(\text{CYP24A1}\) mutations, suggesting that this is one cause for IIH, but a rare one. The studies by Schlingmann et al. and Dauber et al. provided strong evidence for mutations in \(\text{CYP24A1}\) as the basis of certain cases of IIH and suggested a role of \(\text{CYP24A1}\) mutations in other disorders of calcium excess.
CYP24A1

Structure and Action

The enzyme 24-hydroxylase is responsible for the inactivation and degradation of both 25(OH)D and 1,25(OH)$_2$D. It is comprised of three components: ferredoxin, ferredoxin reductase, and a cytochrome P450. Of these, only the cytochrome P450 element, CYP24A1, is specific to the 24-hydroxylation of vitamin D (Jones et al., 2012). The enzyme contains 514 amino acids (Nesterova et al., 2013) in a highly evolutionarily conserved sequence across 57 species from bony fish to man (Jones et al., 2012). Nearly the entire protein is required to maintain the structure, heme-binding, and activity. While the crystal structure of human CYP24A1 has yet to be reported, its DNA sequence shares 82% identity and 90% similarity with rat CYP24A1, the structure of which has been reported (Mugg et al., 2015). Rat CYP24A1 shows the canonical cytochrome P450 structure of helices and β-pleated sheets surrounding a prosthetic heme group and a substrate binding pocket (Jones et al., 2012).

CYP24A1 is found in the mitochondria of vitamin D target tissues, especially kidneys, intestine, bone, and skin (Masuda et al., 2005). It is one of 60 known cytochrome P450s and one of four vitamin D-related cytochrome P450s, alongside CYP27A1, CYP2R1, and CYP27B1. However, while each of three catalyzes only one step in vitamin D metabolism, CYP24A1 catalyzes several different steps and side reactions. Its most important function is to catalyze the five-step breakdown of 1,25(OH)$_2$D to calcitroic acid, an inactive water-soluble product that is excreted in bile. 1,25(OH)$_2$D is the preferred substrate of CYP24A1 and upregulates expression of the
enzyme in order to attenuate its own action. 25(OH)D is converted by CYP24A1 to 24,25-dihydroxyvitamin D (24,25(OH)₂D), a metabolite with negligible physiological function (St-Arnaud et al., 2000). Thus, CYP24A1 modulates vitamin D action by inactivating its two most common forms.

**In vitro Studies**

To study the role of CYP24A1 in vitamin D metabolism, St-Arnaud et al. developed a line of 24-hydroxylase knockout mice (*Cy24a1*⁻/⁻) (St-Arnaud, 1999). Of these, 49% were dead due to hypercalcemia at weaning, showing significant perinatal lethality. The animals that survived were unable to effectively clear 1,25(OH)₂D, with levels that remained abnormally elevated hours to days after a bolus of vitamin D. Furthermore, offspring of homozygous mutant females had mineralization defects and incomplete mineralization at sites of intramembranous ossification, a finding later attributed to elevated levels of 1,25(OH)₂D in the mother (St-Arnaud et al., 2000). Long-term vitamin D treatment of knockout mice led to renal calcium deposition consistent with nephrocalcinosis.

A related study of *Cyp24a1*⁻/⁻ knockout mice by Masuda et al. (2005) supplemented the findings by St-Arnaud et al. In the study, homozygous mutants were unable to completely clear exogenous 1,25(OH)₂D after 96 hours, with levels that remained 20- to 30-fold higher than in their heterozygous *Cyp24*⁺/⁻ littermates. Levels of 1,25(OH)₂D were especially high in the kidneys, indicating a lack of homeostasis in a target tissue. Serum tests of *Cyp24a1*⁻/⁻ mice showed a lack of 24-hydroxylated
metabolites and the side product 1α,25(OH)2D-26,23-lactone, despite having sufficient substrate for enzyme activity. Comparative in vitro studies of CYP24A1 activity in mouse keratinocytes demonstrated an absence of the enzyme products in Cyp24a1<sup>-/-</sup> mouse cells.

*Reported Mutations in CYP24A1*

Since the initial description of CYP24A1 mutations as a cause of IIH, there have been a total of 18 distinct mutations reported in case studies across 25 symptomatic and 3 asymptomatic patients (Figueres et al., 2015), reviewed in (Mugg et al., 2015). Among these, E143del, L409S, R396W, E22K, and E151X have been reported in multiple patients. While some mutations correlate with mild disease, such as asymptomatic nephrocalcinosis, others have been implicated in more severe disease including IIH and recurrent nephrolithiasis, demonstrating the variability of phenotypes in CYP24A1 mutations (Mugg et al., 2015).

In vitro mutations of CYP24A1 led to ablation of CYP24A1 catabolic activity in most tested mutations (Schlingmann et al., 2011). The exception was L409S, which maintains 32% of wild-type activity (Mugg et al., 2015). Leucine 409 is not part of the substrate binding pocket, substrate access channel, or redox partner binding site, and the L409S mutation most likely affects protein conformation by substituting a polar residue for an aliphatic one, thus affecting substrate binding.

In the initial report of CYP24A1 mutations related to IIH, Schlingmann et al. (2011) described four patients with IIH and 2 asymptomatic siblings with medullary
nephrocalcinosis. Four additional patients were identified who had vitamin D toxicity and severe hypercalcemia after receiving multiple large doses of vitamin D as prophylactic pulse therapy, the preferred method in the German Democratic Republic at the time. All symptomatic patients recovered with rehydration and cessation of vitamin D supplementation.

Multiple later case studies described patients with IIH and CYP24A1 mutations. In each case, the patients presented with dehydration and failure to thrive and were found to have high or high-normal 1,25(OH)₂D and suppressed PTH (Castanet et al., 2013; Dauber et al., 2012; Ketha et al., 2015). Some patients were found to have the same mutations reported by Schlingmann et al., while others were found to have novel mutations. In each case, impaired activity of CYP24A1 was confirmed by measuring low levels of 24,25(OH)₂D.

