High Urinary Calcium Excretion and Familial Aggregation of Hypertension, Kidney Stone Disease, Obesity, Excessive Weight Gain and Type 2 Diabetes in Patients with Calcareous Stones

by

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Abstract

High Urinary Calcium Excretion and Familial Aggregation of Hypertension, Kidney Stone Disease, Obesity, Excessive Weight Gain and Type 2 Diabetes in Patients With Calcareous Stones. Doctor of Philosophy, 2007, Andrew Mente, Department of Public Health Sciences, University of Toronto.

Associations between kidney stone disease (KSD), hypertension, obesity, excessive weight gain and type 2 diabetes suggest the possibility that susceptible individuals share a common genetic background and are influenced by similar environmental factors. To test this hypothesis, this thesis examined 1) the aggregation of these conditions in families of KSD patients with hypercalciuria, and 2) the combined effect of dietary potassium (K^+), measured by its urinary excretion, and hypercalciuria on the familial risk of these conditions. Consecutive KSD patients, aged 18-50 years, were recruited from a population-based Kidney Stone Center and collected a 24-hour urine sample. The first-degree relatives of eligible subjects (n=333) and their spouse were interviewed by telephone to collect demographic and health information. Familial aggregation was assessed using Generalized Estimating Equations. Multivariate-adjusted odds ratios (OR) revealed significant associations between hypercalciuria in patients and hypertension and KSD in first-degree relatives and specifically in siblings (OR and 95% confidence interval for hypertension = 2.9, 1.4-6.2 and for KSD = 1.9, 1.03-3.5). No significant associations were found in parents or spouses, or in patients with hyperuricosuria. Similarly, no aggregation with other conditions was observed. When examining the effects of urinary K^+, relatives of low K^+ excretors had a higher frequency of obesity and excessive weight gain, but not hypertension, KSD or diabetes, than those of normal excretors. In hypercalciuric patients, however, low K^+ excretion predicted hypertension

(OR = 3.3, 1.5-7.4) and KSD (OR = 2.9, 1.1-7.3) in first-degree relatives, and diabetes in siblings (OR = 4.1, 1.1-15.6). No conditions aggregated in spouses. In a validation study, increased 24-hour urinary K^+ was associated with a progressive increase in diet quality score, the intake of foods recommended by current dietary guidelines and a lower BMI, diastolic blood pressure and heart rate. While an environmental effect cannot be fully excluded, the findings suggest that the disturbance in calcium metabolism in hypertension and KSD has a genetic basis. Furthermore, the combination of a low K^+ diet, an environmental factor, and hypercalciuria, a suspected inherited trait, identified a subset of patients with an apparent common pathway for the development of KSD, hypertension and possibly diabetes.

Preface

Andrew Mente's contribution to this thesis was in the following capacity: 1) conception of the project, 2) study design, 3) collection and assembly of the data, 4) analysis and interpretation of the data, 5) drafting of manuscripts, 6) critical revision of the articles for important intellectual content, and 7) write-up of the thesis.

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I would like to dedicate this work to my family. My parents have displayed an unbelieveable amount of patience and support over many years, and always had faith in me. My brother, Peter, provided rock-solid motivational support and could be counted on to provide a few laughs along the way.

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Table of Contents

List of Manuscripts

List of Tables

List of Figures

List of Appendices

Abbreviations

α = alpha

BMI = body mass index

BP = blood pressure

Ca^{2+} = calcium

CN = calcium nephrolithiasis

CS = calcareous stones

DASH = Dietary Approaches to Stop Hypertension

DM2 = type 2 diabetes mellitus (non-insulin dependent diabetes mellitus)

EO = European origin

FFQ = food frequency questionnaire

GEE = generalized estimating equation

HC = hypercalciuria

IN = idiopathic nephrolithiasis

K^+ = potassium

KSD = kidney stone disease

μU = microunits

mmol = millimole

μmol = micromole

mm Hg = millimeters mercury

Nephrolithiasis = kidney stone disease

OR = odds ratio

PTH = parathyroid hormone

PO_4 = phosphate

RFS = Recommended Foods Score

SD = standard deviation

SE = standard error

$1,25(OH)_2D_3$ = 1,25-dihydroxyvitamin D_3

$25(OH)_2D_3$ = 25-hydroxyvitamin D_3

Glossary

Sensitivity is the proportion of truly diseased persons in the screened population who are identified as diseased by the screening test.

Specificity is the proportion of truly nondiseased persons in the screened population who are identified as nondiseased by the screening test.

Positive predictive value is the probability that a person with a positive test is a true positive.

Negative predictive value is the probability that a person with a negative test is a true negative.

Positive likelihood ratio is the amount the odds of disease increase when a test is positive. It is given by the formula [sensitivity / (1-specificity)].

Negative likelihood ratio is the amount the odds of disease decrease when a test is negative. It is given by the formula [(1-sensitivity) / specificity].

I. OBJECTIVES

A. Introduction

Hypertension, kidney stone disease (KSD), obesity, excessive weight gain, and type 2 diabetes mellitus (DM2) are major public health problems in Canada that arise likely as a result of the combined effects of genetic and environmental factors. Efforts to identify the genes that confer susceptibility to these conditions have been disappointing, with few causative genes being found. It has been suggested that a well-defined clinical phenotype may reduce genetic heterogeneity and facilitate the search for disease alleles involved in the pathogenesis of these conditions. A number of epidemiological studies have shown associations between KSD and several clinical conditions related to renal stone formation including hypertension, obesity, excessive weight gain and DM2. Disturbances in calcium (Ca^{2+}) metabolism are a common finding in these disorders. The biological basis of these abnormalities and how these clinical disorders are connected, however, are still undefined. This thesis tests the hypothesis that alterations in Ca^{2+} metabolism play a central role in the pathogenesis of these closely linked conditions with excessive renal loss of Ca^{2+} or hypercalciuria being a principal manifestation. One way to identify common etiologic and pathogenetic mechanisms among different diseases is to determine whether the disorders occur more frequently within certain families.

B. Primary Objectives of Thesis

The primary objectives of the thesis are: 1) to determine whether hypertension, KSD, obesity, excessive weight gain and DM2 aggregate in first-degree relatives of hypercalciuric patients with calcareous stones (CS), and 2) to examine the influence of dietary potassium (K^+), measured by its urinary excretion, on the familial risk of these conditions, and its interaction with hypercalciuria, a presumed inherited trait.

C. Secondary Objectives

The secondary objectives are: 1) to assess the influence of proxy reported health information on the observed associations for familial aggregation, and to examine the accuracy of these reports for hypertension, DM2, KSD, obesity and osteoporosis status in first-degree relatives and spouses; 2) to determine whether there are ethnic differences in the propensity for KSD in Canadians of Asian and European origin visiting a population-based treatment facility; and 3) to examine the validity of urinary K^+ excretion as a measure of diet quality and to evaluate its relationship to body mass index, blood pressure and heart rate.

II. STUDY OVERVIEW, RATIONALE AND HYPOTHESIS

A. Background

Hypertension, KSD, obesity, excessive weight gain, and DM2 are common and rapidly growing health problems. They are major risk factors for premature death and/or disability in Canada (Stamatelou et al., 2003; Wolf-Maier et al., 2003), and place a heavy financial and social burden on society, which will continue to grow as the population ages (Clark et al., 1995). Multiple strategies have been used to combat these health problems. One approach involves identifying the genes that confer susceptibility to these conditions and the factors that influence their expression. This information is critically important to gain a better understanding of basic disease mechanisms, to develop new and specific therapeutic interventions, and to diagnose and prevent presymptomatic disease (Khoury et al, 1993). Mendelian forms of these conditions, arising from a defect in a single gene, are rare. In most instances, they are polygenic disorders where the small or moderate effects of several disease alleles (i.e., epistasis) are additive and act in concert with environmental factors to affect the level of blood pressure, plasma glucose or body weight. So far, studies to identify susceptibility genes for hypertension, KSD, DM2 and obesity have yielded few positive results. It has been suggested that better phenotypic characterization of these disorders may reduce genetic heterogeneity and enable investigators to find susceptibility genes and elucidate the molecular mechanisms involved in their pathogenesis. The overall goal of this thesis is to test the hypothesis that associations among these conditions identify susceptible people who share a common genetic background and have similar environmental exposures. Finding an intermediate phenotype that aggregates in families is critically important in designing studies to identify the gene or genes responsible for these conditions.

Abnormalities of calcium (Ca^{2+}) metabolism are a common finding in patients with calcareous stones (CS) (Lemann, 1996) and may play a major role in its etiology

(Monk and Buchinski, 1996). Epidemiological studies have shown associations between KSD and several clinical conditions including hypertension (Borghi et al, 1999; Tibblin, 1967; Cirillo et al., 1988; Cappuccio et al., 1990; Madore et al., 1998; Cappuccio et al., 1999a), obesity (Soucie et al., 1996; Bulusu et al., 1970; Powell et al., 2000; Leonetti et al., 1998; Curhan et al., 1998a), weight gain (Taylor et al, 2005a) and DM2 (Taylor et al, 2005b). Disturbances in Ca^{2+} metabolism are also a common finding in these conditions. While hypercalciuria is the most prominent feature, other abnormalities have been reported including lowered serum ionized Ca^{2+}, raised intracellular Ca^{2+}, suppressed intracellular free magnesium, defective membrane binding and transport kinetics of Ca^{2+}, elevated serum parathyroid hormone (PTH) and increased serum 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) (Lemann, 1996; Monk and Buchinski, 1996). The biological basis of these biochemical disturbances and how these clinical disorders are connected are still undefined.

Dietary factors play an important role in the etiology of these conditions. Several epidemiologic studies have reported an inverse association between low Ca^{2+} intake and increased risk of KSD (Curhan et al., 1997), hypertension (McCarron et al., 1984; Birkett, 1998), obesity (Zemel et al., 2000; Skinner and Carruth, 2001), diabetes (Colditz et al., 1992) and osteoporosis (Hansen et al., 1991). Randomized trials have also shown that high Ca^{2+} diets or supplementation can decrease the risk of kidney stone formation (Williams et al., 2001; Borghi et al, 2002), lower BP (Allender et al., 1996; Bucher et al., 1996; Appel et al., 1997; Sacks et al, 2001), reduce body weight or obesity (Davies et al., 2000), improve insulin sensitivity (Sanchez et al., 1997; Ard et al, 2004), and increase bone mineral density (Baran et al., 1990; Devine et al., 1997).

Other dietary factors are also related to these disorders and have an effect on Ca^{2+} metabolism as well. In metabolic balance studies of healthy adults, Lemann et al found that the consumption of a diet deficient in KCl or $KHCO_3$ increases urinary Ca^{2+}

excretion (Lemann et al., 1991a), and $KHCO_3$ administration reverses this effect and promotes a positive Ca^{2+} balance (Lemann et al., 1989). Prospective dietary studies revealed that a diet rich in K^+ significantly reduces Ca^{2+} excretion (Appel et al., 1997; Osorio et al., 1997; Sacks et al., 2001) and the incidence of KSD (Barcelo et al., 1993), and lowers blood pressure (Appel et al., 1997), mitigates salt sensitivity (Sacks et al., 2001) and improves urinary stone risk profile (Meschi et al., 2004). On the other hand, administration of NaCl increases urinary Ca^{2+} excretion, while its restriction has the opposite effect (Muldowney et al., 1982; Lemann et al., 1991b; Massey and Whiting, 1995). Oral animal protein loading also increases urinary Ca^{2+} excretion in renal stone formers and healthy volunteers (Robertson et al., 1979), and epidemiological studies have demonstrated an association between animal protein intake and KSD (Curhan et al., 1993; Taylor et al., 2004), particularly in subjects with idiopathic hypercalciuria (Goldfarb, 1988). Administration of glucose or sucrose also is accompanied by an exaggerated calciuresis among hypercalciuric stone formers and their relatives (Lemann et al., 1969).

B. Rationale

The conceptual basis for this thesis is outlined in Figure 1 on page 6. It is postulated that a negative balance of Ca^{2+} underlies these metabolic disorders. This arises as a result of increased urinary Ca^{2+} excretion occurring in genetically susceptible people when exposed to one or more dietary factors. Ca^{2+} loss sets into motion a cascade of events that ultimately lead to the development of hypertension, KSD, osteopenia, excessive weight gain, obesity, and DM2. It also increases renal sodium reabsorption leading to extracellular fluid volume expansion and in turn to urinary Ca^{2+} loss, and worsens negative Ca^{2+} balance.

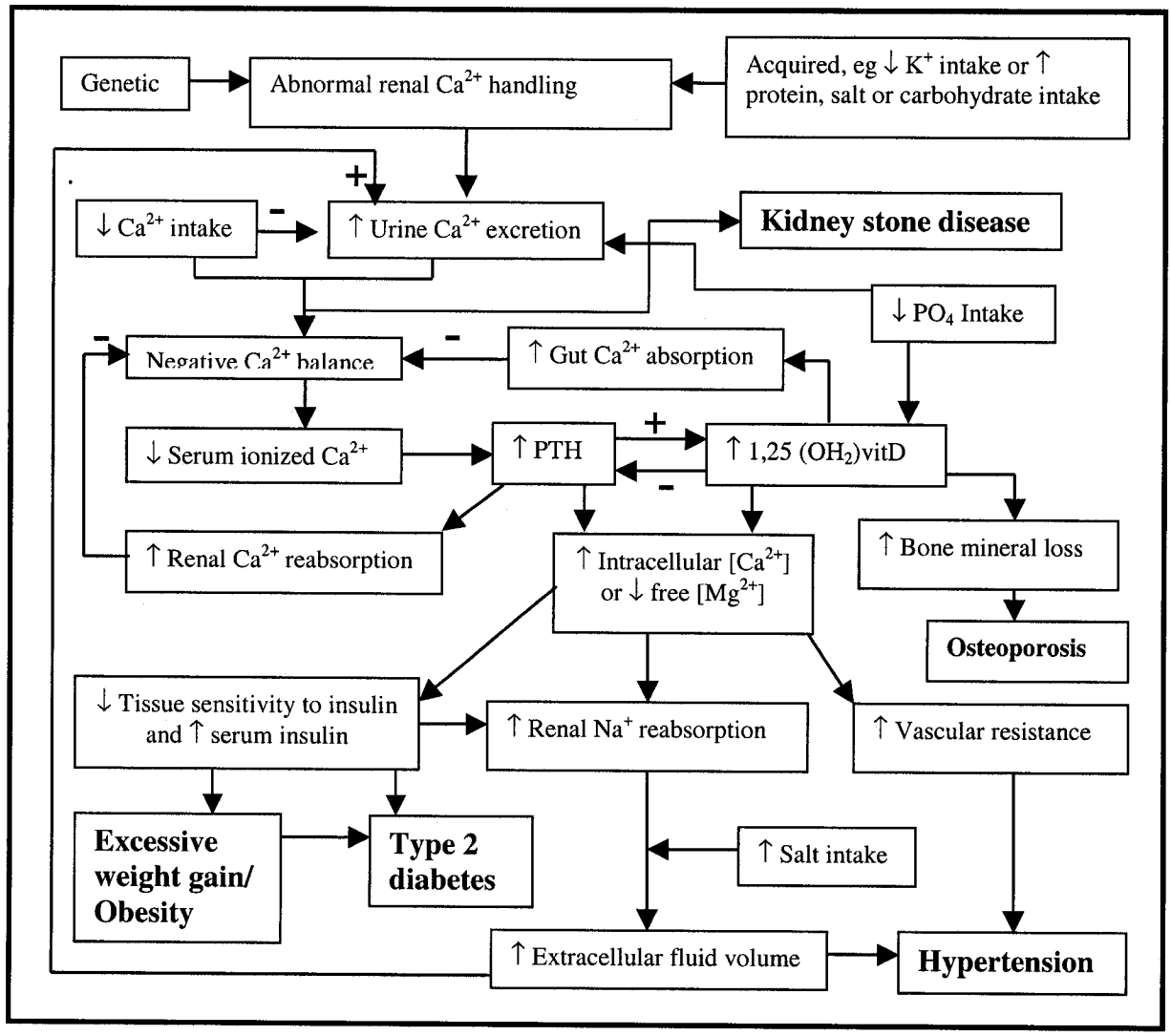

Figure 1. Biological model of a common pathway for a set of related conditions. Negative Ca^{2+} balance via heightened Ca^{2+} excretion and low Ca^{2+} and/or a poor overall diet is a common denominator among metabolic abnormalities leading to the development of KSD, obesity, excessive weight gain, DM2 and hypertension.

One way to identify common etiologic and pathogenetic mechanisms among different diseases is to determine whether the disorders occur more frequently within certain families (Khoury et al., 1993). This approach also permits investigators to discriminate between genetic and environmental factors that may contribute to familial clustering. In this study, a common pathway involving alterations in Ca^{2+} metabolism

that leads to KSD as well as its associated conditions is postulated. This proposition was explored by determining whether these conditions aggregate in the families of KSD patients with hypercalciuria (high urinary Ca^{2+} excretion). Because members of the family share not only genetic information but also tend to adopt similar ways of living, the study also determined the risk of these disorders in the spouses of affected family members. If the data suggest a role for genetic factors, future studies will be directed towards finding susceptibility genes and elucidating the underlying disease mechanisms.

1. Impact of Proxy Reports on Associations for Familial Aggregation

Family health information is often used in studies of familial aggregation of disease. These studies are employed to determine whether a trait or disease occurs more frequently within certain families, and are considered a first step in the dissection of genetic traits (Khoury et al., 1993). Due to cost and logistical issues, however, familial aggregation studies often rely on proxy reported information from probands to ascertain affectation status, an insensitive method of identifying disease status in family members. Misclassification of disease status resulting from random or nondifferential measurement error across exposure groups may lead to attenuated associations between exposure and disease (Khoury et al., 1993). The extent of the attenuation of the effect size in familial aggregation studies that ascertain health information from proxy reports has not previously been evaluated. This thesis, therefore, examines the influence of proxy reported health information on the observed associations for familial aggregation, and evaluates the accuracy of these reports for hypertension, KSD, DM2, obesity and osteoporosis status in first-degree relatives and spouses.

2. Assessment of Ethnic Differences in the Prevalence of Kidney Stone Disease

Studies examining the prevalence of KSD have been limited largely to individuals of European ancestry, while data in populations from other parts of the world and

individuals of non-European ancestry living in Western societies are sparse. In the U.S. the prevalence of KSD is lower in Asians than in Caucasians (Hiatt et al., 1982; Sarmina et al., 1987; Soucie et al., 1994; Stamatelou et al., 2003). The ancestral origin of the Asian subjects, however, was not specified.

Ethnic minority groups make up a rapidly growing proportion of the Canadian population, and visible minorities currently account for 37% of the Greater Toronto Area (GTA) population compared to 26% in 1991 and 14% in 1981 (Canada's Ethnocultural Portrait, 2001). Unlike the U.S., the majority (74%) of visible minorities residing in Canada are of Asian ancestry (Canada's Ethnocultural Portrait, 2001). The greater degree of ethnic diversity in Canada has accentuated the need to identify and eliminate health disparities between minority and Caucasian populations. Because of the unique nature of the treatment facility used to recruit subjects for the main study, the study Centre provides the opportunity to test for differences in the prevalence of KSD among Canadians from ethnic minority populations and those of European ancestry.

3. Validation Study of Urinary Potassium and Diet Quality

One of the primary objectives of the familial aggregation study is to examine the influence of dietary K^+, measured by its urinary excretion, on familial risk of disease, both alone and combined with hypercalciuria. Previous studies have shown that increased dietary K^+ intake is strongly related to 'healthy' dietary patterns (Kant et al., 2000; Trichopoulou et al., 2003; Hu et al., 2000) and a reduced risk of developing a broad range of health problems (McCarron et al., 2001). Moreover, urinary K^+ excretion from a single 24-hour collection is strongly and inversely related to risk of mortality from all-causes (Tunstall-Pedoe et al., 1997). These properties suggest the possibility that 24-hour urinary K^+ would be a powerful marker of diet quality. While studies have repeatedly shown that a high quality diet reduces the risk of mortality and morbid events, there is no

simple, objective and inexpensive measurement tool for assessing diet quality in routine clinical practice. This thesis, therefore, examines the validity of urinary K^+ excretion as a measure of diet quality and evaluates its relationship to body mass index, blood pressure and heart rate.

C. Hypothesis

This study will test the hypothesis that abnormal Ca^{2+} metabolism plays a central role in the pathogenesis of a group of closely linked disorders (hypertension, KSD, obesity, excessive weight gain and DM2) and that its principal manifestation is hypercalciuria. Chronic Ca^{2+} loss, if not compensated by increased gut Ca^{2+} absorption from dietary sources, will result in a negative Ca^{2+} balance. The long-term consequences are 1) KSD from increased urinary Ca^{2+} excretion; 2) hypertension from elevated vascular tone and increased renal sodium reabsorption resulting in extracellular fluid volume expansion; 3) obesity or excessive weight gain and DM2 from diminished peripheral tissue insulin sensitivity. The proposed model of these relationships is outlined in Figure 1 and forms the basis of this thesis.

III. LITERATURE REVIEW

A. Rationale for Familial Aggregation Study

Genetically complex or multifactorial conditions such as hypertension, KSD, obesity and DM2 are the consequence of a complex interplay between multiple genes, environmental factors, and their interactions (Griffin et al 2004; Barlassina et al, 2002; Horenstein et al, 2004; Owen, 1999; Brown and Duncan, 1999). The multitude of physiologic systems that are involved in BP and glucose regulation and body weight create an enormous potential for a variety of mutant genes to have an influence on each of these health problems. However, identifying genes that underlie susceptibility to such complex traits is hampered by several obstacles. Firstly, the expression of a gene may be age-dependent or may have its most pronounced effect at a certain time or developmental stage. Secondly, the presence of a certain mutation may confer susceptibility to the extent dictated by the presence of other mutations, a phenomenon known as epistasis. Thirdly, a gene may exert an adverse effect only when an individual possessing the gene is exposed to a certain environmental stimulus, hence the term 'gene-environment interaction'. Lastly, individuals with the same phenotype (i.e., high BP) may possess mutations of independent genes, or locus heterogeneity (Glazier et al, 2002).

To overcome these problems of the dissection of genetically complex traits, there is a need to find more homogenous patient populations in which the role of a particular gene can be more readily assessed (Timberlake et al, 2001). To achieve this goal, one can select participants from isolated communities, examine characteristics of animal models of the genetic trait and find parallelism to humans, employ pharmacologic, dietary or other interventions to select probands and family members, or use associated clinical features to define the more homogenous selected phenotype. Several such intermediate phenotypes have already been used successfully to analyze genetic

determinants of hypertension (Williams et al, 1992; Hopkins et al, 1996; Litchfield et al, 1998; Williams et al, 2000) and DM2 (Fogarty et al 2000a, 2002b). The premise is that intermediate phenotypes or physiological traits that represent early or intermediate steps in the causal path to disease are more directly determined by the action of a smaller number of genes and, consequently, less influenced by environmental factors than a complex trait such as BP or bone density.

The primary benefit of using selected populations in genetic epidemiologic studies is that they may help to identify key metabolic or regulatory processes that explain the specific phenotype (Kailasam et al, 1996; O'Connor et al, 2000). Genes that are involved in such key processes are primary candidates for linkage and association studies. Another advantage is that they aid in identifying homogenous subgroups within a heterogenous group, and allow for greater sample sizes for genetic analysis because even younger subjects at genetic risk in their preclinical years may be informative. One limitation of using such selected populations is that evidence for linkage or association in a specific patient group does not necessarily mean that the same will be found in unselected patients (Kailasam et al, 1996; O'Connor et al, 2000).

The epidemiologic association between KSD and conditions of disturbed Ca^{2+} regulation creates an opportunity to define and explore an intermediate phenotype. This thesis examines these relationships, with the ultimate goal to help characterize and select homogenous populations for subsequent linkage analysis.

1. Kidney Stone Disease

a. Descriptive Epidemiology

KSD, or nephrolithiasis, is a common health problem worldwide, and its incidence has been increasing in recent decades (Pak, 1998; Stamatelou et al., 2003). Renal stones also cause considerable pain and suffering, and the cost of diagnosis and treatment results

in substantial financial burden (Clark and Thompson, 1995). The cost of KSD in the United States was estimated at $1.83 billion in 1993 (Ross Morton, 2002).

The annual incidence of KSD is estimated to be about 0.5% in North America and Europe (Pak, 1998). The prevalence has doubled in the last 30 years and is increasing steadily in affluent countries (Johnson et al., 1979; Leusmann et al., 1990; Yoshida et al., 1999; Stamatelou et al., 2003). The lifetime risk is about 10-15% in the developed world, but can be as high as 20-25% in the Middle East (Pak, 1998). KSD is largely a recurrent disease with a relapse rate of 50% in 5-10 years and 75% in 20 years (Trinchieri et al., 1999). KSD is more prevalent in the age group, 30 to 69 years (Johnson et al., 1979; Yoshida et al., 1999). It is also more common in men than in women throughout most of adult life until the sixth decade, where the incidence falls in men and rises in women, a trend toward equivalence (Johnson et al., 1979; Soucie et al., 1994).

Epidemiologic investigations in selected communities in the United States have shown that the risk of stone disease is significantly lower among African- and Hispanic-Americans compared to Caucasians (Stamatelou et al., 2003). It is also less common in Asian populations compared to those of European ancestry (Hiatt et al., 1982; Sarmina et al., 1987; Soucie et al., 1994; Stamatelou et al., 2003), although the region of origin of the Asian subjects was not specified in these reports. Time trend studies in Japan have shown a doubling of the annual incidence over the past three decades in parallel with the growing adoption of lifestyles characterizing Western societies (Yoshida et al., 1999).

b. Analytic Epidemiology

(1) Environmental Factors

(a) Introduction

Kidney stones can be classified according to their primary constituents into Ca^{2+} oxalate (60%), Ca^{2+} oxalate and Ca^{2+} phosphate (10%), Ca^{2+} phosphate (brushite) (10%), Mg^{2+} ammonium phosphate (struvite) (5-10%), uric acid (5-10%), cystine (1%), and other (1%) (Ross Morton, 2002). Therefore, more than 75% of renal stones are composed of Ca^{2+} oxalate or phosphate (Coe et al., 1992). Hypercalciuria is a major risk factor for idiopathic Ca^{2+} stone formation, present in 10-50% of patients (Lemann, 1996; Curhan et al., 2001), but its pathogenesis remains controversial (Monk and Buskinsky, 1996). Dietary factors are important in the development of nephrolithiasis. Other factors include being overweight (Soucie et al., 1996), having a family history of renal stones (Resnick et al., 1968; Curhan et al., 1997), living in lower latitudes (Soucie et al., 1994), and ambient temperature and sunlight (Pak, 1991; Curhan et al., 1994; Soucie et al., 1996). The most likely explanation for the geographic variation in risk of kidney stones remains unclear (Stamatelou et al., 2003).

(b) Dietary promoters/inhibitors of calcium stone disease

Several dietary factors affect urinary Ca^{2+} excretion and have been incriminated in the pathogenesis of Ca^{2+} nephrolithiasis (Lemann, 1996; Monk and Buskinsky, 1996).

i) Calcium. Until recently, higher Ca^{2+} intake was thought to increase the risk of stone formation. However, there is currently substantial evidence that a higher Ca^{2+} diet is associated with a reduced risk of stone formation. Recent data from several large prospective studies in men and women consistently support a reduced risk of stone formation with increasing dietary Ca^{2+} intake. Compared to individuals in the lowest

quintile of dietary Ca^{2+} intake, those in the highest quintile had more than a 30% lower risk of forming a stone (Curhan et al., 1993; Curhan et al., 1997; Curhan et al., 2004). The results of these studies were adjusted for multiple factors, including age, body mass index (BMI), use of antihypertensive medications, and intake of nutrients such as K^+, sodium and animal protein.

In a 5-year randomized controlled trial, stone recurrence was compared in patients with a history of Ca^{2+}-oxalate stones and hypercalciuria assigned to a diet low in Ca^{2+} (400 mg/day) or to a diet with normal Ca^{2+} content (1200 mg/day) and low amounts of animal protein and salt (Borghi et al., 2002). At the end of the study, the risk of stone recurrence on the normal Ca^{2+} diet was 51% lower than for the low Ca^{2+} diet (Borghi et al., 2002).

One potential mechanism to explain the apparent paradox that higher Ca^{2+} intake is protective against stone formation is that dietary Ca^{2+} promotes binding with oxalate in the gut, thereby reducing oxalate absorption and urinary excretion. It is also possible that dairy products (the major source of dietary Ca^{2+}) may contain other inhibitory factors.

ii) Potassium. Dietary K^+ restriction increases urinary Ca^{2+} excretion and stimulates tubular citrate reabsorption, thereby decreasing the urinary excretion of citrate, an important inhibitor of Ca^{2+}-oxalate stone formation. K^+ in food also accompanies organic anions such as citrate that are metabolized to bicarbonate. Thus, the consumption of K^+-containing foods such as fruits and vegetables represents an alkali load that increases the urinary excretion of citrate. Higher K^+ intake is inversely associated with incident kidney stones in men and women (Curhan et al., 1993; Curhan et al., 1997).

iii) Sodium. High sodium intake, and a subsequent decrease in proximal sodium reabsorption, reduces renal tubular Ca^{2+} reabsorption (Friedman and Gesek, 1993; Burtis et al., 1994). Randomized trial data confirm the powerful effect of dietary sodium and animal protein restriction on reducing urinary Ca^{2+} excretion (Borghi et al., 2002).

Observational studies found a positive, independent association between sodium consumption and new kidney stone formation in women but not men (Curhan et al., 1993; Curhan et al., 1997).

iv) Animal protein. The metabolism of sulphur-containing amino acids in animal flesh generates sulphuric acid. As such, dietary animal protein represents an acid load that increases urinary Ca^{2+} excretion and reduces urinary citrate excretion (Kok et al., 1990; Trinchieri et al., 2001). Dietary protein may also lead to an increase in $1,25(OH)_2D_3$ production. Moreover, dietary purines contained in animal protein are metabolized into uric acid thereby increasing the risk of the development of both Ca^{2+} and uric acid containing stones (Coe, 1978). A positive association between animal protein consumption and new kidney stone formation has been shown in men but not women (Curhan et al., 1993; Curhan et al., 1997; Curhan et al., 2004).

v) Carbohydrates. Carbohydrate intake results in increased urinary Ca^{2+} excretion, an effect that may be at least partially mediated by insulin. Lemann et al. (1969) found that Ca^{2+} oxalate stone formers and their relatives exhibited higher rates of urinary Ca^{2+} excretion after glucose or sucrose ingestion than control subjects. Also, insulin administration in healthy subjects elicits decreases in urinary sodium, K^+ and phosphate excretion, but increases in urinary Ca^{2+} excretion (DeFronzo et al., 1975). A positive association between sucrose intake and incident kidney stones has been shown in women but not men (Curhan et al., 1993; Curhan et al., 1997; Curhan et al., 2004).

vi) Magnesium. Magnesium complexes with oxalate, potentially decreasing Ca^{2+}-oxalate supersaturation in the urine. Magnesium may also reduce oxalate absorption in the gastrointestinal tract. Higher dietary magnesium was associated with a 30% lower risk of stone formation in men (Taylor et al., 2004), but no association has been observed in women (Curhan et al., 1997; Curhan et al., 2004).

vii) Phosphorus. Phosphorus decreases intestinal absorption of dietary Ca^{2+} and

phosphate supplementation can decrease renal Ca^{2+} excretion (Lemann, 1996). Human data to support the role of dietary phosphorus as a risk factor for Ca^{2+} stones are lacking.

Diet alone, however, is likely not responsible for the altered Ca^{2+} metabolism in KSD patients with hypercalciuria, because even when dietary factors are rigorously controlled, patients continue to exhibit reduced renal tubular Ca^{2+} reabsorption (Lemann, 1996).

(c) Pathophysiology of calcium stone disease

There is substantial evidence that disturbed Ca^{2+} metabolism plays a key role in the development of KSD (Bleich et al., 1979). Hypercalciuria is the most frequent metabolic abnormality encountered among KSD patients, occurring alone or in combination with other abnormalities in up to 60% of cases (Curhan et al., 2001) and in less than 10% of normal subjects (Coe et al., 1992). It is not a demarcated illness, but rather a quantitative departure from normal range. Although hypercalcuria may result from an underlying medical condition (i.e., sponge kidney, sarcoidosis, renal tubular acidosis, Crohn's disease, or primary hyperparathyroidism), it usually results from unknown causes and is often referred to as idiopathic hypercalcuria (Pak et al., 1974). The primary defect in a subset of these patients is the kidney's inability to conserve Ca^{2+} ("renal Ca^{2+}-leak") which results in a moderate decrease in serum ionized Ca^{2+} levels, thereby stimulating PTH and $1,25(OH)_2D_3$ production and increasing intestinal Ca^{2+} reabsorption (Pak, 1979). A prominent feature of renal hypercalciuria is that Ca^{2+} renal stone formers are unable to decrease urinary Ca^{2+} excretion even when Ca^{2+} intake is low (Coe et al., 1982; Trinchieri et al., 1998). Coe et al (1982) showed that severe dietary Ca^{2+} restriction (<400 mg/day) led to only a small decrease in urinary Ca^{2+} in most patients with recurrent renal stones and hypercalciuria, an indication that low Ca^{2+} diets would make Ca^{2+} balance even more negative in hypercalciuric patients. This negative

Ca^{2+} balance may be attributed to elevated PTH and $1,25(OH)_2D_3$ levels typically found in patients with hypercalciuria. Not surprisingly, osteopenia has been reported in hypercalciuric patients with recurrent kidney stones (Bordier et al., 1977), and dietary Ca^{2+} intake has been found to be lower in Ca^{2+} renal stone formers with low bone mineral density than in those with normal bone density (Trinchieri et al., 1998). Since the Ca^{2+} content of bone is approximately 1000 times that of daily Ca^{2+} intake, the importance of bone Ca^{2+} resorption triggered by PTH and $1,25(OH)_2D_3$ action in increasing urinary Ca^{2+} excretion and kidney stone formation is readily recognized (Curhan et al., 1993).

(2) Familial Aggregation and Genetic Factors

(a) Familial aggregation of kidney stone disease

Numerous studies have reported that KSD aggregates in families, although the relative contribution of genes and environmental factors is still not clear (Langman, 2004). The increased prevalence of KSD among family members of stone patients was first observed over 40 years ago. Melick and Henneman (1958) found that 12% of the general stone population had at least one family member with a history of stone passage. Similarly, McGeown et al reported that 20% of those with KSD attending a clinic had a first-degree relative, either parent, sibling or child, with a history of stones (McGeown, 1960). These findings were later confirmed among patients with Ca^{2+} oxalate stones. Resnick et al reported a family history of stone in 14% of the Ca^{2+} oxalate stone formers, which was more frequent than the first-degree relatives of the spouses of the stone formers (Resnick et al., 1968). The authors suggested that the formation of Ca^{2+} oxalate stones was regulated by a polygenic system.

Ljunghall (1979) found a positive family history of KSD, defined as one or more first-degree relatives with renal stones, among 29.6% of middle-aged Swedish male stone formers compared to 17.4% among the controls. In a subsequent study of 380

consecutive patients, Ljunghall et al (1985) found a positive family history present in 64.7% of female patients and 51.0% of male patients. Moreover, family history of stone disease was found to increase in a dose-dependent manner as the number of stone recurrences in probands increased (Ljunghall et al 1985).

Trinchieri et al (1988) reported a higher frequency of Ca^{2+} stones among the first-degree relatives of Ca^{2+}-stone patients (37.9%) compared to the relatives of controls (12.8%) or the relatives of the spouses (18.4%). In addition, the frequency of KSD among the spouses of the cases was similar to that of the general population, which provides support for the role of an underlying genetic predisposition to kidney stones rather than merely a shared environment or lifestyle (Trinchieri et al., 1988).

Wasserstein et al reported that the siblings of stone patients had a 7 times greater prevalence and parents had an almost 4 times greater prevalence of KSD compared to controls. The odds ratios were smaller for more distant relatives (Wasserstein et al., 1987). More recently, using cross-sectional data from the Health Professionals Follow-up Study, Curhan et al reported that the age adjusted odds ratio for KSD among individuals with a positive family history of kidney stones was 3.2 compared to those with a negative family history (Curhan et al., 1997). Their analysis of prospective data over the 8-year follow-up period revealed that the relative risk of incident KSD was 2.6 (95% C.I., 2.2–3.0) for individuals with a positive family history of kidney stones. Furthermore, the relative risk was even higher (RR=2.9, 2.4–3.5) when the analysis was restricted to those below 60 years of age at baseline (Curhan et al., 1997), which suggests that KSD occurring at an earlier age is more likely to be determined by genetic factors.

Taken together, the evidence to date shows that the development of KSD is influenced by familial factors and that these associations may be largely genetically determined. There are a number of candidate genes with vastly different phenotypes that have been investigated for their role in KSD, as summarized in Appendix 1.

(b) Familial aggregation of hypercalciuria

Several early studies demonstrated the presence of idiopathic hypercalciuria in single families (Beilin and Clayton, 1964; De Luca and Guzetta, 1965; Royer et al., 1974). In a larger study, Buckalew et al (1974) described a family in which 13 of 64 members had hypercalciuria, six more had nephrocalcinosis, and four had distal renal tubular acidosis. Sperling et al. (1974) examined a family in which 5 of 24 members had hypercalciuria, decreased bone density and hypouricemia due to increased renal uric acid clearance.

More compelling evidence for the familial aggregation of hypercalciuria may be obtained from studies recruiting multiple families. Three studies measured Ca^{2+} excretion in the families of children with hypercalciuria. In one report (Mehes et al., 1980), among 10 children with hypercalciuria, 8 had one parent with hypercalciuria, and among the 47 family members examined, 23 cases of idiopathic hypercalciuria were identified. Upon observing the increased frequency of hypercalciuria among family members, the authors concluded that hypercalciuria follows an autosomal dominant mode of inheritance.

Aladjem et al (1983) subsequently compared 22 children with hypercalciuria as well as their parents and siblings to 29 control children and their parents and siblings. Urinary Ca^{2+} excretion following Ca^{2+} deprivation or Ca^{2+} loading was significantly higher in parents and siblings of hypercalciuric children than in the corresponding controls. The authors, however, concluded that the elevated urinary excretion of Ca^{2+} was more likely due to nutritional than genetic factors, since significantly higher excretion of sodium, K^+ and phosphate were found in family members of the hypercalciuric group compared to controls.

Finally, in a multicenter study, Stapleton (1990) investigated the frequency and diagnostic importance of hypercalciuria in 215 children with hematuria in a prospective

study. Compared to children with normal Ca^{2+} excretion, a greater number of children with hypercalciuria (46.1% vs 21.6%) had a positive family history of urolithiasis. Four year follow-up revealed that hypercalciuria was predictive of future urolithiasis in children with hematuria.

In their study of adult probands, Coe et al (1979) examined the frequency of hypercalciuria in the families of nine hypercalciuric patients who formed recurrent oxalate renal stones. Hypercalciuria was observed in 26 of 73 relatives. A total of 19 of 44 first-degree relatives (43%) had hypercalciuria, compared to 7 of 29 (29%) other relatives. In addition, renal stones were formed by 19 of the 44 first-degree relatives but by none of the other relatives. Families showed examples of horizontal and vertical patterns of stone and hypercalciuria, with multiple members of individual sibships affected. It was less clear, however, as to whether the hypercalciuria was a direct result of a genetic effect, since stones developed in some family members without hypercalciuria.

The authors explored the possibility that familial patterns of hypercalciuria may have resulted from environmental factors, such as dietary practices, rather than inherited abnormalities in Ca^{2+} metabolism. If diet were an important factor, then the frequency of hypercalciuria among stone formers and their spouses would be expected to be equivalent. Coe and colleagues found that only 2 of 9 spouses of probands were hypercalciuric and that the contribution of their offspring to the overall frequency of hypercalciuria and stone was negligible (Coe et al., 1979). The authors concluded that there is a familial form of hypercalciuria that is consistent with an autosomal dominant mode of inheritance.

Since idiopathic hypercalciuria is comprised of various subcategories, such as 'absorptive' and 'renal' subtypes (Pak, 1979), a number of studies examined the pattern of inheritance associated with specific hypercalciuric subcategories. Pak et al classified

stone patients with hypercalciuria according to their pathogenetic criteria and found family stone history in 45% of patients with absorptive hypercalciuria and in 38% of those with renal hypercalciuria (Pak et al., 1975). Two studies provided evidence that absorptive hypercalciuria is inherited as an autosomal dominant trait, but suggested that environmental factors also play a role in the level of Ca^{2+} excretion (Pak et al., 1981; Hamed et al., 1979). In contrast, one investigation indicated that absorptive hypercalciuria is the consequence of nutritional factors rather than genetic predisposition (Harangi and Mehes, 1993). For renal hypercalciuria, one study indicated that it may be transmitted as an autosomal dominant trait (Harangi and Mehes, 1993), while another investigation provided evidence for an interaction between genetic and environmental factors (Eggert et al., 1998). Finally, another study indicated that both renal and absorptive hypercalciuria are autosomal dominant traits (Nicolaidou et al., 1996). The bulk of the evidence from these studies suggest that both Ca^{2+} excretion subtypes are, to varying extents, the consequence of genetic factors interacting with environmental influences. While the familial aggregation of hypercalciuria is well documented, the pathogenetic basis for such enhanced Ca^{2+} excretion remains uncertain.

2. Epidemiology of Conditions Related to Disturbed Ca^{2+} Homeostasis

a. Hypertension

(1) Descriptive Epidemiology

Hypertension, or high arterial blood pressure, is a substantial public health problem, affecting 1 in 4 adults in industrialized societies (Burt et al., 1995; Wolf-Maier et al., 2003). It is a major risk factor for many common causes of morbidity and mortality including stroke, myocardial infarction, congestive heart failure, and end stage renal disease (Kannel, 2000; Mosterd et al., 1999). Hypertension also represents a

significant financial issue, since it is a leading cause of office visits and use of prescription drugs in Canada (Hamet, 2000).

The annual incidence of hypertension increases steadily with age from less than 1% in the second decade to as high as 8% in the seventh decade. Incidence rates are higher in men in the earlier decades, but the reverse occurs in subsequent years (Whelton, 1994). The prevalence of hypertension also increases steadily throughout adult life from 4% at age 18-29 years to 65% in those aged ≥ 80 years in the United States (National High Blood Pressure Education Program Working Group, 1993). The prevalence of hypertension in Canada is similar to that of the United States, affecting 14% of individuals aged 35 to 44 years, and 53% of those aged 65 to 74 years (Wolf-Maier et al., 2003). The overall age- and sex-adjusted prevalence of hypertension in persons aged 35 to 64 years in Canada and the US is 28% (about 30% in men and 25% in women) (Wolf-Maier et al., 2003).

Epidemiologic investigations in the United States have shown that hypertension is more common in African Americans than in Caucasians, Hispanics, Japanese and American Indians (Haffner et al., 1990; Whelton et al., 1994; Burt et al., 1995). On the other hand, it is less common in Asian/Pacific Islanders and Mexican Americans than in Caucasians (Haffner et al., 1990). The cause of this lower prevalence in these groups is unknown (Haffner et al., 1990). In Canada, individuals of Chinese descent have a higher prevalence of hypertension requiring medication (15.9%) compared with South Asians (13.7%) and Europeans (11.0%) (Anand et al., 2000).

(2) Analytic Epidemiology

Considerable effort has been devoted to defining the pathogenesis of blood pressure variation. The evidence to date suggests that hypertension is the result of a

complex interplay between environmental and genetic factors.

(a) Environmental factors

i) Introduction

Despite the importance of hypertension as a cause of disease, its pathogenesis remains largely unknown. Epidemiologic studies have documented the impact of a variety of factors, including increased age, being overweight (Whelton et al., 1998; He et al., 2000), abdominal obesity (Gillum et al., 1998), sedentary lifestyle (Whelton et al., 2002) heavy alcohol consumption (Xin et al., 2001), smoking (Whelton et al., 1994), hyperinsulinemia (Sattar et al., 2003), and psychological stress (Krantz and Manuck, 1984). Diet has also been implicated, with low dietary K^+ and Ca^{2+}, and excess salt intake suggested as important factors (Appel et al., 1997; Sacks et al., 2001). How these risk factors influence physiology to alter blood pressure has been the subject of extensive investigation (Beilin et al., 1999).

ii) Hypertension and dietary mineral intake

Data from population based studies indicate an inverse association between dietary Ca^{2+} and K^+ intake and mean population blood pressure (McCarron et al., 1984; Cappuccio et al., 1995; Birkett, 1998). This finding is most apparent in those whose sodium to K^+ ratio is high and suggests that an adequate Ca^{2+} and K^+ intake appears to protect against the blood pressure raising action of a high sodium diet (Gruchow et al., 1988). In addition, increased blood pressure found in salt-sensitive individuals (i.e., subjects with marked increases in blood pressure in response to salt loading) does not appear to be associated with elevated salt intake, but rather with decreased ingestion of Ca^{2+} and K^+ (Harlan and Harlan, 1986; Zemel et al., 1988). Recently, findings from the CARDIA study revealed that individuals who consumed dairy products $\geq$35 times per

week had a significantly lower 10-year cumulative incidence of hypertension compared to participants who consumed little or no dairy products (Pereira et al., 2002).

Several clinical studies have also explored the blood pressure lowering capability of Ca^{2+} and K^{+}. Resnick et al (1985) found that only hypertensive patients with salt-sensitive hypertension showed a decrease in serum ionized Ca^{2+} level and an increase in $1,25(OH)_2D_3$ levels during dietary sodium loading (Resnick et al., 1985). Also, only salt-sensitive patients exhibited a fall in blood pressure in response to increases Ca^{2+} intake (Resnick et al., 1986). Weinberger et al (1993) confirmed the blood pressure lowering effect of Ca^{2+} in salt-sensitive hypertensives, and also found that these patients excreted more Ca^{2+} in their urine that was unrelated to differences in dietary Ca^{2+} intake (Weinberger et al., 1993). Clinical and metabolic studies also demonstrated the blood pressure lowering effect of dietary K^{+} (Krishna et al., 1989) and its efficacy in reducing urinary Ca^{2+} excretion in healthy adults on a K^{+}-deficient diet at baseline (Lemann et al., 1989).

The findings from a number of clinical trials indicate that a diet low in dietary Ca^{2+} and K^{+} is associated with an increased prevalence of hypertension (Allender et al., 1996; Bucher et al., 1996). The landmark DASH trial examined the effect of an overall high quality diet on blood pressure (Appel et al., 1997). The results showed that a diet with good sources of Ca^{2+} and K^{+} such as fruits, vegetables, and low-fat dairy products (the combined diet) elicited the greatest fall in blood pressure compared to a 'Western' diet and a diet high in fruits and vegetables only. The blood pressure reduction appeared within two weeks of taking the combination diet and the magnitude of the change was greater in those with high blood pressure. In addition, the change in blood pressure in the DASH trial was 2- to 4-fold greater than that observed in the pooled results of sodium restriction trials (Appel et al., 1997).

Taken together, the findings to date indicate that increased dietary Ca^{2+} and K^{+} might be more effective in lowering blood pressure than dietary salt restriction, particularly in salt sensitive individuals.

iii) Hypertension and calcium metabolism

Many biochemical abnormalities in Ca^{2+} metabolism have been associated with human hypertension. The most consistent finding is increased urinary Ca^{2+} excretion for a given salt intake (Young et al., 1992), but lower serum ionized Ca^{2+} and phosphorus levels, higher serum PTH and $1,25(OH)_2D_3$, raised intracellular Ca^{2+} and/or reduced free magnesium levels, and reduced cellular membrane Ca^{2+} binding have also been reported (Hamet, 1995). These changes are believed to be the consequence of a renal Ca^{2+} leak (McCarron, 1989). Salt-sensitive forms of hypertension are more likely to manifest disturbances in Ca^{2+} metabolism. Resnick et al. (1985) observed that dietary salt loading suppressed serum ionized Ca^{2+} and increased $1,25(OH)_2D_3$ more in salt-sensitive hypertensive patients compared to salt-resistant patients. Salt-sensitive hypertensive patients have also shown a higher urinary Ca^{2+} output than salt-resistant patients in response to an acute intravenous saline load (Galletti et al., 1993). In response to Ca^{2+} supplementation, only salt-sensitive individuals displayed reductions in BP (Weinberger et al., 1993).

There is evidence from experimental studies that these abnormalities in Ca^{2+} metabolism are a prominent feature of salt-sensitive forms of hypertension. Galletti et al (1993) found that intravenous NaCl infusion induced increases in urinary Ca^{2+} excretion only among salt-sensitive hypertensive patients. Similarly, Takada et al (1995) reported that high urinary Ca^{2+} excretion after dietary salt loading was observed almost exclusively among salt-sensitive normotensive men and they suggested that high urinary Ca^{2+} could be a diagnostic indicator of salt-sensitivity. Also, mineralcorticoid

administration both in humans (Haller et al., 1995) and animals (Suki et al., 1968) induces salt-sensitive hypertension and hypercalciuria. Increased urinary Ca^{2+} excretion also is a feature of primary aldosteronism (Rossi et al., 1995).

There is also recent evidence that a subset of individuals with hypertension manifest a disturbed Ca^{2+} metabolic profile compatible with consequent bone loss. A low bone mineral density has been observed consistently in experimental models of hypertension (Izawa et al., 1985; Lucas et al., 1986; Metz et al., 1990; Wang et al., 1993). Metz et al. (1999) found that blood pressure was associated with reduced bone density and content in men, and Cappuccio et al (1999b) reported that higher blood pressure in elderly white women is predictive of future bone loss at the femoral neck.

(b) Familial aggregation and genetic factors

i) Hypertension

Family studies have long established that genetics account for 30 to 50 percent of blood pressure variation in the population (Ward, 1990; Levy et al.,, 2000). However, hypertension is a complex trait, as there is no single gene with large effects across all populations. Instead, multiple genes with smaller effects likely account for the heritability of this disorder (Lifton et al., 2001; Timberlake et al., 2001). The hypothesis of no major single gene effect is supported by blood pressure data in animal and human populations that do not exhibit bimodal distributions (Barlassina et al., 2002).

Some of the evidence for the role of genetic factors in the determination of blood pressure comes from studies showing marked differences in rates of hypertension across ethnic groups living in similar environments (Haffner et al., 1990; Burt et al., 1995; Anand et al., 2000). There is also substantial evidence for genetic influence on blood pressure from family-based studies (Barlassina et al., 2002). Twin studies document greater concordance of blood pressure of monzygotic than dizygotic twins (Feinleib et al.,

1977), and population studies demonstrate greater similarity of blood pressure within families than between families (Longini et al., 1984). This familial aggregation of blood pressure is not simply attributable to shared environmental effects since adoption studies show greater concordance of blood pressure among biological siblings than adoptive siblings living in the same household (Biron et al., 1976; Rice et al., 1989).

Subjects with a positive family history of hypertension have been reported to have higher blood pressure than those without a family history, both in clinic (Watt et al., 1983; Watt, 1986a) and in ambulatory (Ravogli et al, 1990; van Hooft et al., 1993) settings. Most of these studies focused on relatively young subjects (<35 years) because of the view that familial factors are likely to act early (Hunt et al, 1986).

ii) Disturbances in Ca^{2+} metabolism

Abnormalities in Ca^{2+} metabolism have been observed in young normotensive offspring of hypertensive patients, which suggests that these abnormalities are not simply a secondary effect of hypertension (Brands and Hall, 1998). In a Dutch study, normotensive offspring of two hypertensive parents had decreased serum ionized Ca^{2+} and increased serum PTH levels after adjustment for differences in blood pressure compared to subjects with two normotensive parents (van Hooft et al., 1993). Moreover, in a Japanese study, when the offspring of hypertensive parents were exposed to a high salt diet their urinary Ca^{2+} excretion and platelet cytosolic [Ca^{2+}] increased while plasma ionized Ca^{2+} levels decreased more than those with negative family history of hypertension (Yamakawa et al., 1992). These studies strongly suggest a genetic basis for the disturbances in Ca^{2+} metabolism in hypertension.

iii) Conclusions

While hypertension has a genetic component in its pathogenesis, it is not caused

solely by genetic variation. This makes pinpointing genetic causes more difficult than in diseases that are caused strictly by genetic factors (Lifton et al., 2001; Timberlake et al., 2001). A number of candidate genes have been examined for their role in primary hypertension, as summarized in Appendix 2. Several of these studies employed the candidate gene strategy to gene mapping, and, in conjunction with a number of genome-wide scans, have yielded highly promising results suggesting the role of a few genes in blood pressure regulation. The findings of these studies also point to the need for better defined phenotypes that are intermediate in the pathogenetic development of hypertension (Lifton et al., 2001; Timberlake et al., 2001).

b. Obesity and Weight Gain

(1) Descriptive Epidemiology

Obesity is epidemic in Canada and in most industrialized countries (Katzmarzyk, 2002). It is a major risk factor for diabetes, hypertension, dyslipidemia, coronary artery disease, gallbladder disease and osteoarthritis, and almost 40% of obese adults have at least two of these comorbidities (Must et al., 1999). The direct health costs of obesity in the United States (mostly from diabetes, hypertension and cardiovascular disease) have been estimated at 10% of the total health care expenditure (Koplan and Dietz, 1999). Indirect costs, which are far greater than direct costs, include workdays lost, physician visits, disability pensions and premature mortality (Wolf et al., 1996).

The prevalence of obesity has been increasing globally. In recent decades, prevalence rates have doubled, and in some cases tripled, in China (Bell et al., 2001a), the Indian subcontinent (Yajnik, 2004), the Arabian peninsula (Bener, 2006), and the Pacific Islands (Bell et al., 2001b). In the United States, the prevalence of obesity rose from 13% to 27% of the population from 1960 to 1999 (Health, United States, 2000). Similarly, in

Canada, the prevalence of obesity has increased over the past two decades in both adults and children (Tremblay et al., 2000; Tremblay et al., 2002). Recent survey data showed that the prevalence of obesity increased from 9% to 14% in men and from 8% to 12% in women from 1981 to 1996 (Tremblay et al., 2002). In the context of its rising prevalence, obesity is increasingly being recognized as an important medical and public health problem (Must et al., 1999).

In low-income countries, obesity is more common in people of higher socioeconomic status and in those living in urban communities, and is often most apparent among middle-aged women. In more affluent countries, obesity is associated with lower socioeconomic status, especially in women, and is more prevalent in rural communities (Cooper et al., 2000; Park et al., 2003). The gender differences in prevalence are less marked in affluent countries.

(2) Analytic Epidemiology

(a) Environmental factors

i) Introduction

The etiology of most human obesity is still not clearly understood. The most common endocrinological cause of obesity and weight gain, with a predominance of central fat distribution, is Cushing's syndrome. Hypothyroidism is often stated as a cause of obesity, but the fat gain with this condition is usually minor. Polycystic ovarian syndrome is often associated with obesity, but has not been shown to be an etiological factor. Drug-induced weight gain through the use of antipsychotics (Allison et al., 1999), antidepressants (Stanton, 1995), steroids and anti-diabetic drugs including insulin (UK Prospective Diabetes Study Group, 1998) is common. A very common cause of weight gain is cessation of smoking (Flegal et al., 1995). Obesity and weight gain are also strongly related to a sedentary lifestyle and poor dietary habits such as excess energy

intake (James et al., 2001).

ii) Obesity/weight gain and dietary mineral intake

Recent human and animal studies have determined that a higher Ca^{2+} intake is associated with lower body fat. Dietary Ca^{2+} given together with sodium induced weight loss and reduced total body fat in spontaneously hypertensive rats and Wistar-Kyoto rats (Metz et al., 1988). In transgenic mice, high Ca^{2+}, medium dairy, and high dairy diets reduced lipogenesis, stimulated lipolysis and reduced body fat accumulation at equivalent levels of energy intake (Zemel et al., 2000).

In humans, several epidemiologic studies demonstrated a weight reducing effect by dietary Ca^{2+}. Zemel et al (2000) in their analysis of NHANES III data found a dose-responsive reduction in the relative risk of obesity by quartile of Ca^{2+} intake in women, after controlling for energy intake, activity level, age, race and ethnicity (Zemel et al., 2000). In a prospective study of children, Carruth et al (2001) found that higher intakes of Ca^{2+}, monounsaturated fat, and servings of dairy products were associated with lower body fat (Carruth and Skinner, 2001). In young adults, findings from the CARDIA study revealed that individuals who consumed dairy products $\geq$35 times per week had a significantly lower 10-year cumulative incidence of obesity compared to participants who consumed little or no dairy products (Pereira et al., 2002).

Since a low Ca^{2+} diet might be a marker for a poor diet (Barger-Lux et al., 1992), the associations between Ca^{2+} intake and lower body weight reported in observational studies do not themselves establish a causal relationship between Ca^{2+} and weight loss. However, a randomized controlled trial by Recker et al (1996) of post-menopausal women showed that the Ca^{2+} supplementation group (1.2 g/day) lost 0.671 kg/yr while the placebo group lost 0.325 kg/yr ($p<.025$) (Davies et al., 2000). These results indicate

that the associations noted in other observational studies are at least partly due to the differences in Ca^{2+} intake.

Dietary K^+ is also related to lower body weight. An analysis of data from the first NHANES study showed that a low K^+ diet is associated with a higher BMI (McCarron et al., 1984). In addition, recent clinical trial data from the Women's Health Initiative study showed that a diet containing K^+-rich foods such as fruits and vegetables significantly lowered body weight in older women (Howard et al., 2006).

iii) Obesity and calcium metabolism

Alterations in Ca^{2+} homeostasis have been reported in obesity (Zemel, 1998), which is also related to difficulties in renal sodium handling (DeFronzo, 1981). The mechanism by which Ca^{2+} deficiency contributes to increased weight gain or obesity probably involves an underlying impairment in intracellular Ca^{2+} regulation (Zemel, 1998). The levels of serum PTH and $1,25(OH)_2D_3$ have been found to be increased in obese subjects as a consequence of a low serum ionized Ca^{2+} level (Andersen et al., 1986; Bell et al., 1985). An inverse relation between ionized Ca^{2+} and body mass also has been reported (Lind et al., 1993). High blood PTH and $1,25(OH)_2D_3$ levels, as would be evoked by a diet low in several minerals such as Ca^{2+} and K^+, increase cytosolic Ca^{2+} in human adipocytes in culture, switching their metabolism from lipolysis to lipogenesis (Zemel et al., 2000).

(b) Familial aggregation and genetic factors

Genes related to obesity are clearly not responsible for the epidemic of obesity, since the gene pool in Western societies did not change significantly in recent decades. Nevertheless, body weight is strongly influenced by a genetic component. A number of investigations conducted with the collaboration of obese patients, their parents, siblings

and spouses suggest that the genetic contribution to obesity is between 25 and 40% of the individual differences in BMI (Bouchard, 1994).

Several types of studies provide evidence for a genetic influence on body mass. The evolution of the "thrifty geneotype" is postulated to have resulted in selective survival advantage in times of famine by allowing highly efficient storage of calories in plentiful times. However, this thrify genotype has become detrimental when food supplies are constant and abundant and is believed to have led to an increased prevalence of obesity and DM2 in certain populations.

There are marked differences in rates of obesity among ethnic groups residing in the same geographic area. For instance, populations of European, African and South Asian origin tend to have a higher BMI than East Asians, even after taking into account demographic and lifestyle factors (Anand et al., 2000; Cappuccio et al., 2002; McTigue et al., 2002).

A number of family-based studies provided evidence for the importance of genetic factors in the development of obesity (Loos, 2003). Twin studies revealed a higher rate of obesity within monozygotic twins than within dizygotic twins (Stunkard et al., 1986). Studies in Denmark with adopted children found that a tendency toward obesity correlates greater with the genetic than with the adopted parents (Sorensen et al., 1998). In addition, studies of nuclear families demonstrated that the level of risk for a first-degree relative (a son or daughter) of an overweight person is about two to three times higher than the general population (Danielzik et al., 2002).

Several investigations evaluated the single gene hypothesis in human obesity using segregation analysis. In a Swedish study of morbidly obese probands, Rice et al (1999) reported that the major effect dominates the BMI expression before the age of 20 years, but multifactorial effects account for most of the BMI variance after age 20. The authors concluded that the major effect was transmitted in these families, but the pattern did not

appear to be consistent with a simple Mendelian trait (Rice et al., 1999). Similar findings of non-Mendelian transmission were reported for a putative major locus in 432 families in India (Feitosa et al., 2000), and a locus in 400 Nigerian families (Colilla et al., 2000). It appears that there may be several loci (i.e., epistasis) and gene by environment interactions that may account for these findings.

There are many known candidate genes, both for body fatness and also for body fat distribution (see Appendix 3). However, very few cases have been attributed to mutations of a single gene to date.

c. Type 2 Diabetes

(1) Descriptive Epidemiology

DM2 is a common condition in Canada and globally, and its incidence has been increasing rapidly in recent decades (Amos et al., 1997). DM2 is related to improved living conditions, increasing rates of obesity and aging of most societies. It is estimated that 6% of the Canadian population has diabetes (mostly type 2) (Diabetes in Canada, Health Canada, 1999), and that an additional 3-5% have either undiagnosed diabetes or impaired glucose tolerance (Harris et al., 2000). DM2 increases the risk of cardiovascular disease events by 2- to 4-fold (Harris et al., 2000; Sowers et al., 2001) and is the leading cause of renal failure (Mogensen, 2000). It is also the fourth or fifth leading cause of death in most developed countries, mainly due to cardiovascular complications, especially ischemic heart disease and renal disease (Morrish et al., 2001; Sievers et al., 1992). Consequently, DM2 has become a major health problem worldwide.

In populations of European descent in the United States and Europe, the prevalence of DM2 increases with age at least into the seventies (Greenlund et al., 2004). In recent years, however, the prevalence and incidence of DM2 in children and

adolescents have shown dramatic increases (Fagot-Campagna et al., 2000), particularily in Native North Americans, Mexican and African Americans, Chinese, Polynesians, South Asians, and Arabs (Savage et al., 1979; Dabelea et al., 1998).

(2) Analytic Epidemiology

(a) Environmental factors

i) Introduction

The strongest predictor for the risk of DM2, apart from age, is increased BMI (Jung, 1997). Even at a BMI of 25 kg/m^2 the risk of DM2 is significantly higher compared to a BMI of less than 22 kg/m^2, but at BMI over 30 kg/m^2, the relative risks are enormous (Colditz et al., 1995). Asian populations appear to develop DM2 at a lower BMI than other populations (Cockram, 2000).

The distribution of body fat also influences the risk of developing DM2. Central obesity is associated with an increased risk of DM2 in many different ethnic groups, and is associated with an increased incidence of coronary heart disease, hyperinsulinemia, high serum triglyceride, low HDL-cholesterol levels, and hypertension. Insulin resistance is a characteristic of patients with DM2, but also precedes and predicts the development of the disease (Lorenzo, 2003).

Physical inactivity is another important risk factor for DM2 (2-3 times increased risk) (Kriska et al., 1993), and its effect appears to be strongest among obese individuals (Perry, 2002). Dietary factors including a 'Western' diet have also been incriminated (Perry, 2002).

ii) Diabetes and diet

Increased dietary intake of essential minerals appears to exert a protective effect against insulin resistance and diabetes mellitus. In a prospective cohort study, Colditz et

al (1992) examined data from the Nurses' Health study and found that Ca^{2+}, K^{+}, magnesium, and vegetable fat were inversely associated with DM2 risk in a dose-dependent fashion. More recently, findings from the CARDIA study revealed that overweight individuals who consumed dairy products ≥35 times per week had a significantly lower 10-year cumulative incidence of impaired glucose homeostasis (defined as high fasting plasma insulin or glucose, or use of medications to control blood glucose) compared to participants who consumed little or no dairy products (Pereira et al., 2002).

Information on Ca^{2+} supplementation and insulin resistance is limited. Studies of Ca^{2+} as well as magnesium and K^{+} intakes indicate that these elements can increase insulin secretion and reduce blood glucose concentrations in experimental animal models and humans (Karolyi, 1987; Durlach and Collery, 1984; Sjogren et al., 1988). Ismail and Namala (2000) found that Ca^{2+} supplementation normalized intolerance to glucose in vitamin D deficient rats with impaired glucose tolerance, an indication that insulin sensitivity appears to be enhanced by Ca^{2+} supplementation in a vitamin D-deficient state (Ismail and Namala, 2000).

In humans, Sanchez et al (1997) investigated the impact of Ca^{2+} supplementation on insulin resistance in 20 non-diabetic, hypertensive patients. All subjects were placed on standardized diets consisting of approximately 500 mg dietary Ca^{2+} per day for four weeks. Following this period, 1500 mg of either Ca^{2+} or placebo were given daily in a randomized, double-blind fashion for eight weeks. Following the intervention period, treated patients had a decrease in serum PTH, serum $1,25(OH)_2D_3$, and fasting plasma insulin levels and an increase in insulin sensitivity (Sanchez et al., 1997).

A few studies have also examined the potential effect of vitamin D on insulin sensitivity. Boucher et al. (1995) found that vitamin D injection in non-diabetic subjects with impaired glucose tolerance led to an increase in specific insulin, serum vitamin D,

and corrected serum Ca^{2+}, and a decrease in serum PTH, though the abnormal glucose tolerance was not changed by the vitamin D treatment. No changes were observed in subjects with normal glucose tolerance after the 8-12 weeks of follow-up. Also, Kautzky-Willer et al (1995) reported normalization of insulin sensitivity and a marked reduction in serum PTH levels in renal patients on hemodialysis after 12 weeks of intravenous $1,25(OH)_2D_3$ therapy. In a replication study, Mak (1998) found that intravenous $1,25(OH)_2D_3$ therapy corrected glucose intolerance, insulin resistance, hypoinsulinemia as well as hypertriglyceridemia in renal patients on hemodialysis.

iii) Diabetes and calcium metabolism

Disturbances in Ca^{2+} metabolism have been reported in diabetes mellitus and insulin resistance in both animal and human studies (Draznin et al., 1988; Aviv, 1992). These disturbances may involve changes in intracellular Ca^{2+} (Zemel et al., 2000) and/or free magnesium (Resnick et al., 1990) concentration in adipocytes and other responsive tissues. It appears that an excessive increase in intracellular Ca^{2+} concentration induced by elevated serum PTH levels creates a state of insulin resistance (Draznin et al., 1987; Plehwe et al., 1983).

Negative Ca^{2+} balance increases PTH secretion and PTH increases the concentration of Ca^{2+} in adipocytes (Ni et al., 1994) and myocytes (Bogin et al., 1987). This influx of Ca^{2+} in fat and muscle cells may interfere with signal transduction and cellular response to insulin. Infusion of PTH in rats impairs insulin action and causes insulin resistance (Saxe et al., 1995). Moreover, mutations in the mouse Agouti gene caused marked obesity, hyperinsulinemia and insulin resistance as well as a rise of intracellular Ca^{2+} levels in skeletal muscle (Zemel et al., 1995).

In normotensive, glucose-tolerant, and healthy subjects, Chiu et al. (2000) found a relation between high plasma PTH and reduced insulin secretion. Draznin et al (1988)

showed that prevention of the rise of intracellular Ca^{2+} with verapamil alleviated insulin resistance. Moreover, the calcium channel blocker nifedipine attenuated insulin resistance of cardiac myocytes (Segal et al., 1990; Eckel et al., 1991). Draznin et al (1989) pointed out that insulin resistance in DM2 and obesity may be caused by a high basal intracellular Ca^{2+} concentration. Therefore, an excessive increase in intracellular Ca^{2+} concentration induced by elevated serum PTH levels may create a state of insulin resistance (Draznin et al., 1987).

The mechanism by which insulin receptors are blocked by increases in cellular Ca^{2+} concentration has been examined by several investigators. The activation of protein kinase C (PKC) plays a key role in the action of insulin on the cell by stimulating glucose transport, while PKC inhibition reduces the cellular response to insulin (Standaert et al., 1990; McCarty, 2004). The intracellular Ca^{2+} concentration plays a key role in such PKC activation by facilitating the translocation of PKC from the cytoplasm to the plasma membrane. A rise of cellular Ca^{2+} however may impair PKC activation (Takaya et al., 1991), which in turn may lead to impaired insulin sensitivity (McCarty, 2004).

(b) Familial aggregation and genetic factors

DM2 is the result of the interplay between genetic factors and environmental exposures. The disease has been found to aggregate in families of many different populations. The prevalence of DM2 is higher among offspring when either or both of their parents have diabetes (Yki-Jarvinen, 1994). The risk of having DM2 is increased by 2- to 6-fold if a parent or sibling has the disease (Klein et al., 1996). In addition, parental DM2 is associated with an earlier age of onset of the disease in offspring as well as higher LDL-cholesterol levels (Bo et al., 2000).

Further evidence for the importance of genetic factors in the development of DM2 comes from concordance studies in twins. The concordance rate for DM2 in identical

older twins reaches 90%, while the rate is much lower in non-identical (dizygotic) twins or among siblings (Pyke et al., 1979; Barnett et al, 1981; Matsuda and Kuzuya, 1994; Medici et al., 1999). The fact that not all monozygotic twins are concordant for the disease confirms the importance of environemental factors.

Studies of populations of different genetic backgrounds living in similar environments also provide evidence for the the importance of genetic factors in the development of DM2. For instance, in Canada, South Asians have a higher prevalence of treated diabetes (6.2%) compared to ethnic Chinese (2.6%) and Europeans (2.2%) (Anand et al., 2000). Although environmental factors undoubtedly account for some of these differences, it is likely that the differences across ethnic groups are at least partly due to inherited differences in susceptibility.

In short, genetic susceptibility appears to be an important factor in the development of DM2, although the expression of the disease is largely influenced by environmental factors.

A great deal of research has focused on identifying the genes that confer susceptibility to DM2. A number of genes are likely to be involved (see Appendix 4). However, very few of the candidate genes examined so far has been shown to confer substantial risk for DM2 (Horenstein et al., 2004). A better defined phenotype may help to reduce the degree of genetic heterogeneity present in the study sample, and may enable investigators to eludicate the molecular mechanism involved in DM2.

3. Kidney Stone Disease and Conditions of Disturbed Ca^{2+} Homeostasis

a. Biological Plausibility

As outlined in the preceding sections, there is strong evidence that Ca^{2+} metabolism plays an important role in BP regulation and kidney stone formation in humans, and may also affect insulin sensitivity and adiposity. Furthermore, several

epidemiologic studies have reported an inverse association between low Ca^{2+} intake and increased risk of KSD (Curhan et al., 1997), hypertension (McCarron et al., 1984; Birkett, 1998), obesity (Zemel et al., 2000; Skinner and Carruth, 2001) and DM2 (Colditz et al., 1992). Randomized trials have also shown that high Ca^{2+} diets or supplementation can decrease the risk of kidney stone formation (Williams et al., 2001; Borghi et al, 2002), lower BP (Allender et al., 1996; Bucher et al., 1996; Appel et al., 1997; Sacks et al, 2001), reduce body weight or obesity (Davies et al., 2000) and improve insulin sensitivity (Sanchez et al., 1997; Ard et al, 2004). Therefore, a negative balance of Ca^{2+} appears to be the common denominator behind these metabolic disorders. These relationships were outlined in Figure 1 on page 6.

The abnormality responsible for the disturbed Ca^{2+} homeostasis in these metabolic disorders is unknown. We postulate a primary disturbance in Ca^{2+} regulation as the initiating event for the development of hypertension and KSD. The functional expression is hypercalciuria that produces negative Ca^{2+} balance that is amplified if dietary Ca^{2+} intake is inadequate (See Figure 1). The underlying defect may be inherited (e.g., renal tubular defect in Ca^{2+} handling) or acquired (e.g., low dietary intake of K^+, magnesium, or phosphate; high intake of animal protein or sodium). The biochemical changes that arise from Ca^{2+} deficiency lead to increased vascular resistance and hypertension as well as several metabolic defects including insulin resistance, obesity and weight gain. A high salt diet may make the hypertension worse as a result of blunted renal sodium excretion that arises from the negative Ca^{2+} balance, but is insufficient in itself to cause hypertension in the absence of a disturbance of Ca^{2+} homeostasis.

b. Evidence for Associations with Other Conditions

(1) Kidney Stone Disease and Hypertension

There is strong evidence of an independent epidemiologic association between

KSD and hypertension both from cross-sectional (Tibblin, 1967; Cirillo et al., 1988; Cappuccio et al., 1990; Madore et al., 1998) and from longitudinal (Madore et al., 1998; Cappuccio et al., 1999a) studies. The longitudinal evidence also suggests that the development of KSD precedes that of hypertension (Madore et al., 1998), and both conditions show clear familial patterns (Curhan et al., 1997; Hunt et al., 1986).

The pathogenetic link between hypertension and KSD is not well understood. Hypercalciuria is a common finding in hypertensive patients (Borghi et al., 1999; McCarron et al., 1980; Strazzullo et al., 1983; Young et al., 1992), particularly among those with salt-sensitive forms of hypertension (Coruzzi et al., 1993; Galletti et al., 1993). In renal Ca^{2+} stone-formers with hypercalciuria the increment in urinary Ca^{2+} excretion in response to an increase in dietary sodium is 2-fold higher than that in normal subjects (e.g., approximately 2 mmol vs. 1 mmol per 100 mmol increase in sodium intake) (Massey and Whiting, 1995). KSD patients with dietary Ca^{2+}-independent hypercalciuria have exaggerated natriuretic and calciuric responses to hydrochlorothiazide (Sutton and Walker, 1980; Sakhaee et al., 1985), implying a renal tubular defect in this subtype of hypercalciuria.

An important issue that remains unanswered is whether the hypercalciuria of hypertension and KSD are manifestations of a primary disturbance in sodium or Ca^{2+} metabolism. Salt sensitivity has clear familial determinants (Grim et al., 1979; Skrabal et al., 1984; Weinberger et al., 1987; Luft et al., 1987; Sharma et al., 1989; Widgren et al., 1991; Watt, 1991), and many have proposed an inherited defect in renal sodium handling as its underlying cause (MacGregor and Cappuccio, 1993; Woolfson et al., 1996). The results of the DASH trials strongly suggest, however, that salt sensitivity is not a fixed heritable trait, but is often an acquired abnormality that can be modified by improving overall dietary quality (Appel et al., 1997, Sacks et al., 2001). They showed that improving mineral intake without changing dietary sodium reduced urinary Ca^{2+}

excretion, mitigated salt sensitivity and lowered BP. Based on these findings and other evidence, a more plausible hypothesis postulates a primary disturbance in Ca^{2+} regulation.

(2) Kidney Stone Disease and Obesity, Weight Gain and Type 2 Diabetes

Many studies have shown that higher body weight, obesity or weight gain increase KSD risk (Soucie et al., 1996; Bulusu et al., 1970; Powell et al., 2000; Taylor et al., 2005a), although the pathogenic link is poorly understood. Recent prospective data also revealed that DM2 is associated with KSD (Taylor et al., 2005b).

Several alterations in Ca^{2+} homeostasis have been reported in subjects with obesity or DM2 including increased urinary Ca^{2+} excretion (Borghi et al., 1999; Ishihara et al., 1989; Gregorio et al., 1994; Raskin et al., 1978; Schwartz et al., 2003) and elevated levels of $1,25(OH)_2D_3$ and PTH (Andersen et al., 1986; Bell et al., 1985). An inverse relation between BMI and plasma levels of ionized Ca^{2+} and phosphate has also been reported (Lind et al., 1993). Hypophosphatemia is associated with impaired glucose metabolism, arising principally from decreased tissue sensitivity to insulin (DeFronzo and Lang, 1980). These biochemical changes likely account for increased cytosolic Ca^{2+} and/or decreased free intracellular magnesium observed in a variety of cell types including adipocytes and vascular smooth muscle cells (Draznin et al., 1988, Resnick et al., 1991). It has been suggested that these alterations create a state of insulin resistance (Draznin et al., 1988) and may be the common cellular pathway that links hypertension, obesity and DM2 (Draznin et al., 1988; Resnick et al., 1991). Recently, both epidemiological and interventional studies have shown that increased dietary Ca^{2+} intake reduces body weight, supporting an important role of Ca^{2+} in weight control (Zemel et al., 2000; Davies et al., 2000; Carruth and Skinner, 2001).

c. Concluding Remarks

Abnormalities of Ca^{2+} metabolism are common in patients with Ca^{2+} stone disease of the urinary tract, and these defects may play a crucial role in the etiology of this condition (Lemann, 1996; Monk and Buchinski, 1996). A number of studies have shown associations between KSD and several clinical conditions including hypertension (Borghi et al, 1999; Tibblin, 1967; Cirillo et al., 1988; Cappuccio et al., 1990; Madore et al., 1998; Cappuccio et al., 1999a), obesity (Soucie et al., 1996; Bulusu et al., 1970; Powell et al., 2000; Leonetti et al., 1998; Curhan et al., 1998a), excessive weight gain (Taylor et al., 2005a) and DM2 (Lemann, 1996; Taylor et al., 2005b). Disturbances in Ca^{2+} metabolism are also a common finding in these disorders. While the most prominent Ca^{2+} disturbance is hypercalciuria, other abnormalities have also been reported including lowered serum ionized Ca^{2+}, raised intracellular Ca^{2+}, suppressed intracellular free magnesium, defective membrane binding and transport kinetics of Ca^{2+}, elevated serum PTH and increased serum 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) (Lemann, 1996; Monk and Buchinski, 1996). The biological basis of these biochemical disturbances and how these clinical disorders are connected are still undefined.

We suspect that abnormal renal Ca^{2+} handling plays a central role in the pathogenesis of these closely linked disorders (hypertension, KSD, obesity, weight gain and DM2) and that its principal manifestation is excessive renal loss of Ca^{2+}. Chronic Ca^{2+} loss, if not compensated by increased gut Ca^{2+} absorption from dietary sources or intake of other Ca^{2+}-conserving minerals such as K^+, would result in a negative Ca^{2+} balance. The long-term consequences are 1) KSD from increased urinary Ca^{2+} excretion; 2) hypertension from elevated vascular tone and blunted renal sodium excretion; and 3) obesity/weight gain and DM2 from diminished peripheral tissue insulin sensitivity.

One way to identify common etiologic and pathogenetic mechanisms among different diseases is to determine whether the disorders occur more frequently within

certain families (Khoury et al., 1993). This approach also permits investigators to discriminate between genetic and environmental factors that may contribute to familial clustering. In this study, a common pathway involving alterations in Ca^{2+} metabolism that leads to KSD as well as its associated conditions was postulated. This was explored by determining whether these conditions aggregate in the families of KSD patients with hypercalciuria. Because members of the family share not only genetic information but also tend to adopt similar ways of living, the study determined the risk of these disorders in the spouses of affected family members. If the data suggest a role for genetic factors, future studies would be directed towards elucidating the underlying genetic mechanisms.

B. Methodological Issues in Familial Aggregation Studies

1. Introduction

The objective of evaluating the familial aggregation of a trait or disease is to determine whether it occurs more frequently within certain families than in the general population (Khoury et al., 1993). The study of familial aggregation of disease is the first of three steps in the dissection of genetic traits (Farrer and Cupples, 1998). The second step would be to determine whether the familial aggregation of disease is due to genetic or environmental factors by using various design methods, such as twin or adoption studies, or by enrolling spouses along with relatives in a family study. The third step is to identify the specific genetic mechanism involved through segregation analysis (Farrer and Cupples, 1998). The present study, which examines the familial aggregation of a group of closely related conditions in KSD patients with hypercalciuria, deals mainly with the first of these steps, although it also addresses the second as well (see Section 4). The determination of familial aggregation is essential before laboratory studies are undertaken to identify specific genetic mechanisms.

2. Study Design

Familial aggregation studies traditionally begin with the recruitment of individuals with a specific disease and determining whether their relatives have a higher prevalence of the same disease when compared to a reference population (Khoury et al., 1993; Farrer and Cupples, 1998). Similarly, another way to assess the familial aggregation of a certain disease would be to recruit probands with a given disease, such as KSD, and ask relatives about the occurrence of a different disease, such as hypertension, obesity, weight gain and DM2 in order to identify common etiologic or pathogenic mechanisms shared between different diseases (Khoury et al., 1993). Determining the familial occurrence of

a disease other than the one affecting probands has been used previously to identify common etiologic or pathogenetic mechanisms shared between different diseases (Schwartz et al., 1999; Tisler et al., 1999; Tisler et al., 2002). This second approach is of interest in the present study.

Studies of familial aggregation share features of the two major types of epidemiologic designs, namely case-control and cohort studies (Khoury et al., 1993). Therefore, the family health data that is collected in these studies can be examined from two perspectives. First, the disease status of relatives can be considered as an exposure attribute of the probands and incorporated into a case-control analysis. Alternatively, the family health data can also be viewed from a cohort perspective where the disease status of the proband is the exposure variable for the disease status of other family members. In studies of familial aggregation, the usual measure of association is the odds ratio given the ambiguity of the causal and temporal relationship between exposure and disease.

In this study, a cohort design was used to determine whether hypertension, KSD, obesity, weight gain or DM2 aggregate in families of KSD patients with a particular urinary abnormality (hypercalciuria). Specifically, the urinary abnormality of the proband was the primary exposure variable, while the disease status of the relatives was the outcome variable (Khoury et al., 1993; Khoury and Beaty, 1994). The rationale for this approach stems from the observation that HC precedes the development of KSD and hypertension (Madore et al., 1998). Moreover, it was postulated that that the underlying metabolic abnormality that gives rise to KSD and hypertension also precedes the development of obesity, weight gain and DM2 as well.

3. Restriction of Recruitment to Younger Probands

Phenotypes that appear at an earlier age are more likely to have an underlying genetic basis (Childs and Scriver, 1986; Lander and Schork, 1994). Therefore, restricting

recruitment to younger KSD probands in this study would likely increase the chance of finding a genetic basis for disease. In addition, recruiting younger probands would help to enroll more younger siblings with positive affectation status, thereby helping to increase the study's power to detect disease clustering in younger relatives. Another benefit of recruiting younger probands is that parental information on disease status is more likely to be accurate compared to when older probands are used, since parents are more likely to be alive. Lastly, exclusion of older patients would reduce the likelihood that probands are receiving medical treatment that may influence their biochemical measures. In the present study, probands above the age of 50 years would be more likely to be taking blood pressure medication, such as thiazide diuretics or beta blockers, which may influence urinary calcium excretion. Proband recruitment, therefore, was restricted to KSD patients ranging in age from 18 to 50 years.

4. Inclusion of Spouses

The inclusion of spouses in a familial aggregation study may help to differentiate between genetic and environmental influences on disease clustering in families (Mitchell et al., 1996; Knuiman et al., 1996a; Nicolaou et al., 2000; Lee et al., 2003; Wu et al., 2003). Each proband and spouse share a common environment, whereas probands and siblings share 50 percent of their genetic makeup on average. Thus, for example, the presence of an association between the urinary abnormality in probands and disease in first-degree relatives, but not in spouses, would suggest a genetic susceptibility to these conditions. In addition, there are other advantages to enrolling spouses. The recruitment of spouses can be time- and cost-efficient, because of easy accessibility and their ability to provide proxy data on the patients' relatives if necessary. Moreover, a higher response rate can be expected with spouse controls compared to population controls (Verhage et al., 2003). A theoretical drawback is the possibility of assortative mating related to

genetic susceptibility of the disease under study (Knuiman et al., 1996b). However, one recent simulation study demonstrated that even strong assortative mating on a factor that is strongly correlated with a true risk factor under study exerts a negligible effect on the observed extent of familial aggregation (Verhage et al., 2003). Spouses were enrolled in the present study to control for environmental influences.

5. Measurement of Family Health Information

The measurement of disease status in the family members of probands may be approached in three ways. Firstly, family health information may be obtained from the probands themselves concerning the disease in their family members. While this approach is inexpensive and time efficient, it requires prior knowledge about the validity of the reported family information. A second approach is to contact the family members of probands for a direct interview. This approach is more time consuming and costly compared to interviewing proxy respondants, but it also yields more accurate information on the disease status of relatives, as well as the collection of data on exposure and risk factors. It can also be used to validate proxy-reported information (Bensen et al., 1999). An even more detailed evaluation would involve the reviewing of medical records, or a physical and laboratory examination of relatives for clinical signs and genetic markers. This third approach allows for the most thorough examination of the disease in the relatives, as well as the collection of data on exposure and risk factors, and the use of the genotypic information in subsequent genetic analysis. The drawback of this method, however, is its considerably higher cost (Khoury et al., 1993).

The second approach was employed in this study as family members of probands were contacted directly and asked to report on their own health status. Though more time-consuming than proxy reporting, this approach would produce the greatest degree of accuracy in measuring affectation status of family members (Khoury et al., 1993), and is

therefore less likely to dilute the study's odds ratios, since non-differential measurement error would be minimized.

a. Questionnaire Administration

Obtaining health information directly from family members would require the use of a questionnaire. There are three common approaches to questionnaire administration: mail, telephone, and face-to-face (Dillman, 1978). From general epidemiologic principles, one would expect an interviewer administered questionnaire (by telephone or in-person) to be more likely to attain a higher response rate and complete information than a mailed questionnaire, although the latter would be less costly. A number of studies have compared the efficacy of these approaches on a variety of health endpoints, but only one study focused on the conditions of interest in this study. In a prospective study in Potsdam, Bergmann et al (2004) found that agreement between face-to-face interview and mail questionnaire was highest for myocardial infarction, cancer and diabetes mellitus (kappa = 0.83-0.88), lower for hypertension, osteoporosis, kidney stones, and stroke (kappa = 0.68-0.77), and lowest for angina pectoris and hyperlipidemia (kappa = 0.39-0.59). The authors concluded that there was poor agreement between questionnaire methods for diseases that are less severe, poorly defined, or have only intermittent symptoms (Bergmann et al., 2004). The study, however, did not evaluate agreement between the two approaches when disease is defined by use of doctor prescribed medication, nor was there a "gold standard" source of information to directly measure validity.

While in-person interviewing elicits more complete information and a higher response rate than postal questionnaires (Dillman, 1978), this approach would not be feasible in the present study, since a substantial number of relatives would likely reside in distant geographic areas. Telephone interviewing, in contrast, allows for the inclusion of

larger numbers of people over a wider geographic area in a much shorter period of time. Moreover, compared to postal questionnaires, telephone interviewing is more likely to yield complete information and may allow sources of confusion to be cleared up promptly (Brogger et al, 2002). Telephone interviewing is also less suceptible to non-response bias compared to mailed questionnaires (Siemiatycki, 1979). In one study, middle-aged women in Massachusetts responding by mail were more likely to hold professional jobs, have higher incomes, and have more years of education, compared to women who did not respond by mail but were interviewed later by telephone (Brambilla and McKinlay, 1987). Similarly, in health plan subscribers, telephone interviews of mail nonrespondents improved the demographic representativeness of the responding samples (Fowler et al., 2002). Therefore, telephone interviewing may be used to reduce cost compared to in-person interviewing, yet secure a higher response rate than is usually obtained with mail questionnaires.

The participation rate using telephone interviewing in this study is likely to be particularly high since participation only involves a short five-minute interview. Subjects would briefly provide information on the conditions of interest and several demographic factors. Younger relatives are more likely to be home during evening hours, while older relatives can be reached readily at various times throughout the day.

b. Content of Questionnaires

(1) Family Composition

A familial aggregation study requires information on family size and age- and gender-distribution for appropriate analysis, particularly for studies of common and late-onset diseases such as hypertension and diabetes. It is also important to distinguish

between different relative categories including blood relatives, spouses, and adopted relatives.

The enrollment of second-degree relatives may allow one to assess familial aggregation in relatives with a smaller kinship coefficient with respect to the proband, which provides additional information on possible genetic susceptibility (Farrer and Cupples, 1998). However, obtaining information from second-degree relatives also has a number of notable disadvantages: a) the number of telephone interviews required to assess health information would increase substantially, b) the participation rate of second-degree relatives is low, and c) the accuracy of obtaining family health information from proxy respondents (probands) on behalf of second-degree relatives is poor (Hastrup et al., 1985; Silberberg et al., 1994; Ziogas et al., 2003; Murff et al., 2004). Therefore, the present study will focus on first-degree relatives and spouses only.

The participation rate of first-degree relatives is especially high in families where the proband consents to contacting kin (see Appendix 5). To minimize the problem of missing data, therefore, one can restrict enrollment to only probands who are willing to allow family members to be contacted and restrict inquiries to first-degree relatives. In a previous study by our group, approximately two-thirds of all KSD probands gave their consent to participate and agree to allowing first-degree relatives to be contacted directly (Tisler et al., 1999). However, once probands allow family members to be contacted, the participation rate of relatives may approach 85%.

(2) Conditions or Traits of Interest

The definition used to assess self-reported hypertension, DM2 and KSD may vary. Some studies have used a more sensitive definition, such as "do you have (disease)" (Bensen et al., 1999; Murabito et al., 2004), while others have used a more specific definition based on whether the respondent was "ever told by a doctor" (Bergmann et al.,

2004), and still some have used a highly specific definition asking if they were "ever prescribed medications by a doctor" (Hunt et al., 1986; Hunt et al., 2000; Mackenbach et al., 1996; Tisler et al., 2002).

The use of a relatively restrictive definition (high specificity) would be more appropriate for a familial aggregation study, since it would be more likely to identify relatives with more severe manifestations of disease, and it is known that traits with greater severity are more likely to have a genetic origin (Lander and Schork, 1994). Stringent definitions, therefore, help to increase specificity at the expense of sensitivity, which is advantageous in genetic studies because the power to detect familial associations is greatly reduced when specificity is low (Khoury et al., 1993). As an added benefit, individuals are more likely to know whether they take medication for hypertension or DM2 than to be aware of their actual blood pressure or serum glucose level, particularly if untreated.

One of the advantages of obtaining family health data directly from family members is that covariates can be assessed for each individual relative (Khoury et al, 1993). In the present study, this approach would allow for the control for additional potential confounding variables, such as the relative's age, smoking status, and use of Ca^{2+} or vitamin D containing products or multivitamins. Studies with missing relative information typically substitute predictors pertaining to the proband (e.g., age or smoking status) in place of the relative. However, this approach may provide an inadequate adjustment of potential confounders (Kondo et al., 2005).

(3) Age at Diagnosis

In all conditions of postnatal onset, the presence of disease in gene carriers varies with age. For instance, the phenotype of elevated blood pressure has delayed penetrance, typically occurring in the fourth, fifth, or sixth decades of life (Hunt et al., 1986). If

complete information on age of onset is available, survival analysis may be employed to explore the modifying effects of age on familial aggregation of disease (Khoury et al., 1993; Thomas et al, 2004). The rationale is that by evaluating early-onset disease, we may be able to define a subset of all patients that is more likely to be explained by genetic factors.