While infantile hypercalcemia is the most commonly reported presentation of CYP24A1 mutations, it is now apparent that symptoms may first appear later in life especially in milder phenotypes or in the absence of vitamin D supplementation. Older patients may first be identified due to nephrolithiasis or hypercalciuria rather than symptoms of hypercalcemia. In 2013, Nesterova et al. described two adult patients with elevated 1,25(OH)₂D levels and inactivating mutations in CYP24A1 with no history of IIH as infants (Nesterova et al., 2013). One patient was diagnosed with nephrocalcinosis at the age of 3 during ultrasound for a urinary tract infection. The other was a 38-year-old man who had recurrent kidney stones starting at age 25. Dowen et al. (2014) described a man who first presented at age 10 and again at age 45 with kidney stones. Although he
was found to have the same homozygous E143del mutation as patients in the 2011 study, unlike the infants in the study by Schlingmann et al., this patient had never received vitamin D supplementation. This illustrates that, while vitamin D supplementation can lead to more severe problems with CYP24A1 mutations, it is not required for the manifestation of symptoms. Wolf et al. described a patient with recurrent nephrolithiasis and impaired calcium homeostasis who did not receive any vitamin D supplementation (Wolf et al., 2014). He was found to have two previously identified mutations in CYP24A1, and was described as having “late-onset idiopathic infantile hypercalcemia.”

Recently, Mugg et al. described a 21-year-old male patient with bilateral nephrolithiasis, nephrocalcinosis, hypercalcemia, renal insufficiency with a creatinine level of 2 mg/L, and no detectable 24,25(OH)2D (Mugg et al., 2015). He took high doses of vitamin D and consumed 2-3 gallons of milk per week. The patient was found to have the missense mutation of CYP24A1 L409S on a single allele with no other identified mutations. Cessation of vitamin D supplementation and reduction of calcium intake were enough to return his calcium and creatinine levels to high normal.

The mode of inheritance of CYP24A1 mutations is not entirely agreed upon. Most studies have described autosomal recessive inheritance (Dauber et al., 2012; Dinour et al., 2013; Nesterova et al., 2013; Schlingmann et al., 2011). However, Tebben et al. reported a splice site mutation believed to exhibit an autosomal dominant inheritance pattern with incomplete penetrance, thus accounting for variable phenotypes (Tebben et al., 2012).

Nesterova et al. (2013) sought to determine the risk of kidney stones related to CYP24A1 mutations, assuming an autosomal recessive pattern of inheritance. They found
13 deleterious mutations in dbSNP with a total minor allele frequency (MAF) of 0.140, or, disregarding one mutation with an especially high MAF, of 0.065. Applying these to the Hardy-Weinberg equation, they estimated the frequency of \textit{CYP24A1} mutations to be between 420 and 1960 per 100,000. Combined with a lifetime risk of stones estimated at 10\% for the general population, this leads to an estimated frequency of 4-20\% of kidney stones due to CYP24A1 deficiency.

The spectrum of clinical manifestations of \textit{CYP24A1} mutations is not yet fully understood. While early studies focused on the relationship between mutations and \textit{CYP24A1} and IIH, later studies identified patients who had renal manifestations of disease, including nephrolithiasis, hypercalciuria, and nephrocalcinosis. Some patients with \textit{CYP24A1} mutations remained asymptomatic, and many became symptomatic only after vitamin D supplementation. There is a need for studies evaluating the prevalence of \textit{CYP24A1} mutations and the associated clinical characteristics in patients who do not present with idiopathic infantile hypercalcemia. Our study aims to address this issue by analyzing the prevalence and clinical characteristics of one of the most common characteristics of \textit{CYP24A1} mutations, specifically elevated 1,25(OH)$_2$D, among pediatric patients with nephrolithiasis, nephrocalcinosis, and hypercalciuria.
SPECIFIC AIMS

Little is known about the prevalence and clinical spectrum of 24-hydroxylase deficiency, particularly in pediatric patients. Our study aims to determine the prevalence and clinical characteristics of pediatric patients with laboratory findings suggestive of 24-hydroxylase deficiency, specifically high-normal or high serum 1,25(OH)₂D.

Through a retrospective chart review of pediatric patients at Boston Children’s Hospital, we aim to:

1) Determine the prevalence of elevated 1,25(OH)₂D in pediatric patients with nephrolithiasis

2) Compare the clinical outcomes in children with normal (≤ 55 pg/mL) versus elevated (> 55 pg/mL) levels of serum 1,25(OH)₂D, with specific regard to recurrence of nephrolithiasis

3) Compare the biochemical findings in children with normal versus elevated 1,25(OH)₂D, with specific regard to urine calcium/creatinine ratio.

We hope these studies will provide information that will allow for clinical recommendations regarding elevated 1,25(OH)₂D and provide the basis for further studies of CYP24A1 mutations in pediatric nephrolithiasis and related disorders.
PATIENTS AND METHODS

This retrospective chart review was approved by the Institutional Review Board at Boston Children’s Hospital (BCH). Due to the fact that many eligible subjects might not be able to be contacted for consent (e.g. deceased, no longer followed in institution), and the study involved no more than minimal risk, a waiver of consent was approved. We reviewed clinical records from the BCH electronic medical records system between January 1, 1990 and October 15, 2014, including clinic notes, laboratory data, and radiology reports.

Prevalence Study

Using the hospital’s electronic medical records system, we identified all patients who were seen in the Stone Clinic program at Boston Children’s Hospital between January 10, 2007 and October 15, 2014 who were under 18 years of age at the time of symptom onset or initial diagnosis.

The following data were recorded for each patient: sex, date of birth, date of onset of symptoms or incidental diagnosis, and all 1,25(OH)₂D levels that were measured at BCH. Where applicable, we used outside medical records that had been uploaded into BCH electronic medical records in order to determine the date of onset. Outside laboratory results were not included in the study.

Patients were grouped based on their highest measured 1,25(OH)₂D level: Normal (≤55 pg/mL), High-Normal (56-75 pg/mL), or High (>75 pg/mL). Patients who did not
have any record of 1,25(OH)₂D measurements within the study period were placed into a separate group.