However, information on age at diagnosis is generally less complete than for disease status (Khoury et al., 1993; Thomas et al., 2004). In addition, information on age at diagnosis likely varies with the severity of the condition, the extent to which the condition develops gradually, and whether symptoms are present. For instance, DM2 and hypertension develop gradually over a prolonged period of time, are mostly asymptomatic, and are often times not diagnosed until years after onset. For these conditions, age at diagnosis is used merely as a proxy for the actual age of onset, since these conditions may be present but undiagnosed (Wilk et al., 2004). In addition, Wilk et al (2004) recently reported that use of age at diagnosis as a phenotype results in a loss of power because it likely provides an imprecise estimate of hypertension onset (Wilk et al., 2004). Nevertheless, age at diagnosis can be included as part of the questionnaire for assessing hypertension and DM2 in family members.

As discussed earlier, phenotypes that appear at an earlier age are more likely to have an underlying genetic basis (Lander and Schork, 1994). Therefore, in this study, one would expect stronger aggregation of disease in siblings than parents of probands if the intermediate phenotype in question (i.e., the urinary abnormality) has a genetic origin. This approach would allow one to use relative or kinship categories (e.g., parents, siblings) to assess the modifying effects of age on familial aggregation of disease.

6. Analysis Methods

Numerous statistical methods have been developed to examine familial

aggregation of disease. The most ideal approach for cohort studies of familial aggregation is to use survival methods, particularly for evaluating diseases with late-onset and substantial censoring (Thomas et al., 2004). However, a drawback of this approach in the present study is that it requires information on age at diagnosis, and this information is generally less complete than for disease status. In addition, the accuracy of determining time of disease onset (i.e., age at diagnosis) is poor for these clinical conditions, as discussed in the preceding section (Wilk et al., 2004; Khoury et al., 1993; Thomas et al., 2004). In these situations, logistic methods are a feasible alternative (Zhao and Le Marchand, 1992).

An obstacle in the analysis of family data in an epidemiologic study is the lack of independence among relatives, which invalidates many of the assumptions of standard statistical tests (Khoury et al., 1993; Thomas et al., 2004). Newer statistical approaches, however, have been developed that modify classic analytic models and relax the assumption of strict independence among family members.

a. General Estimating Equations for Family Studies

A commonly used statistical approach called GEE (General Estimating Equation) regression is designed to analyze correlated data, as found in family studies, and uses the relative (as opposed to the family) as the unit of observation (Zhao and Le Marchand, 1992; Liang and Zeger, 1986; Zeger and Liang, 1986). Using a cohort design, the disease status of each individual family member is a binary outcome variable defined by whether there is a presence or absence of disease in the family member and is viewed as an attribute of the family member. The exposure variable is the disease status of the proband, or in this study, the urinary status of the proband (e.g., hypercalciuria, no hypercalciuria). A 2x2 contingency table may be constructed and the crude odds ratio computed. In addition, separate contingency tables may be constructed for each relative

type (parent, sibling, spouse) and the prevalence of disease is then compared separately among parents, siblings and spouses of probands for each proband affection strata. In the multivariate analysis, the association between the diagnosis status of the proband and the disease status of the different family members can be modeled, while adjusting for a set of covariates such as proband age, gender, BMI and relative age.

The GEE approach has several strengths compared to regular logistic regression, which uses family history as a binary outcome variable. First, the approach is independent of family size since each relative is analyzed individually. Second, separate contingency tables may be constructed for each relative type (parent, sibling, spouse). This separate analysis provides useful information because different relative types may have different risks for genetic diseases (e.g., siblings vs. spouses). A limitation, however, is that the analysis is more complex and computationally demanding than 'regular' logistic regression. The standard errors of the regression coefficients are underestimated when using standard likelihood methods since observations from the same family are inter-correlated, and hence the need to use GEE methods (Liang and Zeger, 1986; Zeger and Liang, 1986; Zhao and Le Marchand, 1992). The parameter estimates obtained by GEE are similar to those obtained by MLE, but the GEE standard error estimates tend to be larger since the degree of familial correlation is taken into account. The corrected standard error is roughly equivalent to multiplying the standard error obtained under the assumption of independence by a correction factor. This correction factor depends on the degree of correlation among family members, the size of the family, and the distribution of the explanatory variables between and within families (Neuhaus and Segal, 1993).

b. Multiple Testing

The present study includes several exposure variables and multiple outcome

variables, which makes adjustment for multiple statistical testing an issue to consider. The primary concern introduced by multiple comparisons is the increased probability of detecting significant associations where none exist. Some have recommended that adjustment for multiple comparisons are necessary to avoid rejecting the null hypothesis too readily (Ludbrook, 1998).

Others, however, suggest that the possibility of "false positives" owing to multiple comparisons (type I errors) is of particular concern when comparisons are conducted post-hoc (i.e., a 'fishing expedition'), and undue emphasis is given to positive findings (Rothman, 1990; Feise, 2002). The dilemma is that reducing the risk of type I error for null associations also increases the risk of type II error for those associations that are not null. Feise (2002) suggests that one must balance a study's statistical significance with the magnitude of effect, the quality of the study, and with findings from other studies (Feise, 2002). There is a general consensus that, at the very least, it is important to report on all multiple analyses and declare which were prespecified and which were conducted as exploratory activities.

The present study was specifically designed to assess an a priori hypothesis, which stipulates that urinary Ca^{2+} excretion alone or in combination with low K^{+} excretion is related to the familial clustering of hypertension and related conditions. If the biological model of a common metabolic pathway is valid, one would expect significant associations between these urinary profiles in probands and at least two conditions in first-degree relatives. The associations should also be consistent and of sufficient magnitude (e.g., ORs > 2 were predicted in this study). In addition, the number of 'sporadic' associations should be minimal. For instance, we would not expect significant associations between lower-risk urinary measures in probands (e.g., any combination of low urine Ca^{2+} , low uric acid, and high K^{+}) and disease in relatives, nor would we expect associations to be in the opposite direction (e.g., an association between low urine Ca^{2+} in

probands and disease in relatives) of what is predicted by the a priori hypothesis. Such sporadic associations would be indicative of possible 'chance' associations brought about by multiple testing.

7. Bias in Familial Aggregation Studies

Studies of familial aggregation are subject to similar potential biases as classic epidemiologic studies, including selection and information biases, and confounding. Issues specific to the current familial aggregation cohort study are reviewed.

a. Selection Bias

Selection bias can seriously compromise the validity of a study if the probands are selected on the basis of known family history. The use of population controls may create spurious associations for familial aggregation through selection bias. However, if probands are drawn from the same population, this bias is less likely. Moreover, enrollment of the probands before their disease status is known, as was done in the present study, helps to eliminate this problem.

b. Information Bias

(1) Recall Bias

Differential error in the recall of family health information may pose a problem in familial aggregation studies, since cases may consider questions more carefully than non-cases. Recall bias may either enable cases to report more accurately, and therefore increase sensitivity and specificity, or to over-report disease in family members, or decrease specificity relative to controls. Alternatively, obtaining family health information on a different disease than that affecting the proband might reduce the likelihood of this source of error, particularly if all probands are affected by the same disease. This approach was employed in the current study, as all probands had KSD and

the type of kidney stone (i.e., the urinary abnormality) was not known at the time of the interview.

(2) Measurement Error

Non-differential misclassification of either exposure or disease attenuates measures of association toward the null. In a familial aggregation cohort study, the effect of measurement error may occur in the assessment of both the exposure variable (e.g., the proband's urinary group) and the disease outcome (e.g., the relative's disease status). In studies of familial aggregation, the bias introduced by the misclassification of disease status of family members is more influenced by specificity than sensitivity (Khoury et al., 1993), as mentioned previously.

(a) Measurement error in the assessment of urinary Ca^{2+} excretion

Urine has the most direct bearing on the formation of kidney stones than any other body fluid, and thus its composition is of substantial diagnostic value (Zerwekh, 1996). There are four types of urine samples for detecting metabolic disturbances: random, fasting, first-morning and 24-hour (Brunzel, 1994). These are summarized in Appendix 6.

A 24-hour urine specimen is the current benchmark method used to evaluate stone-forming patients in a clinical setting (Zerwekh, 1996). This measure is more accurate than specimens collected over a shorter time. Substances such as hormones, proteins, and electrolytes are variably excreted over 24 hours. In addition, extraneous factors such as exercise, posture, hydration, and body metabolism influence excretion rates. Since analytes are excreted at different rates throughout the day or night, random specimens may miss the time of maximal excretion. A sample collected over a 24-hour period yields an average concentration of the analyte and thereby negates some of these

confounding factors (Zerwekh, 1996). An important limitation of a 24-hour urine collection is that it is burdensome to the patient and may elicit poor compliance when the specimen is not a part of standard medical care. A frequently used approach to identify incomplete urine collections caused by poor compliance is to compare the level of urinary creatinine excretion between study groups (Waterlow, 1986).

The standard measure of urinary Ca^{2+} is a 24-hour urine specimen (Zerwekh, 1996). There is considerable diurnal variation in the excretion of various different urinary constituents, including Ca^{2+} (Ahlstrand et al., 1984; Ebisuno et al., 1986), which makes random or fasting samples less ideal (Brunzel, 1994). Urinary Ca^{2+} excretion is influenced by dietary factors, including Ca^{2+}, K^{+}, animal protein, carbohydrate, and sodium intake (Lemann, 1996; Monk and Bushinsky, 1996). Subjects are often instructed to follow their normal dietary, drinking and lifestyle habits, although there is no guarantee that they comply with these instructions (Hosking et al., 1983; Hofbauer et al., 1994). Results form the INTERSALT study showed that test-retest reliability of 24-hour urinary Ca^{2+} measured 14 days apart is between 0.64 and 0.69 (Dyer AR et al., 1994, Am J Epid). Seasonal changes in urinary values have also been demonstrated. Robertson et al found that there were significant seasonal variations in the urinary excretion of Ca^{2+} and oxalate, each showing a maximum during the summer months and a minimum in the winter (Robertson et al., 1975). Each of these factors may contribute to the variation between repeated samples. In this study, consecutive patients were enrolled during a full 2-year period to minimize the influence of seasonal variations on urinary results. Individuals with a 24-hour urine Ca^{2+} excretion exceeding 0.1 mmol/kg/day while on a nonrestricted diet are considered to have hypercalciuria (Coe et al., 1982; Audran and Legrand, 2000; Lerolle et al., 2002).

In general, under clinical conditions, one urine collection provides useful and sufficient information. Nevertheless, the use of two collections is said to be better. It is

also widely accepted that the more samples that are analyzed, the higher the chance of correct diagnosis of underlying metabolic problems. For practical reasons, however, it is much more feasible to obtain one urine collection (Bek-Jensen and Tiselius, 1998). In addition, it is important that the samples are received for analysis soon after the collection has been completed. The collection of several 24-hour urine specimens may make this routine less likely to be successful (Bek-Jensen and Tiselius, 1998). Since 24-hour urine collections are burdensome to the patient, the use of multiple specimens is not feasible in large-scale epidemiologic studies. In this study, a single 24-hour urine specimen was obtained from each patient.

(b) Measurement error of self-reported disease

Accurate information on disease status is a key element in any epidemiologic study. As discussed earlier (page 51), high specificity in the classification of disease is more advantagious in familial aggregation studies (Lander and Schork, 1994). Several studies have provided information on sensitivity and specificity of self-reported disease for hypertension, diabetes and KSD. Both sensitivity and specificity of self-reported diabetes (Heliovaara et al., 1993; Haapanen et al., 1997; Mackenbach et al., 1996; Bowlin et al., 1993; Kehoe et al., 1994) and KSD (Ljunghall et al., 1979) were high in all studies. Sensitivity of hypertension was more variable, ranging from 40% to 91%, but specificity remained at or above 87% (Heliovaara et al., 1993; Haapanen et al., 1997; Bowlin et al., 1993; Kehoe et al., 1994). Self-reported weight and height obtained from personal interviews, telephone interviews or mail questionnaire have been found to be accurate (Stewart et al., 1982; Stunkard et al., 1981; Jalkanen et al., 1987; Palta et al., 1982). In this study, health information was obtained from relatives directly instead of proxy respondents in order to increase the accuracy of reporting.

c. Potential Confounders

The potential for confounding in this familial aggregation study exists from several sources. From previous familial aggregation studies of hypertension and KSD, proband attributes included age, gender, BMI, and use of antihypertensive medication, as each of these factors is related to both urinary Ca^{2+} excretion and hypertension status (Tisler et al, 1999; Tisler et al, 2002). Relative age is also likely to be an important confounder, since penetrance is age related, meaning that the trait is not expressed in most gene carriers at birth but occurs with increased frequency as the carriers get older. Therefore, the a priori set of confounders chosen for inclusion in all multivariate regression models in this study included proband age, gender, BMI, and use of antihypertensive medication, as well as relative age.

Based on a systematic review of the literature, other potential confounders were also considered for inclusion to the above basic models. These included proband ethnicity (Anand et al., 2000), marital status (Johnson et al., 2000), education (Kaplan et al., 1993), area of residence (inside/outside city) (Anand et al., 2001), country of birth (inside/outside Canada) (Anand et al., 2000), smoking status (Ambrose et al., 2004), use of Ca^{2+} or vitamin D containing products (Curhan et al., 2002), and relative smoking status.

Adjustment for family size is not appropriate in the present study because the analysis method that was employed took this into account, as discussed previously. In the analysis of effect modification, urinary K^+ was examined in combination with urinary Ca^{2+}, based on our a priori hypothesis. In addition, proband age, gender, BMI, and relative age of onset of disease were examined for interactions with proband urinary group as part of exploratory analyses.

8. Rationale for Validation of Proxy Reported Health Information by Probands for Family Members

Family history is an established risk factor for many chronic health problems, such as hypertension, diabetes, KSD and osteoporosis. Individuals with a positive family history of these conditions tend to be at increased risk for developing the condition in the future, independent of other traditional risk factors. In addition, most common diseases such as these occur as a result of a complex interplay between multiple genes and environmental factors, and family history reflects these interactions. Consequently, family history information provides a "genomic tool" that can help to capture interactions between genetic susceptibility and lifestyle factors in the development of disease (Hunt et al., 2003).

The most convenient and commonly used method of assessing family history in both clinic and research settings is by asking probands about the disease status of relatives. However, proxy reported family history information is subject to a greater degree of measurement error and requires prior knowledge about the validity of the reported family information. Alternatively, more detailed approaches may be used to assess family history such as interviewing family members directly, reviewing medical charts, or clinical examination. While these more invasive approaches yield more accurate information on family history, they are also more time consuming and costly, and less feasible compared to interviewing proxy respondents. As a result, proband reported family history information has been used extensively in clinical, epidemiologic and genetic studies.

Despite the usefulness of proxy reported family history, a review of the literature revealed that the accuracy of proxy reported KSD and osteoporosis by probands on behalf of relatives has yet to be examined. Both of these conditions are common in Westernized societies, are a major cause of morbidity, and tend to cluster in families. It

was also recognized that very few studies have evaluated the accuracy of proband reported obesity in family members, and the studies that did so did not provide estimates of sensitivity or specificity in classifying obesity. Furthermore, the accuracy of proband reported hypertension and diabetes in family members showed a great deal of variability across studies, likely reflecting the different methods of collecting information from relatives (face-to-face, telephone, mail survey), the criterion used to validate the information (medical chart review, clinic exam, interview of relatives), or the proband's age (high school or college student, young adult, middle-aged). These studies are summarized in Table 1. In this study, we further examined the accuracy of proband reported information based on two different definitions of each disease endpoint, namely, whether the condition was diagnosed by a physician and whether medications were prescribed. Lastly, there is very little information on whether attributes of the proband or family (e.g., age, sex, ethnicity, family history status, proband-relative relationship) affect the accuracy of proband reported information in relatives.

Therefore, as a component of this thesis, a validation study was conducted to: 1) assess the accuracy of proband reported family health information (KSD, hypertension, obesity, diabetes, osteoporosis) in first-degree relatives and spouses; and 2) examine the factors (e.g., proband characteristics, positive family history, proband-relative relationship) that have the greatest impact on the accuracy of family reporting.

Table 1. Validation studies of proband reported health information for relatives.

Reference	Setting and subjects	Proband reporting method	Standard for comparison	Conditions	Sensitivity (%) †	Specificity (%) †
Napier et al, 1972	Tecumseh, WI; N=243 probands and siblings	in-person interview	in-person interview	**hypertension**	25	97
				diabetes	35	99
Hastrup et al, 1985	Amherst, NY; N=292 college student probands and parents	group questionnaire session	mail questionnaire	**hypertension**	81	94
Bensen et al, 1999	Four communities in U.S.; N=3020 middle-aged probands, parents, siblings & spouses	mail questionnaire	mail questionnaire	**hypertension**		
				parents	76	84
				siblings	56	91
				spouses	77	96
				diabetes		
				parents	87	98
				siblings	72	98
				spouses	83	98
Kahn et al, 1990	San Luis Valley Diabetes Study; N=20 (10 diabetics, 10 controls);	clinic questionnaire	telephone interview	**diabetes**	100	100
Nadalin et al, 2003	Ontario, Canada; N=55 colorectal cancer patients, relatives & spouses	mail questionnaire	mail questionnaire	**diabetes**	100	100
Bochud et al, 2004	Seychelles, Indian Ocean; N=73 families with ≥ 2 hypertensives	in-person interview	in-person interview and clinical exam	**hypertension**		
				vs. clinic exam	90	55
				vs. interview	89	78
				diabetes		
				vs. clinic exam	61	98
				vs. interview	53	98
Murabito et al, 2004	Framingham Heart Study; N=791 men, N=837 women; fathers and mothers	clinic questionnaire	clinical exam	**hypertension**		
				fathers	44	88
				mothers	57	90
				diabetes		
				fathers	56	97
				mothers	65	97
Epstein et al, 1991	Pittsburgh, PA; probands and spouses	method not indicated	self-reported height and weight	**body mass index**	probands vs. spouses r = 0.87	
Reed et al, 1998	Philadelphia, PA; N=94 probands and 1st degree relatives	method not indicated	measured height and weight	**height**	overestimated by proxies (mean=1.4 cm)	
				weight	underestimated by proxies (mean=4.1 kg)	

†, except where indicated otherwise.

C. Assessment of Ethnic Differences in the Prevalence of Kidney Stone Disease

1. Introduction

KSD is a common disorder with a lifetime prevalence of 15% in the United States and Europe (Johnson et al., 1979; Leusmann et al., 1990; Stamatelou et al., 2003). Prevalence data in populations from other parts of the world and individuals of non-European ancestry living in Western societies are sparse. In Japan, the prevalence is lower than in the United States, although time trend studies have shown a doubling of the annual incidence over the past three decades (Yoshida et al., 1999). Studies indicate that KSD is a common problem in the Middle East (Hodgkinson, 1979; Zaidman et al., 1986; Akinci et al., 1991; Mkony et al., 1991; Pak, 1998). In the United States, the prevalence of KSD is lower in Asians than in Caucasians (Hiatt et al., 1982; Sarmina et al., 1987; Soucie et al., 1994; Stamatelou et al., 2003). The ancestral origin of the Asian subjects, however, was not specified.

Ethnic minority groups make up a rapidly growing proportion of the Canadian population (Canada's Ethnocultural Portrait, 2001). The greater degree of ethnic diversity in Canada has accentuated the need to identify and eliminate health disparities between minority and Caucasian populations.

2. Literature on Kidney Stone Disease and Ethnic Origin

a. Studies in the United States

To date the study of KSD and its risk factors has been limited largely to individuals of European ancestry. In the United States, African Americans have the lowest prevalence of KSD, followed by Hispanics and East Asians (Chinese, Japanese and Korean), while non-Hispanic Whites exhibit the highest prevalence (Hiatt et al., 1982; Sarmina et al., 1987; Soucie et al., 1994; Stamatelou et al., 2003). In a recent

nationwide cross-sectional study, the prevalence of KSD among those of African ancestry was 1.7%, compared to 2.6% in Mexican Americans, and 5.9% among Caucasians (Stamatelou et al., 2003).

Time trends in the United States from 1976 to 1994 indicate that the prevalence of kidney stones has increased significantly among Caucasians aged 40 and over, but not among African Americans (Stamatelou et al., 2003). However, in South Africa, the prevalence of KSD in ethnic Africans increased significantly over a 15-year period, most likely in conjunction with changes to dietary patterns, while no change in prevalence was observed in Caucasians (Beukes et al., 1987). Ca^{2+} oxalate stones are the most common calculi in African Americans and Caucasians residing in the United States (Hiatt et al., 1982), sub-Saharan Africa (Beukes et al., 1987; Klufio et al., 1996) and Australia (Baker et al., 1993). The reason for the lower prevalence of KSD among ethnic Africans is not known, though one possible explanation is that they have lower oxalate absorption rates than Caucasians (Lewandowski et al., 2001).

b. Studies in Europe

In Europe, a similar rise in stone rates was reported in an urban population in Sweden, where the prevalence of upper urinary tract stones was 13.7% (Ljunghall and Hedstrand, 1975). Two nationwide surveys in Germany revealed an incidence of 0.54% and a prevalence of 4% (Vahlensieck et al., 1982). In a survey of 1500 people in the Balearic Islands in Spain, the prevalence of urinary stone disease was 14.3% (Grases et al., 1994). Although Ca^{2+} oxalate stones are most common among Europeans, the prevalence of uric acid stones showed considerable variation, from as low as 4% in Scandinavia and 5% in Belgium, to as high as 17% in Germany (Halabe and Sperling, 1994).

c. Studies in Asia

In the far East, renal calculi are becoming increasingly common. In Japan, a recent nationwide survey showed that the annual incidence of KSD has more than doubled from 1965 to 1995, although the incidence is still lower than in the U.S. (Yoshida et al., 1999). A similar trend was observed in Malaysia (Sreenevasan, 1990). Prevalence rates in East and Southeast Asia range from 3.5% among adults in Korea (Kim et al., 2002), 9.6% among adults in Taiwan (Lee et al., 2002) and 10.3 % among adults aged 50 or over in Japan (Iguchi et al., 1996). Ca^{2+} oxalate is the most common stone found in Thailand and Indonesia (Hodgkinson, 1979).

The incidence or prevalence of KSD in South Asians (India, Pakistan, Bangladesh, and Sri Lanka) has never been reported. There is evidence that Ca^{2+} oxalate is the major constituent of renal calculi in Bombay (Hussain et al., 1990) and northern India (Ahlawat et al., 1996), making up about 97% of kidney stones. In contrast, the commonest stones in northeastern Pakistan are uric acid (28.1%), followed by Ca^{2+} oxalate (26.1%), mixed calculi containing Ca^{2+} oxalate and uric acid (21.8%), and Ca^{2+} oxalate mixed with Ca^{2+} phosphate (10.4%) (Rafique et al., 2000).

There is evidence that Middle Eastern and West Asian ethnic groups have a propensity for renal stones. A nationwide survey of KSD in Turkey revealed an overall prevalence of 14.8% and an incidence of 2.2% (Akinci et al., 1991). Trend data in Israel indicate that the prevalence of Ca^{2+} oxalate stones increased by about 6 times and uric acid stones doubled from 1974 to 1982 (Zaidman et al., 1986). Moreover, a disproportionate number of Arabs in Tanzania develop kidney stones at rates that are comparable to Western countries and most of which are Ca^{2+} oxalate (Mkony et al., 1991). Ca^{2+} oxalate stones are also common in Saudi Arabia and Sudan (Hodgkinson, 1979). In Israel, Lebanon, Iran, Iraq and Syria, Ca^{2+} oxalate stones are more common among non-Jewish ethnic groups, whereas uric acid stones are 2-3 times more common in

people of Jewish descent (Zaidman and Pinto, 1976). The prevalence of uric acid stones is estimated at 19.9% in Israel and 37.7% in Iran (Halabe and Sperling, 1994).

3. Ethnicity and Urinary Calcium Excretion

There is substantial evidence that hypercalciuria occurs less frequently in Africans than in Caucasians (Bell et al., 1985; Meier et al., 1991), even when accounting for dietary Ca^{2+} intake, sunlight exposure, muscle strength, body fat, and growth factors (Bikle et al., 1999; Ettinger et al., 1997). It is less clear whether these lower levels of urinary Ca^{2+} excretion occur in other ethnic groups. One study reported that Hong Kong adolescents had a lower level of 24-hour urinary Ca^{2+} than previously published for Caucasian adolescents (Wong et al., 1998). Similarly, in the United Kingdom, South Asians (3.33 mmol/day) were reported to have lower levels of urinary Ca^{2+} than Europeans (4.62 mmol/day) (Blackwood et al., 2001). In Israel, Arab boys aged 10 to 11 had higher urinary Ca^{2+} excretion than Jewish boys (Sokol et al., 1978). However, the results of these studies may have been confounded by differences dietary Ca^{2+} intake between the ethnic groups, since dietary intake was not assessed.

4. Diet, Ethnicity, and Kidney Stone Disease

Apart from studies of Japanese-Americans, there is little information on the dietary patterns and their relationship to disease in Asian and Arabic populations living in Western societies. In the United States, dairy food intake is low in African-Americans, Hispanics and a group simply specified as Asian (Miller et al., 2001; Pereira et al., 2002; Jackson et al., 2001; Aloia et al., 1996; Bell et al., 2001c). There is some evidence that East and Southeast Asians living in different parts of the world consume lower than recommended amounts of dietary Ca^{2+} (Jackson et al., 2001; Aloia et al., 1996; Bell et al., 2001c; Hirota et al., 1992; Kim et al., 1993; Mackelvie et al., 2001; Woo et al., 1998;

Koh et al., 2001). To this point, only one comparative study examined dietary Ca^{2+} intake in ethnic groups of South Asian and West Asian/Arabic origin. In that report, premenopausal women of Indian/Pakistani origin living in the U.S. consumed significantly less dietary Ca^{2+} (683 mg/day) than Caucasian women (960 mg/day) (Alekel et al., 1999).

Currently, the influence of a dairy-rich diet on KSD risk or related conditions in non-European populations is largely unknown (Borghi et al, 2002). A higher intake of dairy products might be beneficial to ethnic minority groups, particularly if they are prone to developing renal stones. Many studies have already demonstrated the beneficial effects of a diet rich in Ca^{2+} in Caucasians, including a reduced risk of stone recurrence, osteoporosis and insulin resistance, as well as a reduction in blood pressure, salt sensitivity and body weight, as discussed earlier.

5. Summary of Ethnic Differences in Kidney Stone Disease

To summarize, the literature suggests that the propensity for renal calculi might be higher in a number of ethnic groups residing in Southeast Asia, the Middle East and West Asia compared to Europe and North America. However, prevalence data for these populations are sparse, and the susceptibility to kidney stones has never been studied in a systematic way in a culturally diverse Western population. Thus, another objective of this thesis was to determine whether there are ethnic differences in the prevalence of KSD in Canadians attending a population-based treatment facility.

D. Urinary Potassium and Diet Quality

1. Dietary Patterns and Disease

Until recently, past studies in diet and health have generally examined the role of single nutrients, foods, or food groups in the etiology of disease (Kant, 1996; Hu, 2002; Kant, 2004). However, very few individual dietary factors have exhibited a strong or consistent association with cardiovascular disease or related endpoints in epidemiologic studies. The weak effects in these studies may be attributed to the fact that complex diets consist of a combination of foods containing multiple nutrients, and the intercorrelation of dietary factors makes it difficult to isolate the effects of single nutrients or foods. Moreover, biological activities of nutrients within the body are interdependent, which is likely to reduce the reproducibility of single nutrient studies. As a result, studies increasingly now focus on the health effects diet quality, or dietary patterns, comprising of multiple interrelated dietary factors (Hu, 2002; Kant, 2004).

Focusing on the overall diet rather than on single dietary factors offers two important advantages. Foremost, the overall dietary pattern may have a greater influence on health than individual nutrients or foods. For example, Trichopoulou et al (2003) reported that adherence to a traditional Mediterranean diet is associated with a significant reduction in total mortality and death due to coronary heart disease compared to low adherence to this diet. In contrast, associations between individual food groups contributing to the Mediterranean diet and mortality were mostly null (Trichopoulou et al., 2003). Thus, the cumulative effect of multiple nutrients and foods can be examined by focusing on the overall diet (Kant, 1996; Hu, 2002; Kant, 2004).

More importantly, a total diet approach better reflects dietary intake in the real world, in which nutrients and foods are consumed in combination. As such, an examination of dietary patterns may provide a comprehensive approach to disease

prevention or treatment, which has been used in several randomized controlled trials including the Dietary Approach to Stop Hypertension (DASH) (Appel et al., 1997) and the Lyon Diet Heart Study (de Lorgeril et al., 1994; de Lorgeril et al., 1999). Studying dietary patterns, therefore, could have important public health implications because the overall patterns of dietary intake may be easy for the public to interpret or translate into diets (National Research Council, 1989; Kant, 1996; Kant et al., 2000).

2. Approaches to Studying Dietary Patterns

Since dietary patterns cannot be measured directly, selective statistical and methodological approaches are often used. The predominant approaches that have been used in the literature are a posteriori and a priori methods (Hu, 2002; Kant, 2004). A third approach, biochemical markers, has also received attention recently.

a. a posteriori Methods

The a posteriori approach makes use of factor analysis to derive eating patterns through statistical modelling of dietary data at hand (Hu, 2002; Kant, 2004). Factor analysis (usually principal component analysis) is a multivariate statistical technique that uses information gathered by food frequency questionnaires (FFQs) or in dietary records to identify common underlying factors or patterns of food composition. It assembles specific food items based on the degree to which food items in the dataset correlate with one another. A summary score for each pattern is then derived.

For example, using data from large prospective studies of men and women, Hu et al (2000) employed factor analysis to identify two major dietary patterns and assessed the cardiovascular disease risk associated with each of them. The 'prudent' diet included higher intake of fruits, vegetables, whole grains, fish and poultry, while the 'Western' pattern was characterized by higher intake of meats, refined grains, high-fat dairy

products, and sweets. The investigators found a strong, inverse and significant association between the 'prudent' dietary pattern scores and cardiovascular disease risk, whereas the exact opposite was observed for the 'Western' pattern (Hu et al., 2000; Fung et al., 2001). Subsequent studies reported that the 'prudent' diet was also associated with a significantly lower risk of incident DM2 (van Dam et al., 2002) and stroke (Fung et al, 2004), whereas again, the reverse was true for the 'Western' pattern. These findings provide compelling evidence for the cardioprotective effect of the 'prudent' diet pattern.

A drawback of the a posteriori approach is that the dietary patterns that are generated do not necessarily represent optimal patterns, since they are based on available empirical data without a priori hypothesis. Furthermore, when using this approach, it is important to evaluate whether the patterns that are generated fit into the commonly recognized eating habits in the population, since these patterns are derived simply on the basis of eating behaviours (Hu, 2002; Kant, 2004).

b. a priori Methods

In the a priori approach, dietary indices are created on the basis of previous knowledge of a 'healthy' diet (Hu, 2002; Kant, 2004). A number of dietary indices have been constructed to assess overall diet quality. For example, the Diet Quality Index (Kim et al., 2003) is a summary score of the degree to which an individual's diet conforms to specific dietary recommendations from Diet and Health (National Research Council, 1989). The Healthy Eating Index (Kennedy et al, 1995) provides a single measure of the extent to which a person's diet conforms to the recommendations of the U.S. Department of Agriculture Food Guide Pyramid (U.S. Department of Health and Human Services, 1992). Another simple score is the Recommended Foods Score, which simply tallies the foods recommended by current dietary guidelines (Kant et al., 2000).

Several recent studies examined associations between health outcomes and dietary patterns, as measured by diet indices constructed according to a priori knowledge. For instance, in a prospective study in Greece (Trichopoulou et al., 2003), a two-point incremental increase in the Mediterranean diet score, which measures adherence to a traditional Mediterranean diet, was associated with a significant reduction in risk of all-cause mortality, and mortality due to coronary heart disease and cancer. On the other hand, associations between individual food groups contributing to the Mediterranean diet score and total mortality were generally not significant. More recently, Knoops et al (2004) reported similar findings in a prospective study of elderly men and women residing in Europe (Knoops et al., 2004). In another prospective study of women, Kant et al (2000) reported that individuals with an RFS diet quality score in the highest quartile had a greater than 30% lower risk of death from all-causes, as well as death from coronary heart disease, stroke and cancer. A subsequent study, which also used the RFS index to assess diet quality, reported similar findings in middle-aged women in Sweden (Michels et al., 2002).

The main limitation of the a priori approach is that a diet scale is limited by current knowledge about the effect of diet on disease. There may also be a great deal of uncertainty in selecting individual components of the diet score and subjectivity in defining cutoff points. Lastly, the construction of a dietary index is based on prevailing dietary recommendations, which may not represent the best available scientific evidence.

In short, both a posteriori and a priori approaches have identified dietary patterns that predict health outcomes.

c. Biochemical Measures

An alternative approach for assessing diet quality is to use biochemical markers of dietary intake, such as blood or urine specimens. The rationale for using this approach

stems from the fact that self-report methods, such as FFQs, 24-hour dietary recall or food diaries, are time consuming for respondants to complete and staff to review, and are susceptible to inaccurate recall of dietary information and reporting bias (Prentice et al., 2002; Heitmann, 1995). Moreover, these approaches require access to nutrient databases that need frequent and costly updating. These limitations impede on the ability of physicians to provide effective dietary counseling to patients in a clinical setting. To address some of the problems of self-report instruments, biochemical markers are now being evaluated as a new alternative approach to assess nutrient intake and diet quality (Neuhouser et al., 2003).

3. 24-hour Urinary Potassium

One potentially powerful marker of a healthy all-around diet is 24-hour urinary K^+. The 'healthy' dietary patterns derived in the literature are characterized by increased fruit, vegetable, whole grain, low-fat dairy product, fish and poultry consumption (e.g., Hu et al., 1999; Kant et al., 2000; Trichopoulou et al., 2003). Each of these foods or food groups is a good source of K^+ (see Appendix 7). Moreover, increased dietary K^+ intake is associated with a reduced risk of developing a broad range of health problems, including hypertension (McCarron et al., 1984; Geleijnse et al., 2003), stroke (Ascherio et al., 1998), KSD (Curhan et al., 1993; Taylor et al., 2004), obesity (McCarron et al., 1984), DM2 (Colditz et al., 1992) and osteoporosis (Sebastian et al., 1994). Furthermore, data from the Scottish Heart Study showed that a single 24-hour urinary K^+ measure is strongly and inversely related to risk of death from all-causes (Tunstall-Pedoe et al., 1997). The day-to-day, within-person variability of K^+ intake is relatively low (Willett, 1999) and urinary K^+ excretion strongly reflects dietary K^+ intake (Caggiula et al., 1985; Bingham et al., 1995).

Thus, as part of this thesis, the objectives of this study were to examine the validity of urinary K^+ excretion as a measure of diet quality, to evaluate its relationship to BMI, blood pressure and heart rate, and to determine an optimal 24-hour urinary K^+ cutoff point to identify individuals consuming a poor quality diet.

IV. Study Outline

The following is a brief summary of the study procedures:

- Young KSD patients (aged 18-50 years) were recruited.
- All KSD patients provided a 24-h urine collection (while on an unrestricted diet), a fasting urine sample, and a fasting blood sample.
- Based on the 24-h urine sample, KSD patients were classified into urinary groups (e.g., 'hypercalciuria' or 'normocalciuria').
- Structured interview was used to obtain sociodemographic and health information from probands and to identify family members (first degree relatives and spouse) for participation.
- Probands completed FFQs; dietary data were reviewed to ensure full and proper completion of FFQs.
- The probands' first degree relatives and spouse were interviewed to assess demographic variables and health outcomes.
- The familial aggregation of hypertension, KSD, obesity, excessive weight gain and DM2 were analyzed using GEE regression.
- A validation substudy was conducted to assess the accuracy of family health information reported by KSD probands on behalf of first-degree relates.
- Ethnic differences in KSD propensity were examined using the frequency counts of patients attending the Centre during the two-year study period and denominator data from the 2001 Canada Census.
- 24-hour urinary K^+ was examined as a valid, clinically useful marker of diet quality as measured by FFQ.

V. METHODS

A. Selection of Participants and Initial Assessment

Consecutive idiopathic calcareous renal stone formers, aged 18 to 50 years, attending the St. Michael's Hospital's Kidney Stone Centre between February 2002 to March 2004 were eligible for recruitment. The Centre, which contains one of only three shockwave lithotriptors in Ontario, serves the health needs of about six million people including the Greater Toronto Area community and may be considered a population-based treatment facility. Thus for the purposes of this study, referral bias is unlikely.

The age restriction increases the chance of finding a genetic basis of disease, parents who are alive and probands off treatments that may influence urinary Ca^{2+} excretion (Lander and Schork, 1994). Age-eligible patients were contacted by telephone before their scheduled lithotripsy appointment to describe the nature of the study and determine their willingness to participate. A condition for participation was the willingness of patients to allow the study staff to contact first-degree relatives. Reasons for non-participation were documented. Patients were also excluded if they reported having any of the following conditions: inflammatory bowel disease, bowel resection, sponge kidney, sarcoidosis, renal tubular acidosis, primary hyperparathyroidism, primary hyperoxaluria, or enteric hyperoxaluria (Trinchieri et al., 1988).

Potential participants were asked to collect a single 24-hour urine specimen, starting the morning prior to their lithotripsy treatment, and were not provided with any specific dietary instruction. The 24-hour urine sample, collected on a free choice diet, was used to classify KSD patients into urinary groups (e.g., hypercalciuria or normocalciuria).

At the Centre, patients, after giving informed consent, were interviewed to collect personal and family information about sociodemgraphic characteristics and family

composition, personal and family history of hypertension, diabetes mellitus and KSD, and weight and height estimates in first-degree relatives (excluding offspring) and spouses. They then completed a FFQ that was reviewed by trained study staff. Missed items or ambiguities in recorded food items were clarified by discussing responses with subjects at the visit or later by telephone. Weight (measured in a light hospital gown) and height (without shoes) were measured to calculate BMI (weight [kg] / height $[m]^2$). Sitting blood pressure (average of two readings using a mercury sphygmomanometer) was assessed in a standard manner (Khan et al., 2004). Routine peripheral venous blood samples (after 14 hours of fasting) were also drawn and a fasting urine sample was collected as well.

B. Interview of Family Members

Family members of the probands were contacted by telephone. With their consent, each relative and spouse was asked to provide information on the presence of hypertension, KSD and DM2, their current height and weight, and their weight five years ago. Weight change was calculated by subtracting their current weight from their weight of five years previously. Both the interviewer and the family member were 'blind' as to the results of the probands' metabolic analysis at the time of the interview.

It was recognized that obtaining family health information directly from first-degree relatives would require additional time and effort, and introduce additional burden to participants. To minimize the problem of missing data, we enrolled only probands who allowed us to contact family members and restricted inquiries to first-degree relatives and spouse. Previous studies have shown that the participation rate of first-degree relatives is high in families where the proband consents to contacting kin (Rocca et al., 2004) (also see Appendix 5).

Only full first-degree relatives were included in the study. Offspring were excluded, however, since most of them would likely have been aged less than 18 years, and almost all of the outcomes of interest in this study have a low prevalence among pre-adults. Half-siblings, adopted siblings, or step-parents were also excluded.

C. Measurement of Variables

In the assessment of disease clustering in families, the primary exposure variable was the urinary abnormality of the proband (e.g., the level of urinary Ca^{2+} excretion), while the principal outcome variables were the presence or absence of hypertension, KSD, obesity, weight gain and DM2 among the first-degree relatives and spouse of the patient.

1. Classification of Urinary Variables

Standard definitions of hypercalciuria and hyperuricosuria were used. Hypercalciuria was defined as a 24-hour urine Ca^{2+} excretion exceeding 0.1 mmol/kg/day on a nonrestricted diet (Coe et al., 1982; Audran and Legrand, 2000; Lerolle et al., 2002). Hyperuricosuria was defined as a 24-h urine uric acid value > 4.7 mmol/day in males, and >4.3 mmol/day in females (Pak et al., 2002). These pre-defined cutpoints for urinary Ca^{2+} and uric acid corresponded with approximately the upper quartile of the distribution of values for these parameters in the Kidney Stone Centre database.

Low urinary K^+ was defined as a 24-h urine K^+ value < 43.0 mmol/day in males and < 31.0 mmol/day in females. These cutoff values are the first quartile of the age- and gender specific distributions of urinary K^+ values of the Kidney Stone Centre database, which contains the collected urine results from over 2000 consecutive age-eligible patients over the previous two years. Moreover, these cutpoint values relate to the mean excretion value of 39 mmol/day in subjects consuming the 'control diet' (a poor quality

diet) in the DASH trial (Appel et al., 1997).

Patients were classified by their urinary Ca^{2+} and K^+ status into one of four study groups: high Ca^{2+} and low K^+ (HC/LK), HC and normal K^+, normal Ca^{2+}/LK, and normal Ca^{2+} and K^+ (reference group). Similar groups were also created to examine the effects of urinary Ca^{2+} with urinary uric acid excretion.

2. Clinical Measurements in KSD Patients

The two blood pressure readings were averaged. Patients were classified as having hypertension if their untreated systolic or diastolic readings were $\geq$ 140/90 mm Hg, or if they required antihypertensive medications to control their BP (Chobanian et al., 2003). Patients with a BMI of $\geq$ 30 kg/m^2 were classified as obese (World Health Organization, 1997; National Institutes of Health, 1998). BMI is used extensively in epidemiologic studies to measure percentage body fat (Gallagher et al., 1996).

3. Measurement of Diet Quality in KSD Patients

A 166-item quantitative FFQ was used to estimate usual nutrient and energy intake (Jain et al., 1982; Jain et al., 1996). Participants were asked to report their usual frequency of intake and portion size of foods during the previous 12 months. The frequencies were reported as the number of times per month, week or day that a food item was consumed. Portion size was reported as a multiple of a standard portion size, by reference to food model photographs. Nutrients were based on the 2005 Canadian Nutrient File (Canada Nutrient File, 2005). Standard definitions were used to classify foods and food groups (Hu et al., 1999; Hu et al., 2000). The FFQ was also used to derive the Recommended Foods Score (RFS), a measure of diet quality that is related to all-cause and cause-specific mortality (Kant et al., 2000). The overall score is the unweighted sum of the value of one given for each of 23 recommended food items that a

person consumes at least once weekly (see Appendix 8). Scores range from 0 to 23, and a value of 9 or higher was previously shown to be associated with a significant reduction in risk for all-cause and cause-specific mortality in a large population-based cohort study of women (Kant et al., 2000). In the diet study, we used a RFS score of <9 to indicate a poor quality diet in women, which represented the 25th percentile of the scores. For men, the value was <8. The scores were adjusted for age, gender, ethnicity, urinary creatinine and energy intake.

4. Classification of Disease Outcome in Family Members

To increase specificity, as recommended for aggregation studies (Khoury et al., 1993), restrictive definitions for outcome variables were employed (Lander and Schork, 1994). In addition, individuals are more likely to know whether they take medication for hypertension or DM2 than to be aware of their actual blood pressure or serum glucose level, particularly if untreated.

Accordingly, hypertension was defined as being treated with antihypertensive medications to lower BP. This restrictive definition identifies those with BP values at the upper 10% of the BP distribution, and consequently those with the most severe forms of hypertension (Watt, 1986b). Similarly, DM2 was defined as being treated with oral hypoglycemic agents at any age or with insulin after the age of 40 years, which identifies individuals with more severe diabetes (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2000). Obesity in relatives, as with probands, was defined as a BMI of 30 kg/m^2 or higher (World Health Organization, 1997; National Institutes of Health, 1998). Weight gain was arbitrarily considered excessive if the change exceeded the 75th percentile of the gender-specific 5-year weight change distribution, as there is no standard definition (Choi et al., 2005). For men, this was >5.1

kg and for women, >6.8 kg. This cutpoint translates into a weight gain that is about twice the average for the general population (Choi et al, 2005).

D. Potential Confounding Variables

Multivariate analyses were used to adjust for potential confounders (Khoury et al., 1993; Zhao and Le Marchand, 1992) identified by a systematic review of the literature. The main covariates were patient age, gender, BMI, personal history of disease, use of antihypertensives and relative age (Lemann, 1996; Borghi et al., 1999; Tisler et al., 2002).

Other potential confounders included ethnicity (Anand et al., 2000), marital status (Johnson et al., 2000), education (Kaplan et al., 1993), area of residence (inside/outside city) (Anand et al., 2001), country of birth (inside/outside Canada) (Anand et al., 2000), smoking status (Ambrose et al., 2004), use of Ca^{2+}/vitamin D products (Williams et al., 2001) and relative gender and smoking status. In the analysis of effect modification, proband age, gender, BMI, and relative age of onset of disease were examined for interactions with proband urinary group.

E. Statistical Analyses

Generalized estimating equations (GEE) were used to test for familial aggregation of disease among urinary groups, as measured by a log odds ratio, assuming an exchangeable correlation matrix (Zhao and Le Marchand, 1992). The GEE algorithm is a valid statistical approach for analyzing correlated data in family studies (Zhao and Le Marchand, 1992; Liang and Zeger, 1986; Zeger and Liang, 1986). The primary exposure variable was the urinary status of the patient, while the binary outcome variable was the disease status of an individual family member. Urinary variables (Ca^{2+}, K^{+}, uric acid)

were also assessed individually as continuous, ordinal, and binary (i.e., hypercalciuria, no hypercalciuria) exposure variables. The odds ratio (OR) quantified the association between the metabolic abnormality of the proband and the disease status of the relative, while controlling for potential confounders. Parameter estimates were obtained for each relative type (fathers, mothers, brother, sisters, and spouse) and first-degree relatives overall. Relative types with similar parameter estimates were analyzed together as a combined group to increase statistical precision, where appropriate (e.g., brothers and sisters combined into a siblings group).

Assessment of confounding and effect modification was conducted using a hierarchical backwards elimination approach (Kleinbaum and Klein, 2002). Two-way interaction terms between proband urinary parameters (e.g., urinary Ca^{2+} and K^{+}) or between a urinary predictor and effect modifier (e.g., patient gender) were assessed first. Wald and likelihood ratio tests were performed to identify terms that made significant contributions to the model ($p<0.05$). Potential confounders were then considered and only those that changed the OR estimate by more than 10 percent were retained.

The mean values ± S.D. for continuous variables and the rates for categorical variables were computed for each urinary group. Unpaired t-tests or analyses of variance (ANOVAs) were conducted to compare groups on continuous variables (urine and serum data, anthropometric measures and blood pressure). Post hoc pairwise comparisons were made using Tukey's approach to adjust for multiple comparisons. Chi-square contingency tables were used to compare urinary groups on categorical variables including sociodemographic and health characteristics.

The analyses were conducted using SAS version 8. PROC FREQ was used to obtain cross-tabs and χ^2 tests or Fisher Exact tests, PROC GLM was used for unpaired t-tests, while PROC ANOVA was used for ANOVA tests. For the GEE regression analyses, PROC GENMOD was employed with the inclusion of a REPEATED statement

(Horton and Lipsitz, 1999; Ziegler and Gromping, 1998). All p-values below 0.05 were considered statistically significant.

F. Sample Size Determination

The primary purpose of this study, viewed in the long-term, was to identify the genetic basis of a complex phenotype. To determine sample size, an odds ratio of 3.0 was used as the minimally important value since a smaller effect would make it highly unlikely that the gene or genes conferring susceptibility would be isolated with present day methodology. It was also assumed that about 25% of the stone patients would have hypercalciuria (Tisler et al., 1999; Tisler et al., 2002) and that 25% of first-degree relatives would have hypertension (Wolf-Maier et al., 2003). With alpha=0.05 (two-sided), power to detect an odds ratio of 3 was determined to be greater than 80% with a sample size of 308.

H. Ethical Issues

The study was approved by the St. Michael's Hospital's and Mount Sinai Hospital's Research Ethics Board. Standard techniques were used to help participants understand the protocol. These included discussion with the recruiter, written information on the consent form, and the availability of the investigator at any time to answer questions during the course of the study. Participants were informed that participation is completely voluntary and confidential. Written consent was obtained from all patients prior to participation in the study. All study material containing personal identifiers were stored in locked filing cabinets or in password-protected computers to maintain confidentiality.

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VI. Manuscripts

High urinary calcium excretion and genetic susceptibility to hypertension and kidney stone disease

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ABSTRACT

Increased urinary calcium excretion is commonly found in patients with hypertension and kidney stone disease (KSD). This study investigates the aggregation of hypertension and KSD in families of KSD patients with hypercalciuria and explores whether obesity, excessive weight gain and diabetes mellitus, commonly related conditions, also aggregate in these families. Consecutive KSD patients, aged 18-50 years, were recruited from a population-based Kidney Stone Center and collected a 24-hour urine sample. The first-degree relatives of eligible subjects (n=333) and their spouse were interviewed by telephone to collect demographic and health information. Familial aggregation was assessed using Generalized Estimating Equations. Multivariate-adjusted odds ratios (OR) revealed significant associations between hypercalciuria in patients and hypertension and KSD in first-degree relatives and specifically in siblings (OR and 95% confidence interval for hypertension = 2.9, 1.4-6.2 and for KSD = 1.9, 1.03-3.5). No significant associations were found in parents or spouses, or in patients with hyperuricosuria. Similarly, no aggregation with other conditions was observed. In an independent study of siblings of hypercalciuric KSD patients, the adjusted mean first-morning urinary calcium/creatinine ratio was significantly higher in the hypertensive siblings compared to normotensive siblings (0.60 ± 0.32 vs. 0.46 ± 0.28 mmol/mmol, $p<.05$), and both sibling groups had significantly higher values than the unselected study participants ($p<.001$). Urinary sodium/creatinine and uric acid/creatinine ratios were not different among the groups. While an environmental effect cannot be fully excluded, our findings suggest that the disturbance in calcium metabolism in hypertension and KSD has a genetic basis.

Keywords: Hypercalciuria; Hypertension; Nephrolithiasis; Familial Aggregation

INTRODUCTION

Disturbances in calcium (Ca^{2+}) metabolism have been reported in hypertension (1,2), obesity (1,3) and kidney stone disease (KSD) (4,5), and there are strong associations among these conditions (6-8). Normotensive offspring of hypertensive patients also have disturbed Ca^{2+} metabolism, suggesting a possible genetic basis for these abnormalities (9,10). We previously reported familial aggregation of hypertension in KSD patients with hypercalciuria (HC) and hyperuricosuria (HUA), but not with either urinary abnormality alone (11,12). Hypertension in first-degree relatives, however, was ascertained from reports by patients, an insensitive method of identifying disease status in family members. Moreover, spousal information, which may help to control for environmental effects, was not collected (11,12).

This study investigates the aggregation of HC with hypertension and separately with KSD in families of KSD patients, taking into account the shortcomings of earlier studies (11,12). It also explores whether obesity, excessive weight gain and type 2 diabetes mellitus, commonly associated with hypertension and KSD, aggregate in families with Ca^{2+} abnormalities. We hypothesized that HC would be associated with familial aggregation of hypertension, KSD, obesity, weight gain and diabetes in KSD patients. We further tested our hypothesis by comparing urinary Ca^{2+} excretion in KSD patients and an independent sample of siblings of KSD patients with HC. Lastly, the study explores whether the associations for disease aggregation in families vary by patients' body mass index (BMI), age, gender and relatives' age of disease onset.

MATERIALS AND METHODS

Selection of Participants and Assessment

Consecutive patients, aged 18 to 50 years, attending the St. Michael's Hospital's Kidney Stone Center between February 2002 to March 2004 were eligible for recruitment. The Center, which contains one of only 3 shockwave lithotriptors in Ontario, serves the health needs of about six million people including the Greater Toronto Area community and may be considered a population-based treatment facility. The hospitals' Research Ethics Board approved the study.

Age-eligible patients were contacted by telephone before their scheduled lithotripsy appointment to describe the nature of the study and determine their willingness to participate and allow the study staff to contact family members. Reasons for non-participation were documented. Potential participants were asked to collect a single 24-hour urine specimen, starting the morning prior to their lithotripsy treatment, and were not provided with any specific dietary instruction. At the Center, patients, after giving informed consent, were interviewed to collect personal and family information about sociodemographic characteristics and family composition, personal and family history of hypertension, diabetes mellitus and KSD, and weight and height estimates in first-degree relatives (excluding offspring) and spouses. Weight (measured in a light hospital gown) and height (without shoes) were measured to calculate BMI. Sitting blood pressure (average of two readings using a mercury sphygmomanometer) was assessed in a standard manner (13). Routine peripheral venous blood samples were also drawn.

With their consent, relatives and spouse were interviewed by telephone to collect information about the presence of hypertension, diabetes and KSD, their current height and weight, and their weight five years ago. Weight change was calculated by subtracting their current weight from their weight of five years previously. Both the interviewer and the family

member were 'blind' as to the results of the patients' metabolic analysis at the time of the interview.

Classification of Urinary Variables and Disease Status

Standard definitions of HC (14,15) and HUA (16) were used. To increase specificity, as recommended for aggregation studies (17), restrictive definitions for outcome variables were employed. Accordingly, hypertension was defined as being treated with antihypertensive medications to lower blood pressure (18) and type 2 diabetes mellitus, as being treated with oral hypoglycemic agents at any age or with insulin after the age of 40 years (19). Individuals with a BMI of 30 kg/m^2 or higher were classified as obese (20). Weight gain was arbitrarily considered excessive if the change exceeded the 75^{th} percentile of the gender-specific 5-year weight change distribution, as there is no standard definition (21). For men, this was > 5.1 kg and for women, > 6.8 kg.

Biochemical Procedures

All 24-hour urine collection bottles contained thymol crystals, dissolved in isopropanol as a preservative. Urinary Ca^{2+}, uric acid (UA) and creatinine were analyzed on commercially available analyzers. Urine specimens were considered to have been collected properly when the creatinine value was in the daily reference range of 8.8 to 22 mmol for men and 4.5 to 16 mmol for women, and patients with a value outside the range were excluded from the study.

Statistical Analyses

Based on odds ratios reported in studies of intermediate phenotypes to assess genetic

susceptibility (11,12), power to detect an odds ratio of 3 was determined to be greater than 80% with a sample size of 308, assuming a=0.05 (two-sided). In making this calculation, we estimated that 25% of KSD patients would have HC (11,12) and that 25% of first-degree relatives would have hypertension (22).

For the primary analysis, patients were categorized in binary groups, based on the presence of elevated Ca^{2+} or UA excretion. They were further divided into four groups (HC/HUA, HC/normal UA, normal Ca^{2+}/HUA, and normal Ca^{2+} and UA) with the latter subgroup acting as the reference group. Mean values (±SD) for continuous variables and percentages for categorical variables were computed.

Generalized estimating equations (GEE) were used to test for familial aggregation of disease among urinary groups as measured by a log odds ratio (23). The primary exposure variable was the urinary status of the patient, while the binary outcome variable was the disease status of an individual family member. The covariates were patient age, gender, BMI, personal history of disease, use of antihypertensives and relative age (11). Other potential confounders included patient ethnicity, marital status, education, area of residence (inside/outside city), country of birth (inside/outside Canada), smoking status, and use of Ca^{2+}/vitamin D products. These factors were included in analytic models if singly they changed the point estimate by 10% (24). Regression parameter estimates were computed for first-degree relatives, individual relative categories and spouses. Brothers, sisters, mothers and fathers were analyzed separately and as siblings and parents. Stratified analyses were undertaken to assess for effect modification using strata defined by the median values for age, BMI or time of disease onset to avoid sparse data within urinary categories. Likelihood ratio tests were conducted to test for homogeneity. PROC GENMOD with a REPEATED statement in SAS software version 8.1 (SAS Institute Inc,

Cary, North Carolina) was used for the GEE regression analyses (25).

Independent Study of Siblings

We further tested our hypothesis of a genetically determined abnormality in Ca^{2+} metabolism by assessing urinary Ca^{2+} excretion in study participants and an independent sample of siblings of patients with HC. The latter sample came from a previous study that recruited 75 KSD patients with HC, aged 18 to 50 years, using the same protocol as employed in the current study (26). The urinary results have never been reported. With their consent, siblings were interviewed by telephone to collect information on demographic characteristics, the presence of hypertension and KSD, the names of all prescribed medications and supplementary health products, and their current weight and height. Individuals taking Ca^{2+} supplements or multivitamins were excluded. A urine bottle and instructions to collect a first morning urine sample were mailed to the eligible subjects. A total of 114 siblings (63 brothers and 51 sisters) returned the urine sample by an overnight courier delivery service in a pre-paid self-addressed envelope. The results of the first morning urinary Ca^{2+}/creatinine ratio in study participants and the independent sample of siblings were compared. Urinary sodium/creatinine and uric acid/creatinine ratio values were also evaluated to assess for a possible environmental effect. GEE regression was used to compare the main study participants to the siblings of HC patients, as well as the normotensive and hypertensive siblings, on each of the urinary variables, while adjusting for age, gender and BMI. Quantile plots were performed within study groups.

RESULTS

Response Rate

Figure 1.1 displays a flowchart summary of the recruitment process at the Kidney Stone Center. A total of 414 patients met our eligibility criteria and of these, 356 were enrolled in the study for a participation rate of 86%. We were unable to contact the first-degree relatives of 23 patients who agreed to participate, leaving a final total of 333 study subjects. There were no significant differences in age, gender, ethnic origin, marital status, education, BMI and blood pressure level between participants and those who declined or whose first-degree relatives could not be contacted. We obtained health information on 345 parents, 477 siblings and 193 spouses.

Baseline Characteristics

The baseline characteristics of the KSD patients are presented separately by urinary Ca^{2+} status and UA status in Table 1.1. The groups are not mutually exclusive. Patients with HC had a significantly lower BMI and prevalence of obesity than those with normal Ca^{2+} excretion. Patients with HUA were significantly older and more likely to be male than patients with normal UA excretion. They also had a significantly higher BMI, prevalence of obesity, treated hypertension, treated diabetes and mean serum glucose levels.

Disease Aggregation in First-Degree Relatives

Table 1.2 shows the multivariate adjusted odds ratios of disease aggregation in first-degree relatives by patients' urinary Ca^{2+} and UA status. There was a significant association between HC in patients and hypertension (OR = 1.8, 95% confidence interval [CI]: 1.1-2.8) and KSD (OR = 1.8, 95% CI: 1.1-3.0) in their first-degree relatives. In patients with HC and HUA, aggregation of hypertension in first-degree relatives was more pronounced. There were no

significant associations for HUA, for obesity, excessive weight gain or diabetes mellitus, or for spouses.

Disease Aggregation by Relative Type

The multivariate adjusted odds ratios for hypertension and KSD by relative type are presented separately in Figure 1.2a and 1.2b for patients with increased urinary Ca^{2+} excretion. HC in patients was associated with hypertension (OR = 2.9, 95% CI: 1.4-6.2) and KSD (OR = 1.9, 95% CI: 1.03-3.5) in siblings. HUA in patients was not associated with hypertension or KSD in any relative type. Moreover, no significant associations were found in spouses. In patients with HC and HUA, the odds ratio for hypertension in siblings was 4.5 (95% CI: 1.5-13.8). There was no association with hypertension in parents or spouses.

Effect Modifiers

In a series of exploratory analyses, we examined whether the associations between patients' urinary status and disease in relatives vary across strata of patient age, gender and BMI. When patients were aged 40 years or over, there were significant associations between HC in patients and hypertension in mothers (OR = 5.9, 95% CI: 1.6-21.2) and siblings (OR = 3.4, 95% CI: 1.4-8.1) that were not observed in younger patients. The opposite was found for KSD. The associations were stronger in mothers (OR = 4.7, 95% CI: 1.3-16.8) and siblings (OR = 2.9, 95% CI: 1.2-7.0) of HC patients <40 years of age. Female gender was a significant effect modifier for KSD in siblings of patients with HC. For hypertension, late disease onset in mothers and patients' BMI $\geq$ 27 were significant effect modifiers. For patients with HUA, the only significant effect modification of the associations was hypertension in siblings for patients' BMI $\geq$ 27. Only patients' age modifying the association between HC and hypertension in mothers

was significant for interaction. No significant aggregation of disease was found in spouses in the stratified analyses.

Urine Comparisons

The results of the 24-hour urinary values of the KSD patients are displayed in Table 1.3. After adjusting for age, gender, body weight and urinary creatinine, the HC group exhibited significantly higher excretions of magnesium, sodium, phosphate and urea, greater dietary protein intake, and higher ion activity product for Ca^{2+}-oxalate compared to the normal Ca^{2+} group. When patients were assessed by UA status, after adjustment HUA patients showed significantly higher excretions of potassium, sodium, oxalate, phosphate and urea, and a higher protein intake and urine pH. When each of the urinary variables was included in the main multivariate GEE models as covariates, associations between the urinary predictors and disease in relatives did not change.

Independent Study of Siblings

Figure 1.3 displays the quantile plots for urinary Ca^{2+}, sodium and uric acid excretion from the first void urine specimens in both study participants and an independent sample of normotensive and hypertensive siblings of KSD patients with HC. For Ca^{2+} excretion (Figure 1.3a), the adjusted mean Ca^{2+}/creatinine ratio was significantly higher in the siblings of the HC patients than the unselected study patients (0.49 ± 0.30 vs. 0.22 ± 0.15 mmol/mmol, $p<.001$). Moreover, the hypertensive siblings had a significantly higher mean Ca^{2+}/creatinine ratio compared to normotensive siblings (0.60 ± 0.32 vs. 0.46 ± 0.28 mmol/mmol, $p<.05$). There were no differences in sodium/creatinine or uric acid/creatinine ratio among the groups (Figures 1.3b and 1.3c).

DISCUSSION

Our study identified a strong, positive and significant relationship between hypertension in first-degree relatives and HC in KSD patients. The association was even stronger in those with both HC and HUA. These relationships were independent of patients' age, gender, BMI, use of antihypertensives and relatives' age, and took into account the correlated nature of family data. Our findings are relevant to a broad Ca^{2+}-containing KSD population because they were found in a study of consecutive KSD patients under the age of 50 years who were referred to a population-based Kidney Stone Center.

In an earlier study, we reported that hypertension aggregates in families of KSD patients with HC and HUA, but not with either urinary abnormality alone (11,12). However, family health information was ascertained from reports by patients, an insensitive method of identifying disease in family members (27), and spouses were not evaluated. In the present study, we contacted family members directly and used their health information to test for associations with several related conditions. The aggregation of hypertension in first-degree relatives, but not the spouse of patients with HC, suggests a genetic basis for the disturbance in Ca^{2+} metabolism (17).

Further support for a genetic link between HC in families of KSD patients and hypertension was our finding that siblings of KSD with HC have significantly higher Ca^{2+} excretion than unselected KSD patients, and that hypertensive siblings have a significantly higher mean Ca^{2+} excretion value than normotensive siblings, irrespective of age, gender and BMI. Several previous studies showed that hypertensive patients, particularly those who are salt sensitive, excrete a greater amount of Ca^{2+} compared to controls (1,2,28,29) and that normotensive offspring of hypertensive patients show disturbances in Ca^{2+} metabolism (9,10).

Our findings extend these earlier observations by directly linking hypertension to Ca^{2+} excretion in families.

Our findings do not support the possibility that other dietary and environmental factors influenced urinary Ca^{2+} excretion. Urinary sodium and estimated protein intake were virtually identical in the HC and HUA groups, yet aggregation of hypertension and KSD was observed only in families of HC patients. In the independent sibling study, urinary sodium and uric acid excretion were not significantly different among the three study groups, despite marked differences in urinary Ca^{2+} excretion. Finally, the group with HC had a significantly lower BMI and prevalence of obesity than patients with normal Ca^{2+} excretion, suggesting that weight was not a significant factor in these individuals.

Results from the Nurses Health Study cohort demonstrated that KSD is a risk factor for hypertension (6). In a stratified analysis of our study population, we observed a stronger aggregation of KSD in relatives of younger patients with HC, and for hypertension the aggregation was stronger in families of older HC patients. These findings support the proposition that KSD is manifested at a younger age before the development of hypertension (6).

We observed significant independent aggregation of KSD in first-degree relatives of KSD patients with HC. Many studies reported that Ca^{2+}-containing KSD aggregates in families (30-35). Several examined associations between urinary abnormalities in unselected populations of KSD patients and the presence of KSD in first-degree relatives (30,32-35). Most showed no associations with increased urinary Ca^{2+} excretion. Curhan et al (35), however, found in the Health Professionals Follow-up Study that men with incident KSD and a positive family history of KSD had significantly higher mean 24-hour urinary Ca^{2+} excretion. Some studies specifically

selected KSD patients with HC to explore familial relationships (36-38). Like our study, they found that their first-degree relatives were more likely to have HC or KSD episodes.

We did not find any associations between HC or HUA in KSD patients and obesity, excessive weight gain, or diabetes in first-degree relatives. These conditions are commonly related to hypertension and KSD, and the negative results may indicate the importance of environmental factors in these relationships.

Our study provides some insights into the possible role of HUA in the etiology of Ca^{2+}-containing kidney stones. KSD patients with HUA had a significantly higher BMI and were likely to be obese. Moreover, they had a higher prevalence of treated hypertension and diabetes and higher serum glucose and systolic blood pressure levels. These characteristics are components of the 'metabolic syndrome', a condition attributed to lifestyle factors (39). We also observed an increase in urinary oxalate excretion and others have shown that both urinary UA and oxalate are positively associated with obesity (4,40,41). These findings in conjunction with the negative familial aggregation results underscore the importance of environmental factors in these individuals, with HUA being the common link for hypertension, obesity, diabetes and possibly hyperoxaluria.

We purposefully selected younger patients for study since abnormal urinary phenotypes that appear at an earlier age are more likely to have an underlying genetic basis (42). In way of support for this approach, the strongest association for hypertension that we observed was in siblings of probands. The lack of association in parents suggests that environmental factors play a more prominent role as age increases (43). An important caveat in our study is the use of restrictive definitions in classifying health problems in relatives. This restriction identifies relatives with more severe manifestations of diseases, which are thus more likely to have a

genetic origin (42). Such stringent phenotypic definitions also help to increase specificity at the expense of sensitivity, an advantage in familial aggregation studies, since bias introduced by the misclassification of disease status of relatives is substantially greater when specificity is low (42).

The study has some limitations. Family members were not personally evaluated, nor were their physicians contacted to verify the information collected. Nonetheless, 89% of first-degree relatives that reported being told by a doctor to have hypertension were also treated with antihypertensive medications to lower blood pressure. Moreover, when we used 'ever told by a doctor' as the criterion for hypertension, the associations between the urinary measures in patients and hypertension in relatives remained steadfast. Finally, errors in ascertainment of disease status are likely random, and this would dilute rather than magnify associations.

The study was conducted in a single center in primarily Caucasian kidney stone patients. Thus, the results may only be applicable to this population. A single 24-hr urine specimen was used to determine the urinary status of patients. While this reduces the reliability of diagnostic classification (44), repeated collections were generally not feasible. If the 24-hour urine collection has substantial measurement error, this would weaken associations and under-estimate true associations.

Patients with HC or HUA had values for several urinary measures that were higher than those in patients with normal Ca^{2+} or UA excretion. However, controlling for these differences including creatinine in the multivariate GEE analyses did not influence the associations. These findings and the exclusion of patients with daily urinary creatinine values outside the reference range make it unlikely that the results were biased due to under- or over-collection of the urine specimens.

Ideally, a 24-hour urine collection in siblings of HC patients would have permitted us to control for dietary influences such as sodium and animal protein intake on urinary Ca^{2+} excretion. However, this was not feasible in our study. As an alternative strategy, we compared the first morning urinary sodium/creatinine and uric acid/creatinine ratios among the study groups. We observed no differences, suggesting that dietary factors did not account for the differences in urinary Ca^{2+} excretion.

Drug treatment for hypertension can alter the urinary excretion of minerals (45) and 45 patients in our study were taking antihypertensive medications. In a sensitivity analysis that excluded these patients, the estimates for familial aggregation were unaltered.

We did not observe a dose-response relationship between urinary Ca^{2+} in patients and disease in first-degree relatives. These observations are consistent with the findings of Lerolle et al (15), who found a threshold effect for the relationship between urinary Ca^{2+} excretion and risk of KSD. In their study, there was no association observed at urinary levels below the cutpoint of 0.1 mmol/kg/day used to classify HC.

In summary, the familial aggregation results and the findings of the independent sibling study suggest that the disturbance in Ca^{2+} metabolism in hypertension and KSD has a genetic basis.

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Table 1.1. Baseline characteristics of kidney stone patients (n=333) presented separately by urinary calcium (Ca^{2+}) and uric acid (UA) excretion status.

	Urinary Ca^{2+} status		**Urinary UA status**	
	Normal Ca^{2+} (n = 262)	High Ca^{2+} (n = 71)	Normal UA (n = 256)	High UA (n = 77)
Mean age (years) ± SD	38.5 ± 7.9	40.5 ± 7.1	38.0 ± 7.9	42.2 ± 6.2***
Male gender (%)	135 (51.5)	39 (54.9)	121 (47.3)	53 (68.8)***
European origin (%)	210 (80.2)	60 (84.5)	213 (83.2)	57 (74.0)
Born in Canada (%)	187 (71.4)	52 (73.2)	191 (74.6)	48 (63.2)
Toronto area resident (%)	214 (81.7)	59 (83.1)	207 (80.9)	66 (85.7)
Married (%)	177 (67.6)	45 (63.4)	166 (64.8)	56 (72.7)
≤ High school education (%)	87 (33.2)	23 (32.4)	85 (33.2)	25 (32.5)
Unemployed (%)	17 (6.5)	2 (2.8)	15 (5.9)	4 (5.2)
Income <$30000 (%)	24 (14.8)	5 (12.2)	25 (16.3)	4 (8.0)
Current smoker (%)	75 (29.1)	20 (28.2)	77 (30.3)	18 (24.0)
Taking Ca^{2+}/vitamin D (%)	100 (38.2)	21 (29.6)	96 (37.5)	25 (32.5)
Gout (%)	6 (2.3)	1 (1.4)	6 (2.3)	1 (1.3)
Previous kidney stone (%)	155 (59.6)	44 (63.8)	153 (60.5)	46 (60.5)
Mean BMI (kg/m^2) ± SD	28.0 ± 5.6	25.5 ± 4.6***	26.9 ± 5.3	29.6 ± 5.8***
Obesity (%)	77 (29.4)	9 (12.9)**	58 (22.7)	28 (36.8)*
Mean SBP (mm Hg) ± SD	123.1 ± 18.6	119.8 ± 18.2	121.0 ± 19.1	127.0 ± 15.8*
Mean DBP (mm Hg) ± SD	75.7 ± 12.4	72.4 ± 11.0	74.2 ± 12.4	77.5 ± 11.3*
Treated hypertension (%)	34 (13.1)	11 (15.7)	25 (9.8)	20 (26.7)***
Treated diabetes (%)	12 (4.7)	2 (2.9)	5 (2.0)	9 (12.0)***
Mean serum glucose (mmol/L)	6.47 ± 1.92	6.40 ± 1.56	6.33 ± 1.71	6.86 ± 2.17*

Abbreviations: Ca^{2+} , calcium; UA , uric acid; SD , standard deviation; BMI , body mass index; SBP , systolic blood pressure; DBP , diastolic blood pressure; mm Hg , millimeters of mercury; mmol/L , millimoles per liter.
* p<.05; ** p<.01; *** p<.001

Table 1.2. Disease aggregation in first-degree relatives by patients' urinary calcium (Ca^{2+}) and uric acid (UA) status.

	Patients' urinary group		
	† High Ca^{2+}	† High UA	‡ High Ca^{2+} + High UA
	OR (95% C.I.) §	OR (95% C.I.) §	OR (95% C.I.) §
Hypertension	1.8 (1.1, 2.8)*	1.4 (0.9, 2.3) [4]	2.5 (1.1, 5.4)*
Kidney stone disease	1.8 (1.1, 3.0)* [1]	1.4 (0.9, 2.4)	1.9 (0.9, 3.9)
Obesity	0.9 (0.6, 1.4) [3]	0.7 (0.4, 1.1) [3]	0.6 (0.3, 1.3) [3]
Excessive weight gain	1.3 (0.8, 1.9) [3]	0.9 (0.6, 1.3)	1.5 (0.8, 2.7) [3]
Diabetes mellitus	1.6 (0.7, 3.5) [2]	1.4 (0.7, 2.7) [5]	2.3 (0.9, 6.3) [5]

Abbreviations: Ca^{2+}, calcium; UA, uric acid; OR, odds ratio; 95% C.I., 95 percent confidence interval.
* $p < .05$;
†, reference group is patients with normal levels of the urinary marker in question.
‡, reference group is patients with normal urinary Ca^{2+} and UA.
§, adjusted for patients' age, gender, body mass index, antihypertensive medication use or personal history status, and relative age (also adjusted for other potential confounders based on a 10% change in the point estimate).
[1], also adjusted for patients' smoking status.
[2], also adjusted for patients' ethnicity and Ca^{2+}/vitamin D supplementation.
[3], also adjusted for patients' education.
[4], also adjusted for patients' area of residence.
[5], also adjusted for patients' ethnicity.

Table 1.3. Comparison of mean 24-hour urinary values of kidney stone patients (n=333) presented both by urinary calcium (Ca^{2+}) and uric acid (UA) excretion status.

	Urinary Ca^{2+} status ‡		**Urinary UA status ‡**	
	Normal Ca^{2+} (n = 262) Mean (SD)	High Ca^{2+} (n = 71) Mean (SD)	Normal UA (n = 256) Mean (SD)	High UA (n = 77) Mean (SD)
Calcium (mmol/d)	4.8 (2.0)	9.0 (2.8) ***	5.6 (2.6)	6.1 (2.9)
Uric acid (mmol/d)	3.5 (1.3)	3.6 (1.4)	3.2 (0.9)	4.6 (1.1) ***
Citrate (mmol/d)	2.5 (1.5)	2.5 (1.3)	2.4 (1.3)	2.7 (1.9)
Creatinine (mmol/d)	12.8 (4.6)	14.0 (4.6) **	12.6 (4.0)	14.6 (4.2) ***
Potassium (mmol/d)	52.2 (22.8)	56.6 (23.6)	51.9 (20.0)	57.6 (24.6) *
Magnesium (mmol/d)	3.6 (1.5)	4.4 (1.6) ***	3.8 (1.5)	3.6 (1.6)
Sodium (mmol/d)	149 (66)	183 (79) ***	149 (58)	180 (77) ***
Oxalate (μmol/d)	378 (167)	378 (154)	366 (157)	415 (143) *
Phosphate (mmol/d)	25.6 (11.6)	28.1 (12.2) *	25.4 (10.2)	28.7 (12.3) *
Urea (mmol/d)	324 (127)	357 (134) **	315 (104)	387 (122) ***
pH	6.3 (0.6)	6.4 (0.5)	6.3 (0.6)	6.4 (0.5) *
γ Protein intake (g)	113 (73)	145 (90) ***	112 (58)	143 (94) ***
δ IAP calcium-oxalate	1314 (611)	1753 (853) ***	1371 (652)	1518 (740)
ε IAP calcium-phosphate	12.5 (26.8)	19.0 (22.8)	13.2 (27.9)	16.1 (19.2)

Abbreviations: IAP, ion activity product; * $p < .05$; ** $p < .01$; *** $p < .001$

† , means are adjusted for patients' age, gender, body weight and urinary creatinine.

‡ , reference group for each abnormality is relatives of patients without the urinary abnormality in question.

γ , estimate of dietary protein intake from 24-hour urine is expressed as: [urea (mmol/L) x volume (L) x 0.18) +13] (46).

δ , estimate of ion activity product for calcium oxalate in 24-hour urine is expressed as: 1.9 x $calcium^{0.84}$ (mmol/L) x oxalate (mmol/L) x $citrate^{-0.22}$ (mmol/L) x $magnesium^{-0.12}$ (mmol/L) x $volume^{-1.03}$ (L) (47).

ε , estimate of ion activity product for calcium phosphate in 24-hour urine is expressed as: 0.0027 x $calcium^{1.07}$ (mmol/L) x $phosphate^{0.70}$ (mmol/L) x $(pH - 4.5)^{6.8}$ x $citrate^{-0.20}$ (mmol/L) x $volume^{-1.31}$ (L) (47).

Figure Legends

Figure 1.1. A flowchart summary of the recruitment process at the St. Michael's Hospital Kidney Stone Center. A total of 333 patients and families participated in the study.

Figure 1.2. Adjusted odds ratios † and 95 percent confidence intervals for (a) hypertension and (b) kidney stone disease in relatives of patients with hypercalciuria. * $p<.05$, ** $p<.01$;
reference group = normocalciuria; F = fathers; M = mothers; B = brothers; S = sisters;
Parents = middle bar between F and M; Siblings = middle bar between B and S;
† , adjusted for patients' age, gender, body mass index, antihypertensive medication use or personal history status, relative age, and other potential confounders (see Methods for details).

Figure 1.3. Quantile plots of (a) urinary Ca^{2+} to creatinine ratio*, (b) sodium to creatinine ratio*, and (c) uric acid to creatinine ratio* from the first void urine specimens in study patients, and in a separate sample of normotensive and hypertensive siblings of hypercalciuric patients. Dark lines indicate the distributions for main study participants.
* , values are adjusted for age, gender, BMI and creatinine excretion using GEE regression.
† , statistically significant vs. main study patients; ‡ , statistically significant vs. normotensive siblings.
O = main study participants; Δ = normotensive siblings of HC patients;
◊ = hypertensive siblings of HC patients.

Figure 1.1

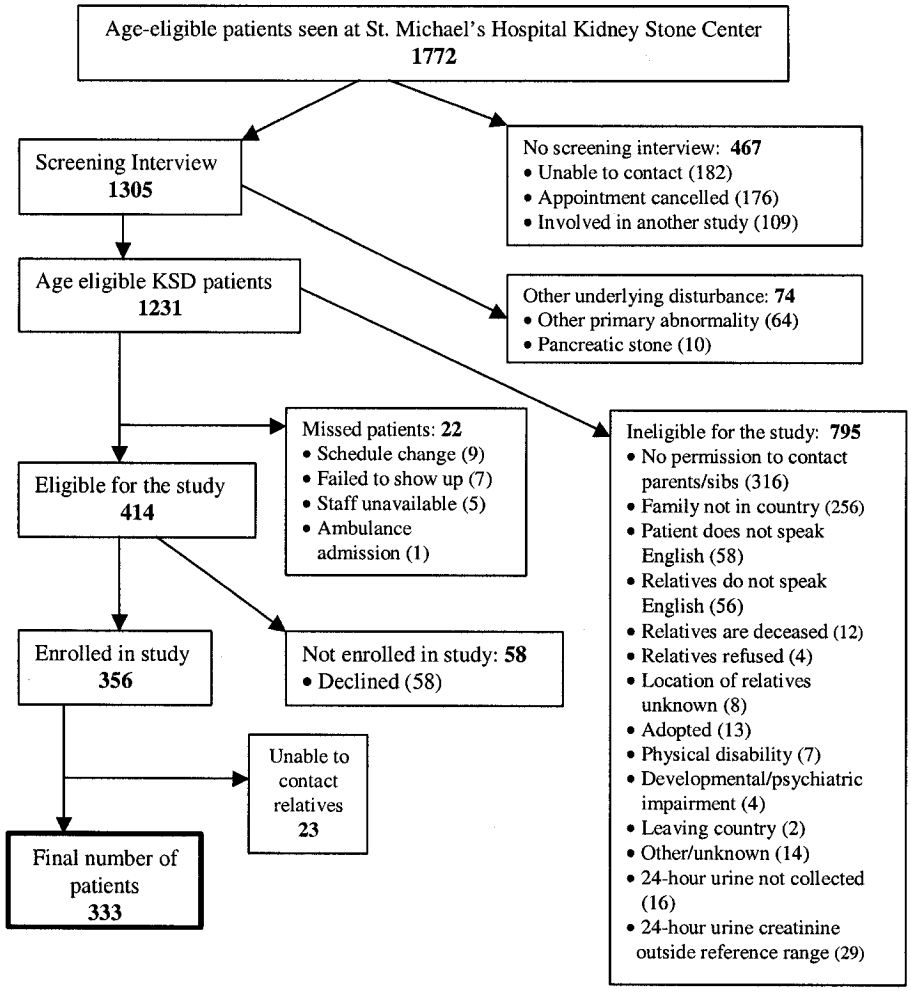

Age-eligible patients seen at St. Michael's Hospital Kidney Stone Center
1772
Screening Interview
1305
No screening interview: 467
• Unable to contact (182)
• Appointment cancelled (176)
• Involved in another study (109)
Age eligible KSD patients
1231
Other underlying disturbance: 74
• Other primary abnormality (64)
• Pancreatic stone (10)
Missed patients: 22
• Schedule change (9)
• Failed to show up (7)
• Staff unavailable (5)
• Ambulance admission (1)
Ineligible for the study: 795
• No permission to contact parents/sibs (316)
• Family not in country (256)
• Patient does not speak English (58)
• Relatives do not speak English (56)
• Relatives are deceased (12)
• Relatives refused (4)
• Location of relatives unknown (8)
• Adopted (13)
• Physical disability (7)
• Developmental/psychiatric impairment (4)
• Leaving country (2)
• Other/unknown (14)
• 24-hour urine not collected (16)
• 24-hour urine creatinine outside reference range (29)
Eligible for the study
414
Enrolled in study
356
Not enrolled in study: 58
• Declined (58)
Unable to contact relatives
23
Final number of patients
333

Figure 1.2a

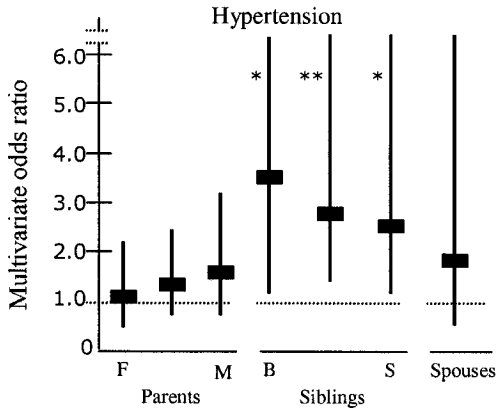


Figure 1.2b

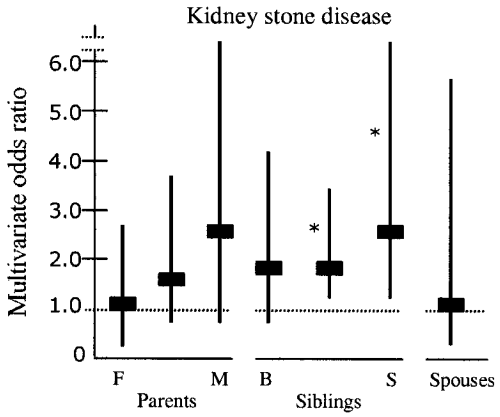


Figure 1.3a

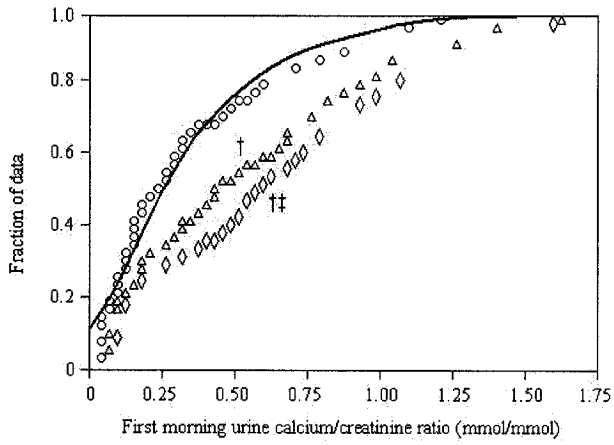


Figure 1.3b

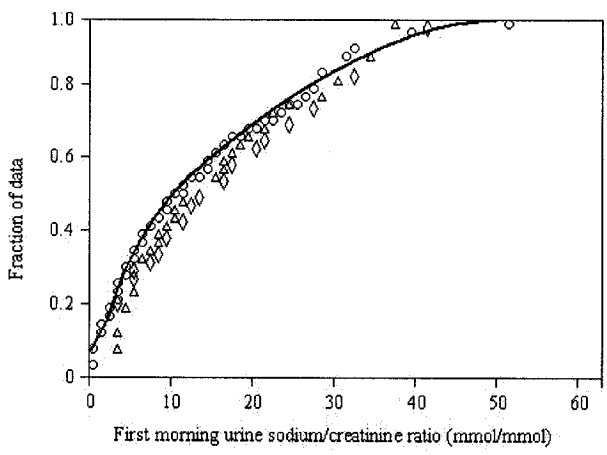
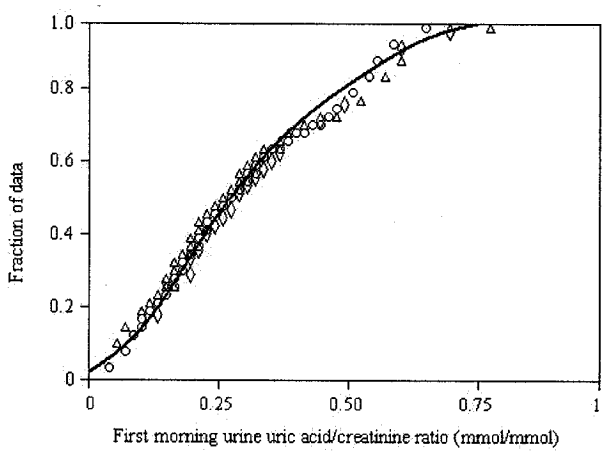


Figure 1.3c



Dietary potassium and familial risk of hypertension and kidney stone disease

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Word count: 3727; Number of references: 49; Number of tables: 3; Number of figures: 3

ABSTRACT

Previous familial aggregation studies suggested that the well-documented association of hypertension and kidney stone disease is related to an underlying disturbance in calcium metabolism. The cause of the calcium abnormalities, however, was not defined. This study was undertaken to assess the contribution of dietary factors that increase urinary calcium excretion. Consecutive patients with idiopathic calcium nephrolithiasis (n=328), aged 18-50 years, with and without hypercalciuria were stratified as normal or low urinary excretors of potassium or phosphorus, or normal or high excretors of sodium or urea. Familial clustering of hypertension and kidney stone disease was observed only in the first-degree relatives of hypercalciuric patients with low urinary potassium excretion (odds ratio [OR]=3.3, 1.5-7.4; OR=2.9, 1.1-7.3, respectively). Hypercalciuria in high urinary sodium excretors predicted kidney stone disease, but not hypertension in families. Urea and phosphate excretion were not associated with familial disease risk. Neither condition aggregated in spouses. The influence of genetic background was further assessed by comparing fasting urine results of consecutive patients with those of siblings of hypercalciuric patients from another sample. After adjusting for differences in urinary potassium and other potential confounders, the mean calcium/creatinine ratio was significantly higher in siblings of hypercalciuric patients (0.44±0.28 vs. 0.23±0.15 mmol/mmol, $p<0.0001$), and in hypertensive than normotensive siblings. Our results indicate that a low potassium diet contributes to, but does not fully account for, familial aggregation of hypertension and kidney stone disease in patients with idiopathic calcium nephrolithiasis and hypercalciuria. The disturbance in calcium metabolism appears to have a strong genetic component.

Keywords: Dietary Potassium; Hypercalciuria; Nephrolithiasis; Hypertension; Familial Aggregation

INTRODUCTION

The association of hypertension and kidney stone disease (1, 2) raises the possibility that susceptible individuals share common genetic variants, which have modest effects and are influenced by the same environmental factors. To date, efforts to identify the genes that confer susceptibility and influence disease expression to these conditions have yielded few positive results (3, 4). It has been suggested that better understanding of the interaction between genetic and environmental factors may facilitate the search for disease alleles involved in their pathogenesis (5).

Previously we postulated that disturbances in calcium metabolism play a central role in the pathogenesis of hypertension and kidney stone disease with hypercalciuria being a principal manifestation (6). One way to identify common etiologic and pathogenetic mechanisms among different diseases is to determine whether the disorders occur more frequently within certain families (7). This approach also permits investigators to discriminate between genetic and environmental factors that may contribute to familial clustering. In a recent study we reported that hypertension and kidney stone disease aggregated in families of patients with idiopathic nephrolithiasis and hypercalciuria. No significant aggregation was observed in spouses (8). While the findings of our study suggested a genetic basis for the disturbance in calcium metabolism, an environmental effect on urinary calcium excretion could not be fully excluded.

It is well known that several dietary factors increase urinary calcium excretion including high intake of sodium and animal protein and low potassium and phosphate diets (9). Because members of the family share not only genetic information but also tend to adopt similar ways of eating (10), it is important to determine whether nutritional habits may contribute to the familial occurrence of hypertension and kidney stone disease. This study was undertaken to determine

the role of dietary elements known to affect urinary calcium excretion on the familial aggregation of these conditions. Because obesity, excessive weight gain and type 2 diabetes mellitus are strongly associated with hypertension and kidney stone disease (11, 12), we also assessed whether these disorders occurred more frequently in families with abnormal calcium metabolism.

MATERIALS AND METHODS

Selection of Participants and Assessment

Consecutive patients with idiopathic calcium nephrolithiasis (CN), aged 18 to 50 years, attending the St. Michael's Hospital's Kidney Stone Center between February 2002 to March 2004 were eligible for recruitment. The Center contains a lithotripsy unit that serves the regional needs of approximately six million people in the Greater Toronto Area. Patients are referred to the Centre for extracorporeal shockwave lithotripsy and post-treatment care. Patients with acute manifestations of kidney stone disease such as renal colic were excluded. The Hospital's Research Ethics Board approved the study prior to its initiation.

Patients were contacted by telephone before their scheduled lithotripsy appointment to describe the nature of the study and determine their willingness to participate and to allow the study staff to contact their family members. Reasons for non-participation were documented. Potential participants were asked to collect a single 24-hour urine specimen, starting the morning prior to their lithotripsy treatment, and were not provided with any specific dietary instruction. They were advised to fast overnight before their procedure. At the Center, patients, after giving informed consent, were interviewed to collect personal and family information about sociodemographic characteristics and family composition, personal and family history of hypertension, diabetes mellitus and kidney stone disease, and weight and height estimates in first-degree relatives (excluding offspring) and spouses. Weight (measured in a light hospital gown) and height (without shoes) were measured and used to calculate BMI. Sitting blood pressure (average of two readings using a mercury sphygmomanometer) was assessed in a standard manner (13). A fasting urine specimen was collected and routine peripheral venous blood samples were drawn.