**Clinical Characteristics Study**

Patients were eligible who had a diagnosis based on ICD-9 code of calculus of the kidney and ureter (592), calculus of lower urinary tract (594), or other disorder of calcium metabolism (including nephrocalcinosis or hypercalciuria) (275.49); who were under 18 years of age at the time of diagnosis or onset of symptoms; and who had at least one recorded laboratory value for 1,25(OH)₂D. Potential subjects were identified via automated query of the electronic medical records, and eligibility was confirmed by review of the medical records. Exclusion criteria included a diagnosis of hypoparathyroidism (ICD-9 252.1) or premature birth at less than 31 weeks gestation. Hypoparathyroidism is often treated with calcium and vitamin D, which would affect laboratory values for our review. Premature infants are often treated with vitamin D to prevent osteopenia and furosemide to aid in breathing. Furosemide treatment has a known association with hypercalciuria and nephrolithiasis.

Our initial cohort consisted of 52 patients who met the study criteria and were enrolled in a related genetics study in the Department of Nephrology at BCH. We expanded our cohort based on the results of our i2b2 queries. Priority was placed on patients who had been seen in Stone Clinic and had a diagnosis that was confirmed by radiology studies or urine solute analysis.
All collected data were stored in the REDCap secure data system at BCH. Each patient was de-identified and assigned a study number, and study ID linkages were maintained separately from data collection. Patient information was obtained from the BCH electronic medical record system, including laboratory test results, radiology studies, and clinic notes. Outside medical records were included in our review only when available through the BCH electronic medical record system. Due to the retrospective nature of the study, no additional tests or records were requested to complete missing data.

Data collected included demographic information; diagnosis of nephrolithiasis, nephrocalcinosis, or hypercalciuria; family history of these conditions; treatments administered; and radiographic findings from ultrasound or CT. Laboratory data included serum calcium, phosphate, PTH, 1,25(OH)$_2$D, and 25(OH)D, and urine calcium and creatinine. Urine calcium/creatinine ratio was calculated exclusively for samples obtained prior to the initiation of treatment. We preferentially used 24-hour urine samples if available within 6 months of the reference serum date. Otherwise, a spot urine sample was used. Twenty-four hour urine studies were available for 46 of the 83 patients who were included in statistical analysis.

**Statistical Analysis**

Our primary outcomes were prevalence of elevated 1,25(OH)$_2$D in pediatric patients with nephrolithiasis; recurrence of nephrolithiasis in patients with normal versus elevated 1,25(OH)$_2$D; and urine Ca/Cr ratios in patients with normal versus elevated
1,25(OH)\(_2\)D. Patients were grouped based on their highest measured 1,25(OH)\(_2\)D level. We defined elevated 1,25(OH)\(_2\)D as levels in the upper third of normal range or higher (>55 pg/mL), while patients with 1,25(OH)\(_2\)D ≤55 pg/mL were defined as normal.

Recurrence of nephrolithiasis was defined as the formation of a new stone after an initial diagnosis of nephrolithiasis. Patients who had no history of nephrolithiasis were excluded from the analysis of recurrence. Recurrence in patients with normal versus elevated 1,25(OH)\(_2\)D was analyzed using the chi squared test. Logistic regression was used to analyze the relationship between 1,25(OH)\(_2\)D levels and recurrence of nephrolithiasis.

We conducted univariate logistic regression analyses to identify additional variables that were associated with recurrence of nephrolithiasis. Significant predictive variables by univariate analysis were included in a multivariate logistic regression analysis to determine which variables were independent predictors of recurrence of nephrolithiasis.

Student’s t-test was used to compare the mean urine Ca/Cr ratio in patients with normal versus elevated 1,25(OH)\(_2\)D. The relationship between 1,25(OH)\(_2\)D as a continuous variable and urine Ca/Cr ratio was further analyzed via linear regression.

We conducted univariate linear regression analyses to determine whether other variables were associated with urine Ca/Cr ratio. A multivariate model was then constructed to identify independent predictors of urine Ca/Cr. Because of the known association between age and urine Ca/Cr ratio, a multivariate linear regression was conducted to determine whether there was a confounding effect.
For secondary outcomes, we analyzed the relationship between 1,25(OH)$_2$D levels and age of onset, family history, 25(OH)D, PTH, serum calcium, serum phosphorus, and urine calcium mg/kg/day. Patients with missing values were excluded from the associated analysis. Continuous outcomes were analyzed by Student’s t-test, and categorical variables were analyzed by chi squared test.

Changes to Planned Methods:

Based on the results of our regression analyses, we conducted post-hoc analysis of urine Ca/Cr ratio and recurrence of nephrolithiasis in patients with normal versus high 1,25(OH)$_2$D, defined as above the upper limit of normal 1,25(OH)$_2$D (>75 pg/mL). We therefore conducted new analyses to compare urine Ca/Cr ratios and recurrence rates between patients with 1,25(OH)$_2$D levels ≤75 pg/mL versus 1,25(OH)$_2$D levels >75 pg/mL. As in the prior analysis, urine Ca/Cr ratio was analyzed by Student’s t-test, and recurrence of nephrolithiasis was analyzed by chi squared test.

Many of the patients included in our study were also enrolled in a genetic study analyzing the frequency of $CYP24A1$ mutations in pediatric patients with nephrolithiasis, nephrocalcinosis, or hypercalciuria. We had hoped to received genetic results from this study in order to analyze and describe the clinical presentation of patients with an identified mutation in $CYP24A1$. Unfortunately, the genetic testing was not completed in time for submission and so could not be included in our analysis.
RESULTS

Prevalence of Elevated 1,25(OH)_2D in Pediatric Nephrolithiasis

We identified a total of 435 patients who were under 18 years old at the time of initial presentation with nephrolithiasis. 346 of these patients had at least one recorded measurement of 1,25(OH)_2D (Table 1). The prevalence of high 1,25(OH)_2D was 28.9%, and the prevalence of high-normal 1,25(OH)_2D was 34.7%.

Overall, more girls than boys were evaluated in Stone Clinic. 28.8% of girls had high 1,25(OH)_2D and 37.2% had high-normal 1,25(OH)_2D compared to 29.0% and 30.5%, respectively, in boys. However, there was no statistically significant relationship between sex and 1,25(OH)_2D levels (p = .37).