With their consent, relatives and spouse were interviewed by telephone to collect information about the presence of hypertension, kidney stone disease and diabetes, their current height and weight, and their weight five years ago. Weight change was calculated by subtracting their current weight from their weight of five years previously. Both the interviewer and the family member were unaware of the results of the patients' urinary analysis at the time of the interview.

Classification of Urinary Variables

A standard definition of hypercalciuria was used (14, 15). Using the urinary values in the Kidney Stone Center's database of over 2000 consecutive age-eligible patients, the lowest or highest quartiles of age and gender specific distribution of urinary potassium, sodium, urea and phosphate were determined. Patients were further classified into 4 categories for each element based on high or normal 24-hour urinary calcium. Urinary measures were used as indicators of dietary intake.

Classification of Disease Status

Hypertension was defined as being treated with antihypertensive medications to lower blood pressure (16) and type 2 diabetes mellitus, as being treated with oral hypoglycemic agents at any age or with insulin after the age of 40 years (17). Individuals with a BMI of 30 kg/m^2 or higher were classified as obese (18). Weight gain was arbitrarily considered excessive if the change exceeded the 75^{th} percentile of the gender-specific 5-year weight change distribution, as there is no standard definition (19). For men, this was > 5.1 kg and for women > 6.8 kg. These restrictive definitions were employed to increase specificity, as recommended for aggregation

studies (7).

Biochemical Procedures

All 24-hour urine collection bottles contained thymol crystals, dissolved in isopropanol as a preservative. Urine specimens were considered to have been collected properly when the creatinine value was in the daily reference range of 8.8 to 22 mmol for men and 4.5 to 16 mmol for women, and patients with a value outside the range were excluded from the study. The concentration of calcium, potassium and creatinine was measured in the fasting urine samples and the results were expressed as mmol per mmol creatinine. All biochemical parameters were analyzed on commercially available analyzers.

Statistical Analyses

Based on odds ratios previously reported in studies of intermediate phenotypes to assess genetic susceptibility (20, 21), the power to detect an odds ratio of 3 was estimated to be greater than 80% with a sample size of 308, assuming a two-sided alpha of 0.05 (two-sided). In making this calculation, we estimated that 25% of kidney stone patients would have hypercalciuria (20, 21) and that 25% of first-degree relatives would have hypertension (22).

Mean values (±SD) for continuous variables and percentages for categorical variables were computed. Generalized estimating equations (GEE) were used to test for familial aggregation of disease among urinary groups as measured by a log odds ratio (23), with the subgroup with normal urinary values acting as the reference group. The primary exposure variable was the urinary status of the patient, while the binary outcome variable was the disease status of an individual family member. The covariates were patient age, gender, BMI, personal

history of disease, use of antihypertensive drugs, and relative age (21). Other potential confounders included patient ethnicity, marital status, education, area of residence (inside/outside city), country of birth (inside/outside Canada), smoking status, use of calcium/vitamin D products and, where appropriate, urinary potassium, sodium and urea. These factors were included in the analytic models if singly they changed the point estimate by 10% (24). Regression parameter estimates were computed for first-degree relatives, individual relative categories and spouses. Likelihood ratio tests were conducted to test for interactions between the urinary predictors. PROC GENMOD with a REPEATED statement in SAS software version 8.1 (SAS Institute Inc, Cary, North Carolina) was used for the GEE regression analyses (25).

Study of Genetic Background

The contribution of genetic background to hypertension and kidney stone disease was further tested by comparing urinary potassium and calcium values as ratios with creatinine in fasting urines from consecutive patients and siblings of hypercalciuric patients. The siblings were enrolled in another study that recruited 75 CN patients with hypercalciuria, aged 18 to 50 years, using the same protocol as that employed in the current study (26). With their consent, siblings were interviewed by telephone to collect information on demographic characteristics, the presence of hypertension and kidney stone disease, the names of all prescribed medications and supplementary health products, and their current weight and height. Individuals taking multivitamins were excluded. A urine bottle and instructions to collect a fasting urine sample were mailed to the eligible subjects. A total of 108 siblings (55 brothers and 53 sisters) returned the urine sample by an overnight courier delivery service in a pre-paid self-addressed envelope.

GEE regression was used in these analyses, while adjusting for age, gender, BMI, and potentially confounding urinary measures (sodium, uric acid and, where appropriate, potassium). Quantile plots were also performed to evaluate familial aggregation of dietary habits.

RESULTS

Response Rate

The flowchart summary of the recruitment process at the Kidney Stone Center was previously reported (8). In brief, 409 patients met the eligibility criteria and of these, 351 were enrolled in the study representing a participation rate of 86%. We were unable to contact the first-degree relatives of 23 patients who agreed to participate, leaving a final total of 328 study subjects. There were no significant differences in age, gender, ethnic origin, marital status, education, BMI and blood pressure level between participants and those who declined or whose first-degree relatives could not be contacted. We obtained health information on 335 parents, 465 siblings and 190 spouses.

Baseline Characteristics

Study patients were younger (mean age = 38.9 years), more likely to be male (52.4%), and predominantly of European origin (81.4%), residents of the Greater Toronto Area (82.3%), married (66.8%), completed high school (67.1%), non-smokers (71%), and non-users of calcium or vitamin D supplements (63.7%). A majority also had a previous kidney stone (59.9%). The mean BMI was 27.5 ± 5.5 kg/m^2, 26.0% were obese, 13.6% had treated hypertension, and 4.3% had treated diabetes.

Disease Aggregation in First-Degree Relatives by Urinary Status

Multivariate analyses revealed that the first-degree relatives of low urinary potassium excretors had an excess frequency of obesity (OR = 1.8, 95% confidence interval [CI]: 1.2-2.7) and excessive weight gain (OR = 1.7, 95% CI: 1.2-2.4) compared to those of normal potassium

excretors (Table 2.1). No significant associations were observed for hypertension, kidney stone disease or diabetes. High urinary sodium was associated with an excess frequency of diabetes in first-degree relatives, but no other conditions (Table 2.1). High urinary urea and low urinary phosphate were not associated with increased familial risk of disease.

Disease Aggregation in First-Degree Relatives by Urinary Calcium Status

In multivariate analyses using the normal excretion group as the reference, there were significant associations between hypercalciuria and low urinary potassium excretion in patients and the presence of hypertension (OR = 3.3, 95% CI: 1.5-7.4), kidney stone disease (OR = 2.9, 95% CI: 1.1-7.3) and excessive weight gain (OR = 3.4, 95% CI: 1.7-6.8) in first-degree relatives (Table 2.2). The presence of hypercalciuria combined with high urinary sodium was associated with kidney stone disease in first-degree relatives (OR = 2.2, 95% CI: 1.1-4.3), but not with hypertension or other conditions. The combined effect of hypercalciuria and high urinary urea or low urinary phosphate was not associated with increased familial risk of disease.

Disease Aggregation by Relative Type

Multivariate analyses showed that siblings of patients with the combined abnormality of hypercalciuria and low urinary potassium were more likely to have hypertension (OR = 5.3, 95% CI: 2.1-13.4), obesity (OR = 3.1, 95% CI: 1.5-6.7), excessive weight gain (OR = 4.4, 95% CI: 1.8-10.7) and diabetes (OR = 4.1, 95% CI: 1.1-15.6) compared to siblings of patients with normal calcium and potassium excretion. The combined abnormality group was also more likely to have parents who reported the presence of kidney stone disease (OR = 7.9, 95% CI: 2.5-24.8).

No conditions aggregated in spouses nor in relatives of patients with high calcium/normal potassium or normal calcium/low potassium excretion.

The siblings of patients with hypercalciuria combined with high urinary sodium were more likely to have kidney stone disease (OR = 2.5, 95% CI: 1.1-6.0) compared to siblings of patients with normal calcium and sodium excretion. No other condition aggregated in relatives of these patients, nor in hypercalciuric patients with high urea or low phosphate excretion.

Characteristics of Family Members by Urinary Potassium and Calcium Status

Table 2.3 shows the characteristics of first-degree relatives of stone patients by potassium and calcium excretion status. The first-degree relatives of low potassium excretors were younger and had a significantly higher adjusted BMI and prevalence of obesity and excessive weight gain compared to the relatives of normal potassium excretors. Compared to first-degree relatives of patients with normal calcium and potassium excretion, the relatives of patients with hypercalciuria and low urinary potassium had a significantly greater BMI and prevalence of hypertension, kidney stone disease, obesity and excessive weight gain.

Study of Genetic Background

The quantile plot of the fasting urinary potassium/creatinine ratio in consecutive CN patients differed from that in the siblings of CN patients with hypercalciuria (Figure 2.1a). The adjusted mean value in the siblings (Figure 2.1b) was significantly lower than that in the consecutive CN patients (3.9 ± 2.0 vs. 5.6 ± 2.6 mmol/mmol, $p<.001$). Consecutive CN patients (n=77) with a low urinary potassium had a significantly higher calcium/creatinine ratio compared to patients

(n=251) with normal urinary potassium (0.25 ± 0.11 vs. 0.20 ± 0.16 mmol/mmol, p=.009) (Figure 2.2).

Figure 2.3 displays the adjusted mean calcium/creatinine ratio of consecutive CN patients compared to siblings of patients with hypercalciuria. After adjusting for differences in urinary potassium and other potential confounders, the mean calcium/creatinine ratio was significantly higher in the siblings of the hypercalciuric patients than the unselected study patients (0.44 ± 0.28 vs. 0.23 ± 0.15 mmol/mmol, p<.0001).

In an analysis of siblings of patients with hypercalciuria, there was no significant difference in urinary potassium/creatinine ratio between hypertensive and normotensive siblings (3.9 ± 2.1 vs. 3.9 ± 1.8 mmol/mmol, p=.935), despite the significantly higher mean calcium/creatinine ratio in hypertensive siblings (0.55 ± 0.23 vs. 0.42 ± 0.27 mmol/mmol, p<05).

DISCUSSION

In this study familial aggregation of hypertension and kidney stone disease was observed in first-degree relatives of hypercalciuric patients with low urinary potassium excretion. The associations were independent of patients' age, gender, BMI, use of antihypertensive medications and relatives' age. Hypercalciuria in high urinary sodium excretors predicted kidney stone disease, but not hypertension in families. Urinary urea and phosphate reflecting the intake of other dietary elements were not associated with familial disease risk. Furthermore, in the absence of stratification by urinary calcium excretion, neither low potassium nor high sodium excretion in patients predicted familial risk of hypertension or kidney stone disease. Our findings suggest that in the presence of hypercalciuria, specific dietary patterns contribute to the familial expression of these conditions.

Dietary factors alone can account for familial aggregation. Mitchell et al (10) recently showed that eating habits aggregate in families. In our study, low urinary potassium excretion in patients, regardless of urinary calcium status, was associated with increased familial risk of obesity and excessive weight gain, and diabetes clustered in families of patients with high urinary sodium excretion. Evidence from observational studies and clinical trials has documented an inverse relationship between vegetable and fruit consumption, good sources of dietary potassium, and BMI and the prevalence of obesity (27-29). Moreover, a recent study showed that high sodium excretion is associated with an increased risk of incident diabetes (30). These results support the notion of clustering of eating habits in families and indicate the importance of applying dietary interventions to the whole family.

Familial aggregation can also be attributed to genetic factors interacting with environmental influences. There is considerable evidence to suggest a genetic basis for

hypercalciuria in hypertension and CN. Studies of calcium metabolism in normotensive offspring of hypertensive patients found several abnormalities including hypercalciuria (31, 32). Moreover, the heritability of hypercalciuria in families of patients with calcium-containing kidney stones is well documented (33, 34). We recently reported that hypercalciuria in CN patients was predictive of hypertension and kidney stone disease in first-degree relatives, but not spouses. Moreover, family members of CN patients with hypercalciuria had significantly higher urinary calcium levels than unselected patients with CN (8).

In this study, we assumed that urinary potassium largely reflects dietary potassium intake, based on findings from studies such as the DASH trial showing that urinary potassium excretion mirrored the level of potassium in the assigned diet determined by chemical analysis of the menus (35). It is well known that a low potassium diet increases urinary calcium excretion (35-38) and our results in consecutive patients are in agreement with this observation. However even after controlling for difference in urinary potassium excretion the adjusted urinary calcium/creatinine ratio in the siblings of patients with hypercalciuria was still significantly higher than that in the consecutive CN patients. Moreover, hypertensive siblings had a greater calciuric response than normotensive siblings with equivalent urinary potassium measures. Thus, a low potassium diet contributes to, but does not fully account for, familial aggregation of hypertension and kidney stone disease in patients with idiopathic calcium nephrolithiasis and hypercalciuria. The disturbance in calcium metabolism appears to have a strong genetic component.

Dietary potassium is strongly associated with hypertension and kidney stone disease (39, 40) and influences disease expression (35, 38). Akita et al demonstrated that the introduction of a diet rich in potassium-containing foods largely mitigated salt sensitivity in pre-hypertensive

and hypertensive subjects on a potassium deficient diet (41). In a randomized trial of hypercalciuric calcium-oxalate stone formers, a high quality diet containing potassium-rich foods such as fruits and vegetables significantly reduced kidney stone recurrence by 51% compared to a control diet (42). More recently, a clinical study in healthy adults found that the elimination of fruits and vegetables from the diet for two weeks significantly reduced the 24-hour excretion of urinary potassium by 62% and increased urinary calcium by 49% (43). Moreover, one-third of subjects with normal urinary calcium excretion had calcium levels definable as hypercalciuria after fruit-vegetable withdrawal (43). In our study, familial risk of hypertension and kidney stone disease in patients with hypercalciuria was apparent only when dietary potassium intake was low.

Our study provides new information about the relationship of urinary abnormalities and diabetes. While we previously reported no significant association between hypercalciuria and familial risk of diabetes (8), this study showed that hypercalciuria combined with a low potassium diet in patients was predictive of diabetes in siblings. Abnormalities in calcium metabolism have been reported in patients with type 2 diabetes (44-46), and lower dietary potassium intake is associated with an increased risk of incident diabetes (47). Our finding, however, while statistically significant, was based on a modest number of diabetic cases among relatives and needs to be replicated in a larger dataset to ensure that the findings were not due to chance.

An obstacle in the dissection of genetically complex traits is genetic heterogeneity, which substantially reduces power in identifying disease genes (5). Our observation of an increased familial risk of disease associated with hypercalciuria and low dietary potassium, but not hypercalciuria and a normal potassium diet, suggests that an assessment of dietary potassium

intake may help to identify a more homogenous subset of hypercalciuric patients with an increased propensity for disease. In further support for this view, an analysis of the baseline measurements showed that patients with hypercalciuria on a low potassium diet had a significantly greater BMI and prevalence of obesity, and a trend for a higher prevalence of hypertension, than hypercalciuric patients with normal potassium intake (data not shown). A wealth of evidence has shown that hypercalciuria is a multifactorial trait with both genetic and environmental determinants (33). By defining a more homogenous patient population, genetic studies would be more efficient in identifying a group of subjects with a common set of genetic abnormalities, and elucidating the underlying molecular mechanisms.

The study has several strengths. Firstly, our findings are relevant to a broad calcium-containing CN population because they were found in a study of consecutive stone formers under the age of 50 years who were referred to a population-based Kidney Stone Center. Secondly, patients were selected without knowledge of family history or biochemical findings and health information was gathered from relatives and spouses before the urine chemistry results were known. These safeguards eliminated the potential for selection and ascertainment bias. Thirdly, the data were collected prospectively and information from spouses allowed us to control for environmental effects. Lastly, health information for family members was obtained directly from relatives rather than relying on proxy reporting by probands, thereby reducing measurement error.

The study also has shortcomings. The prevalence of the conditions studied may be inaccurate as family members were not personally evaluated, nor were their physicians contacted to verify the information collected. However, errors in ascertainment of disease status were likely random and would dilute rather than magnify associations. Extrapolation of our findings

to a general kidney stone population must take into account that the study subjects were referred to a large regional center for extracorporeal shockwave lithotripsy and that the majority were Caucasian. A single 24-hour urine specimen was used to determine the urinary status of patients because repeated collections were generally not feasible. While this reduces the reliability of diagnostic classification (48), measurement error from a single collection would weaken associations and under-estimate true associations. Finally, it is possible that patients may have altered their diet in response to having renal colic or other acute problem caused by kidney stone disease. Nonetheless, patients in the acute phase of their condition were excluded from the study.

Drug treatment for hypertension can alter the urinary excretion of minerals (49) and 44 patients in our study were taking antihypertensive medications. In a sensitivity analysis that excluded these patients, the estimates for familial aggregation were unaltered.

In summary, a low potassium diet significantly predicted hypertension and kidney stone disease in first-degree relatives of CN patients with hypercalciuria, whereas no familial aggregation was found in those on a normal potassium diet. High urinary sodium combined with hypercalciuria predicted kidney stone disease, but not hypertension. Urinary urea and phosphate were not associated with familial risk. Familial disease clustering of these conditions was not solely related to dietary potassium. After adjusting for differences in urinary potassium and other potential confounders, the mean calcium/creatinine ratio was significantly higher in siblings of hypercalciuric patients than consecutive CN patients, and in hypertensive than normotensive siblings. These findings implicate an interaction between a presumptive inherited abnormality in calcium metabolism, manifested as hypercalciuria, and a low potassium diet, a lifestyle behavior.

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Table 2.1. Disease aggregation in first-degree relatives by patients' urinary potassium, sodium, urea and phosphate status.

	Patients' urinary group (OR [95% CI]) †§			
	Low potassium	High sodium	High urea	Low phosphate
Hypertension	1.1 (0.7-1.8) [1]	1.0 (0.7-1.5) [1]	1.0 (0.6-1.6) [1]	0.7 (0.5-1.2) [1]
Kidney stone disease	1.1 (0.7-1.9)	1.3 (0.8-2.2)	1.1 (0.7-1.8) [2]	0.8 (0.5-1.2)
Obesity	1.8 (1.2-2.7)* [3]	0.9 (0.6-1.4) [4]	1.1 (0.7-1.8) [4]	0.9 (0.6-1.6) [4]
Excessive weight gain	1.7 (1.2-2.4)* [3]	0.9 (0.6-1.3) [4]	1.1 (0.7-1.6) [4]	0.8 (0.5-1.2) [4]
Diabetes mellitus	1.1 (0.5-2.4) [5]	2.4 (1.3-4.5)* [6]	0.8 (0.4-1.7) [5]	1.2 (0.6-2.3) [5]

OR , odds ratio; C.I. , confidence interval.

* $p < .01$.

† , reference group is patients with normal levels of the urinary marker in question (eg, normal potassium).

§ , odds ratio and 95% confidence interval adjusted for patients' age, gender, body mass index, antihypertensive medication use or personal history status, and relative age (also adjusted for other potential confounders based on a 10% change in the point estimate).

[1] , also adjusted for patients' area of residence.

[2] , also adjusted for patients' smoking status.

[3] , also adjusted for patients' education.

[4] , also adjusted for patients' education and urinary potassium.

[5] , also adjusted for patients' ethnicity and urinary sodium.

[6] , also adjusted for patients' ethnicity.

Table 2.2. Disease aggregation in first-degree relatives of patients with hypercalciuria by their potassium, sodium, urea and phosphate status.

	Patients' urinary group (OR [95% CI]) †§			
	Low potassium	High sodium	High urea	Low phosphate
Hypertension	3.3 (1.5-7.4)** [1]	1.4 (0.7-2.8) [2]	1.2 (0.4-3.1) [2]	1.7 (0.8-3.7) [2]
Kidney stone disease	2.9 (1.1-7.3)*	2.2 (1.1-4.3)* [3]	1.9 (0.9-4.2) [4]	0.9 (0.4-2.1) [3]
Obesity	2.1 (0.9-4.8) [5]	0.7 (0.4-1.5) [5]	0.8 (0.3-2.4) [5]	1.1 (0.5-2.7) [5]
Excessive weight gain ψ	3.4 (1.7-6.8)*** [5]	1.1 (0.6-2.1) [6]	1.4 (0.7-2.7) [6]	0.9 (0.5-1.6) [6]
Diabetes mellitus	2.7 (0.7-10.5) [7]	2.4 (0.9-6.0) [7]	0.8 (0.2-3.5) [7]	1.6 (0.4-5.7) [7]

OR , odds ratio; C.I. , confidence interval.

* $p < .05$; ** $p < .01$; *** $p < .001$;

† , reference group is patients with normocalciuria and normal levels of the urinary marker in question (eg, normal calcium and potassium)..

§ , odds ratio and 95% confidence interval adjusted for patients' age, gender, body mass index, antihypertensive medication use or personal history status, and relative age (also adjusted for other potential confounders based on a 10% change in the point estimate).

[1] , also adjusted for patients' area of residence.

[2] , also adjusted for patients' area of residence and urinary potassium.

[3] , also adjusted for patients' urinary potassium.

[4] , also adjusted for patients' smoking status and urinary potassium.

[5] , also adjusted for patients' education.

[6] , also adjusted for patients' education and urinary potassium.

[7] , also adjusted for patients' ethnicity.

ψ , test for interaction between urinary calcium and potassium statistically significant by Wald test (p=.04).

Table 2.3. Characteristics of first-degree relatives of kidney stone patients by potassium (K^+) and calcium (Ca^{2+}) excretion status.

	Patients' urinary group		
	Normal K^+ (N = 608)	Low K^+ (N = 192)	Low K^+/ High Ca^2 (N = 35)
Mean age (years) ± SD	52.0 ± 14.6	49.1 ± 14.6 †	49.2 ± 13.8
Male gender	271 (44.6)	88 (45.8)	15 (42.9)
Current smoker	120 (19.8)	46 (24.0)	6 (17.1)
Mean BMI (kg/m^2) ± SD	26.4 ± 4.3	28.2 ± 5.9 †††	30.1 ± 6.8 ‡‡‡
Obesity	110 (18.4)	63 (33.0) †††	15 (42.9) ‡‡
Excessive weight gain	129 (21.9)	62 (32.6) ††	18 (51.4) ‡‡‡
Kidney stone disease	103 (17.1)	34 (17.9)	12 (35.3) ‡‡
Treated hypertension	157 (25.8)	56 (29.2)	17 (48.6) ‡
Treated diabetes	41 (6.8)	16 (8.3)	5 (14.3)

K , potassium; Ca^{2+} , calcium; SD , standard deviation.

† $p < .05$; †† $p < .01$; ††† $p < .001$ vs. normal K^+ group after adjusting for age and gender;

‡ $p < .05$; ‡‡ $p < .01$; ‡‡‡ $p < .001$ vs. normal K^+/normal Ca^{2+} group after adjusting for age and gender.

Figure Legends

Figure 2.1. (a) Quantile plot of potassium to creatinine ratio* from the fasting urine specimens in consecutive CN patients (○) and siblings of hypercalciuric patients (Δ). The dark line indicates the distribution for the consecutive CN patients. (b) Plot of mean ± SE urinary potassium to creatinine ratio* in consecutive CN patients (n=328) [□] and siblings of hypercalciuric patients (n=108) [■].

CN , idiopathic calcium nephrolithiasis; HC , hypercalciuria; SE , standard error.

* , value are adjusted for age, gender, BMI and creatinine excretion using general estimating equation regression.

† , statistically significant versus consecutive CN patients (p<.001).

Figure 2.2. Plot of mean ± SE urinary calcium to creatinine ratio values* from the fasting urine specimens in consecutive CN patients with normal (n=251) [□] and low (n=77) [■] urinary potassium.

SE , standard error; CN , idiopathic calcium nephrolithiasis.

* , values are adjusted for age, gender, BMI, urinary sodium and uric acid using GEE regression.

Figure 2.3. Plot of mean ± SE urinary calcium to creatinine ratio values from the fasting urine specimens in consecutive CN patients (n=328) [□] and siblings of hypercalciuric patients (n=108) [■] , after adjusting for urinary potassium and other potential confounders*.

SE , standard error; CN , idiopathic calcium nephrolithiasis; HC , hypercalciuria.

* , values are adjusted for age, gender, BMI, urinary potassium, sodium and uric acid using GEE regression.

Figure 2.1a.

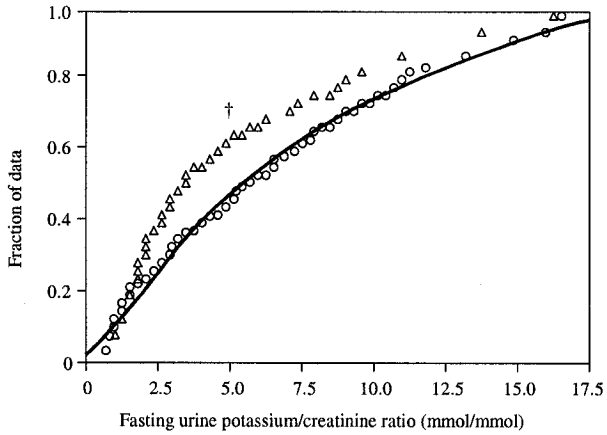

1.0
0.8
0.6
0.4
0.2
0
Fraction of data
0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
Fasting urine potassium/creatinine ratio (mmol/mmol)

Figure 2.1b.

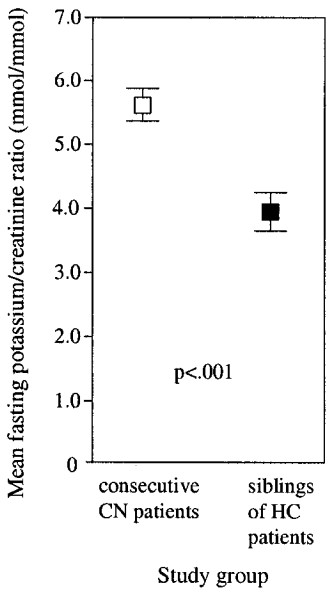
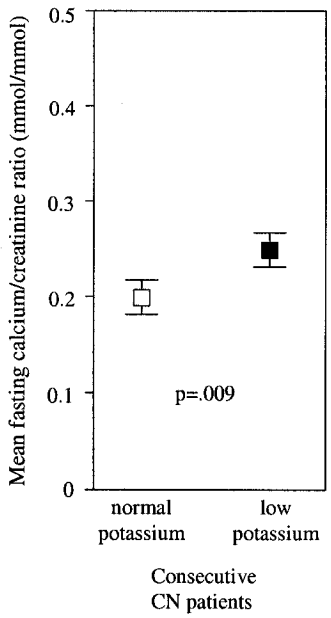

Mean fasting potassium/creatinine ratio (mmol/mmol)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
p<.001
consecutive CN patients
siblings of HC patients
Study group

Figure 2.2.


Mean fasting calcium/creatinine ratio (mmol/mmol)
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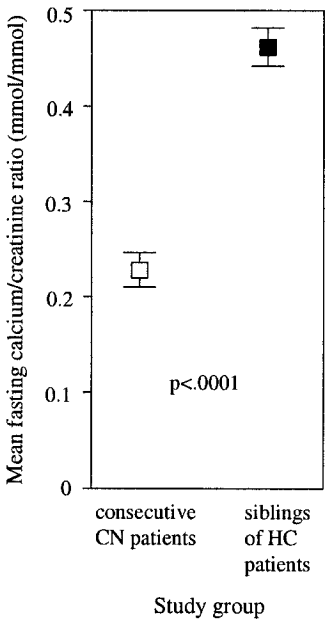
0.3
0.2
0.1
0
p=.009
normal potassium
low potassium
Consecutive CN patients

Figure 2.3.


Mean fasting calcium/creatinine ratio (mmol/mmol)
0.5
0.4
0.3
0.2
0.1
0
p<.0001
consecutive CN patients
siblings of HC patients
Study group

Impact of proxy reported health information on associations of familial aggregation

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ABSTRACT

Purpose: Familial aggregation studies often rely on proxy reports to ascertain affectation status, an insensitive method of identifying disease status in family members. This study examined whether the use of proxy reported information attenuates associations of disease aggregation in a study of 378 families of probands with kidney stone disease (KSD).

Methods: Consecutive KSD patients (n=378), aged 18-50 years, were recruited from a population-based Kidney Stone Center and asked to provide demographic and health information on several conditions (KSD, hypertension, diabetes, osteoporosis and obesity) for their parents, siblings and spouse. Relatives were subsequently contacted by telephone to report on these conditions. Familial aggregation was assessed using Generalized Estimating Equations.

Results: Proxy respondents reported lower prevalence rates of each condition compared to the reports from relatives. The parameter estimates for familial aggregation were consistently weaker when the affectation status of kin was ascertained from the proxy reports than from relatives, regardless of whether sensitive or stringent case definitions were employed. Negative predictive values were high for all conditions and in all relative categories. Positive predictive values were high for reports of KSD, hypertension and diabetes in parents and siblings, whereas positive likelihood ratios were strongest in spouses. Proband demographic variables were associated with errors in reporting.

Conclusions: The use of proxy reported health information from probands attenuates parameter estimates and may mask associations of disease aggregation in families. Our findings support the use of relative reports to ascertain disease status in familial aggregation studies, and have implications for assessing outcome variables in candidate gene or genome-wide association studies.

INTRODUCTION

Family health information is often used in studies of familial aggregation of disease. These studies are employed to determine whether a trait or disease occurs more frequently within certain families, and are considered a first step in the dissection of genetic traits (1). Due to cost and logistical issues, however, familial aggregation studies often rely on proxy reported information from probands to ascertain affectation status, an insensitive method of identifying disease status in family members (2-8). Misclassification of disease status resulting from random or nondifferential measurement error across exposure groups may lead to attenuated associations between exposure and disease (1). The extent of the attenuation of the effect size in familial aggregation studies that ascertain health information from proxy reports has not previously been evaluated.

We recently reported that hypertension and kidney stone disease aggregate in first-degree relatives of kidney stone patients with high urinary calcium excretion (hypercalciuria) (9). In that study, health information was determined directly from interviews with relatives to improve the accuracy of reporting. In contrast, previous studies used proband reports to assess disease status in stone patients enrolled from the same facility and found no significant aggregation of disease in relatives of these patients (10, 11). The discordant findings from these studies raise the possibility that measurement error from the proband reported information may attenuate the associations of familial aggregation.

The primary objective of this study was to examine the influence of proxy reported health information on the observed associations for familial aggregation. We hypothesized that the increased measurement error from the use of proband reports would substantially dilute the effects toward the null. The secondary objectives were: 1) to assess the accuracy of proxy

reported kidney stone disease and osteoporosis status, which to our knowledge, has never been reported previously; 2) to evaluate the accuracy of proxy reported information for hypertension, diabetes and osteoporosis using two different case definitions; and 3) to explore proband or family attributes (e.g., age, sex, ethnicity, family history status, proband-relative relationship) with the greatest impact on the accuracy of family reporting.

METHODS

Selection of Participants and Assessment

Consecutive patients, aged 18 to 50 years, attending the St. Michael's Hospital's Kidney Stone Center between February 2002 to March 2004 were eligible for recruitment. The Center, which contains one of only 3 shockwave lithotriptors in Ontario, serves the health needs of about six million people including the Greater Toronto Area community and may be considered a population-based treatment facility. The hospitals' Research Ethics Board approved the study.

Age-eligible patients were contacted by telephone before their scheduled lithotripsy appointment to describe the nature of the study and determine their willingness to participate and allow the study staff to contact family members. Reasons for non-participation were documented. Potential participants were asked to collect a single 24-hour urine specimen, starting the morning prior to their lithotripsy treatment. At the Center, patients, after giving informed consent, were interviewed to collect personal and family information about sociodemographic characteristics and family composition, personal and family history of hypertension, diabetes mellitus and KSD, and weight and height estimates in first-degree relatives (excluding offspring) and spouses. Weight and height were measured to calculate BMI.

With their consent, relatives and spouse were interviewed by telephone to collect information about the presence of hypertension, diabetes and KSD, and their current height and weight. The interviewer, proband and family member were each 'blind' as to the results of the probands' urinary analysis at the time of the interview. The interviewer and the family member were also unaware of the proband reported information. A subsample of these relatives (n=95) were randomly selected between 12 and 24 months later and telephoned by a different 'blinded' interviewer to assess reproducibility of relative reports.

Classification of Urinary Variables and Disease Status

Standard definitions of hypercalciuria (12, 13) and hyperuricosuria (14) were used. Family members were classified as having KSD if they answered 'yes' to the question, "Have you ever had a kidney stone?" (15). Physician diagnosed hypertension, diabetes, or osteoporosis was defined as a 'yes' response to the question, "Have you ever been told by a doctor that you have _____ (name of disease)?". Medically treated hypertension was defined as an affirmative response to the question, "Have you ever been prescribed a medication for high blood pressure?". Similarly, treated diabetes was defined as a 'yes' response to, "Have you ever been prescribed a medication for high blood sugar?". Those who were prescribed diabetic medications were asked to provide the name(s) of the medication(s) to determine whether they were given an oral hypoglycemic agent or insulin. Only three relatives were prescribed insulin before the age of 40 years, which indicates that almost all of the diabetic relatives had type 2 diabetes (16). Lastly, medically treated osteoporosis was defined as a 'yes' response to the question, "Have you ever been prescribed medication (e.g., a biphosphonate, hormone replacement therapy) or told to take calcium pills to treat thinning bones?". Relatives informed about calcium supplements for preventive purposes were not considered to have medically treated osteoporosis. Individuals with a BMI of 30 kg/m^2 or higher were classified as obese (17).

Statistical Analyses

Based on odds ratios reported in studies of intermediate phenotypes to assess genetic susceptibility (10, 11), power to detect an odds ratio of 3 was determined to be greater than 80% with a sample size of 308, assuming a=0.05 (two-sided). In making this calculation, we estimated that 25% of KSD patients would have hypercalciuria (10, 11) and that 25% of first-

degree relatives would have hypertension (18).

Generalized estimating equations (GEE) were used to test for familial aggregation of disease among urinary groups as measured by a log odds ratio (19). The primary exposure variable was the urinary status of the patient, while the binary outcome variable was the disease status of an individual family member. The covariates were patient age, gender, BMI, personal history of disease, use of antihypertensives and relative age (10). Other potential confounders included patient ethnicity, marital status, education, area of residence (inside/outside city), country of birth (inside/outside Canada), smoking status, and use of Ca^{2+}/vitamin D products. These factors were included in analytic models if singly they changed the point estimate by 10% (20).

The family health data that was gathered from family members was used as the criterion, or "gold standard", to validate the family health data that was obtained from probands (proxy respondents) on behalf of relatives. For the binary variables (hypertension, diabetes, obesity, osteoporosis, KSD), the sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios for each condition were computed. Similarly, for the continuous variables (weight, height and BMI), Pearson correlation coefficients were calculated. Test-retest reliability was evaluated using the kappa statistic or intraclass correlation coefficient where appropriate.

Several explanatory variables were evaluated to determine whether they affect the accuracy of proxy-reported family data. These variables included (1) characteristics of the proband (e.g., age, gender, ethnicity, marital status, education, smoking status, disease status), (2) family history of disease, and (3) the relationship of the family member to the proband (e.g., parent, sibling or spouse). Agreement (yes/no) between the proband and each relative was the

response variable. The odds ratio was used to quantify the association between the explanatory variable and the likelihood of disagreement. Analyses were conducted using GEE regression to account for within family clustering of responses (19).

PROC GENMOD with a REPEATED statement in SAS software version 8.1 (SAS Institute Inc, Cary, North Carolina) was used for the GEE regression analyses (21). PROC LOGISTIC with the CTABLE option in the MODEL statement was used to determine sensitivity, specificity and predictive values.

RESULTS

Response Rate

Figure 1 displays a flowchart summary of the recruitment process at the Kidney Stone Center. A total of 459 patients met our eligibility criteria and of these, 401 were enrolled in the study for a participation rate of 87%. We were unable to contact the first-degree relatives of 23 patients who agreed to participate, leaving a final total of 378 study subjects. There were no significant differences in age, gender, ethnic origin, marital status, education, BMI and blood pressure level between participants and those who declined or whose first-degree relatives could not be contacted. We obtained health information on 407 parents, 541 siblings and 222 spouses.

Proband Characteristics

Table 1 presents the characteristics of probands in the study. The mean age was 38.7 years. Males were slightly more represented than females and participants were predominantly of European ancestry and Toronto area residents. More than half (60.8%) of the patients were recurrent stone formers. The 'told by a doctor' definition of hypertension, diabetes and osteoporosis produced only slightly higher prevalence rates of disease than the 'prescribed medications' definition.

Relative Characteristics

Table 2 presents the proxy and self-reported health characteristics of relatives. Siblings and spouses were similar in age and gender distribution. Proxy respondents reported lower BMI and prevalence rates of each condition compared to the relative self-reports. Parents and siblings had a higher prevalence of KSD compared to spouses. Parents also displayed the highest

prevalence of hypertension and diabetes, followed by siblings and then by spouses, regardless of whether disease was defined by 'doctor diagnosis' or 'prescribed medication'. The prevalence of obesity was similar in siblings and spouses.

Example of Attenuated Associations from Measurement Error

Table 3 shows the adjusted odds ratios of disease aggregation in first-degree relatives of patients for both proxy and relative reported disease. The parameter estimates for familial aggregation were consistently of larger magnitude when the affectation status of kin was ascertained from the relative reports than from proxy respondents. With disease status determined from relative reports, both urinary abnormalities in patients were significantly associated with hypertension in first-degree relatives, and hypercalciuria alone was predictive of KSD. However, when disease status was determined from the proxy reports, none of the urinary phenotypes was significantly associated with hypertension in relatives. Moreover, while the association between hypercalciuria and KSD was statistically significant, the magnitude of the effect was smaller.

Accuracy of Reporting

Table 4 displays the accuracy of proband reported disease status in relatives as compared to relative self-reported disease status. Kappa values were highest for KSD and diabetes in parents and siblings, and hypertension in spouses. Negative history reports were reliable for all relative categories, with negative predictive values of 80% to 99% and negative likelihood ratios significantly less than 1.0.

Positive history reports showed more variability both within and between relative categories. Positive predictive values were high for reports of KSD, hypertension and diabetes in parents and siblings. For spouses, the positive predictive value of doctor-diagnosed hypertension reporting was also high, but predictive values for KSD and diabetes were relatively lower. However, the positive likelihood ratios for these conditions were higher in spouses than in parents and siblings.

The positive predictive value for proband reports of maternal osteoporosis (doctor-diagnosed) was only 71% (CI: 58%-84%). The positive likelihood ratio, however, was 7.0 (CI: 4.1-12.0), indicating that a positive proxy report was 7.0 times more likely to come from a proband with an osteoporotic mother than from a proxy with a mother free of the condition.

The more restrictive definitions of hypertension, diabetes and osteoporosis generally were associated with higher positive predictive values without compromising the accuracy of negative reports. Positive reports of hypertension in siblings and spouses, diabetes in spouses and osteoporosis in mothers were more in agreement with the relative reports when the 'prescribed medication' rather than the 'told by a doctor' definition of disease was used.

For reporting of BMI-derived obesity, the positive predictive value (92%, CI: 81%-103%) and positive likelihood ratio (47.6, CI: 11.8-193) were both high for spouses, but markedly lower for parents and siblings.

The specificity of proband reported information for each disease endpoint was high ($\geq$ 90%) in all relationship categories, whereas the sensitivity of proband reported information was generally lower. For KSD, the sensitivity of proband reporting was high for parents and siblings, but lower for spouses. The sensitivity of doctor diagnosed and medically treated diabetes was also high in parents but lower in siblings and spouses. In contrast, the sensitivity of both doctor

diagnosed and medically treated hypertension was higher in spouses than in parents and siblings. Reporting of BMI-derived obesity had poor sensitivity for all relative types, with values ranging from 55% to 64%. For maternal osteoporosis, the sensitivity of doctor diagnosed and medically treated disease was only 63% and 59%, respectively.

Table 5 presents the correlations between the proband reported age, weight, height, BMI and number of previous stone episodes in relatives compared to relative self-reported values. There was nearly perfect agreement of age reporting, particularly for siblings and spouses. There was good agreement in the reporting of height, weight and BMI. Weight and BMI showed stronger correlations for spouses than for parents and siblings. Information on number of previous stone episodes showed strong agreement in parents and siblings (r=.81 to .85), but was noticeably lower for spouses (r=.71).

Test-Retest Reliability of Relative Reports

Table 6 shows the kappa values and intraclass correlation coefficients for test re-test reliability of relative reports. Reports of KSD showed perfect reproducibility in all three relative categories. Reports of other conditions also showed very good consistency, but kappa values for diabetes reporting in siblings were lower, largely due to a number of new cases diagnosed since the first interview. Weight and height estimates also showed excellent reproducibility, whereas derived BMI showed less consistency in spouses than in parents or siblings.

Influence of Proband Characteristics

Table 7 illustrates the influence of proband characteristics on the accuracy of proband-reported health information for relatives. Under-reporting by probands was the primary reason

for the disagreements in reported disease status of relatives. Positive family history was a strong predictor of disagreement between probands and first-degree relative types for every condition, except for diabetes 'told by a doctor' in parents and siblings.

For parents, higher proband age was predictive of disagreement in the reporting of hypertension. Being unmarried predicted disagreement of obesity reporting. Lower education was associated with more disagreement for diabetes. Smoking was strongly related to disagreement in the reporting of obesity.

For mothers, female gender was associated with less disagreement in the reporting of hypertension. Non-European ethnicity was related to more disagreement in the reporting of KSD. Lower education was related to more disagreement in the reporting of obesity, but higher education predicted more disagreement in the reporting of osteoporosis. Being born outside Canada predicted disagreement on hypertensive status.

For siblings, smoking was associated with disagreement in the reporting of hypertension. Proband characteristics were not significantly associated with disagreement in reported health information for spouses.

DISCUSSION

This study is the first to examine the influence of proxy reported health information on observed associations for familial aggregation. The health status of family members was determined from both proxy respondents and directly from relatives before the results of the urine chemistries were known, so errors in ascertainment of disease status were likely to be random, which would dilute rather than magnify associations. The consecutive patients were recruited from a population-based treatment facility serving a large geographically defined area, thereby minimizing the likelihood of referral bias. The results showed that increased measurement error from the use of proxy reports led to weaker associations of familial aggregation for each urinary abnormality and for all of the clinical outcomes. This was most apparent for hypertension. Our findings suggest that interviewing family members directly would help to minimize measurement error in the assessment of disease status and produce stronger associations of disease aggregation in families.

Several investigators have suggested that the ability to detect familial aggregation of disease may largely depend on the accuracy of the health information obtained on family members (1). Previous studies that used proxy reports to assess disease status in relatives of patients found no significant association between hypercalciuria in kidney stone patients and hypertension in first-degree relatives (10, 11). Our study confirms these observations, as the use of proxy reports resulted in weaker and nonsignificant parameter estimates. In contrast, ascertainment of affectation status by relative reports revealed stronger and significant clustering of hypertension in relatives of patients with hypercalciuria alone or with hyperuricosuria, as we reported recently (9). Our data are also consistent with previous observations showing that proxy reporting is an insensitive approach for assessing hypertension status in parents and

siblings (2, 4, 8). This high degree of underreporting appears to play an important role in attenuating the associations of familial aggregation.

Despite the measurement error introduced by proxy reports in aggregation studies, our findings showed that proband reports have high predictive ability in parents, siblings and spouses. Negative history reports were reliable for all conditions and in all relative categories, as reported previously for hypertension and diabetes (3, 8). Positive predictive values were high for reports of KSD, hypertension and diabetes in parents and siblings, whereas positive likelihood ratios were strongest in spouses, an indication that the lower positive predictive values for spousal history may reflect the low prevalence of these conditions in spouses rather than primarily the low predictive value. Hastrup et al reported a lower positive predictive value for hypertension reporting in parents, possibly because of the lower prevalence of hypertension in these relatives compared to the parents of stone formers in our study (3). No previous study has used self-reported information from relatives as the criterion to validate the predictive ability of proband reported diabetes. From a clinical standpoint, our findings suggest that proband reporting for these conditions may be an important clinical tool to identify disease in relatives and to predict future risk in currently unaffected family members.

This study is also the first to report sensitivity and specificity measures of proxy reported KSD for family members, as we observed accurate reporting for all relative categories. Resnick et al reported frequency counts of false positives and negatives in first-degree relatives by stone forming probands, but did not examine accuracy in different relative types nor did they report the values for sensitivity, specificity or predictive accuracy (15). Using the frequency counts from the study by Resnick et al (15), we derived these values and found a sensitivity of 81%, a

specificity of 99%, a positive predictive value of 96% and a negative predictive value of 97%, which are similar to our findings.

Reporting of KSD for spouses showed high specificity, but lower sensitivity than reports for first-degree relatives. This finding might be due to an awareness that KSD is largely genetically determined and that probands were more likely to 'try' to recall KSD in blood relatives. Also, the kidney stone in some spouses may have occurred prior to meeting the proband, which may reduce the probands' awareness of stone history in spouses.

We also observed that probands are accurate in reporting the number of previous stone episodes in first-degree relatives and, to a lesser extent, in their spouse. To our knowledge, this information was never reported previously.

The accuracy of proxy reported osteoporosis among family members also was not reported previously. We found high negative predictive ability and specificity for osteoporosis reporting in mothers, but a lower positive predictive value and sensitivity. However, using a more restrictive definition of osteoporosis (eg, "taking medication") increased the positive predictive value from 70.8% to 82.9% and the positive likelihood ratio from 7.0 to 15.2. Our results show that negative reports of osteoporosis are highly accurate, and that positive reports may be improved by asking whether mothers take osteoporosis medication rather than whether they were told by a doctor to have osteoporosis. The prevalence of self-reported osteoporosis in mothers was 25%, which is the same as the prevalence rate in women aged >50 years in the general population. This finding indicates that women likely did not under-report their own osteoporosis.

Different case definitions may affect the accuracy of proxy reported family health information. Most validation studies that evaluated family history of hypertension and diabetes

ascertained whether the relatives had the condition or were ever informed by a doctor to have the condition, not whether they were prescribed medication for the condition. Nelson et al, however, reported that serious health events or chronic conditions that require daily medications to be accurately reported by proxy respondents (22). As with osteoporosis, we found that using a more specific definition of hypertension or diabetes, defined by being prescribed medication for each condition rather than being 'told by a doctor', increased both positive predictive ability and sensitivity without compromising the accuracy of negative reports, and the benefit was most apparent in siblings and spouses. However, even the use of this more stringent definition of hypertension or diabetes did not prevent substantial attenuation of the parameter estimates for familial aggregation, as mentioned earlier.

Reporting of BMI-derived obesity had a high positive predictive value and positive likelihood ratio in spouses, whereas both values were lower in parents and siblings. Sensitivity, however, was poor for all relative categories. Previous studies did not evaluate the accuracy of proxy reported obesity on behalf of relatives. The correlation between proxy reported and relative reported BMI (derived from reported weight and height) was also higher for spouses than first-degree relatives. Similarly, Epstein et al found good agreement of body mass index measures as derived from proxy and spousal reported weight and height (r=0.87) (23). Other studies did not examine this correlation in first-degree relatives. Reed and Price (24) found strong correlations between BMI measures derived from proxy reports and direct measurements of weight and height in first-degree relatives. We also found similar correlations in the reporting of height in first-degree relatives as in spouses, whereas a stronger correlation in the reporting of weight was found for spouses. Spouses may be more likely to weigh themselves or confide about each others weight than are parents and siblings.

Personal characteristics may affect the accuracy of proxy reported health information. The accuracy of hypertension reporting by probands for parents significantly declined as proband age increased. Bensen et al (4) made similar observations, but only for sibling reports. The oldest probands in our study may have been the least likely to share a household with parents, yet not old enough to have parents that require care. Additional caregiving would likely help to increase contact with parents and awareness of their health status, particularly if the health condition requires the use of medication or lifestyle changes.

Female probands were more likely to agree on the hypertension status of mothers than male probands. Similarly, Bensen et al (4) found that male respondents provided less accurate reports of the hypertensive status of family members than female respondents. Bochud et al (7) reported that twice as many males than females were unaware of the hypertensive status of siblings. Women are frequently the main caregiver in the family and therefore more familiar with the health status of family members.

Higher education was associated with greater accuracy in reporting of parental diabetes and obesity, but lower accuracy in reporting of osteoporosis in mothers. Higher education has been shown to be positively associated with healthier eating and a lower BMI (25). These individuals may be more keen or conscious of body weight issues. The under-reporting of osteoporosis in mothers by more educated probands was surprising, but suggests that affluent families may be selective about what health information is passed on to family members, particularly conditions related to women's health.

Probands who smoked reported obesity less accurately in parents and hypertension less accurately in siblings. Smoking status may be an indicator of health awareness (26), and smokers are more likely to be of lower socioeconomic status (27), which may be related to

reduced communication about health issues between family members. Non-Europeans reported maternal KSD less accurately, while Non-Canadian born respondents reported maternal hypertension less accurately. Possibly cultural factors are related to reduced communication about the mother's health information among family members.

A positive family history of KSD, hypertension, diabetes, obesity and osteoporosis was associated with disagreement in reported disease status of different first-degree relative types. In each case, the lack of agreement was due to under-reporting by probands. This finding is not surprising considering that proxy reports for each condition are lower in sensitivity than specificity.

This study has several limitations. The study was conducted in a single center in primarily Caucasian kidney stone patients. Thus, the results may only be applicable to this population. Moreover, information was not collected in non-stone formers. The family members were not personally evaluated, nor were their physicians contacted to verify the health information collected. However, past studies have shown that the accuracy of self-reported hypertension, diabetes, KSD, obesity and osteoporosis is reasonably high (28-31).

In summary, the use of proxy reported health information from probands results in attenuated parameter estimates and may mask associations of disease aggregation in families. These diluted effects occurred regardless of whether more sensitive or stringent case definitions were employed. Our findings support the use of relative reports to ascertain the disease status of family members in aggregation studies, and have implications for the assessment of outcome variables in candidate gene or genome-wide association studies.

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Table 3.1. Sociodemographic and health characteristics of probands.

	Number †	Percent †
Number of probands	378	100
Mean age ± SD	38.7	± 7.7
Male gender	201	53.2
European origin	310	82.0
Born in Canada	277	73.3
Toronto area resident	309	81.7
Married	250	66.1
≤ High school education	123	32.5
Unemployed	22	5.8
Income <$30000	32	14.3
Current smoker	107	28.3
Recurrent kidney stone	230	60.8
Mean BMI (kg/m^2) ± SD	27.7	± 5.6
Obesity	102	27.4
Hypertension		
told by doctor	71	18.8
prescribed medication	52	13.8
Diabetes		
told by doctor	22	5.8
prescribed medication	16	4.2
Osteoporosis		
told by doctor	6	1.6
prescribed medication	3	0.8

Abbreviations: SD, standard deviation; BMI , body mass index.

† , except where indicated otherwise.

Table 3.2. Proxy and self-reported health characteristics by relationship category.

	Parents (n=407)		**Siblings (n=541)**		**Spouses (n=222)**	
	Proxy reported	Self reported	Proxy reported	Self reported	Proxy reported	Self reported
Characteristic	number (%)†	number (%)†	number (%)†	number (%)†	number (%)†	number (%)†
Mean age (years) ± SD	64.3 ± 9.5	64.6 ± 9.5	41.3 ± 9.3	41.7 ± 9.4	40.6 ± 7.8	40.6 ± 7.6
Male gender	------	176 (43.2)	------	257 (47.5)	------	105 (47.3)
Kidney stone disease	74 (18.5)	75 (18.8)	83 (15.6)	92 (17.1)	7 (3.2)	7 (3.2)
Kidney stone episodes						
0	326 (81.7)	325 (81.3)	448 (84.5)	447 (83.1)	215 (96.9)	215 (96.9)
1-3	49 (12.4)	61 (15.3)	48 (9.1)	70 (13.0)	7 (3.2)	7 (3.2)
> 3	23 (5.9)	14 (3.5)	34 (6.4)	21 (3.9)	0 (0)	0 (0)
Mean BMI (kg/m^2) ± SD	26.7 ± 5.9	27.2 ± 4.7	25.3 ± 4.5	26.5 ± 4.8	25.2 ± 4.8	25.9 ± 4.6
Obesity (BMI>30)	61 (19.8)	96 (23.9)	57 (13.5)	104 (19.5)	25 (13.4)	41 (18.6)
Hypertension						
told by doctor	150 (42.3)	192 (47.2)	43 (8.7)	86 (15.9)	21 (9.6)	20 (9.0)
prescribed medication	118 (35.3)	181 (44.6)	30 (6.2)	67 (12.4)	14 (6.4)	14 (6.3)
Diabetes mellitus						
told by doctor	69 (17.0)	73 (17.9)	17 (3.1)	23 (4.3)	6 (2.7)	5 (2.3)
prescribed medication	50 (12.6)	55 (13.5)	15 (2.8)	20 (3.7)	4 (1.8)	4 (1.8)
Osteoporosis						
told by doctor	47 (12.5)	65 (16.1)	3 (0.6)	7 (1.3)	1 (0.5)	1 (0.5)
prescribed medication	33 (8.9)	61 (15.1)	1 (0.2)	6 (1.1)	1 (0.5)	1 (0.5)
Osteoporosis (mothers)						
told by doctor	45 (21.7)	60 (26.1)				
prescribed medication	33 (16.4)	58 (25.1)				

Abbreviations: SD, standard deviation; BMI , body mass index.

† , except where indicated otherwise.

Table 3.3. Impact of proband reported health information for first-degree relatives on associations of familial aggregation.

	High calcium excretion †		High calcium + high uric acid excretion ‡	
	proband reported disease	relative reported disease ϕ	proband reported disease	relative reported disease ϕ
	OR (95% C.I.) φ	OR (95% C.I.) φ	OR (95% C.I.) φ	OR (95% C.I.) φ
Hypertension	1.0 (0.6, 1.6) [1]	1.8 (1.1, 2.8)* [1]	1.2 (0.7, 2.1) [1]	2.5 (1.1, 5.4)* [1]
Kidney stone disease	1.5 (1.03, 2.3)* [2]	1.8 (1.1, 3.0)* [2]	1.2 (0.6, 2.3)	1.9 (0.9, 3.9)
Obesity	1.0 (0.6, 1.5) [3]	0.9 (0.6, 1.4) [3]	0.7 (0.4, 1.3) [3]	0.6 (0.3, 1.3) [3]
Diabetes mellitus	1.1 (0.6, 2.0) [4]	1.6 (0.7, 3.5) [4]	1.5 (0.7, 3.2) [5]	2.3 (0.9, 6.3) [5]

Abbreviations: OR , odds ratio; C.I. , confidence interval.

* $p < .05$;

† , reference group is relatives of patients with normal urinary calcium.

‡ , reference group is relatives of patients with normal urinary calcium and uric acid.

ϕ , reported in Mente et al (9).

φ , adjusted for patients' age, gender, body mass index, antihypertensive medication use or personal history status, and relative age.

[1] , also adjusted for patients' area of residence.

[2] , also adjusted for patients' smoking status.

[3] , also adjusted for patients' education.

[4] , also adjusted for patients' ethnicity and Ca^{2+}/vitamin D supplementation.

[5] , also adjusted for patients' ethnicity.

Table 3.4. Accuracy of proband reported disease status in relatives compared to relative self-reported disease status.

	No. of pairs	Kappa † (95% CI)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive predictive value, % (95% CI)	Negative predictive value, % (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Proband vs. Parents								
Kidney stone disease	400	.85 (.79-.92)	87 (78-93)	98 (95-99)	89 (82-96)	97 (95-99)	35.2 (17.6-70.1)	0.14 (0.08-0.24)
Obesity (BMI>30)	309	.55 (.43-.66)	59 (47-69)	93 (89-95)	72 (61-83)	88 (83-92)	8.1 (4.9-13.3)	0.45 (0.34-0.59)
Hypertension								
told by doctor	359	.66 (.58-.73)	76 (69-82)	90 (84-93)	88 (82-93)	80 (74-85)	7.4 (4.8-11.4)	0.27 (0.21-0.35)
prescribed medication	337	.68 (.60-.76)	73 (65-80)	94 (89-96)	90 (85-95)	82 (76-87)	11.5 (6.6-20.0)	0.29 (0.22-0.38)
Diabetes Mellitus								
told by doctor	407	.81 (.74-.89)	82 (72-89)	97 (95-99)	87 (79-95)	96 (94-98)	30.5 (15.9-58.6)	0.18 (0.11-0.30)
prescribed medication	396	.87 (.80-.95)	90 (78-96)	98 (96-99)	88 (79-97)	99 (97-100)	51.9 (23.4-115.4)	0.10 (0.05-0.24)
Osteoporosis (mothers)								
told by doctor	210	.56 (.43-.69)	63 (50-75)	91 (86-95)	71 (58-84)	88 (83-93)	7.0 (4.1-12.0)	0.41 (0.29-0.58)
prescribed medication	203	.61 (.48-.75)	59 (45-72)	96 (92-98)	83 (70-95)	88 (83-93)	15.2 (6.7-34.4)	0.43 (0.30-0.60)
Proband vs. Siblings								
Kidney stone disease	535	.84 (.77-.90)	83 (74-90)	98 (96-99)	89 (83-96)	97 (95-98)	41.2 (21.4-79.2)	0.17 (0.11-0.27)
Obesity (BMI>30)	430	.57 (.47-.67)	55 (44-65)	96 (93-98)	77 (66-88)	89 (86-93)	13.4 (7.8-23.2)	0.47 (0.38-0.60)
Hypertension								
told by doctor	495	.54 (.43-.65)	47 (36-59)	98 (96-99)	80 (68-91)	91 (89-94)	22.1 (11.1-44.1)	0.54 (0.43-0.67)
prescribed medication	484	.65 (.52-.77)	54 (40-67)	99 (98-100)	90 (79-100)	95 (93-97)	78.1 (24.6-248)	0.46 (0.34-0.63)
Diabetes Mellitus								
told by doctor	541	.84 (.72-.97)	74 (54-88)	100 (99-100)	100 (100-100)	99 (98-100)	infinity	0.26 (0.13-0.52)
prescribed medication	539	.91 (.80-1.0)	83 (61-94)	100 (99-100)	100 (100-100)	99 (99-100)	infinity	0.17 (0.06-0.47)
Proband vs. Spouse								
Kidney stone disease	223	.71 (.43-.98)	71 (36-92)	99 (97-100)	71 (38-105)	99 (98-100)	77.1 (18.0-331)	0.29 (0.09-0.93)
Obesity (BMI>30)	185	.71 (.57-.84)	64 (48-78)	99 (95-100)	92 (81-103)	92 (88-96)	47.6 (11.8-193)	0.37 (0.24-0.57)
Hypertension								
told by doctor	220	.81 (.68-.95)	85 (64-95)	98 (95-99)	81 (64-98)	99 (97-100)	42.5 (15.8-114)	0.15 (0.05-0.44)
prescribed medication	220	.92 (.82-1.0)	93 (69-99)	99 (97-100)	93 (79-106)	99.5 (99-100)	191 (26.9-1359)	0.07 (0.01-0.47)
Diabetes Mellitus								
told by doctor	222	.72 (.42-1.0)	80 (38-96)	99 (97-100)	67 (29-104)	99.5 (99-100)	86.8 (20.4-369)	0.20 (0.04-1.17)
prescribed medication	222	.75 (.41-1.0)	75 (30-95)	99 (98-100)	75 (33-117)	99.5 (99-100)	164 (21.4-1252)	0.25 (0.05-1.37)

†, all kappa statistics are significant at $p<0.0001$.

Table 3.5. Correlation coefficients* between the proband reported values in relatives compared to relative self-reported values.

Relative category	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Number of stone episodes†
Parent	0.97	0.80	0.76	0.60	0.85
Sibling	0.99	0.76	0.78	0.64	0.84
Spouse	0.99	0.82	0.88	0.79	0.71

Abbreviations: BMI , body mass index; * all correlations are statistically significant at p<.0001;

† , based on Spearman rank correlation coefficients, while all other correlations are based on Pearson coefficients.

Table 3.6. Test-retest reliability of self reported information from relatives. †

	Parents	**Siblings**	**Spouses**
Kidney stone disease	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)
Hypertension			
told by doctor	0.80 (0.60-1.01)	0.79 (0.57-1.01)	1.0 (1.0-1.0)
prescribed medication	0.81 (0.61-1.01)	0.82 (0.59-1.06)	1.0 (1.0-1.0)
Diabetes Mellitus			
told by doctor	0.89 (0.68-1.10)	0.65 (0.03-1.28)	1.0 (1.0-1.0)
prescribed medication	0.87 (0.62-1.12)	0.65 (0.03-1.28)	1.0 (1.0-1.0)
Osteoporosis (mothers)			
told by doctor	0.77 (0.34-1.20)	--------	--------
prescribed medication	0.77 (0.34-1.20)	--------	--------
Obesity (BMI>30)	0.82 (0.57-1.06)	0.78 (0.56-1.02)	0.77 (0.35-1.20)
Weight ‡	0.97 (0.94-0.98)	0.95 (0.91-0.97)	0.96 (0.90-0.98)
Height ‡	0.98 (0.96-0.99)	0.98 (0.97-0.99)	0.98 (0.96-0.99)
Body mass index ‡	0.96 (0.92-0.98)	0.91 (0.83-0.95)	0.80 (0.58-0.91)

† , all values are kappa statistics and 95% confidence interval unless indicated otherwise;

‡ , values are intraclass correlation coefficient and 95% confidence interval.

Table 3.7. The influence of proband characterictics on the accuracy of proband-reported health information for family members. The values are odds ratios that quantify the association between each proband characterictic and the likelihood of disagreement between the proband reported information as compared with the relative's own self-report.

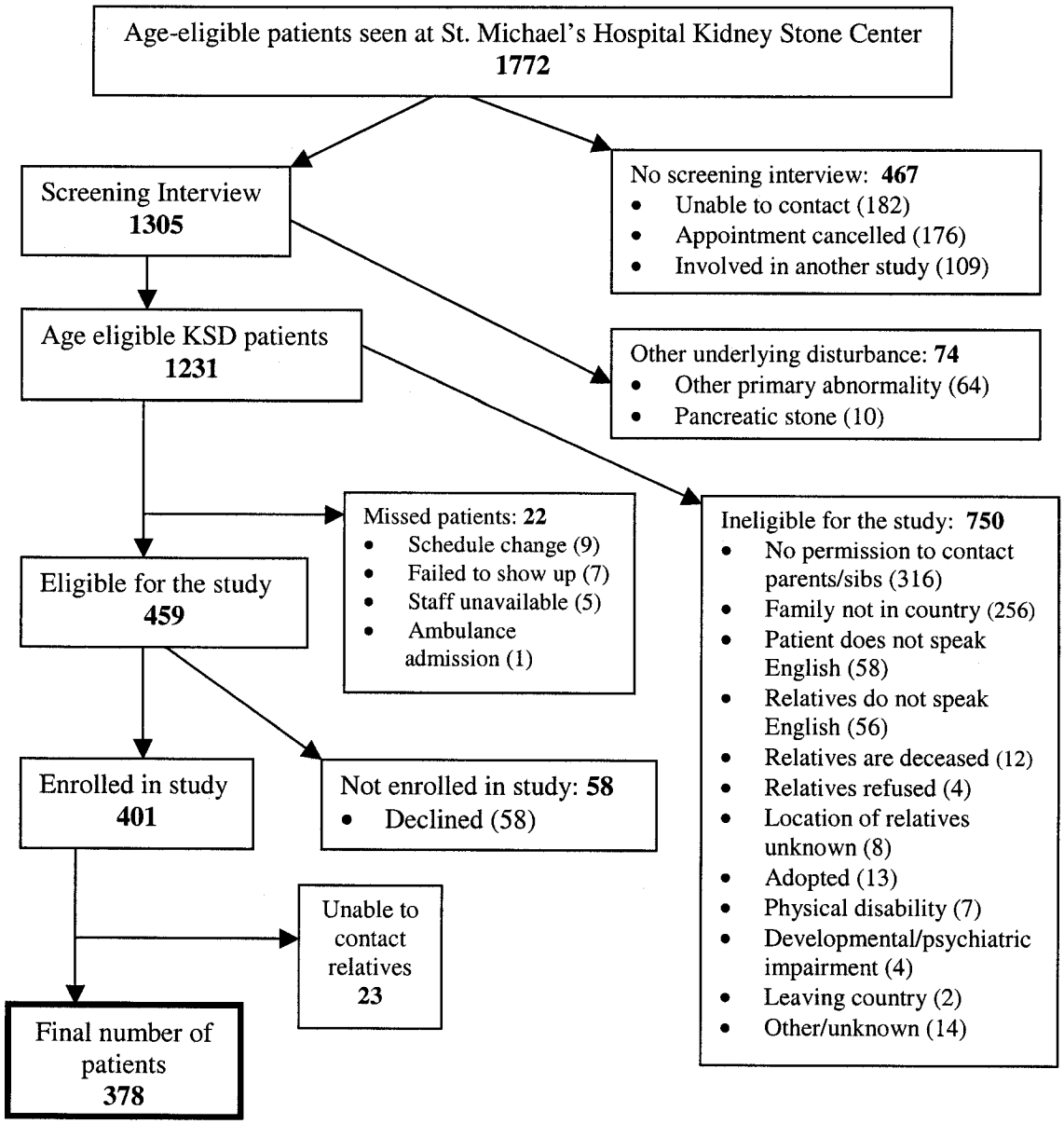
Proband Characterictics	Kidney stone disease	Obesity	Hypertension		Diabetes		Osteoporosis (mothers)	
			Told by doctor	Prescribed medication	Told by doctor	Prescribed medication	Told by doctor	Prescribed medication
Proband vs. Parents								
Age								
18-30	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
31-40	0.88	1.07	2.17φ	2.87φ	6.62φ	2.74	1.09	0.97
41-50	1.02	1.52	2.49*	5.13**	5.41	3.03	1.69	1.91
Presently unmarried	0.70	2.00*	0.80	0.74	1.53	3.24φ	1.03	1.20
Education								
≤ High school	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Post high-school or college degree	1.28	0.43	0.74	0.68	0.43	0.21*	4.22*	4.49*
> College degree	1.51	0.39	0.80	0.70	0.75	0.35	2.90	1.94
Current smoker	0.32	3.33***	1.28	1.00	1.00	0.60	1.78	1.32
Positive family history	3.06*	4.93***	2.25**	2.97***	1.49	4.11*	5.29***	16.33***
Proband vs. Mothers								
Female gender	0.42	0.83	0.26**	0.24**	0.26	-----	0.86	0.79
Non-European ethnicity	4.91*	0.62	1.63	1.88	0.74	6.96φ	0.84	0.51
Education								
≤ High school	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Post high-school or college degree	2.67	0.37*	0.97	0.64	0.49	-----	4.22*	4.49*
> College degree	3.29	0.19	1.26	0.96	1.57	-----	2.90	1.94
Born outside Canada	1.91	1.10	2.70*	2.78*	1.28	5.37	0.62	0.38
Positive family history	2.41	5.21***	2.59*	3.23*	2.28	3.46	5.29***	16.33***
Proband vs. Siblings								
Current smoker	0.91	1.67φ	2.58**	5.86***	2.18	4.33	0.30	-----
Positive family history	6.94***	3.91***	5.33***	8.16***	3.42	-----	8.28**	25.23**

* $p < .05$; ** $p < .01$; *** $p < .001$.

Figure Legends

Figure 3.1. A flowchart summary of the recruitment process at the St. Michael's Hospital Kidney Stone Center. A total of 378 probands and families participated in the validation study.

Figure 3.1.


Age-eligible patients seen at St. Michael's Hospital Kidney Stone Center
1772
Screening Interview
1305
No screening interview: 467
• Unable to contact (182)
• Appointment cancelled (176)
• Involved in another study (109)
Age eligible KSD patients
1231
Other underlying disturbance: 74
• Other primary abnormality (64)
• Pancreatic stone (10)
Missed patients: 22
• Schedule change (9)
• Failed to show up (7)
• Staff unavailable (5)
• Ambulance admission (1)
Ineligible for the study: 750
• No permission to contact parents/sibs (316)
• Family not in country (256)
• Patient does not speak English (58)
• Relatives do not speak English (56)
• Relatives are deceased (12)
• Relatives refused (4)
• Location of relatives unknown (8)
• Adopted (13)
• Physical disability (7)
• Developmental/psychiatric impairment (4)
• Leaving country (2)
• Other/unknown (14)
Eligible for the study
459
Enrolled in study
401
Not enrolled in study: 58
• Declined (58)
Unable to contact relatives
23
Final number of patients
378

Ethnic differences in risk of idiopathic calcium nephrolithiasis in North America

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ABSTRACT

Purpose: Data on the risk of kidney stone disease are sparse in individuals of non-European ancestry residing in North America. The objective of this study was to determine the risk of calcium nephrolithiasis among people of different ethnic backgrounds living in the same geographic region.

Materials and Methods: Using a cross-sectional design, consecutive patients with idiopathic calcium nephrolithiasis (n=1128), aged 18 to 50 years, were recruited from a population-based Kidney Stone Center in Toronto. Age- and gender-adjusted odds ratios (OR) and 95% confidence intervals were calculated by logistic regression using the 2001 Canada Census population data.

Results: Compared to Europeans, the risk of calcium nephrolithiasis was significantly higher in individuals of Arabic (OR=3.8, 2.7-5.2), West Indian (OR=2.5, 1.8-3.4), West Asian (OR=2.4, 1.7-3.4) and Latin American (OR=1.7, 1.2-2.4) origin and significantly lower in those of East Asian (OR=0.4, 0.3-0.5) and African (OR=0.7, 0.5-0.9) background. Several ethnic groups had kidney stone risk factors that were significantly different from the European group including higher urinary uric acid and urea excretion and estimated protein intake and lower urinary citrate, potassium, magnesium and phosphate excretion. None, however, explained the variation in stone risk overall.

Conclusions: Ethnic groups in North America differed markedly in their risk to develop calcium nephrolithiasis. This variability may reflect the influence of genetic susceptibility as there was no dominant environmental factor to account for the differences in stone risk.

INTRODUCTION

Kidney stone disease (KSD) is a common disorder with a lifetime prevalence of 15% in the United States and Europe.[1-2] Data on rates of kidney stones, however, are sparse for other populations and for individuals of non-European ancestry living in Western societies. Prevalence studies from Japan and in Asians living in the United States as well as in Black South Africans, African Americans and Hispanic Americans found lower risks of stones compared to non-Hispanic Caucasian populations.[2-5] On the other hand, KSD is highly prevalent in the Middle East.[1,6-8] Past studies did not distinguish among different types of stones nor, to our knowledge, examine the risk of KSD in stone formers from multiethnic backgrounds living in the same geographic region and exposed to the influences of a Western society.

The primary objective of this study was to determine whether there are differences in the risk of idiopathic calcium nephrolithiasis (CN) among ethnic minorities residing in North America compared to those of European origin attending a population-based Kidney Stone Center. Particular attention was given to defining the ancestral origin of all subjects, a limitation of previous studies of minorities living in Western societies. The secondary objective was to examine whether there are ethnic differences in demographic characteristics, clinical measures, urine volume, and urine and stone composition.

MATERIALS AND METHODS

Selection of Participants and Assessment

Patients with idiopathic CN, aged 18 to 50 years, were recruited consecutively from the St. Michael's Hospital's Kidney Stone Center. The Center, which contains one of only 3 shockwave lithotriptors in Ontario, is a regional treatment facility serving principally the Greater Toronto Area (GTA). The hospitals' Research Ethics Board approved the study.

Patients with GTA telephone area codes were contacted before their scheduled lithotripsy appointment to describe the nature of the study, determine their willingness to participate in a separate family study, and obtain information on sociodemographic characteristics including ethnic origin. All attendees of the Center are asked to collect a single 24-hour urine specimen. At the Center, nursing staff routinely measure weight, height, and sitting blood pressure. The weight and height measures were used to calculate BMI. Obesity rates were not computed due to the lack of a universally accepted BMI cutoff point for each ethnic minority group. Routine peripheral venous blood samples were also drawn.

Classification of Ethnic Origin

The ethnic origin of patients was determined jointly from information gathered on self-reported ethnicity, country of birth and surname.[9] We excluded individuals with a mixed ethnic background. Standard ethnicity categories used in the National Census by Statistics Canada were employed. The ethnic groups consisted of East Asians (Chinese, Taiwanese, Japanese, Koreans), Southeast Asians (Vietnamese, Malays, Indonesians, Burmese), Filipinos, South Asians (Indians, Pakistanis, Bangladeshis, Sri Lankans), non-African West Indians (Trinidad, Guyana), West Asians (Turks, Iranians, Afghanis, Israelis), Arabs (Egyptians, Syrians,

Jordanians, Lebanese, Iraqis, Saudi Arabians), Africans, Latin Americans and Europeans (Caucasians).

Biochemical Procedures

A single 24-hour urine sample was collected and patients with a urinary creatinine value outside the daily reference range were excluded (8.8-22 mmol for men and 4.5-16 mmol for women). The intake of dietary protein was estimated from the 24-hour urine specimens using the formula by Mitch et al.[10] Estimated ion activity product for calcium oxalate and calcium phosphate in 24-hour urine were each determined by the formula of Fine et al.[11]

Statistical Analyses

Mean values (±SD) for continuous variables and percentages for categorical variables were computed. Analysis of covariance was used to compare ethnic groups on continuous variables while adjusting for age, gender and BMI where appropriate. Post hoc pairwise comparisons were made using Tukey's approach to adjust for multiple comparisons. Chi-square contingency tables were used to compare ethnic groups on categorical variables. The ethnic specific rates of CN were computed by using the number of calcareous stone patients as the numerator and the population total for the 18 to 50 years age group in the GTA (as determined from the 2001 Canada Census) as the denominator. Age- and gender-specific population denominators were also ascertained to adjust for these covariates. The adjusted odds ratios (95% confidence intervals) for CN across ethnic groups were computed using logistic regression, with European ancestry as the reference category.

SAS software version 8.1 (SAS Institute Inc, Cary, North Carolina) was used for the statistical analyses.

RESULTS

Participant Selection

There were 1375 age- and region-eligible patients attending the Kidney Stone Center during the recruitment period, and 1128 met all eligibility criteria (Figure 1). Of these 850 had urinary data, 838 blood results and 516 stone analyses. There were no significant differences in age, gender, ethnic origin, marital status, education, BMI and blood pressure level between participants with biochemical or stone information and those with no results.

Baseline Characteristics

The sociodemographic and clinical characteristics of patients by ethnic group are presented in Table 1. Compared to Europeans, patients of West Asian origin were more likely to be male, while patients of African and West Indian descent were less likely to be educated beyond high school. South Asians were more likely to be married than Europeans, whereas Africans were less likely. Europeans had a significantly higher adjusted BMI compared to Africans, East Asians, South Asians, West Indians, Filipinos and Southeast Asians. Patients of Arabic origin had a significantly lower systolic and diastolic blood pressure compared to Europeans.

Ethnic Differences in Calcium Nephrolithiasis Risk

Figure 2 presents the age- and gender-adjusted odds ratios and 95% confidence intervals of calcareous stones for each of the ethnic groups compared to Europeans. Individuals of Arabic, West Indian, West Asian and Latin American ancestry were significantly more likely to have CN, whereas ethnic East Asians and Africans had a significantly lower risk. The odds

ratios of South Asians, Filipinos and Southeast Asians were similar to that of European Canadians.

Urine and Serum Comparisons

Several ethnic groups had urinary kidney stone risk factors, which differed significantly from the European group (Table 2). West Indians, for example, had a lower urinary excretion of citrate, potassium and magnesium that might explain their increased risk for CN. Offsetting this risk was a significant decrease in urinary calcium excretion. East Asians, despite a lower risk of CN, had an increased urinary excretion of uric acid and higher estimated protein intake. Their urine volumes, however, were significantly higher than Europeans, a factor that would reduce the risk. Urinary excretion values for the groups of African, West Asian and Arabic origin did not differ from the European group, even though their stone risk was significantly different than that of Europeans.

There were few serum values that differed between Europeans and the other ethnic groups (detailed information available from authors). Compared to European Canadians, West Asians and Arabs had a lower mean serum creatinine, and Africans, Latin Americans, South Asians and Arabs had significantly lower serum urea.

Stone Composition

The distribution of stone composition in the CN patients by ethnicity are displayed in Table 3. There were no significant differences between Europeans and other ethnic groups in their stone composition. Africans, South Asians and West Asians had a greater proportion of patients with calcium oxalate stones, whereas East Asians and Latin Americans displayed a

greater propensity for calcium oxalate combined with calcium phosphate/apatite stones. Europeans, West Indians, Filipinos, Southeast Asians and Arabic patients had approximately an equal number of each stone type.

DISCUSSION

This is the first study to examine prospectively the risk of idiopathic CN in stone formers from multiethnic backgrounds living in the same geographic area and exposed to the influences of a Western society. Previous studies on ethnic minorities came from the United States, were based on self-reported history of kidney stones or retrospective review of stone formers and focused primarily on African- and Mexican-Americans.[2,4,12] In this study, consecutive patients were recruited from a population-based treatment facility serving the entire Metropolitan GTA, thereby minimizing the likelihood of referral bias. Restricting the admission criteria to younger stone formers reduced the possibility of differential immigration patterns among ethnic groups and likely increased the probability that differences in genetic background may influence the propensity for renal stone formation.

The results revealed that individuals of Arabic, West Indian, West Asian and Latin American ancestry were significantly more likely to have idiopathic CN than Europeans, while the risk in East Asians and Africans was significantly less. The marked variability of the risk of CN in Asian populations underscores the need for more specific information about the region of origin. By implication, the lower prevalence rate in Asian Americans[4] suggests that the stone formers were predominantly of East Asian background.

Prevalence studies of populations residing in the Arabian Peninsula, Middle East and West Asia have shown an increased propensity for renal stones, although none directly compared their rates to stone formers of European origin living in the same region.[1,6-8] A nationwide survey of KSD in Turkey revealed an overall prevalence of 14.8% and an incidence of 2.2%,[7] while the lifetime incidence in Saudi Arabia was estimated at 20 to 25%.[1] A disproportionate number of Arabs developed calcium oxalate stones compared to non-Arabs living in Tanzania.[8]

In addition, trend data in Israel indicate that the prevalence of calcium oxalate stones increased by about 6 times and uric acid stones doubled from 1974 to 1982.[6]

Our finding of an increased risk in non-African West Indians is in keeping with a recent report from Trinidad showing a 3-fold higher prevalence of renal calculi in this population compared to those of African ancestry.[13] We also observed that non-African West Indians had a greater propensity for stone formation than South Asians, suggesting the importance of environmental influences as these groups likely share a similar genetic and possibly cultural background.[14] Our observation that South Asians have a similar risk of CN as individuals of European ancestry is a novel finding. To our knowledge, the rate of CN among South Asians (India, Pakistan, Bangladesh and Sri Lanka) has not been reported previously for individuals living in these countries or in Western societies.

The increased risk of CN in Latin Americans that we observed differs from previous studies in the United States that found a lower prevalence of stones among Hispanic participants in nationwide surveys.[2,4] In the study by Soucie et al, the majority of the Hispanics lived in Southwest and South-central regions of the United States, and by inference were of Mexican origin.[4] Hispanic participants in the study by Stematelou et al were categorized as Mexican-Americans based on self-reported information of race/ethnicity.[2] In our study, only 8.6% of Latin American subjects originated from Mexico. In keeping with our findings, Soucie et al reported a high prevalence of KSD among men, and to a lesser extent, women living in Puerto Rico.[4] The apparent heterogeneity in risk of kidney stones among subgroups of Latin American populations needs further study.

The results showing a decreased risk of CN in ethnic Africans are also consistent with those of previous studies.[2,4,8,13] Kidney stones are uncommon in black South Africans compared

to whites.[5] In the United States, people of African ancestry have a lower prevalence of KSD than Caucasians (1.7% vs. 5.9%), and the frequency in African-Americans over the age of 40 years has not risen in the period between 1976 and 1994 in contrast to the marked rise in older Caucasian subjects.[2] The reason for the lower prevalence of KSD in blacks is unknown, although urinary calcium excretion is lower in blacks with KSD than white stone formers.[12]

A strength of our study is the control of climatic conditions. Regional variation in the prevalence and incidence of KSD was documented in two American studies.[4,15] They reported that individuals living in the southern latitudes of United States had a greater risk of stone formation. It has been posited ambient temperature and length of sunlight exposure as independent risk factors for kidney stones. The importance of these climatic factors in determining stone risk remains to be determined given the marked difference in stone risk of people from different ethnic backgrounds living in the same warm geographic area.[8,13]

It is well known that obesity and weight gain are risk factors for nephrolithiasis.[16] Weight, however, was not a factor in this study. The BMI in all ethnic groups was either significantly lower or did not differ from that of Europeans. However, obesity may account, at least in part, for the difference in stone risk between non-African West Indians and South Asians, who share a common remote ancestry.[14] Using a threshold value of ≥ 30 kg/m^2 to define obesity, non-African West Indians were 4 times more likely to be obese than South Asians, although their age- and gender-adjusted BMI was only slightly higher.

Information on urine volumes and urinary biochemical profiles may provide insights into possible mechanisms behind ethnic differences in CN risk. East Asians, South Asians and Filipinos had significantly higher urine volumes than Europeans. Similarly, in the United States, Maloney et al reported that a group identified as 'Asian' had a higher urine volume than

whites.[12] There were also several differences in urinary biochemical profiles between the European Canadians and the various ethnic groups that individually might account for differences in stone risk. Strikingly, however, there was no overriding or dominant abnormality or pattern of urinary excretion that would explain the variability in stone risk among ethnic groups. In an analysis examining the relation between urinary stone risk factors and stone risk across ethnic groups we found no significant associations (results not shown). Taken together, these findings suggest the importance of genetic susceptibility, possibly amplified or weakened by environmental factors, in explaining the differences in stone risk among ethnic groups.

There are very few previous studies that have examined stone composition in ethnic populations. Similar to our study, previous studies showed that South Asians living in India or South Africa have an increased propensity to form calcium oxalate rather than apatite stones.[17,18] These findings may be attributed to the lower intake of protein in South Asian diets,[19] since a higher intake of protein is related to apatite stone formation.[20] The diets of East Asians, on the other hand, are higher in protein,[19] which we also observed in conjunction with a significantly higher prevalence of apatite stones compared to South Asians.

This study has several limitations. Firstly, information on dietary intake and acculturation, which is a function of length of residency in a new country, was not gathered in study participants. Secondly, we did not collect information on the country of birth of parents and grandparents, their "mother tongue" or the parental surnames. Additionally, the study findings are relevant only to patients with idiopathic CN referred for lithotripsy treatment. Finally, information was not collected in non-stone formers.

CONCLUSIONS

Ethnic groups in North America differed markedly in their risk to develop idiopathic CN. There was no overriding or dominant environmental factor that explained the variability in stone risk among ethnic groups. This would suggest that genetic susceptibility accounts for the differences that we observed. Further exploration of this possibility would require multistage sampling of a multiethnic population in candidate gene or genome-wide association studies.

Abbreviations: KSD , kidney stone disease; CN , calcium nephrolithiasis; GTA , Greater Toronto Area; BMI , body mass index; SD , standard deviation.

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Conflict of Interest Statement: We declare that we have no conflict of interest.

Internal Review Board: The study was approved by the Mount Sinai Hospital's and St. Michael's Hospital's Research Ethics Board.

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Titles and legends to figures

Figure 1. A flowchart summary of the recruitment process at the St. Michael's Hospital Kidney Stone Center. A total of 1128 patients participated in the study.

GTA , Greater Toronto Area; CN , calcium nephrolithiasis.

Figure 2. Age and gender adjusted odds ratios and 95% confidence intervals for idiopathic calcium nephrolithiasis by ethnic origin in subjects living in the Greater Toronto Area.

■ Europeans are the reference group for comparison; ● statistically significant vs. Europeans.

Table 4.1. Sociodemographic and clinical characteristics of kidney stone patients (n=1128) by ethnic origin.

Characteristic	Ethnic origin									
	European (n=696)	**African (n=50)**	**East Asian (n=48)**	**Latin American (n=34)**	**South Asian (n=126)**	**West Indian (n=42)**	**Filipino (n=44)**	**Southeast Asian (n=14)**	**West Asian (n=34)**	**Arabic (n=40)**
Mean age (years) ± SD	39.8 ± 7.4	37.8 ± 8.1	41.4 ± 7.5	39.4 ± 8.4	38.1 ± 6.9	41.1 ± 7.1	40.4 ± 7.3	40.9 ± 6.9	40.0 ± 7.4	39.0 ± 6.6
Male gender, n (%)	430 (58.4)	22 (44.0)	31 (64.6)	16 (47.1)	85 (67.5)	26 (61.9)	30 (68.2)	11 (78.6)	28 (82.4) **a**	28 (70.0)
Married, n (%)	472 (69.4)	26 (53.1) **b**	38 (82.6)	23 (67.7)	107 (85.6) **a**	32 (76.2)	35 (79.6)	8 (57.1)	26 (76.5)	24 (63.2)
≤ High school educ., n (%)	224 (34.6)	28 (57.1) **a**	17 (39.5)	14 (42.4)	40 (33.6)	29 (69.1) **a**	5 (11.4)	3 (23.1)	11 (32.4)	8 (21.6)
Unemployed, n (%)	39 (6.0)	5 (10.2)	4 (9.3)	3 (9.1)	7 (5.9)	5 (11.9)	0 (0)	0 (0)	2 (5.9)	5 (13.5)
†Mean BMI (kg/m^2) ± SD	28.2 ± 5.8	26.0 ± 5.1 **b**	24.0 ± 3.6 **b**	27.0 ± 5.6	25.5 ± 3.3 **b**	26.0 ± 4.8 **b**	25.0 ± 6.2 **b**	23.9 ± 3.4 **b**	26.7 ± 3.8	26.9 ± 3.6
‡ Mean SBP (mm Hg) ± SD	124 ± 18	128 ± 23	123 ± 18	120 ± 16	124 ± 19	121 ± 19	123 ± 16	124 ± 21	120 ± 14	116 ± 17 **b**
‡ Mean DBP (mm Hg) ± SD	75 ± 12	75 ± 13	76 ± 13	74 ± 9	77 ± 12	75 ± 11	77 ± 10	76 ± 12	72 ± 10	69 ± 8 **b**
‡ Mean heart rate (beats/minute) ± SD	76 ± 13	73 ± 12	76 ± 13	70 ± 10 **b**	76 ± 13	76 ± 14	78 ± 11	79 ± 14	76 ± 11	79 ± 13

Abbreviations: SD , standard deviation; SBP, systolic blood pressure; DBP, diastolic blood pressure; mm Hg, millimeters of mercury.
† , means adjusted for age and gender; ‡ , means adjusted for age, gender and body mass index;
a , significantly higher than Europeans;
b , significantly lower than Europeans.

Table 4.2. Comparison of mean † 24-hour urinary excretion values of kidney stone patients (n=850) by ethnic origin.

	Ethnic origin										
Urinary measure	**European (n=533)**	**African (n=33)**	**East Asian (n=31)**	**Latin American (n=30)**	**South Asian (n=93)**	**West Indian (n=35)**	**Filipino (n=36)**	**Southeast Asian (n=11)**	**West Asian (n=23)**	**Arabic (n=25)**	**p-value**
Calcium (mmol/d)	5.9 ± 2.9	4.8 ± 2.8	6.1 ± 3.1	5.6 ± 2.2	5.0 ± 2.4 **a**	4.2 ± 1.8 **a**	5.3 ± 2.7	4.8 ± 2.7	6.2 ± 2.7	5.2 ± 2.4	0.001
Citrate (mmol/d)	2.4 ± 1.5	2.2 ± 1.4	1.9 ± 1.4	2.1 ± 1.4	2.1 ± 1.4	1.6 ± 1.1 **a**	1.3 ± 1.0 **a**	1.6 ± 0.9	2.1 ± 0.8	2.4 ± 1.3	<0.001
Creatinine (mmol/d)	13.5 ± 3.9	13.5 ± 4.3	13.1 ± 4.5	12.4 ± 3.8	11.4 ± 3.4 **a**	11.4 ± 3.6 **a**	12.5 ± 4.3	11.1 ± 4.3	13.7 ± 3.3	12.1 ± 3.7	<0.001
Potassium (mmol/d)	55.4 ± 22.8	48.5 ± 20.5	49.6 ± 17.9	49.8 ± 19.3	49.6 ± 17.3	40.6 ± 17.6 **a**	40.2±15.0 **a**	33.8±10.1 **a**	49.9 ± 17.9	52.9 ± 18.1	<0.001
Magnesium (mmol/d)	3.8 ± 1.5	3.4 ± 1.6	3.3 ± 1.3	3.2 ± 1.3	3.5 ± 1.3	3.0 ± 1.3 **a**	3.2 ± 1.4	2.2 ± 0.8 **a**	4.1 ± 1.2	3.3 ± 1.3	<0.001
Sodium (mmol/d)	159 ± 66	154 ± 82	172 ± 75	165 ± 65	156 ± 67	162 ± 63	154 ± 65	158 ± 48	161 ± 44	176 ± 57	0.912
Oxalate (µmol/d)	380 ± 151	415 ± 167	451 ± 160	340 ± 95	373 ± 150	355 ± 215	417 ± 161	380 ± 125	393 ± 122	370 ± 138	0.075
Phosphate (mmol/d)	27.3 ± 10.7	24.6 ± 9.4	26.0 ± 9.5	23.8 ± 10.0	23.7 ± 8.9 **a**	24.0 ± 10.0	23.5 ± 10.8	17.8 ± 8.4 **a**	27.9 ± 8.5	22.5 ± 8.7	<0.001
Uric acid (mmol/d)	3.5 ± 1.1	3.2 ± 1.0	4.0 ± 1.3 **b**	3.4 ± 1.1	3.3 ± 1.1	3.4 ± 1.5	4.0 ± 1.4 **b**	3.3 ± 0.9	3.9 ± 1.1	3.5 ± 0.9	0.006
Urea (mmol/d)	348 ± 116	328 ± 110	363 ± 124	327 ± 103	294 ± 100 **a**	328 ± 147	365 ± 157	303 ± 93	366 ± 101	330 ± 115	0.001
Sodium/potassium ratio	3.2 ± 1.3	3.5 ± 1.5	3.7 ± 1.4	3.4 ± 1.3	3.2 ± 1.2	4.2 ± 1.6 **b**	4.0 ± 1.4 **b**	4.7 ± 1.4 **b**	3.4 ± 1.0	3.5 ± 1.6	<0.001
Urea/potassium ratio	6.9 ± 2.6	7.6 ± 2.4	7.8 ± 2.7	7.7 ± 3.6	6.3 ± 2.3	8.6 ± 3.2 **b**	9.1 ± 3.1 **b**	9.0 ± 2.4	7.7 ± 2.7	7.0 ± 2.7	<0.001
Volume (L)	1.6 ± 0.7	1.6 ± 0.7	2.1 ± 0.7 **b**	1.7 ± 0.5	1.9 ± 1.0 **b**	1.4 ± 0.7	2.0 ± 0.8 **b**	1.6 ± 0.7	1.7 ± 0.8	1.6 ± 0.7	<0.001
pH	6.2 ± 0.6	6.4 ± 0.7	6.4 ± 0.5	6.3 ± 0.5	6.3 ± 0.5	6.2 ± 0.4	6.3 ± 0.5	6.7 ± 0.6	6.3 ± 0.5	6.3 ± 0.7	0.142
Protein intake (g) ‡	121 ± 71	112 ± 54	155 ± 82 **b**	117 ± 41	120 ± 74	106 ± 83	160 ± 109 **b**	106 ± 61	124 ± 56	112 ± 70	0.007
IAP Ca-oxalate §	1475 ± 686	1370 ± 621	1553 ± 841	1217 ± 428	1225±548 **a**	1277 ± 582	1458 ± 673	1495 ± 580	1688 ± 931	1371 ± 724	0.011
IAP Ca-phosphate φ	12.1 ± 22.6	19.5 ± 39.7	19.4 ± 36.7	9.1 ± 13.6	9.2 ± 19.8	5.5 ± 6.1	8.4 ± 11.5	26.2 ± 31.6	10.6 ± 20.3	9.5 ± 18.0	0.060

Abbreviations: mmol, millimoles; µmol, micromoles, pmol, picomoles; IAP , ion activity product.