<table>
<thead>
<tr>
<th>Normal 1,25(OH)_2D</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>53</td>
<td>73</td>
<td>126 (36.4%)</td>
</tr>
<tr>
<td>High-Normal 1,25(OH)_2D</td>
<td>40</td>
<td>80</td>
<td>120 (34.7%)</td>
</tr>
<tr>
<td>High 1,25(OH)_2D</td>
<td>38</td>
<td>62</td>
<td>100 (28.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>131</td>
<td>215</td>
<td>346</td>
</tr>
</tbody>
</table>

Clinical Characteristics

We identified 83 children with nephrolithiasis, nephrocalcinosis, or hypercalciuria who fulfilled the criteria for inclusion in the study (Table 2). Seventy-five of these children were diagnosed with nephrolithiasis, 12 with nephrocalcinosis, and 34 with hypercalciuria. There were more girls than boys (49 vs. 34). Forty-seven reported a family history of nephrolithiasis, nephrocalcinosis, or hypercalciuria; four were adopted.
and had an unknown family history. Twelve patients reported the use of vitamin D supplementation prior to presentation.

Table 2. Demographics and clinical characteristics of patients with normal (≤55 pg/mL) versus elevated (>55 pg/mL) 1,25(OH)₂D

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>Normal 1,25(OH)₂D</th>
<th>Elevated 1,25(OH)₂D</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group total</td>
<td>83</td>
<td>40</td>
<td>43</td>
<td>-</td>
</tr>
<tr>
<td>Sex M/F</td>
<td>34/49</td>
<td>18/21</td>
<td>16/28</td>
<td>.38</td>
</tr>
<tr>
<td>Mean (±SEM) age at presentation (years)</td>
<td>10.04 (± 0.56)</td>
<td>11.60 (± 0.77)</td>
<td>8.60 (± 0.77)</td>
<td>.01</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-4.9</td>
<td>18</td>
<td>6</td>
<td>12</td>
<td>.111</td>
</tr>
<tr>
<td>5-9.9</td>
<td>21</td>
<td>7</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>10-14.9</td>
<td>24</td>
<td>13</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>15-18</td>
<td>20</td>
<td>13</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Diagnosis of nephrolithiasis, N (%)</td>
<td>75 (90.3)</td>
<td>38 (95.0)</td>
<td>37 (86.0)</td>
<td>.27</td>
</tr>
<tr>
<td>Recurrence of nephrolithiasis</td>
<td>34</td>
<td>9</td>
<td>25</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Diagnosis of nephrocalcinosis, N (%)</td>
<td>12 (14.5)</td>
<td>2 (5.0)</td>
<td>10 (23.2)</td>
<td>.03</td>
</tr>
<tr>
<td>Diagnosis of hypercalciuria, N (%)</td>
<td>34 (40.9)</td>
<td>12 (30.0)</td>
<td>23 (53.5)</td>
<td>.05</td>
</tr>
<tr>
<td>Family history*, N (%)</td>
<td>47 (56.6)</td>
<td>20 (50.0)</td>
<td>27 (62.8)</td>
<td>.37</td>
</tr>
<tr>
<td>Vitamin D supplementation</td>
<td>12</td>
<td>7</td>
<td>5</td>
<td>.45</td>
</tr>
</tbody>
</table>

*Family history of nephrolithiasis, nephrocalcinosis, or hypercalciuria

The 83 children in our cohort have been followed for a mean (±SEM) of 3.4 (±0.3) years, with a range of 0 to 14.6 years. Children with elevated 1,25(OH)₂D were younger at presentation (8.60 ± 0.77 vs. 11.60 ± 0.77 years, p = .01), and were more likely to have a diagnosis of nephrocalcinosis (23.2% vs. 5.0%, p = .03) or hypercalciuria (53.3% vs. 30.0%, p = .05). There was no significant difference in the diagnosis of nephrolithiasis between the two groups.

Among the 75 patients who were diagnosed with nephrolithiasis, 34 (45.3%) had at least one new stone during follow-up. There was a significantly higher rate of
recurrence among patients with elevated 1,25(OH)\textsubscript{2}D compared to normal 1,25(OH)\textsubscript{2}D (p < .001). Univariate logistic regression demonstrated that 1,25(OH)\textsubscript{2}D levels were a significant predictor of recurrence of nephrolithiasis (Odds Ratio (OR) = 1.025, Nagelkerke \(R^2 = 0.135\), p = .01).

We also investigated additional clinical variables associated with recurrence of nephrolithiasis (Table 3). Univariate binary logistic regression analysis demonstrated that serum calcium (p = .01) and PTH (p = .04) were significant predictors of recurrence of nephrolithiasis in addition to 1,25(OH)\textsubscript{2}D; however, in multivariate analysis, only 1,25(OH)\textsubscript{2}D (OR = 1.02, p = .04) and serum calcium (OR = 5.26, p = .04) remained independent predictors of nephrolithiasis.

Table 3. Analysis of clinical variables predicting recurrence of nephrolithiasis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds Ratio ± SEM</td>
<td>(R^2)</td>
</tr>
<tr>
<td>1,25(OH)\textsubscript{2}D (pg/mL)</td>
<td>1.03 ± 0.01</td>
<td>0.009</td>
</tr>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>1.04 ± 0.02</td>
<td>0.062</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>0.96 ± 0.02</td>
<td>0.083</td>
</tr>
<tr>
<td>Serum Calcium (mg/dL)</td>
<td>7.24 ± 2.83</td>
<td>0.174</td>
</tr>
<tr>
<td>Serum Phosphorus (mg/dL)</td>
<td>1.16 ± 0.30</td>
<td>0.004</td>
</tr>
<tr>
<td>Age of Onset</td>
<td>0.97 ± 0.05</td>
<td>0.010</td>
</tr>
<tr>
<td>Family history</td>
<td>1.50 ± 0.46</td>
<td>0.013</td>
</tr>
<tr>
<td>Urine Ca/Cr ratio (mg/mg)</td>
<td>2.55 ± 1.15</td>
<td>0.011</td>
</tr>
<tr>
<td>Urine Calcium (mg/kg/day)</td>
<td>1.09 ± 0.16</td>
<td>0.007</td>
</tr>
</tbody>
</table>