† , means are adjusted for age and gender;

‡, estimate of dietary protein intake from 24-hour urine is expressed as: [urea (mmol/L) x volume (L) x 0.18) + 13]; [10]

§ , estimate of ion activity product for calcium oxalate in 24-hour urine is expressed as: 1.9 x $calcium^{0.84}$ (mmol/L) x oxalate (mmol/L) x $citrate^{-0.22}$ (mmol/L) x $magnesium^{-0.12}$ (mmol/L) x $volume^{-1.03}$ (L); [11]

φ , estimate of ion activity product for calcium phosphate in 24-hour urine is expressed as: 0.0027 x $calcium^{1.07}$ (mmol/L) x $phosphate^{0.70}$ (mmol/L) x $(pH - 4.5)^{6.8}$ x $citrate^{-0.20}$ (mmol/L) x $volume^{-1.31}$ (L); [11]

a , significantly lower than Europeans; b , significantly higher than Europeans.

Table 4.3. Stone composition of kidney stone patients by ethnic origin. Values are number (percent).

	Ethnic origin									
Stone type	**European (n=314)**	**African (n=16)**	**East Asian (n=27)**	**Latin American (n=16)**	**South Asian (n=56)**	**West Indian (n=24)**	**Filipino (n=20)**	**Southeast Asian (n=6)**	**West Asian (n=13)**	**Arabic (n=24)**
Calcium-oxalate	145 (46.2)	11 (68.8) **a**	9 (33.3)	6 (37.5)	40 (71.4) **b**	13 (54.2)	11 (55.0)	2 (33.3)	9 (69.2) **a**	14 (58.3)
Calcium-oxalate + calcium phosphate or apatite	169 (53.8)	5 (31.3) **a**	18 (66.7)	10 (62.5)	16 (28.6) **b**	11 (45.8)	9 (45.0)	4 (66.7)	4 (30.8) **a**	10 (41.7)

a , significantly different compared to East Asians;
b , significantly different compared to East Asians and Latin Americans.

Figure 4.1.

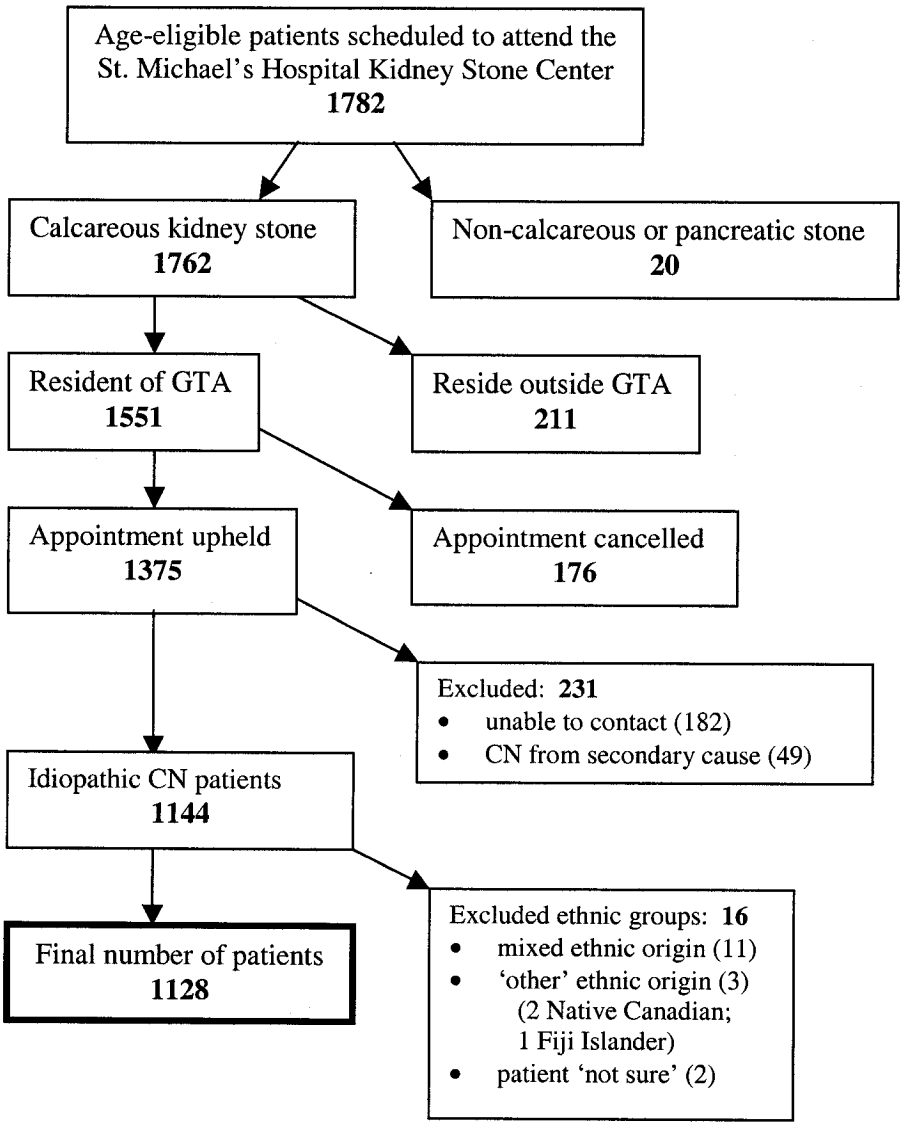
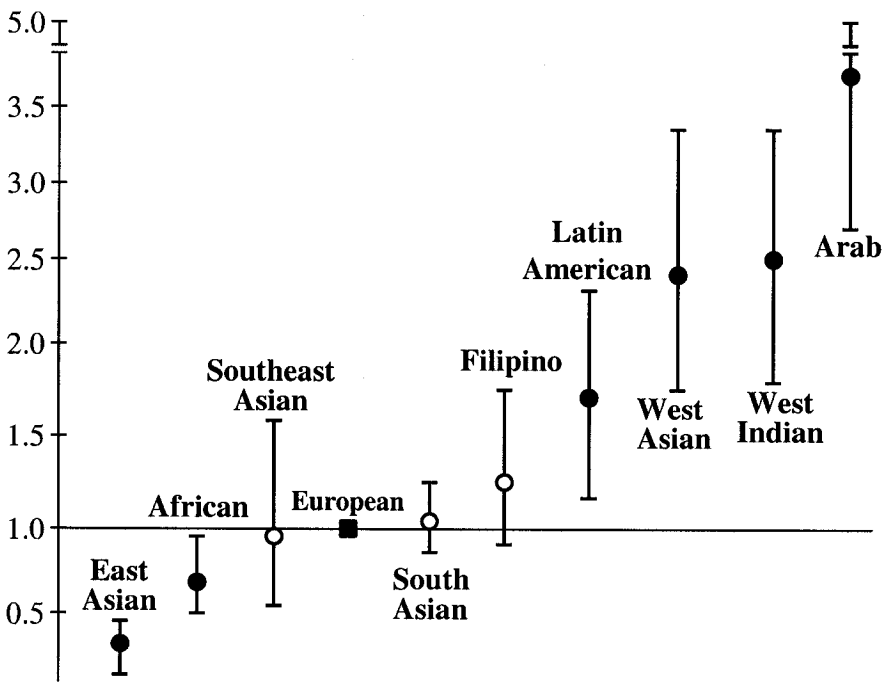

Age-eligible patients scheduled to attend the St. Michael's Hospital Kidney Stone Center
1782
Calcareous kidney stone
1762
Non-calcareous or pancreatic stone
20
Resident of GTA
1551
Reside outside GTA
211
Appointment upheld
1375
Appointment cancelled
176
Excluded: 231
• unable to contact (182)
• CN from secondary cause (49)
Idiopathic CN patients
1144
Final number of patients
1128
Excluded ethnic groups: 16
• mixed ethnic origin (11)
• 'other' ethnic origin (3)
(2 Native Canadian;
1 Fiji Islander)
• patient 'not sure' (2)

Figure 4.2.


5.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
East Asian
African
Southeast Asian
European
South Asian
Filipino
Latin American
West Asian
West Indian
Arab

Urinary potassium as a single objective measure of diet quality

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Word count: 2838; Number of references: 25; Number of tables: 6; Number of figures: 2

ABSTRACT

Background: Healthier dietary patterns are strongly related to health outcomes, yet diet quality is rarely measured because current assessment tools are cumbersome to use in a clinical setting. This study examines the use of urinary potassium as a new simple objective method to assess diet quality.

Methods: Consecutive patients with kidney stones (n=220), aged 18-50 years, from a population-based lithotripsy unit, collected a single 24-hour urine sample and completed a food frequency questionnaire (FFQ) to derive the Recommended Foods Score (RFS), a strong predictor of heath outcomes. Blood pressure, heart rate, weight and height were also measured.

Results: There was a strong, progressive relationship between RFS and 24-hour urinary potassium excretion (p for trend <.001). Increased urinary potassium was significantly and positively associated with the intake of foods recommended by current dietary guidelines, and negatively associated with those generally not recommended. Higher urinary potassium was inversely and significantly related to BMI, diastolic blood pressure and heart rate, after controlling for potential confounders. Using a receiver operating characteristic curve, potassium excretion values below the gender-specific median (men: 60 mmol/d; women: 41 mmol/d) were the optimal cutoff values to identify a poor diet quality, with a sensitivity of 87% and specificity of 61%.

Conclusions: Urinary potassium is an inexpensive and readily available biomarker of diet quality that is related to BMI, blood pressure and heart rate, and to RFS. This urinary marker may act as an aid for physicians to provide effective dietary counseling and facilitate serial monitoring of dietary interventions.

Key words: Urine potassium, diet quality, clinical marker, body mass index, blood pressure, heart rate, screening test

INTRODUCTION

Clinical trials have shown that interventions promoting healthier dietary patterns reduce cause-specific and all-cause mortality in patients with coronary heart disease (1). These interventions also lower blood pressure (2, 3) and largely mitigate salt-sensitivity in people consuming a poor quality diet (3). Population-based cohort studies have demonstrated the protective effect of a healthy diet against heart disease, stroke, cancer and all-cause mortality (4-8), and these benefits are additive with other lifestyle activities aimed at improving health (6).

While diet quality is strongly related to health outcomes, it is rarely measured in routine clinical practice. A major barrier is the lack of a simple, objective test. Present approaches rely on self-report methods, such as food frequency questionnaires (FFQ), 24-hour dietary recall or food diaries, which are time consuming for patients to complete and staff to review and require access to nutrient databases that need continuing and costly updating. One potentially powerful marker of diet quality is urinary potassium (K^+). It is a readily available biochemical test, is strongly related to 'healthy' dietary patterns (4-8), and increased dietary K^+ intake is associated with improved health outcomes (9).

The objectives of this study were to examine the validity of urinary K^+ excretion as a measure of diet quality, to evaluate its relationship to BMI, blood pressure and heart rate, and to find an optimal urinary K^+ cutoff point to identify individuals consuming a poor quality diet. We also examined other urinary variables as indicators of diet quality and their association with intermediate health variables.

METHODS

Subjects

Consecutive patients, aged 18 to 50 years, attending the St. Michael's Hospital's Kidney Stone Centre between February 2002 and March 2004 were eligible for recruitment. The Centre serves the health needs of approximately six million people and may be considered a population-based treatment facility. The hospital's Research Ethics Board approved the study.

Study design

Age-eligible patients who were willing to participate in the study were asked to collect a single 24-hour urine specimen, starting the morning prior to their lithotripsy treatment, and were not provided with any specific dietary instructions. At the Centre, patients, after giving written informed consent, were interviewed to assess sociodemographic variables, health characteristics and the use of vitamins and nutritional supplements. They then completed a food frequency questionnaire (FFQ) that was reviewed by trained study staff. Missed items or ambiguities in recorded food items were clarified by discussing responses with subjects at the visit or later by telephone. Weight, height and blood pressure were assessed in a standard manner.

Dietary assessment

A 166-item quantitative FFQ was used to estimate usual nutrient and energy intake during the previous 12 months (10) and derive the Recommended Food Scores (RFS) (4). Nutrients were based on the 2005 Canadian Nutrient File (11). A RFS value of 9 or higher has been shown previously to be associated with a significant reduction in risk for all-cause and cause-specific mortality in a large population-based cohort study of women (4). We used a RFS score of <9 to

indicate a poor quality diet in women, which represented the 25th percentile of the scores. For men, the comparable value was <8. The RFS scores were adjusted for age, gender, ethnicity, urinary creatinine and energy intake.

Biochemical procedures

A single 24-hour urine sample was collected and patients with a urinary creatinine value outside the daily reference range were excluded (8.8-22 mmol for men and 4.5-16 mmol for women). The laboratory coefficient of variation for the urinary K^+ measurement was 1.5%.

Statistical analyses

Based on correlations reported in previous studies which used biomarkers to validate diet quality measures (12), the power to detect a correlation of 0.20 was determined to be greater than 80% with a sample size of 200, assuming a two-sided test with alpha=0.05.

Patients were categorized into quartile groups, both by sex-specific distribution of 24-hour urinary K^+ excretion and urinary K^+/creatinine ratio. Mean values (± SD) for continuous variables and percentages for categorical variables were computed for each urinary group. The primary exposure variable was the urinary K^+ quartile group, an ordinal predictor. Diet quality score, food intake and the intermediate health variables of BMI, blood pressure and heart rate were separately evaluated as the dependent variable and found to be approximately normally distributed within quartile groups. Covariates of interest included age, gender, ethnicity, urinary creatinine and energy intake. Analysis of covariance was conducted, with tests for linear trend, to compare the mean values for outcome variables among quartile groups, while adjusting for the above covariates. In secondary analyses, urinary K^+/creatinine ratio and FFQ-derived dietary K^+

intake were assessed as predictor variables. The area under the receiver operating characteristic curve (ROC) was used to assess the discriminative ability of urinary K^+ as an indicator of poor diet quality. Model calibration was determined by the Hosmer and Lemeshow goodness of fit test (13). In selecting the optimal cutoff point, greater emphasis was placed on attaining a relatively higher sensitivity than specificity because of the potential harm associated with poor quality diet and the relatively fewer drawbacks of misdiagnosis. Lastly, analysis of covariance was conducted, with tests for linear trend, to compare the mean values for other 24-hour urinary variables among quartile groups of diet quality score. Urinary measures that were associated with diet quality were examined further for associations with intermediate health variables. All results, with the exception of the sociodemographic data, are reported based on multivariate analyses.

The analyses were conducted using SAS software version 8.1 (SAS Institute Inc, Cary, North Carolina). P-values below 0.05 (two-tailed) were considered statistically significant.

RESULTS

Study population

Of 321 potentially eligible patients, 17 did not provide a 24-hour urine collection and 19 had a 24-hour urinary creatinine value outside the reference range, leaving 285 patients with valid urinary data. Of those, 220 provided written informed consent and completed the FFQ, generating a response rate of 77.2%, and all results presented below relate to these subjects. Response rates were similar across all urinary K^+ quartile groups. There were no significant demographic or clinical differences between participants and non-participants. The final item non-response rate for the FFQs was 1.7% and did not vary by K^+ quartile group.

The characteristics of participants, overall and by category of urinary K^+ excretion, are displayed in Table 5.1. The mean age of participants was 39.7 years, and males and females were evenly represented. Subjects with higher K^+ levels were older and mostly of European descent. Women had a significantly higher mean urinary K^+/creatinine ratio compared to men (4.4 ± 1.4 vs. 3.9 ± 1.2 mmol/mmol, $p=.005$). There was no correlation between mean energy intake and K^+ quartile group. Urinary K^+ excretion was positively and significantly correlated with the FFQ-derived dietary K^+ intake ($r=.260$, $p<.001$).

Recommended Food Score

Table 5.2 displays the main findings related to the RFS. The median RFS for the whole group was 9 and scores ranged from 0 to 20. Women had significantly higher mean RFS values than men (10.0 ± 4.3 vs. 8.7 ± 3.6, $p=.009$). Unlike urinary K^+, there was a positive and significant correlation between energy intake and the RFS ($r=.389$, $p<.001$). A higher RFS was associated with a lower BMI ($p=.03$) and heart rate ($p=.001$), but not blood pressure.

Urinary K^+ and diet quality

Figure 5.1 shows the strong, positive and significant association between urinary K^+ excretion and diet quality score (p for trend <.001). The association was equally robust when K^+/creatinine ratios were used instead of urinary K^+ level (data not shown). As outlined in Table 5.3, increased urinary K^+ excretion was significantly and positively associated with the intake of most recommended foods, and negatively associated with those generally not recommended.

Urinary K^+ and intermediate outcome variables

A higher K^+ excretion, illustrated in Table 5.4, was associated with a progressive decline in BMI (p=.03), diastolic blood pressure (p=.04) and heart rate (p=.006) (all p-values are for trend). The same associations were observed when K^+/creatinine ratios were used in place of urinary creatinine excretion (data not shown). For dietary K^+ derived from FFQ, a higher intake was also associated with a lower BMI (p=.005), diastolic blood pressure (p=.03) and heart rate (p=.007).

ROC curves for urinary K^+ and diet quality

The ROC curve comparing different urinary K^+ cutoff values as an indicator of poor diet quality is shown in Figure 5.2. The 50^{th} percentile of the 24-hour urinary K^+ values provided a high sensitivity (0.87, 95% CI: 0.76-0.94) and acceptable specificity (0.61, 95% CI: 0.53-0.68). The corresponding urinary values for these cutpoints were 60 mmol/d in men and 41 mmol/d in women. The mean urinary K^+ for these subjects was 40.7 mmol/d. The area under the ROC curve for urinary K^+ was 0.76 (95% CI: 0.64 – 0.88, p<.001), and there was good calibration as indicated by the Hosmer and Lemeshow goodness of fit test (p=0.49).

Diet quality and other urinary measures

Table 5.5 shows the mean 24-hour urinary values by RFS quartile. Although the magnitude of each association was not as strong as for urinary K^+, there were positive and significant associations between the RFS and 24-hour urinary magnesium (p=0.031 for trend), urea (p=0.032), phosphate (p=0.030) and urine pH (p=0.016). These 24-hour urinary parameters were also significantly and positively correlated with urinary K^+. The RFS was not associated with urinary calcium, citrate, sodium, oxalate, uric acid or creatinine excretion.

Other urinary measures and intermediate outcomes variables

As shown in Table 5.6, both urinary magnesium and urine pH were inversely related to BMI. Higher urinary urea was associated with a lower systolic blood pressure and heart rate. There were no associations with urinary phosphate.

DISCUSSION

We found that a single 24-hour urinary K^+ measurement was a valid, quantitative measure of diet quality. This is the first paper to examine the use of urinary K^+ for this purpose. Our study was based on the premise that 'healthy' dietary patterns, described in previous studies, have typically included foods that are good sources of dietary K^+ (1, 2, 4-8). We hypothesized that urinary K^+ excretion would be strongly associated with the dietary nutrients and foods that reflect a healthy all-around diet. The dietary assessment was conducted using a previously validated quantitative FFQ developed for Canadian populations (10), and our median RFS values were similar to those previously reported (4). Our findings suggest that urinary K^+ excretion is a clinically useful instrument to gauge diet quality.

The health benefits of a high diet quality have been demonstrated in intervention trials (1-3) and observational studies (4-8). From studies examining the health effects of single nutrients, it is estimated that over half of North American adults consume less than the recommended amounts of a wide array of nutrients in their diet including K^+ (recommended intake, 4.7g or 120 mmol per day) (14). Despite mounting evidence that a high quality diet is strongly and broadly related to improved health outcomes, there is no simple, objective test of diet quality to aid physicians in providing effective dietary counseling. Traditional measures such as the FFQ, 24-hour dietary recall or food diaries have not met the needs of practicing clinicians who require a readily available, inexpensive test that can be performed on almost all patients.

Urinary K^+ excretion fulfills several important criteria for a useful measure of diet quality. First, we found that increased urinary K^+ was associated with a higher intake of foods that are most recommended (15), and a lower excretion was correlated with foods not considered part of a 'prudent' eating pattern (7). Secondly, our time horizon for estimating dietary intake

was one year, suggesting that K^+ excretion may be used to assess long-term intake. Third, we demonstrated a strong association between urinary K^+ excretion and RFS, which is inversely related to mortality (4, 5). Fourthly, urinary K^+ was not correlated with energy intake, thus avoiding the potential confounding effect of energy intake, observed when self-report dietary instruments are used to measure diet quality such as the RFS (4). Previous studies have shown that the day-to-day, within-person variability of K^+ intake is relatively low (16) and that urinary K^+ excretion strongly reflects dietary K^+ intake (17), findings which we also observed. Moreover, the DASH trial reported that improving diet quality produced a parallel increase in urinary K^+ excretion (2). Importantly, determination of urinary K^+ excretion from a single 24-hour collection showed a strong protective relationship with risk of death from all-causes in a large population-based cohort study (18). Finally, the cost of a single 24-hour urine K^+ measure is $7.50 in Canada.

The gender-specific RFS cutoff points used in the ROC analysis were derived from the results of a large population-based cohort study showing that all-cause and cause-specific mortality rates were significantly lower in women with a RFS above the 25^{th} percentile of values (4). While there are no corresponding mortality data in men, the DASH trial indicated that men and women derived similar health benefits on a high quality diet (2), and the Scottish Heart Study showed that a 24-hour urinary K^+ was inversely related to all-cause mortality in both genders (18). We found that urinary K^+ values below the gender-specific medians (60 mmol/d in men and 41 mmol/d in women) were predictive of a poor quality diet. The application of such testing would identify individuals at greatest risk, establish therapeutic goals, and facilitate serial monitoring of dietary interventions.

Our observation of an independent, negative correlation between urinary K^+ and BMI is consistent with past findings (19). It is also congruent with new clinical trial data showing greater weight loss in women consuming a diet that promotes increased vegetable and fruit intake, good sources of dietary K^+ (20). Our blood pressure results also confirm the findings of others (2, 3, 21). Experimental studies have shown an increase in blood pressure in normotensive individuals on a low K^+ diet that returns to normal upon repletion (21). The DASH trials demonstrated that switching from a low K^+ diet (39.2 mmol/d) to one rich in K^+-containing foods (74.6 mmol/d) lowered blood pressure markedly (2, 3), and the effect was independent of change in body weight and dietary salt intake. The mean K^+ excretion value of 40.7 mmol/d in subjects consuming a poor quality diet in our study is similar to that noted in control diet subjects in the DASH trial. The mechanism behind the inverse association of resting heart rate with K^+ excretion and RFS that we observed is unexplained. Possibly it is related to a lower BMI that can dampen sympathetic nerve activity (22).

Our finding that women reported consuming a higher quality diet than men is consistent with previous observations (23) and is congruent with the significantly higher urinary K^+/creatinine ratio in women compared to that in men. These gender differences suggest that women may be more knowledgeable about healthy food choices and make better food selections.

Several other urinary measures were associated with a healthier diet and in keeping with past observations they were also related to BMI or blood pressure (24, 25). These relationships, however, were not as strong as those of urinary K^+. Nonetheless, their associations with urinary K^+ as well as intermediate outcome variables suggested that urinary K^+ reflects the cumulative effect of multiple nutrient components acting in concert as part of a high quality diet rather than the actions of a single dietary nutrient.

An important strength of the present study was that subjects were recruited consecutively from a population-based treatment facility. Moreover, no specific dietary advice was provided to study participants before they completed all evaluations, including the dietary assessment. The similar proportion of patients with recurrent kidney stones across the urinary K^+ quartiles suggests that any possible prior dietary instructions had no long-term impact on dietary choices. Finally, the dietary assessment was conducted before the results of the urine tests were known.

The study also has some limitations. It was conducted in a single center predominantly in Caucasian kidney stone patients. Thus, the results may only be applicable to this population. Further study in a broader group will be needed to test the generalizability of the results. A single 24-hour urine specimen was used to determine the K^+ excretion of our study patients. However, a single urinary collection has been shown to predict health outcomes in a large cohort study (18), supporting the validity of obtaining only one sample. If the 24-hour urine collection has substantial measurement error, the observed correlations would likely have underestimated the true correlations.

In summary, increased urinary K^+ was associated with a healthier diet quality score, better adherence to current dietary recommendations and a lower BMI, diastolic blood pressure and heart rate. It was also the strongest predictor of diet quality. A K^+ excretion value below 60 mmol/day in men and 41 mmol/day in women indicated the consumption of a poor quality diet. We suggest that a single 24-hour urinary K^+ measure is a clinically valid, simple and inexpensive test of diet quality that may aid physicians in providing effective dietary counseling to patients at greatest risk because of poor food choices.

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Conflict of Interest Statement: We declare that we have no conflict of interest. We have no financial interests related to the material in the manuscript. The work in this article is original and all authors meet the criteria for authorship, including acceptance of responsibility for the scientific content of the manuscript.

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Figure Legends

Figure 5.1. Mean diet quality score*† by quartile of urinary potassium excretion. Bars are SE.

* diet quality scores derived using the Recommended Food Score (4);

† adjusted for age, gender, ethnicity, energy intake and urinary creatinine excretion.

Figure 5.2. Receiver operating characteristic curve for urinary potassium excretion in the assessment of a poor quality diet. Black dots represent normality cutoff points for 24-hour urinary potassium in percentiles. White dot represents the optimal cutoff point. The potassium excretion cutoff points, in mmol/d, for men and women, respectively, at each percentile value (given in parenthesis) are:

30 and 19 (5^{th}), 36 and 23 (10^{th}), 41 and 29 (20^{th}), 45 and 31 (25^{th}), 49 and 31 (30^{th}), 55 and 35 (40^{th}), 60 and 41 (50^{th}), 67 and 45 (60^{th}), 74 and 51 (70^{th}), 77 and 55 (75^{th}), 81 and 60 (80^{th}), 90 and 68 (90^{th}), 106 and 77 (95^{th}).

Table 5.1. Characteristics of participants overall and by category of urinary potassium intake.

		Urinary Potassium Quartile Group, demarcation points (meq/day)				
		Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Men		≤ 45	46 – 60	61 – 77	≥ 78	
Women	All	≤ 30	31 – 41	42 – 54	≥ 55	p-value
Characteristic *	(n = 220)	(n = 55)	(n = 54)	(n = 55)	(n = 56)	†‡
Age, years, mean (SD)	39.7 (7.7)	37.5 (8.6)	38.0 (7.8)	41.4 (6.8) ‖§	41.8 (6.3) ‖§	0.003
Male gender	114 (51.8)	30 (54.6)	28 (51.2)	27 (49.1)	29 (51.8)	0.96
Caucasian	179 (81.4)	44 (80.0)	44 (81.5) **	38 (69.1)	53 (94.6) **§	0.007
Currently married	154 (70.0)	34 (61.8)	35 (64.8)	41 (74.6)	44 (78.6)	0.18
≤ High school education	62 (28.2)	11 (20.0)	18 (33.3)	18 (32.7)	15 (26.8)	0.20
Household income <$30000	29 (14.7)	11 (23.4)	10 (20.0)	5 (10.2)	3 (5.9)	0.19
Current smoker	51 (23.3)	12 (22.2)	13 (24.1)	12 (21.8)	14 (25.0)	0.98
Multivitamin use	63 (28.6)	13 (23.6)	17 (31.5)	17 (30.9)	16 (28.6)	0.80
Recurrent kidney stone	128 (58.5)	33 (60.0)	30 (56.6)	34 (61.8)	31 (55.4)	0.90
Energy intake, kilocalories, mean (SD)	2046 (835)	2147 (974)	2006 (941)	2044 (693)	1986 (714)	0.46
Urinary K^+/Cr, mean (SD), meq/meq						
men	3.9 (1.2)	2.6 (0.6)	3.5 (0.7)	4.4 (0.8)	5.0 (1.1) ††	<0.001
women	4.4 (1.4)	2.9 (0.6)	4.1 (1.0)	4.5 (0.8)	6.0 (1.1) ††	<0.001

* values are number (percent) except where indicated otherwise.

† p-values for all variables are from chi-square test, except for age and energy intake (p for trend).

‡ post hoc pairwise comparisons of means were made using Tukey's approach to adjust for multiple comparisons.

§ statistically significant vs. quartile 1; ‖ statistically significant vs. quartile 2; ** statistically significant vs. quartile 3;

†† statistically significant vs. all other quartile groups.

meq = milliequivalents; SD = standard deviation; Cr = creatinine.

Table 5.2. Recommended Food Score (RFS), energy intake and clinical measures, overall and by RFS quartiles.

			Recommended Food Score Quartiles				
Characteristics *		**Overall**	**Quartile 1 (n = 55)**	**Quartile 2 (n = 54)**	**Quartile 3 (n = 55)**	**Quartile 4 (n = 56)**	**p-value for trend**
Recommended Food Score, median (range)	Total	9 (0-20)	5 (0-7)	8 (7-10)	11 (9-13)	14 (12-20)	<0.001
	Men	8 (1-19)	5 (1-6)	7 (7-8)	10 (9-11)	14 (12-19)	<0.001
	Women †	10 (0-20)	5 (0-7)	9 (8-10)	12 (11-13)	15 (14-20)	<0.001
Energy intake, kilocalories, mean (SD)		2046 (835)	1614 (765)	2087 (717)	2255 (885)	2370 (739)	<0.001
BMI, kg/m^2, mean (SD)		27.5 (5.6)	28.7 (6.5)	27.1 (5.0)	28.0 (5.6)	25.8 (4.7)	0.03
Systolic BP, mm Hg, mean (SD)		123.0 (17.6)	122.0 (18.0)	122.2 (17.8)	123.1 (17.3)	124.8 (18.2)	0.67
Diastolic BP, mm Hg, mean (SD)		75.2 (11.9)	75.3 (11.3)	75.1 (11.9)	74.6 (10.9)	75.5 (14.0)	0.81
Heart rate, beats/min, mean (SD)		76.1 (12.5)	79.6 (14.1)	78.9 (12.9)	73.6 (11.6)	71.8 (9.9)	<0.001

* means are adjusted for age, gender, BMI, energy intake and urinary creatinine excretion, where applicable.

† median Recommended Food Score values were similar to those reported previously (4).

SD = standard deviation; BMI = body mass index; BP = blood pressure; mm Hg = millimeters of mercury.

Table 5.3. Mean (SD) daily intake of selected foods or food groups by quartile of potassium excretion.

	Urinary Potassium Quartile Group, demarcation points (meq/day)					
	Quartile 1	Quartile 2	Quartile 3	Quartile 4		
men	≤ 45	46 – 60	61 – 77	≥ 78		Pearson
women	≤ 30	31 – 41	42 – 54	≥ 55	p-value	correlation
Food/food groups †	(n = 55)	(n = 54)	(n = 55)	(n = 56)	for trend	coefficient ‡
Vegetables, total (g)	217.1 (202.4)	190.4 (127.2)	257.8 (174.6)	293.9 (202.4)	0.02	0.141*
Dark-yellow (g)	16.3 (23.7)	16.8 (20.5)	30.5 (42.8)	36.2 (52.0)	<0.001	0.175**
Green-leafy (g)	23.4 (22.1)	25.1 (28.2)	32.3 (27.7)	36.8 (33.6)	0.02	0.129*
Cruciferous (g)	33.0 (32.9)	26.1 (29.1)	41.4 (48.9)	44.5 (44.0)	0.08	0.156*
Tomatoes (g)	41.2 (55.3)	38.6 (35.7)	39.3 (39.1)	58.3 (57.5)	0.14	0.122
Legumes (g)	22.0 (38.8)	21.3 (21.3)	28.7 (36.3)	28.4 (28.2)	0.24	0.057
Other (g)	57.5 (66.5)	48.2 (44.5)	65.6 (78.7)	72.1 (63.3)	0.18	0.076
Fruits (g)	153.9 (167.8)	155.1 (120.2)	196.1 (170.6)	272.1 (163.5)	<0.001	0.255***
Whole grains (g)	28.8 (44.8)	34.8 (38.7)	38.8 (43.6)	48.2 (45.5)	0.046	0.182**
Refined grains (g)	102.9 (74.8)	130.0 (152.5)	107.1 (97.5)	118.6 (101.7)	0.72	0.016
Fish and poultry (g)	39.3 (41.5)	48.1 (47.1)	58.8 (57.0)	58.9 (49.4)	0.046	0.185**
Low-fat dairy (g)	106.5 (183.6)	87.6 (161.1)	120.4 (212.0)	241.0 (336.2)	0.008	0.211**
Regular-fat dairy (g)	176.3 (294.2)	222.5 (349.2)	195.4 (168.5)	193.3 (173.5)	0.89	0.019
Nuts (g)	3.10 (9.9)	2.76 (5.0)	10.30 (18.3)	9.85 (21.4)	0.01	0.107
Wine (g)	12.4 (32.8)	14.8 (33.3)	25.8 (42.8)	29.9 (46.2)	0.03	0.166*
Red meat (g)	76.1 (70.8)	54.3 (41.7)	56.0 (50.7)	44.7 (47.5)	0.01	–0.135*
Processed meat (g)	6.74 (11.5)	4.49 (4.1)	4.20 (3.9)	4.79 (5.6)	0.20	–0.064
Sweets and desserts (g)	70.5 (68.3)	71.3 (61.1)	62.5 (48.0)	62.4 (58.4)	0.36	–0.066
High-energy drinks (g)	353.5 (608.2)	162.0 (191.4)	156.7 (240.4)	42.9 (138.0)	<0.001	–0.245***
Fast food (g)	83.7 (72.3)	81.0 (90.5)	62.7 (51.5)	58.7 (57.8)	0.04	–0.167*

* $p < .05$; ** $p < .01$; *** $p < .001$;

† all mean values are adjusted for age, gender, ethnicity, energy intake and urinary creatinine excretion;

‡ correlation of urinary K^+ excretion with each dietary variable while controlling for age, gender, ethnicity, energy intake and urinary creatinine excretion using linear regression. SD = standard deviation; meq = milliequivalents.

Table 5.4. The relationship of intermediate health variables to urinary potassium quartiles.

	Urinary Potassium Quartile Group, demarcation points (meq/day)				
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	
men	**≤ 45**	**46 – 60**	**61 – 77**	**≥ 78**	
women	**≤ 30**	**31 – 41**	**42 – 54**	**≥ 55**	**p-value**
Characteristic *†	**(n = 55)**	**(n = 54)**	**(n = 55)**	**(n = 56)**	**for trend ‡**
BMI, kg/m^2	28.7 (6.5)	28.1 (5.9)	26.6 (4.9)	26.5 (4.8) §	0.03
Systolic BP, mm Hg	123.9 (15.6)	124.1 (20.1)	125.3 (19.9)	119.0 (14.0)	0.27
Diastolic BP, mm Hg	77.3 (11.5)	75.8 (13.4)	76.3 (13.5)	71.4 (9.0) §	0.04
Heart rate, beats/min	80.2 (11.6)	76.6 (14.5)	76.4 (12.6)	71.4 (11.1) §	0.006

* values are mean (SD);

† means are adjusted for age, gender, ethnicity, education, energy intake and urinary creatinine excretion;

‡ post hoc pairwise comparisons of means were made using Tukey's approach to adjust for multiple comparisons.

§ statistically significant vs. quartile 1.

SD = standard deviation; meq = milliequivalents; BMI = body mass index; BP = blood pressure;

mm Hg = millimeters of mercury.

Table 5.5. Mean (SD) 24-hour urinary values by RFS quartiles.

	Recommended Food Score Quartiles					
Urinary measure †	**Quartile 1 (n = 55)**	**Quartile 2 (n = 54)**	**Quartile 3 (n = 55)**	**Quartile 4 (n = 56)**	**p-value for trend**	**Pearson correlation coefficient ‡**
Potassium (meq/d)	49.2 (19.6)	51.8 (24.5)	54.1 (24.2)	59.6 (19.5)	0.002	0.204**
Calcium (meq/d)	5.6 (2.6)	5.7 (2.7)	5.6 (2.6)	6.1 (2.7)	0.378	0.060
Citrate (meq/d)	2.6 (1.5)	2.5 (1.5)	2.3 (1.2)	2.6 (1.6)	0.941	0.006
Creatinine (meq/d)	12.7 (4.3)	13.2 (4.0)	13.2 (4.1)	13.2 (3.8)	0.389	0.060
Magnesium (meq/d)	3.4 (1.6)	3.8 (1.5)	3.9 (1.6)	4.0 (1.5)	0.031	0.148*
Sodium (meq/d)	151 (75)	151 (69)	153 (64)	166 (61)	0.231	0.058
Oxalate (μeq/d)	365 (160)	377 (168)	376 (158)	417 (155)	0.085	0.118
Phosphate (meq/d)	24.6 (10.8)	25.2 (11.2)	26.8 (10.3)	28.1 (10.3)	0.030	0.149*
Uric acid (meq/d)	3.4 (1.2)	3.6 (1.3)	3.4 (1.2)	3.6 (1.1)	0.622	0.033
Urea (meq/d)	317 (117)	327 (125)	338 (124)	352 (113)	0.032	0.147*
pH	6.3 (0.5)	6.2 (0.5)	6.4 (0.5)	6.5 (0.5)	0.016	0.165*

* $p < .05$; ** $p < .01$;

† all mean values are adjusted for age, gender, ethnicity, energy intake and urinary creatinine excretion;

‡ correlation of RFS with each urinary variable while controlling for age, gender, ethnicity, energy intake and urinary creatinine excretion using linear regression. SD = standard deviation; meq = milliequivalents; μeq = microequivalents.

Table 5.6. The relationship of intermediate health variables to urinary measures. †

	Intermediate health variables			
Urinary measure	**Body mass index**	**Systolic BP**	**Diastolic BP**	**Heart rate**
Magnesium (meq/d)	r = –.141 (p=.040)	r = –.011 (p=.873)	r = –.065 (p=.346)	r = –.133 (p=.055)
Phosphate (meq/d)	r = .047 (p=.493)	r = .038 (p=.585)	r = –.018 (p=.791)	r = –.060 (p=.389)
Urea (meq/d)	r = –.031 (p=.650)	r = –.161 (p=.019)	r = –.123 (p=.074)	r = –.186 (p=.007)
pH	r = –.147 (p=.034)	r = –.002 (p=.973)	r = –.067 (p=.337)	r = –.040 (p=.568)

† all r values are Pearson correlation coefficients adjusted for age, gender, ethnicity, energy intake and urinary creatinine excretion;

BP = blood pressure; meq = milliequivalents; μeq = microequivalents.

Figure 5.1

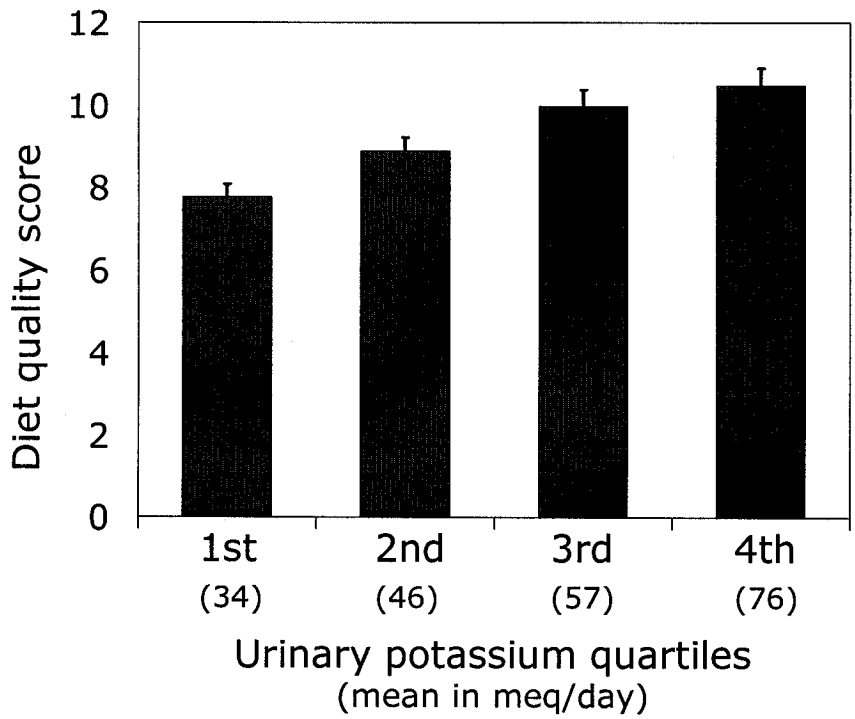
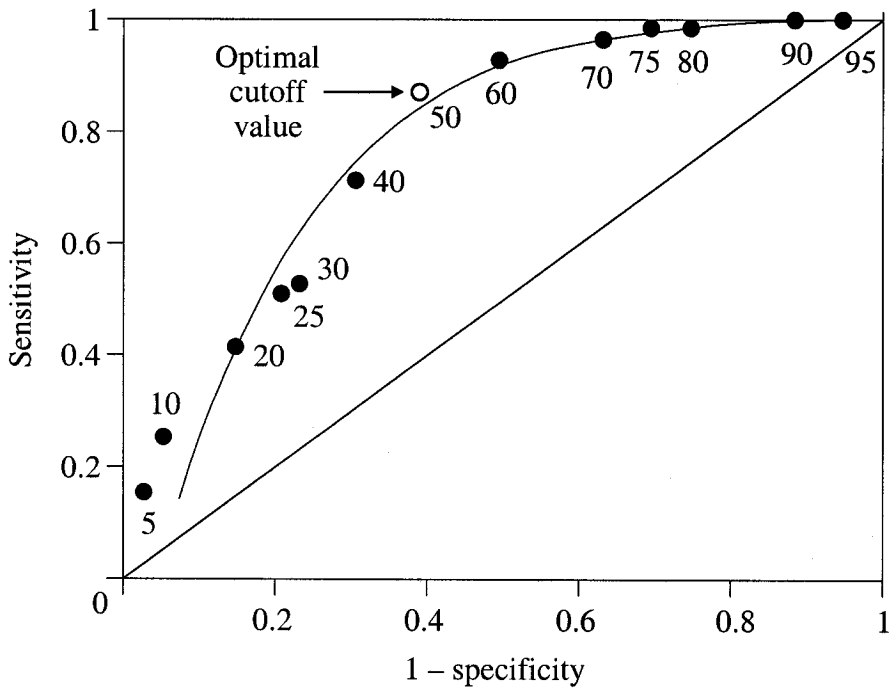

12
10
8
6
4
2
0
Diet quality score
1st
(34)
2nd
(46)
3rd
(57)
4th
(76)
Urinary potassium quartiles
(mean in meq/day)

Figure 5.2


Optimal
cutoff
value
Sensitivity
1 – specificity
0
0.2
0.4
0.6
0.8
1
5
10
20
25
30
40
50
60
70
75
80
90
95

VII. DISCUSSION

A. Overview of Main Findings

Considerable research has been undertaken to identify the genes that confer susceptibility to genetically complex traits such as hypertension, KSD, obesity/weight gain and DM2. To date, few candidate genes have been found. It has been suggested that a well-defined phenotype may facilitate the search for disease alleles involved in the pathogenesis of these conditions. Previous studies have shown associations among these conditions, but the underlying metabolic abnormality behind these associations is not known. One way to identify common etiologic and pathogenetic mechanisms among different diseases is to determine whether the disorders occur more frequently within certain families.

The first of the two familial aggregation studies investigated the aggregation of hypertension and related conditions (KSD, obesity, excessive weight gain and DM2) in families of KSD patients with hypercalciuria. The main objective was to determine whether these conditions share a common disturbance in Ca^{2+} metabolism, manifested as hypercalciuria, and whether this disturbance has a genetic basis. The study showed for the first time that hypercalciuria in KSD patients is predictive of hypertension in first-degree relatives, independent of patients' age, gender, BMI, use of antihypertensive medication and relative age. Unlike previous studies (Tisler et al., 1999; Tisler et al., 2002), family health information was ascertained directly from family members, and spouses were evaluated to control for environmental influences. The aggregation of hypertension in first-degree relatives, but not the spouses, of patients with hypercalciuria suggests a genetic basis for the disturbance in Ca^{2+} metabolism (Khoury et al., 1993).