We did not find a statistically significant difference in urine Ca/Cr ratio between patients with normal versus elevated 1,25(OH)\textsubscript{2}D (0.020 ± 0.03 vs. 0.27 ± 0.04 mg/mg, p = .17) (Table 4). However, a scatterplot of 1,25(OH)\textsubscript{2}D vs. urine Ca/Cr ratio suggested a positive correlation. Linear regression analysis suggested that a statistically significant
proportion of the variation in urine Ca/Cr ratios was predicted by 1,25(OH)$_2$D ($\beta = 0.002 \pm 0.001$, $R^2 = 0.11$, $p = .002$). Because of this statistically significant relationship between 1,25(OH)$_2$D level and urine Ca/Cr ratio, we conducted a post-hoc t-test of urine Ca/Cr ratio in patients with normal versus high 1,25(OH)$_2$D, with a cut-off of 75 pg/mL between these two groups. Patients with 1,25(OH)$_2$D levels >75 pg/mL had higher urine Ca/Cr ratios than patients with 1,25(OH)$_2$D levels $\leq$ 75 pg/mL (0.33 ± 0.06 vs. 0.19 ± 0.02 mg/mg, $p = .04$).

**Table 4. Laboratory data** Laboratory serum values and urine solute analyses for patients with normal ($\leq$55 pg/mL) versus elevated (>55 pg/mL) 1,25(OH)$_2$D. Variations in group totals (N) reflect the absence of associated tests for a portion of our cohort.

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>Normal 1,25(OH)$_2$D</th>
<th>Elevated 1,25(OH)$_2$D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highest 1,25(OH)$_2$D (pg/mL)</td>
<td>83 68.4 ± 3.7</td>
<td>40 43.5 ± 1.2</td>
<td>43 91.6 ± 4.6</td>
</tr>
<tr>
<td>Urine Ca/Cr Ratio (mg/mg)</td>
<td>83 0.24 ± 0.02</td>
<td>40 0.20 ± 0.03</td>
<td>43 0.27 ± 0.04</td>
</tr>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>83 29.4 ± 1.3</td>
<td>40 23.8 ± 1.4</td>
<td>43 34.7 ± 1.8</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>83 27.2 ± 1.7</td>
<td>40 30.8 ± 2.4</td>
<td>43 23.8 ± 2.2</td>
</tr>
<tr>
<td>Serum Calcium (mg/dL)</td>
<td>67 9.9 ± 0.1</td>
<td>32 9.8 ± 0.8</td>
<td>35 9.9 ± 0.1</td>
</tr>
<tr>
<td>Serum Phosphorus (mg/dL)</td>
<td>63 4.1 ± 0.1</td>
<td>32 4.1 ± 0.1</td>
<td>31 4.1 ± 0.2</td>
</tr>
<tr>
<td>Urine Calcium (mg/kg/day)</td>
<td>46 3.40 ± 0.28</td>
<td>26 2.96 ± 0.32</td>
<td>20 3.98 ± 0.47</td>
</tr>
</tbody>
</table>

Because urine calcium excretion is likely to be a risk factor for recurrence of nephrolithiasis and exacerbation of nephrocalcinosis, we sought to determine what clinical variables were predictive of elevated urine Ca/Cr ratio in our cohort (Table 5). Univariate analysis demonstrated that 1,25(OH)$_2$D ($p = .002$), 25(OH)D ($p = .04$) and serum calcium ($p < .001$) were significantly associated with urine Ca/Cr ratio. Subsequent multivariate linear regression showed that both 1,25(OH)$_2$D ($\beta = 0.001 \pm$
.001, \( R^2 = 0.546, p = .01 \) and serum calcium (\( \beta = 0.255 \pm 0.037, R^2 = 0.546, p < .001 \)) were independent predictors of urine Ca/Cr ratio.

### Table 5. Analysis of clinical variables predicting urine Ca/Cr ratio

<table>
<thead>
<tr>
<th></th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta ± SEM</td>
<td>( R^2 )</td>
</tr>
<tr>
<td>( 1,25(\text{OH})_2\text{D} ) (pg/mL)</td>
<td>0.002 ± 0.001</td>
<td>0.114</td>
</tr>
<tr>
<td>( 25(\text{OH})\text{D} ) (ng/mL)</td>
<td>0.004 ± 0.002</td>
<td>0.039</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>-0.002 ± 0.002</td>
<td>0.014</td>
</tr>
<tr>
<td>Serum Calcium (mg/dL)</td>
<td>0.290 ± 0.036</td>
<td>0.496</td>
</tr>
<tr>
<td>Serum Phosphorus (mg/dL)</td>
<td>0.024 ± 0.037</td>
<td>0.007</td>
</tr>
<tr>
<td>Age of Onset</td>
<td>-0.017 ± 0.004</td>
<td>0.149</td>
</tr>
<tr>
<td>Family history</td>
<td>-0.029 ± 0.053</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Additionally, univariate linear regression showed a significant relationship between age at onset and urine Ca/Cr ratio (\( p < .001 \)), although the significance was lost in multivariate analysis. There is a known relationship between younger age and higher urine Ca/Cr ratio. Because the date of onset was not necessarily associated with the date on which the urine solute analysis was conducted, we removed all subjects in whom the difference between the two dates was six months or greater, after which 60 subjects remained. A linear regression analysis was conducted to determine an interaction effect between \( 1,25(\text{OH})_2\text{D} \) and age on urine Ca/Cr ratio among the 60 eligible subjects and was found to be significant (\( \beta = -0.356, R^2 = 0.127, p = .005 \)), suggesting that age may confound the effect of \( 1,25(\text{OH})_2\text{D} \) on urine Ca/Cr ratio. In a multivariate analysis of the effect of both \( 1,25(\text{OH})_2\text{D} \) and age on urine Ca/Cr ratio, both variables remained independently associated with urine Ca/Cr ratio, demonstrating that both variables are independent predictors.
DISCUSSION

*CYP24A1* mutations have been shown to have a wide range of clinical phenotypes. In all reported cases, patients have had high-normal or high 1,25(OH)$_2$D. Symptoms were related to abnormal calcium metabolism and included hypercalcemia, nephrolithiasis, and nephrocalcinosis. In this single-center retrospective study, we sought to determine the prevalence and clinical characteristics of patients with findings suggestive of *CYP24A1* mutations, specifically high-normal or high 1,25(OH)$_2$D and a diagnosis of nephrolithiasis, nephrocalcinosis, or hypercalciuria. We determined the prevalence of elevated 1,25(OH)$_2$D in pediatric kidney stone patients and found a positive association between elevated 1,25(OH)$_2$D and recurrence of nephrolithiasis. We found no statistically significant difference in mean urine calcium/creatinine ratio between our two study groups.

We identified 346 children from Boston Children’s Hospital Stone Clinic with nephrolithiasis in whom a serum assay of 1,25(OH)$_2$D was conducted. We found that 100 (28.9%) of the children had high 1,25(OH)$_2$D, exceeding 75 pg/mL, and an additional 120 (34.7%) of the children had high-normal 1,25(OH)$_2$D between 56 and 75 pg/mL. To date, there have been no prevalence studies of elevated serum 1,25(OH)$_2$D in the general pediatric population or among children with nephrolithiasis. Normal clinical ranges should encompass 95% of the general population, so only 2.5% of the general healthy population should have 1,25(OH)$_2$D levels greater than normal. Our study provides a novel finding of the prevalence of elevated 1,25(OH)$_2$D among pediatric patients with nephrolithiasis. Furthermore, because almost one-third of patients with nephrolithiasis
had 1,25(OH)₂D levels above the normal clinical range, this suggests that 1,25(OH)₂D plays some role in the disease process.

Our analysis demonstrates that children with 1,25(OH)₂D >55 pg/mL have a higher rate of recurrence of nephrolithiasis than children with lower 1,25(OH)₂D levels. This is a novel finding, as no other studies have evaluated the effect of 1,25(OH)₂D on recurrence of nephrolithiasis. This finding is clinically important, as it aids in understanding the prognosis of children with nephrolithiasis and allows for recommendations regarding treatment and follow-up. Importantly, our finding can aid clinicians in determining the necessity for treatments that reduce the likelihood of stone formation, such as the use of thiazide or amiloride diuretics, magnesium, and citrate in patients with no other identified cause for nephrolithiasis.

Another important clinical finding was the association of elevated 1,25(OH)₂D with age of onset in our cohort. In the adult population, nephrolithiasis is positively correlated with increasing age (Scales et al., 2012). In our study, the mean age of onset was 3.0 years younger in patients with 1,25(OH)₂D levels >55 pg/mL than in patients with lower 1,25(OH)₂D. This may reflect a general phenomenon toward higher 1,25(OH)₂D levels with decreasing age, or the fact that younger patients are more likely than older patients to have a defect in vitamin D metabolism underlying their diagnosis of nephrolithiasis, nephrocalcinosis, or hypercalciuria.

Contrary to our hypothesis, we found only a weak relationship between 1,25(OH)₂D levels and urine Ca/Cr ratio. We found no statistically significant difference in mean urine Ca/Cr ratio in patients with 1,25(OH)₂D levels >55 pg/mL versus
1,25(OH)\(_2\)D \leq 55\,\text{pg/mL}. However, linear regression demonstrated a weak but statistically significant effect of 1,25(OH)\(_2\)D on urine Ca/Cr ratio. A post-hoc analysis showed a significantly higher mean urine Ca/Cr ratio in patients with 1,25(OH)\(_2\)D >75 pg/mL (the assay upper limit of normal) than in patients with normal 1,25(OH)\(_2\)D (\leq 75 pg/mL). This suggests an alternative hypothesis of a threshold effect in which patients with 1,25(OH)\(_2\)D levels above clinical normal limits had higher urine Ca/Cr ratios than patients with 1,25(OH)\(_2\)D within clinical normal limits.

One difficulty in considering the effects of other factors on urine Ca/Cr ratio was the effect of age. It is well reported that younger age is associated with higher urine Ca/Cr ratios in children. In children under 2 years of age, a normal ratio is 0.6-0.8 mg/mg versus 0.2 mg/mg in children over 2 years. We did find that a younger age of onset was associated with both higher 1,25(OH)\(_2\)D and higher urine Ca/Cr ratio. Analysis of the interaction variable suggested that 1,25(OH)\(_2\)D level modifies the effect of age on urine Ca/Cr ratio, or vice-versa. However, both variables remained significant in a multivariate model, demonstrating that both 1,25(OH)\(_2\)D levels and age are also independent predictors of urine Ca/Cr ratio.

In considering the effects of phosphorus and PTH, it is important to note that no patients had a phosphorus deficiency or hyperparathyroidism. Serum phosphorus levels are largely dependent on diet and, because samples were not obtained at a standard time relative to meals, were therefore highly variable between patients. Phosphorus deficiency drives the production of 1,25(OH)\(_2\)D. No association was found between 1,25(OH)\(_2\)D level and serum phosphorus. Patients with elevated 1,25(OH)\(_2\)D had lower serum PTH
than patients with normal 1,25(OH)₂D, which is consistent with suppression of PTH by an abnormality in vitamin D or calcium metabolism, not with increased activation of vitamin D by excess PTH action as might be seen in mild primary hyperparathyroidism.

Forty-seven (56.6%) children in our study had family members with a history of nephrolithiasis, nephrocalcinosis, or hypercalciuria (4 were adopted). This number is higher than the 40-45% reported in other studies (Milliner, 2009; Nesterova et al., 2013). It is possible the patients presenting with these conditions in childhood rather than adulthood are more likely to have a familial cause as opposed to an environmental one. However, we found no significant difference in the frequency of family history between patients with normal or elevated 1,25(OH)₂D. Additionally, family history showed no correlation with urine Ca/Cr ratio or recurrence of nephrolithiasis.

**Study Limitations**

Due to the retrospective nature of our study, many eligible patients did not have sufficient data for analysis. On chart review, we found that many patients lacked the necessary studies for comparative analyses, particularly pre-treatment urine solute analyses. Especially among patients who transferred from another provider, many urine solute analyses were only conducted after the initiation of treatment, thus excluding them from our analyses of urine calcium excretion.

Our prevalence analysis was affected by the number of patients in whom no assay of 1,25(OH)₂D was conducted. In addition to the 346 children in whom a serum assay of 1,25(OH)₂D was conducted, there were 89 children for whom no record of 1,25(OH)₂D
levels was available. It is possible that 1,25(OH)$_2$D levels are checked more frequently in patients in whom the clinician has a high suspicion, leading to selection bias and overestimation of the prevalence of elevated 1,25(OH)$_2$D levels in our cohort. We would therefore suggest future prospective studies to better determine the prevalence of elevated 1,25(OH)$_2$D among children with nephrolithiasis.

Our study inclusion criteria did not define a minimum follow-up period or require that a patient have any follow-up after the initial visit, which limited our ability to detect recurrence of nephrolithiasis. Although this limitation exists, we believe its effect should be small because nephrolithiasis is typically symptomatic and often painful, and therefore patients experiencing recurrent symptoms are likely to present for medical care in the emergency setting or in clinic follow-up. In addition, loss to follow-up in unlikely to be related to 1,25(OH)$_2$D level, so this limitation is unlikely to affect the finding that elevated 1,25(OH)$_2$D is associated with recurrence of nephrolithiasis.

Ideally, 24-hour urine studies would have been used exclusively to determine urine Ca/Cr ratios in all patients. However, completing a 24-hour urine study at home requires patients to be toilet-trained (or use a catheter) and not to have nighttime enuresis, which would exclude many of the younger patients from our study. Additionally, not all patients complete a 24-hour urine study when advised by their doctors, and so availability of these studies was highly dependent on patient compliance. In several of our patients, no 24-hour urine study was available, and so a random urine solute analysis was used instead. These may be less accurate, which would affect our analysis of 1,25(OH)$_2$D and urine calcium/creatinine ratios. We tested the extent of this inaccuracy and found no
A statistically significant difference in the means between 24-hour urines and random urine solute analysis.

**Vitamin D supplementation**

Nephrolithiasis is not an absolute contraindication to vitamin D supplementation. A study by Leaf et al. (2012) of vitamin D repletion in kidney stone formers found no significant difference in mean urinary calcium excretion before and after vitamin D repletion. In otherwise healthy children, vitamin D supplementation via fortified foods or other-the-counter supplements should not be sufficient to cause elevated 1,25(OH)_{2}D, due to the tight control of the activation process. Additionally, adults with osteoporosis and a history of nephrolithiasis have a better outcome when receiving the RDA of vitamin D and calcium in conjunction with treatment for hypercalciuria.

In our study, 7 out of 40 children with normal 1,25(OH)_{2}D (17.5%) and 5 out of 43 children with elevated 1,25(OH)_{2}D (11.6%) reported the use of vitamin D supplementation, with no significant difference between the two groups. Only 3 children (2 normal, 1 high) reported the use pharmaceutical vitamin D supplementation at the time of their highest 1,25(OH)_{2}D level. None of these patients had a laboratory measurement of 1,25(OH)_{2}D when they were not using vitamin D supplementation, so it was not possible to make a comparative analysis.

The appropriate levels and the benefits of vitamin D supplementation remain a highly debated topic among health professionals. The IOM finds that a serum 25(OH)D level of 20 ng/mL is sufficient to meet the needs of 97.5% of the population across all age
groups regardless of geographic locations and advises an RDA of 600 IU for children and adults through 70 years of age (Institute of Medicine, 2011). It is possible to meet this requirement without pharmaceutical supplementation. In the United States, milk is fortified on a voluntary basis with 400 IU per quart (Institute of Medicine, 2011). Other fortified food products include soy milk, orange juice, yogurt, and cereal (Manson & Bassuk, 2015). Vitamin D is also found naturally in fatty fish, fish liver oil, and egg yolks. In addition, humans are able make vitamin D via sunlight exposure, although this is reduced with the use of sunscreen. In many cases, it is possible to obtain the RDA of vitamin D through dietary means alone and without the need for vitamin D pills.

In all cases, the benefits of treatment must be balanced with associated risks, and some patients may be at higher risk than others for adverse effects of vitamin D supplementation. In particular, vitamin D supplementation may put patients with CYP24A1 mutations at risk for toxicity. Schlingmann et al. (2011) identified four patients with IIH who became symptomatic only after vitamin D pulse therapy. Figueres et al. (2015) found that prolonged sunlight exposure correlated with increases in serum calcium and urine calcium among 7 patients with CYP24A1 mutations. Consideration of CYP24A1 mutations is therefore important when considering vitamin D repletion among children with nephrolithiasis, nephrocalcinosis, or hypercalciuria. Genetic testing can give conclusive evidence of CYP24A1 mutations but is not a viable option for all patients, and there is currently no enzyme assay to determine the functionality of CYP24A1. Therefore, other clinical methods of assessing for this deficiency are necessary to guide management decisions. We recommend serum assays of 1,25(OH)\(_2\)D and PTH and 24-
hour urine solute analysis prior to and after initiation of vitamin D repletion in children with nephrolithiasis and related disorders in order to identify patients who may have \textit{CYP24A1} mutations and be at risk of worsening symptoms. It is important for clinicians to counsel patients in achieving the RDA of vitamin D without resulting in elevated vitamin D and recurrence of nephrolithiasis, especially for patients in whom the normal regulatory mechanisms do not function properly.

\textit{Future Studies}

Understanding of the prevalence and clinical characteristics of elevated 1,25(OH)$_2$D would be greatly enhanced by a multi-center prospective study. Future studies should include measures of 1,25(OH)$_2$D and 24-hour urine studies at the time of presentation with nephrolithiasis, nephrocalcinosis, or hypercalciuria and prior to the initiation of treatment. Furthermore, genetic analysis would help to determine the prevalence of \textit{CYP24A1} mutations among pediatric patients with nephrolithiasis and related disorders and assess the validity of elevated 1,25(OH)$_2$D as a primary consideration for \textit{CYP24A1} mutations. An obstacle in determining the prevalence and clinical manifestations of \textit{CYP24A1} mutations is that there is currently no commercially-available method to measure CYP24A1 activity. As genetic testing of \textit{CYP24A1} becomes more widely available in the clinical setting, it will provide a better clinical understanding of \textit{CYP24A1} mutations.

Additionally, future studies are needed to determine the impact of nutritional vitamin D supplementation in these at-risk populations. Specifically, studies are needed
to determine the risk of increased 1,25(OH)$_2$D and nephrolithiasis in conjunction with nutritional vitamin D supplementation in patients with CYP24A1 mutations.

**Conclusion**

Mutations in CYP24A1 are associated with elevated 1,25(OH)$_2$D. In this study, we found that patients with 1,25(OH)$_2$D levels in the upper third of the normal range or higher (>55 pg/mL) were statistically younger at onset of nephrolithiasis, nephrocalcinosis, or hypercalciuria and were more likely to have recurrent nephrolithiasis than those with lower 1,25(OH)$_2$D levels. Additionally, we found that they had higher urine calcium/creatinine ratios, although the difference only became significant at 1,25(OH)$_2$D levels above the upper limit of normal (>75 pg/mL). Although CYP24A1 mutations do not account for the majority of cases of elevated 1,25(OH)$_2$D, we hope that this study will raise awareness of the clinical significance of elevated 1,25(OH)$_2$D and prompt further investigation into its underlying cause among pediatric patients with nephrolithiasis and related disorders.
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CURRICULUM VITAE

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EDUCATION

Boston University School of Medicine, Boston, MA
• Graduate Medical Sciences, MS in Medical Sciences expected May 2015
• GPA: 3.67 on a 4.0 scale
• Coursework in biochemistry, physiology, histology, pathology, biostatistics, and embryology

Georgia State University, Atlanta, GA
• Urban Accelerated Certification and Masters Program, certification coursework completed May 2012
• GPA: 3.69 on a 4.0 scale
• Coursework in early childhood education, literacy, child development and culturally responsive pedagogy

Tufts University, Medford, MA
• Bachelor of Arts in Music awarded May 2010
• GPA: 3.41 on a 4.0 scale, Dean’s List 3 semesters
Study Abroad: Eberhard-Karls Universität Tübingen, Tübingen, Germany April-July 2014
• Coursework in German including linguistics, music history, and intercultural communication

RESEARCH AND HEALTHCARE

Boston Children’s Hospital, Boston, MA
Research Assistant, July 2014 – Present
• Analyze clinical characteristics of pediatric kidney stone through retrospective chart review
• Recruit patients for ongoing genetic research on pediatric kidney stones

Tufts Emergency Medical Services
Emergency Medical Technician, November 2006 – May 2010
• Served as a volunteer on a student-run ambulance
• Trained EMTs by running practice call in office hours and evaluating performance on emergency calls
• Promoted to preceptor, the top of 5 rankings, in December 2009
TEACHING EXPERIENCE

George A. Towns Elementary School, Atlanta, GA
First Grade Team Leader, August 2012 – June 2013
• Coordinated curriculum, data analysis, and instructional planning of all first grade classes
• Advocated for first grade students and teachers in school-wide leadership meetings
First Grade Teacher, August 2011 – June 2013
• Developed and implemented research-based lessons to educate students in a classroom comprised of over 30% Early Intervention Program (EIP) students
Third Grade Teacher; Early Intervention Program Teacher, August 2010 – June 2011
• Coordinated with an experienced teacher to team teach 23 third grade students
• Provided additional, small-group assessment and instruction in math for students in the Early Intervention Program for low-achieving students

Classroom 2 Community, Atlanta, GA
Elementary Classroom Resource, December 2012 – May 2013
• Presented classroom sessions on elementary education to 20 Masters of Public Health students and Teach For America corps members
• Provided feedback on lesson planning and execution to MPH students teaching in elementary classrooms
Health Education Advisor, December 2011 – May 2012, December 2012 – May 2013
• Mentored a public health student to educate elementary school students about nutrition, hygiene, and public health

Teach For America, Atlanta, GA
Corps Member, June 2010 – May 2012
• Coordinated with program staff through seminars and individual meetings to increase student achievement in a Title I elementary school
• Analyzed data to create curriculum and lessons and set goals based on individual student needs

Writing Fellows, Tufts University, Medford, MA
Head Fellow, August 2009 – May 2010
• Trained new Fellows in fundamentals of English grammar and tutoring techniques
• Coordinated the program of 45 Fellows, including training and quality improvement
• Instituted a mentoring program for all new Fellows, including regular meetings between new and returning Fellows to discuss tutoring techniques and to build a Fellowing community

Boston University Emergency Medical Services, Boston, MA
Lead Instructor, December 2008 – May 2010
• Taught emergency medical care techniques at Boston University, Tufts, and Harvard
Let’s Get Ready!, Somerville, MA

Head Coach, September – December 2009

• Organized lesson plans and curriculum for Critical Reading/Writing coaches
• Served as liaison between coaches and program directors

Critical Reading/Writing Coach, Fall 2008, Fall 2009

• Taught an SAT and college preparation class for juniors and seniors at Somerville High School
• Assisted students with college application essay writing

ADDITIONAL VOLUNTEER EXPERIENCE

bWell Center, Boston Medical Center, Boston, MA

bWell Center Volunteer, January – June 2014

• Coordinated hourly activities, such as jump rope clinics, yoga classes, and cooking classes for pediatric patients at Boston Medical Center
• Provided patients’ families with educational and community resources to promote healthy lifestyles

ConnectEd 4 Health, Atlanta, GA

Leadership Team Member, May 2012 – July 2013

• Collaborated with educators and public health leaders to develop programs that strive to remove health issues as a barrier to education in low-income communities

HealthSTAT, Steppin’ for Health, Atlanta, GA

Elementary Education Advisor, January – June 2013

• Trained volunteers and review curriculum to adapt nutrition and obesity lessons for elementary students

Rollins School of Public Health, Emory University, Atlanta, GA

Curriculum Project Primary Contact, January – May 2013

• Facilitated communications between Masters of Public Health students and school stakeholders to assist in designing health curriculum
• Reviewed and provided feedback on health curriculum created by MPH students

PUBLICATIONS/PRESENTATIONS


SKILLS AND INTERESTS

German- Written and oral fluency
Piano- Classical and jazz performance