The second study extended this finding by examining the interaction of hypercalciuria, a manifestation of suspected genetic susceptibility, with low urinary K^+,

an environmental factor and a marker of diet quality, on familial aggregation of these conditions. This study identified significant aggregation of hypertension, obesity, excessive weight gain and DM2 in the siblings, but not the spouses, of KSD patients with low dietary K^+ combined with hypercalciuria. The findings implicate an interaction between a suspected genetically determined abnormality in Ca^{2+} metabolism, manifested as hypercalciuria, and a low K^+ diet, an environmental factor. Overall, this study identified a subset of patients that may share a common pathway leading to the development of KSD, hypertension and possibly diabetes.

B. Methodological Issues

Proper interpretation of the findings of the familial aggregation studies depends on whether the associations are real, a consequence of bias or confounding, or due to chance. This section examines the relevant methodological issues that may affect the interpretation of the resulting associations for the main effects and possible effect modification. It also addresses other methodological issues that pertain to the study including approaches to help find genetic effects, analysis methods, study power and generalizability.

1. Selection Bias

Familial aggregation cohort studies are subject to classic selection bias if probands are selected on the basis of known family history (Khoury et al, 1993). In this study, probands were recruited consecutively from a single treatment facility that serves the needs of people living in south-central Ontario and without knowledge of their family history or biochemical test results. Thus, the potential for classic selection bias was minimized.

The participation rate among eligible KSD patients was 86%, and there were no significant differences on the demographic factors or clinical measures between participants and those who declined or whose first-degree relatives could not be contacted. These observations suggest that non-response bias was unlikely to have been an issue in this study.

2. Information Bias

a. Ascertainment bias

It is unlikely that the results were biased by differential recall of family health information, since health information was obtained on different conditions than that affecting the proband, and all of the probands had the same disease. Moreover, the ascertainment of health information from relatives was conducted before the results of the urine chemistries were known. Thus, errors in ascertainment of disease status were likely random, which would dilute rather than magnify associations.

b. Measurement error

Measurement error may have occurred in the assessment of both the exposure variable (e.g., the probands' urinary status) and the disease outcome (e.g., the relatives' disease status).

(1) Assessment of urinary status of probands

A single 24-hr urine specimen was used to determine the urinary status of patients. While this reduces the reliability of diagnostic classification (Parks et al., 2002), repeated collections were generally not feasible since even a single 24-hour urine collection is a burdensome procedure. If the 24-hour urine collection has substantial measurement error, this would under-estimate true associations. A similar urine collection procedure

was used recently in cohort studies to examine kidney stone risk profiles (Curhan et al., 2001; Lerolle et al., 2002). Finally, the results showing familial aggregation of hypertension in siblings of probands with hypercalciuria and hyperuricosuria confirm those previously reported by Tisler et al (2002), indicating the reproducibility of the results.

Our findings depended upon the reliability of urinary creatinine being a valid indicator of an accurate 24-hour urine collection. Laboratory studies indicate that the day-to-day variability of its daily excretion is small and assay methods have acceptably low coefficients of variation (Dyer et al., 1994). Nonetheless, the amount excreted in the urine can be influenced by constitutional factors such as muscle bulk and by external influences such as meat consumption. Its reliability also depends on patients following instructions for a time sample to avoid over- or under-collection. While patients whose urinary creatinine level was outside the reference range for a 24-hour collection were excluded from the study, this does not provide a safeguard for improper collections with creatinine values within the reference range. Accordingly, urinary creatinine was controlled for in the multivariate GEE analyses, and this did not influence the results.

Drug treatment for hypertension can alter the urinary Ca^{2+} excretion (Quereda et al., 1996). This pharmacological effect introduces the possibility that probands taking these medications were misclassified as to group allocation. To reduce this problem, enrollment was restricted to younger patients. Nonetheless, even then a small number of probands (45 in total) were taking antihypertensive medications. Accordingly, in a sensitivity analysis in which these patients and their families were excluded, estimates of familial aggregation were unaltered. For instance, for hypercalciuria alone, the odds ratio and 95% confidence interval changed from 1.9 (1.03, 3.5) to 2.2 (1.1, 4.4) for the aggregation of KSD in siblings, and from 2.9 (1.4, 6.2) to 2.7 (1.1, 6.8) for hypertension

in siblings. It is unlikely, therefore, that the conclusions were biased due to treatment effects.

There is significant seasonal variation in urinary Ca^{2+} excretion, which has been attributed to the effect of sun exposure on vitamin D levels (Robertson et al., 1974; Vanderschueren et al., 1991). However, probands in this study were enrolled continuously over a full two-year period with no peak enrollment periods. Thus, seasonal variation is an unlikely explanation for our findings.

(2) Assessment of disease status in relatives

The ability to detect aggregation of disease in families is largely dependent on the accuracy of the health information obtained on family members. In the validation study, the information obtained from relatives of probands was used to assess the accuracy of family health information obtained from KSD patients. It was noted that the use of proxy reported health information resulted in an attenuation of the effect size for each health condition. Therefore, interviewing family members directly substantially reduced measurement error and as a consequence strengthened associations.

Family members were not personally evaluated, nor were their physicians contacted to verify the information collected. While this approach allows for the most thorough examination of the disease as well as the collection of data on exposure and risk factors in the relatives, it was not feasible. Past studies have shown that the accuracy of self-reported hypertension, DM2, KSD and obesity is reasonably high (Heliovaara et al., 1993; Haapanen et al., 1997; Mackenbach et al., 1996; Bowlin et al., 1993; Kehoe et al., 1994; Ljunghall et al., 1979; Stewart et al., 1982; Stunkard et al., 1981; Jalkanen et al., 1987; Palta et al., 1982). Moreover, errors in the self-reporting of these conditions are more often due to under-reporting (Heliovaara et al., 1993; Haapanen et al., 1997;

Mackenbach et al., 1996; Bowlin et al., 1993; Kehoe et al., 1994), which is more likely to reduce statistical precision.

An important caveat in the familial aggregation studies was the use of restrictive definitions in classifying health problems in relatives. For instance, the outcome of hypertension was defined as "being treated with antihypertensive medications to lower blood pressure" rather than "being told by a doctor to have high blood pressure". This restriction identified relatives with more severe forms of diseases or conditions, which are more likely to have a greater genetic contribution to their development (Lander and Schork, 1994). As shown in the validation study of this thesis, misclassification of disease outcomes is reduced substantially when conditions are defined by being on medications rather than by a positive diagnosis made by a physician. Stringent phenotypic definitions increase specificity at the expense of sensitivity, an advantage in familial aggregation studies, since bias introduced by the misclassification of disease status of relatives is substantially greater when specificity is low (Khoury et al., 1993).

A drawback of defining hypertension by medication status is the failure to capture hypertensive individuals who are untreated or who are treated by modifying lifestyle modification alone. In the latter instance, misclassification may not be random, and could potentially bias the results of the study. As shown in Table 1.3 of the first paper (page 108), stone formers without hypercalciuria ate less animal protein and sodium than their hypercalciuric counterparts. This more desirable eating pattern might be shared with their first-degree relatives, and subjects with these "healthier" lifestyles may control their hypertension with diet and exercise. In this scenario, the study would understate the prevalence of hypertension in first-degree relatives of stone patients with "normal" levels of urinary Ca^{2+} excretion. To address this concern, sub-analyses were performed whereby relatives with treated and untreated hypertension (i.e., "told by a doctor to have hypertension") were combined into one group, and the results were compared to the main

findings (i.e., "treated hypertension"). Of the first-degree relatives that reported being told by a doctor to have hypertension, 89% were also treated with antihypertensive medications to lower blood pressure. Moreover, when "ever told by a doctor" was used as the criterion for hypertension, the associations between the urinary measures in patients and hypertension in relatives remained steadfast. This reduces the likelihood that the prevalence of hypertension in first-degree relatives of stone patients with normal Ca^{2+} excretion is underestimated.

In assessing of disease status of relatives, "don't know" answers could have resulted in loss of information, thereby reducing study power. However, since health information was obtained directly from relatives rather than proxy respondents, the number of these responses was minimized. In total, there were only two "don't know" responses for KSD and eight for obesity and weight gain (out of 1015 interviews). None was recorded for hypertension and DM2.

3. Confounding

The associations found in the two familial aggregation studies were independent of patient age, gender, BMI, use of antihypertensive medication and relative age. Each of these potential confounders was related to both urinary Ca^{2+} excretion and disease status in previous familial aggregation studies (Tisler et al., 1999; Tisler et al., 2002). Other possible confounders were also considered for inclusion in the multivariate models based on a systematic review of the literature, including ethnicity, marital status, education, area of residence (inside/outside city), country of birth, smoking status, and use of Ca^{2+} or vitamin D containing products. They were included in the analytic models if, singly, they changed the point estimate by 10% or more (Kleinbaum and Klein, 2002).

Information was also obtained on covariates from relatives, which permitted controlling for additional potential confounding variables, aside from relative's age, such

as smoking status and use of Ca^{2+} or vitamin D containing products or multivitamins. Other studies with missing relative information typically substituted predictors pertaining to the proband (e.g., age) in place of the relative. However, Kondo et al (2005) reported that this approach may not be adequate to control for potential confounders, since proband and relative information is often discordant.

The patients' urine chemistries showed marked dietary differences between the groups, especially in regards to protein and sodium intake. One might, therefore, argue that since families may eat in the same manner, diet could be part of the explanation for the higher rates of stones and hypertension in the family members. However, if the observed associations were due to diet, there would probably be aggregation of disease in spouses as well, since spouses share a common environment with the patient. In addition, there is recent evidence that different relative pairs (ie, proband-parent vs. proband-sibling vs. proband-spouse) share similar correlations of dietary nutrient and energy intake (Mitchell et al., 2003). Since dietary habits appear to aggregate similarly in each relative category, it is unlikely that the stronger aggregation of hypertension in siblings than in spouses can be explained by dietary influences. Moreover, controlling for urinary sodium and protein did not influence the associations, as the parameter estimates were virtually identical when the urinary measures were included in the regression models.

Ideally, a 24-hour urine collection in siblings of patients would permit controlling for other dietary influences such as sodium, K^+ and animal protein intake on urinary Ca^{2+} excretion. However, a 24-hour urine was not feasible, mainly because of poor compliance in collecting the specimen. Accordingly a fasting urinary specimen was collected instead and measured for creatinine, Ca^{2+}, sodium, K^+ and uric acid. The sodium/creatinine, K^+/creatinine and uric acid/creatinine ratios were used to control for the effects of these dietary variables in the familial aggregation analysis. The fasting

urines also allowed us to compare the urinary Ca^{2+}/creatinine ratio in consecutive patients with calcareous renal stones and siblings of stone formers with hypercalciuria. The use of fasting urine provided valuable information and was feasible to collect.

4. Effect Modification

In the first paper, stratified analyses were conducted by proband characteristics and relative age of disease onset to shed light on possible pathogenetic mechanisms behind the familial aggregation of disease. These analyses for effect modification were exploratory in nature, and it is conceivable that some of the associations were statistically significant by chance. However, the associations were consistent (ie, familial aggregation of disease was always linked to hypercalciuria or hyperuricosuria rather than normal urinary Ca^{2+} or uric acid) and in the same strata of the effect modifier (i.e., familial aggregation of disease was always linked to probands with higher BMIs). Moreover, the associations were found to be compatible with putative biological mechanisms derived from earlier studies or supportive of recent epidemiologic data. Thus, it is unlikely that the associations are due to type 1 error. Given the exploratory nature of this part of the analysis, the effects of multiple testing were not taken into account. Instead, these findings require confirmation in another sample of patients.

The second paper examined the potential interaction between hypercalciuria and a low K^+ diet as predictors of disease in relatives. The likelihood ratio tests showed only one significant interaction between hypercalciuria and low K^+ intake out of the five tested, specifically for excessive weight gain in first-degree relatives. Nevertheless, the magnitude of each association for all five conditions was consistently strongest for the combined group (low K^+ diet + hypercalciuria), and this abnormality was a significant predictor of hypertension, KSD and weight gain in first-degree relatives. These findings

are also consistent with our a priori expectations, further reducing the likelihood that they were due to chance.

5. Methodological Approaches to Help Find Genetic Effects

A number of methodologic approaches were employed to increase the likelihood of finding genetic effects.

a. Enrollment of younger probands

Younger patients were purposefully selected for study since abnormal urinary phenotypes that appear at an earlier age are more likely to have an underlying genetic basis (Lander and Schork, 1994). In way of support for this approach, the strongest associations for each condition were in siblings of probands, whereas no associations were found in parents. As mentioned previously, the lack of association in parents reflects the fact that environmental factors play a more prominent role as age increases (Hunt et al., 1986; Lander and Schork, 1994).

b. Inclusion of spouses

The inclusion of spouses greatly strengthened the familial aggregation studies by providing some insights into the importance of genetic and environmental influences on disease aggregation in families (Mitchell et al., 1996; Knuiman et al., 1996; Nicolaou et al., 2000; Lee et al., 2003; Wu et al., 2003). Earlier studies in KSD populations implied that familial factors were behind the associations between urinary phenotypes and disease, but were not able to differentiate between genetic or common environmental effects (Tisler et al., 1999; Tisler et al., 2002). In this study, the discordance of disease

aggregation between first-degree relatives and spouses was strongly suggestive of a genetic effect in the case of hypertension and KSD in particular.

c. Interviewing relatives to obtain health information

The advantages of obtaining health information directly from relatives rather than probands, in relation to measurement error and attenuated parameter estimates, was discussed earlier in the Measurement Error section.

d. Restrictive definitions of disease

Restrictive definitions of disease help to identify individuals with more severe manifestations of disease, an advantage in genetic studies (Lander and Schork, 1994). This issue was also addressed in the Measurement Error section.

6. Analysis Methods

GEE regression models were used to test for familial aggregation of disease among urinary groups (Zhao and Le Marchand, 1992). This approach is designed to analyze correlated data, as found in family studies, and permits the use of all data from study participants. A key drawback of GEE regression is that age at diagnosis cannot be considered. Cox regression, on the other hand, allows for age at diagnosis and censoring to be considered as long as age at diagnosis is known by the relatives. In this study, subjects were not asked about age at diagnosis for obesity or KSD, as these estimates were deemed to be unreliable, while the question was not applicable for weight gain over the past 5 years. The study did, however, assess age at diagnosis for hypertension and DM2. This information was reported by 96% of interviewed relatives. Although there is evidence that age at diagnosis is not reported accurately for hypertension and DM2 (Wilk

et al., 2004), Cox regression was used on data with available information on age at diagnosis to compare with the results from the GEE regression (Appendix 13). Overall, similar results were observed using both analysis methods. As with the GEE regression, the strongest associations for hypercalciuria, as well as for hypercalciuria combined with a low K^+ diet, were observed for siblings, while no significant aggregation of hypertension or DM2 was found in parents or spouses. In general, the magnitude of the associations were stronger for GEE regression than Cox regression, possibly because of the error associated with the reporting of age at diagnosis by relatives, which would likely attenuate associations toward the null. Thus, it appears that information on age at diagnosis did not affect the results.

Many GEE regression models were run, given that we examined the aggregation of several different conditions in different relative types for several different phenotypes. This may have increased the probability of detecting significant associations where none existed (type I error). However, the study was specifically designed to assess the hypothesis that hypercalciuria alone or in combination with low dietary K^+ or hyperuricosuria is related to the familial aggregation of hypertension and related conditions. Since this hypothesis was based on a theoretical framework, the analyses were pre-specified as opposed to post hoc or exploratory in nature. Moreover, if our biological model of a common pathway is correct, it would be expected that at least two conditions would aggregate in first-degree relatives of patients with hypercalciuria. This was the case in the current thesis, as hypertension and KSD aggregated in first-degree relatives of patients with hypercalciuria, and hypertension, KSD and weight gain aggregated in first-degree relatives of patients with hypercalciuria on a low K^+ diet. Furthermore, the effect sizes observed in this study were strong (often with OR > 3.0 for siblings) and in the expected urinary groups, so it is highly unlikely that they may be attributed to sampling error or multiple testing.

We did not observe a dose-response relationship between urinary Ca^{2+} in patients and disease in first-degree relatives. These observations are consistent with the findings of Lerolle et al (2002), who found a threshold effect for the relationship between urinary Ca^{2+} excretion and risk of KSD. In their study, there was no association observed at urinary levels below the cutpoint of 0.1 mmol/kg/day used to classify hypercalciuria.

7. Study Power

The sample size calculations conducted before starting the study provided similar power to detect associations for hypertension, KSD, obesity and excessive weight gain, since these conditions had similar prevalence rates. However, there was less power to detect familial aggregation of DM2 due to its lower prevalence. In addition, power in this study was further limited when the data were stratified to detect effect modification by proband age, gender and BMI. Moreover, the sample size calculations were conducted without the intention of finding a statistically significant interaction between hypercalciuria and a low K^+ diet. These findings require confirmation with a larger sample of patients and families.

The number of patients with combined hypercalciuria and low K^+ intake was modest (n=12), and consequently there were a limited number of spouses of patients with this urinary abnormality. However, none of these spouses was found to have hypertension or KSD, a finding that would be exceedingly unlikely if spouses had the same disease propensity as siblings. The associations between diabetes and urinary Ca^{2+} and K^+ in particular need further study with a larger number of families.

8. Generalizability

The study was conducted in a single stone Centre that specialized in treating only KSD patients requiring extracorporeal shockwave lithotripsy. Our results likely can be

generalized to other KSD populations requiring this treatment. Since the Centre does not see the full spectrum of patients with KSD, there are concerns about the generalizability of the findings to KSD patients not referred for extracorporeal shockwave lithotripsy. To assess the external validity of this study, the characteristics of our probands were compared to those of participants in other population-based studies. In our study, there were slightly more males than females (52% vs. 48%), which is consistent with previous findings (Johnson et al., 1979; Stematelou et al., 2003). The female participants in our study were compared to the female stone formers in the second Nurses Health Study (NHS-II) (Curhan et al., 2001). Our study subjects were younger (37.9 vs. 41.6 years) and had a lower mean body weight (72.4 kg vs. 73.8 kg), although both study populations had the same mean BMI (27.4 kg/m^2). They also had lower mean 24-hour excretion values for Ca^{2+} (4.9 mmol/d vs. 5.8 mmol/d), uric acid (2.9 mmol/d vs. 3.4 mmol/d), sodium (132 mmol/d vs. 164 mmol/d) and K^+ (46 mmol/d vs. 52 mmol/d) than the female stone formers in NHS-II. On the other hand, the mean 24-hour urinary creatinine excretion values (9.8 mmol/d vs. 10.1 mmol/d) were similar in the two samples, suggesting that the populations were roughly comparable. Thus, our findings are likely generalizable to other female stone populations. Unfortunately, there are no similar comparison datasets in male renal stone formers.

The requirement that relatives had to be contacted directly for health interviews reduced the number of ethnic minorities that were eligible for the study. The main reasons for excluding these patients were that relatives resided overseas and many did not speak English. This may have limited the generalizability of the findings to populations of European ancestry. However, when non-European families were excluded from the analyses, the odds ratios for disease clustering in families remained the same or increased.

There was no comparison group from the general population in this study, and thus the results represented differences in familial aggregation of disease within a KSD population only. However, the prevalence of treated hypertension among parents (44%) corresponded well to the prevalence estimates of hypertension in those aged 55-74 years in the general Canadian population (45%) (Wolf-Maier et al., 2003). The prevalence of hypertension among spouses (7%) was also similar to that of the general population in the 18-50 years age group (~10%) (Hajjar and Kotchen, 2003). Meanwhile, the prevalence of hypertension among siblings (13%) was comparable to the 18-50 years age strata (~10%) (Hajjar and Kotchen, 2003), but the prevalence was noticeably higher in the siblings of probands with hypercalciuria (24%). These findings suggest that the prevalence of hypertension is increased only among siblings of hypercalciuria patients in comparison to the general population, and that the prevalence estimates of hypertension relate to the general population of the same age.

C. Primary Metabolic Disturbance

The study hypothesis was that disturbed Ca^{2+} metabolism with both genetic and environmental determinants plays a central role in the development of hypertension and associated conditions. The main findings of this thesis support this hypothesis. The first study provided support for a disturbance in Ca^{2+} metabolism with an underlying genetic basis, as hypercalciuria in KSD patients was predictive of hypertension and KSD in siblings, but not spouses. Moreover, siblings of KSD patients with hypercalciuria had higher urinary Ca^{2+} than unselected KSD patients, even when controlling for environmental factors such as salt or animal protein intake. Nevertheless, the second study demonstrated the importance of dietary K^+ in these relationships, as hypercalciuria, a manifestation of suspected genetic susceptibility, combined with a low K^+ diet strongly predicted hypertension, obesity, weight gain and DM2 in siblings, but not spouses. There

was no familial aggregation in probands with hypercalciuria consuming a normal K^+ diet. Thus, disturbed Ca^{2+} metabolism with an underlying genetic basis acting together with environmental influences such as low dietary K^+, may be the common denominator behind these linked metabolic disorders. These factors may play an important role in the pathogenesis of this group of related conditions.

An alternative hypothesis to explain the increase in urinary Ca^{2+} excretion found in hypertension and other associated conditions is that there is an inherited defect in renal sodium handling that impairs the kidney's ability to eliminate sodium effectively (Cappuccio et al., 1993; De Wardener, 1996). According to this hypothesis, when dietary salt intake is high, sodium is retained causing central blood volume expansion and, in turn, essential hypertension, and increased urinary Ca^{2+} excretion and negative Ca^{2+} balance resulting in activation of compensatory mechanisms (Cappuccio et al., 1993; De Wardener, 1996). While the current study was not designed to test these competing hypotheses, some observations from this work may be relevant in this regard. We observed that high sodium excretion, alone or combined with hypercalciuria, in KSD patients did not predict hypertension in relatives (see Tables 2.1 and 2.2). The DASH trial showed that improving diet quality results in a markedly lower blood pressure, even with no change in sodium intake (Appel et al., 1997). Moreover, in the DASH Sodium trial, large changes in sodium intake resulted in little or no change in blood pressure when subjects consumed a high quality diet (Sacks et al., 2001). These findings suggest that salt sensitivity (i.e., substantial changes in blood pressure in response to marked changes in salt intake) is not an immutable trait, but the consequence of one or more acquired factors including poor diet quality (Sacks et al., 2001; Akita et al., 2003).

D. Supplementary Studies

1. Accuracy of Proband Reports Study

The methodologic study of this thesis is the first to examine the influence of proxy reported health information on observed associations for familial aggregation. The health status of family members was determined from both proxy respondents and directly from relatives before the results of the urine chemistries were known, so errors in ascertainment of disease status were likely to be random, which would dilute rather than magnify associations. The consecutive patients were recruited from a population-based treatment facility serving a large geographically defined area, thereby minimizing the likelihood of referral bias. The results showed that increased measurement error from the use of proxy reports led to weaker associations of familial aggregation for each urinary abnormality and for all of the clinical outcomes. This was most apparent for hypertension. Our findings suggest that interviewing family members directly would help to minimize measurement error in the assessment of disease status and produce stronger associations of disease aggregation in families.

This study is also the first to report on the accuracy of proband reported KSD and osteoporosis, and further examined the predictive accuracy of hypertension, diabetes and obesity reporting, while using relative self-reports as the criterion for comparison. The study showed that negative history reports were reliable for all conditions and in all relative categories, whereas positive history reports showed more variability both within and between relative categories. Overall, the predictive accuracy of patients' reports of KSD, hypertension and diabetes in all relative categories was high, whereas predictive accuracy of obesity and osteoporosis reporting was lower. Accurate reporting makes family history information a good clinical tool to identify disease in relatives and predict future risk in currently unaffected family members.

2. Ethnicity and Kidney Stones Study

The ecologic study of this thesis found that several ethnic groups in Canada, namely West Asians, Arabs, West Indians and Latin Americans, are at increased risk for developing KSD, while East Asians and Africans are at lower risk. Although there is previous information to support these findings (Hodgkinson, 1979; Zaidman et al., 1986; Akinci et al., 1991; Mkony et al., 1991; Pak, 1998), this study is the first to report on the propensity for KSD in various ethnic populations originating from different parts of Asia and residing in the same geographic region. In contrast, previous studies in the U.S. focused primarily on African- and Mexican-Americans (Hiatt et al., 1982; Sarmina et al., 1987; Soucie et al., 1994; Stamatelou et al., 2003), and none of these studies included Asians in sufficient numbers to analyze their results independently.

The reason(s) for the differences in kidney stone rates between ethnic groups are not known, but are likely the result of a mixture of lifestyle and genetic factors. Geographic variation in the prevalence of KSD has been documented in several cohort studies, and living in southern locations of the United States confers a greater risk of stone formation (Soucie et al., 1994; Curhan et al., 1998b). Several factors have been thought to account for this variation (Shuster and Finlayson, 1982; Coe, 1974), but the most prominent is excess sunlight and heat (Parry and Lister, 1975; Soucie et al., 1996). In this study, the ethnic differences in stone susceptibility likely cannot be explained by exposure to ambient temperature and sunlight, since several of the ethnic groups with lower rates of KSD originate from warm climates (e.g., Africans, South Asians). Length of time residing in Canada was not assessed, however. Increased body mass also did not appear to account for the disparity in risk for KSD. For instance, West Asians and Arabs had a significantly lower BMI and prevalence of obesity compared to Europeans, but higher rates of KSD. Urinary risk factors, including Ca^{2+} excretion, or stone types also could not explain the heightened risk in these groups. Dietary intake, which may

influence urinary measures, was also not evaluated in this study. Diet is known to play a role in the development of KSD (Borghi et al., 2002), and variation in the intake of several dietary factors (e.g., calcium, sodium, animal protein, oxalate and overall diet quality), may help explain ethnic differences in stone risk.

Studies of culturally diverse populations can help to identify new clues in the pathogenesis of disease, since these populations are heterogeneous in genetic and lifestyle characteristics (Anand et al., 2000; Anand et al., 2005). The use of an efficacious intermediate phenotype, such as urinary Ca^{2+} excretion, ultimately may contribute to a better understanding of the role of lifestyle and genetic factors, and better define individuals at risk for developing the disease of interest. Since KSD is likely the result of genetic and environmental factors acting in concert (Griffin et al., 2004), a key question for future studies is whether intermediate phenotypes are affected by environmental exposures such as diet in different ways among ethnic groups, and whether the relationships are influenced by underlying genetic factors. Future investigations need to confirm the findings in this study using more rigorously designed observational studies, and examine whether the disparities in KSD risk may be explained by differences in diet such as Ca^{2+} intake and overall diet quality, as well as biochemical indicators of disturbed Ca^{2+} metabolism including urinary Ca^{2+} excretion. If data show that a low Ca^{2+} diet or poor diet quality increase the risk of developing KSD, future studies could be directed toward improving dietary Ca^{2+} intake and diet quality in a culturally sensitive way to reduce the prevalence of this condition.

3. Potassium and Diet Quality Study

The dietary study of this thesis showed that a single 24-hour urinary K^+ measure is a valid indicator of diet quality and is inversely related to BMI. In keeping with our results, the first NHANES study showed a significant, inverse relationship between

dietary K^+ intake and BMI in the general U.S. population (McCarron et al., 1984). More recent clinical data showed that a diet rich in K^+-containing foods triggers weight loss (Howard et al, 2006). The findings are also congruent with the results of the second study, which showed that a low K^+ diet in probands was predictive of obesity and weight gain in first-degree relatives. Our observations are also consistent with the notion of familial clustering of eating patterns (Mitchell et al., 2003), and suggest that urinary K^+ may be used as a dietary marker in familial aggregation studies of conditions that are related to dietary K^+ intake.

E. Implications

A substantial amount of research has focused on identifying the genes that confer susceptibility to genetically complex traits, but few candidate genes have been found. This study defined an intermediate phenotype that might explain the overlap among several related conditions. A better-defined phenotype may reduce the degree of genetic heterogeneity in these complex conditions, and enable investigators to identify disease alleles and elucidate the molecular mechanisms involved in their pathogenesis. The findings may also be utilized to assess possible interactions between genetic and environmental influences such as dietary K^+ or diet quality.

From a clinical perspective, the results may help to identify individuals whom, when consuming a poor quality diet, are susceptible to developing conditions related to disturbances in Ca^{2+} metabolism such as hypertension, KSD, obesity, weight gain and DM2. Eventually, susceptible individuals who are prone to such metabolic disturbances may be identified at a younger age and encouraged to eat a quality diet that emphasizes fruit, vegetables, low-fat dairy products, whole grains, and lean meats. The findings also suggest that the development and use of Ca^{2+}-conserving medications may be beneficial in patients showing signs of disturbed Ca^{2+} metabolism.

An encouraging aspect of this study was that hypercalciuria in patients did not predict familial aggregation of disease when K^+ excretion was normal, but only when urinary K^+ was low, an indication that a healthy diet may protect against the development of these conditions among genetically susceptible individuals. These findings are congruent with characteristics of common genetically complex traits, which are highly influenced by environmental factors. Given the actual and potential benefits of the DASH diet and other healthy dietary patterns, dietary intervention should be encouraged as part of the standard of practice in the nonpharmacologic prevention and management of these closely related conditions.

F. Recommendations for Future Research

The current study may help in selecting genetically more homogenous populations to identify genes responsible for several related conditions. This is now considered an important first step in studies designed to identify susceptibility genes of complex traits and disorders. These conditions likely arise as a result of the additive effects of many disease alleles acting in concert with environmental factors. One approach to identify disease alleles involves evaluating a large number of highly informative markers located at strategic sites throughout the genome and testing for linkage in patients and their family members. Studies may also utilize the candidate gene approach, where genes that are thought to affect renal Ca^{2+} transport, directly or indirectly, are given primary consideration. Whole genome association studies offer a new approach that involves analyzing hundreds of thousands of highly informative SNPs strategically located throughout the genome and testing for associations in patients with well characterized phenotypes that demonstrate heritability and control subjects (International HapMap Consortium, 2005). Future studies will also likely investigate whether gene variants are related to variability in responsiveness to specific pharmacologic or non-pharmacologic

therapy. These findings would have important implications for tailoring primary and secondary preventive efforts.

G. Concluding Remarks

A detailed search of the literature as a background for this research revealed associations between several common clinical conditions such as hypertension, KSD, obesity, weight gain and DM2. However, it was not apparent from the literature as to why these conditions are linked. These relationships suggested that susceptible individuals share common genetic or environmental influences, or both. We therefore formulated a biological model whereby a common genetic background combined with environmental factors explained the associations between these conditions. This model guided our studies. Two familial aggregation studies were carried out to test our hypothesis that alterations in Ca^{2+} metabolism play a central role in the pathogenesis of these closely related conditions with hypercalciuria being a principal manifestation. The findings of this work pave the way for new studies, possibly using whole gene association approach, to find disease alleles that may be common among these related conditions.

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IX. Appendices

Appendix 1. Genes implicated in kidney stone disease.

Gene	Function	Pathogenetic expression
Chloride channel genes (CLCN5) (Thakker RV 2002)	Renal ion transport	Hypercalciuria; Proteinuria (sometimes); Dent's disease
Na^+-K^+-$2Cl^-$ cotransporter (NKCC2) (Naesens M 2004)	Renal ion transport	Hypercalciuria; Bartter syndrome
K^+ channel (ROMK) (Naesens M 2004)	Renal ion transport	Hypercalciuria; Bartter syndrome
Chloride channel (CLCNKB) (Zelikovic I 2003)	Renal ion transport	Hypercalciuria and Bartter syndrome; Hypocalciuria and Gitelman syndrome
Thiazide-sensitive Na-Cl cotransporter (SLC12A3) (Keszei, 2002)	Renal Na^+ and Cl^- transport	Hypercalciuria; Hypertension; Excess Na^+ reabsorption; Salt sensitivity
Calcium sensing receptor (CaSR) (Vezzoli G 2002)	Renal Ca^{2+} reabsorption	Hypercalciuria; PTH inhibition; Reduced intestinal Ca^{2+} absorption
Vitamin D receptor (VDR) (Nishijima S 2002)	Ca^{2+} absorption and excretion	Hypercalciuria; Increased bone resorption
WNK kinases (Mayan H 2002)	Renal Na^+ and Cl^- transport	Hypercalciuria; Hypertension; Low bone density; Pseudohypoaldosteronism; Hyperkalemia; Metabolic acidosis
ATP6B1 (Ruf R et al 2003)	Renal ATPase pump	Hypercalciuria; hypocitraturia; Metabolic acidosis;
Sodium-phosphate cotransporter (NPT2a) (Prie D 2002)	Renal phosphate transport	Hypophosphatemia; Hypercalciuria; $1,25(OH)_2D_3$; Low PTH; Low bone density
Alanine glyoxylate aminotransferase (AGT) (Amoroso A 2001)	Glyoxylate metabolism	Primary hyperoxaluria
Glyoxylate reductase (GRHPR) (Johnson SA 2002)	Glyoxylate metabolism	Primary hyperoxaluria
Amino acid transporter (SLC3A1) (Schmidt C 2004)	Renal cystine transport	Cystinuria; Pediatric stones
Amino acid transporter (SLC7A9) (Schmidt C 2004)	Renal cystine transport	Cystinuria; Pediatric stones
ZNF365 (Gianfrancesco F 2003)	Unknown	Hyperuricosuria
Xanthine dehydrgenase (XDH) (Gok F 2003)	Purine oxidative metabolism	Hyperuricosuria; Pediatric stones
Uromodulin (UMOD) (Turner JJ 2003)	Urinary glycoprotein	Hypoururicosuria; Hyperuricemia; Juvenile; hyperuricemic nephropathy

Appendix 2. Genes implicated in human hypertension.

Gene	Function	Pathogenetic expression
Angiotensinogen (Kunz R 1997; Staessen JA 1999)	RAA system	High plasma angiotensinogen; Severe early onset hypertension
Angiotensin-converting enzyme (Staessen JA 1997)	RAA system	High plasma ACE levels
Aldosterone synthase (Davies E 1999)	RAA system	Hyperaldosteronism
Epithelial sodium channel (Shimkets RA 1994)	Renal Na transport	Excess Na^+ reabsorption; Liddle's syndrome
α-adrenergic receptor (Kailasam MT 1998)	Catecholaminergic	Increased pressor responsiveness
β2-adrenergic receptor (Bray MS 2000)	Catecholaminergic	Salt sensitivity; Increased peripheral blood flow
G-proteins (Siffert W 1998)	Catecholaminergic	Increased vasoconstriction
Catecholamine synthetic enzyme (Sharma P 1998)	Catecholaminergic	Higher plasma catecholamine levels
Kallikrein (Parmer RJ 1999)	Renal kallikrein-kinin system	Lowered urinary kallikrein; Reduced renal blood flow; Reduced natriuresis
α-adducin (Cusi D 1997)	Renal ion transport	Salt sensitivity
Lipoprotein lipase (Wu DA 1996)	Lipid metabolism	Dyslipidemia
Apolipoprotein B (Frossard PM 1999)	Lipid metabolism	Dyslipidemia
Prostacyclin synthase (PTGIS) (Iwai N 1999)	Vasodilation and platelet aggregation	Higher pulse pressure and systolic BP
Glucagon receptor (Brand E 1999)	Glucose metabolism	High plasma glucose
Transforming growth factor β (Suthanthiran M 2000)	Bone regulation	Low bone mineral density
Glucocorticoid receptor (Lin RC 1999)	Glucocorticoid regulation	Increased plasma cortisol
Thiazide-sensitive Na-Cl cotransporter (Keszei A 2002)	Renal Na^+ and Cl^- transport	High urinary calcium; Excess Na^+ reabsorption; Salt sensitivity
WNK kinases (Chao-Ling Y 2003)	Renal Na^+ and Cl^- transport	Pseudohypoaldosteronism; high serum K^+; suppressed plasma renin

Appendix 3. Genes implicated in human obesity.

Gene	Function	Pathogenetic expression
Leptin gene (Montague CT et al 1997, Nature)	Appetite; Energy expenditure	Obesity
Leptin receptor (Clement K et al 1998, Nature)	Appetite; Energy expenditure	Obesity
Peroxisome proliferator-activated receptor γ (PPARgamma2) (Ristow M et al 1998)	Adipocyte differentiation	Obesity; hyperinsulinemia
B3-adrenergic receptor (ADRB3) (Walston J et al 1995)	Resting metabolic rate; lipolysis	Obesity; insulin resistance, hypertension
Uncoupling protein 3 (Argyropoulos G, Brown AM, et al 1998)	Basal fat oxidation	Obesity, DM2
Adiponectin (APM) (Zietz B 2001)	Insulin sensitivity and anti-inflammatory effects	Obesity; DM2; high LDL-cholesterol

Appendix 4. Genes implicated in type 2 diabetes mellitus.

Gene	Function	Pathogenetic expression
Intestinal fatty acid binding protein (FABP2) (Damcott CM 2003)	Intestinal absorption of fatty acids	Insulin resistance; Hyperinsulinemia
Regulatory subunit of protein phosphatase type 1 (PPP1R3A) (Doney AS 2003)	Synthesis and storage of glycogen from glucose in muscle and liver	Decreased insulin stimulated glycogen synthesis; Hyperinsulinemia; Reduced glucose uptake
β3-adrenergic receptor (ADRB3) (Walston J 1995)	Lipolysis of adipose tissue	Early onset DM2; Insulin resistance; Lower metabolic rate; Higher BMI and WHR; Hypertension
Beta-cell adenosine triphosphate-sensitive K+ channel (KCNJ11) (Gloyn AL 2003)	Insulin secretion	Hyperinsulinemic hypoglycemia in infancy; Subsequent impairment of insulin secretion
Peroxisome proliferator activated receptor-gamma (PPARG) (Doney AS 2004)	Adipogenesis and insulin signaling in fat and muscle	Decreased insulin sensitivity; Hyperinsulinemia
Peroxisome proliferator activated receptor-gamma coactivator-1 (PGC-1) (Koo SH 2004)	GLUT4 expression in skeletal muscle; Gluconeogenesis in the liver	Hyperinsulinemia; Variety of defects seen in DM2
Adiponectin (APM) (Zietz B 2001)	Insulin sensitivity and anti-inflammatory effects	Reduced plasma adiponectin in DM2 and obesity; Hypercholesterolemia
Plasma cell glycoprotein (PC1) (Marzban L 2004)	Possible role in insulin receptor kinase activity	Insulin resistance; Hyperinsulinemia
Insulin receptor substrate-1 (IRS1) (Kovacs P 2003)	Possible role in insulin signalling	Insulin resistance; Hyperinsulinemia

Appendix 5. High participation rate among first-degree relatives when probands give permission to contact relatives.

Participation rate of a colorectal cancer study being conducted at the Samuel Lunenfeld Research Institute. In this study, first-degree relatives were contacted after probands provided permission to contact kin. The numbers show that the participation rate of first-degree relatives is high in families where the proband consents to contacting kin.

Total number of first-degree relatives that probands granted permission to contact	1456
Total number of first-degree relatives that participated after telephone contact	1236
Total number of first-degree relatives that refused participation	220
Final participation rate among first-degree relatives who were contacted after probands gave permission to contact kin	**84.9%**

Appendix 6. Four types of urine specimens.

Specimen type	Description	Advantages	Disadvantages
Random	Subject simply urinates into a nonsterile container; usually obtained during daytime hours, without prior patient preparation	Ease and convenience; ideal when the substance to be tested does not have significant diurnal variation; usually adequate for routine screening	Least rigorous method; high fluid intake, exercise and diet can directly affect urine composition
First morning	Subject voids before going to bed, and then, immediately on rising, collects a urine specimen	Overnight urine represents urine from past 6-8 hours; sample is concentrated, which is ideal for detecting substances such as proteins and nitrates	Not the most convenient to obtain; subject must be given instructions and container before specimen is needed; sample must be preserved if not sent to lab within hours
Fasting	Subject eats nothing after the evening meal, and a single specimen is obtained the following day (excluding the first morning urine)	Less likely to be distorted by dietary intake, since the sample does not contain any food solutes or metabolites from the time prior to the fast	Not feasible when fasting is not a part of the subject's routine medical care; most ideal when testing for substances with minimal diurnal variation (as with a random urine)
24-hour	Subject discards the first void upon waking, the time is noted (e.g., 8 am), and then collects all urine voided up to and including that at 8:00 am the following morning (day 2)	The current benchmark method; more accurate than specimens collected over a shorter time, since substances such as hormones, proteins, and electrolytes are variably excreted over 24 hours, plus extraneous factors such as exercise, posture, hydration, and body metabolism influence excretion rates	Burdensome to the subject; not feasible for large-scale epidemiologic studies; accuracy of results may be compromised by incomplete urine collection; day-to-day variation in diet may affect reproducability

Appendix 7. Some good sources of K^+ in foods.

Source	Serving Size	Amount (mg)
Orange juice	1 cup	496
Cantaloupe	1 cup	494
Banana	1 medium	467
Halibut	3 ounces	490
Broccoli	1 cup	456
Almonds	2 ounces	412
Milk, low fat	1 cup	376
Salmon	3 ounces	319
Turkey	3 ounces	262
Chicken	3 ounces	220

Source: U.S. Department of Agriculture Food Database (http://www.vaughns-1-pagers.com/food/potassium-foods.htm)

Appendix 8. Determining the RFS Score for Diet Quality.

Food items included in the Recommended Foods Score (RFS) to determine diet quality (based on Kant et al. [2000]):

apples or pears
oranges
cantaloupe
orange or grapefruit juice
grapefruit
other fruit juices
dried beans
tomatoes
broccoli
spinach
mustard, turnip or collard greens
carrots or mixed vegetables with carrots
green salad
sweet potatoes, yams
other potatoes
baked or stewed chicken or turkey
baked or broiled fish
dark breads like whole wheat, rye, or pumpernickel
cornbread, tortillas, and grits
high-fiber cereals, such as bran, granola, or shredded wheat
cooked cereals
2% milk and beverages with 2% milk
1% or skim milk.

The items that are consumed at least once weekly are added up for a maximum score of 23 and a minimum of 0. Therefore, the maximum RFS score of 23 represents the highest quality diet.

Appendix 9. Baseline characteristics of study participants and non-participants.

Variable	Participants†	Non-participants†	p
Total number	333	58	
Age, years (mean ± SD)	38.9 ± 7.8	39.4 ± 7.9	0.653
Male gender (%)	174 (52.3)	32 (55.2)	0.788
Ethnic origin (%)			
European	270 (81.1)	46 (79.3)	0.892
Non-European	63 (18.9)	12 (20.7)	
Born in Canada (%)	239 (71.8)	40 (69.0)	0.780
Marital status (%)			
Married	222 (66.7)	41 (70.7)	0.652
Single	111 (33.3)	17 (29.3)	
Education (%)			
≤ High school	110 (33.0)	24 (41.4)	0.230
Post high-school or college degree	193 (58.0)	32 (55.2)	
> College degree	30 (9.0)	2 (3.4)	
Unemployed (%)	19 (5.7)	4 (6.9)	0.957
Body mass index, kg/m^2 (mean ± SD)	27.5 ± 5.5	28.1 ± 6.9	0.462
Obesity (%)	86 (25.9)	17 (29.3)	0.693
Blood pressure, mm Hg			
Systolic (mean ± SD)	122.4 ± 18.6	123.1 ± 19.1	0.792
Diastolic (mean ± SD)	75.0 ± 12.2	74.0 ± 11.3	0.561
Pulse pressure, mm Hg (mean ± SD)	47.4 ± 12.5	49.1 ± 13.5	0.346
Heart rate, beats/min (mean ± SD)	76.0 ± 12.8	78.1 ± 12.2	0.246

† , values are percent (frequency) unless otherwise indicated; Means are ± SD.
SD , standard deviation; mm Hg , millimeters of mercury

Appendix 10a. Response rates of relatives by patients' urinary Ca^{2+} excretion status.

	Parents		Siblings		Spouses	
	Normal Ca^{2+} Group	**High Ca^{2+} Group**	**Normal Ca^{2+} Group**	**High Ca^{2+} Group**	**Normal Ca^{2+} Group**	**High Ca^{2+} Group**
Overall total number	**524**	**142**	**561**	**156**	**177**	**45**
Deceased	123	42	7	2	0	0
Mean age at death (SD)	65.1 (12.2)	62.5 (12.3)	23.9 (15.1)	38.0 (12.0)	N/A	N/A
Total number alive	**401 (76.5%)**	**100 (70.4%)**	**554 (98.8%)**	**154 (98.7%)**	**177 (100%)**	**45 (100%)**
Permission to contact	**276 (68.8%)**	**74 (74.0%)**	**368 (66.4%)**	**116 (75.3%)†**	**156 (88.1%)**	**42 (93.3%)**
No permission to contact	125	26	186	38	21	3
Reason:						
proband refused	44	8	69	16	11	1
contact unknown	11	1	11	0	0	0
other*	70	17	106	22	10	2
Interviewed	**271 (98.2%)**	**74 (100%)**	**362 (98.4%)**	**115 (99.1%)**	**152 (97.4%)**	**41 (97.6%)**
Not interviewed	5	0	6	1	4	1
Reason:						
relative refused	3	0	3	1	0	1
other	2	0	3	0	4	0

* 'other' reason was usually 'relative residing overseas' or 'not English speaking';
† significantly higher than normal Ca^{2+} group.

Appendix 10b. Response rates of parents by patients' urinary Ca^{2+} and K^+ excretion status.

	Normal Ca^{2+}/ Normal K^+	**Normal Ca^{2+}/ Low K^+**	**High Ca^{2+}/ Normal K^+**	**High Ca^{2+}/ Low K^+**
Overall total number	**372**	**142**	**118**	**24**
Deceased	91	32	35	7
Mean age at death (SD)	65.7 (13.0)	63.5 (9.9)	62.0 (12.4)	64.8 (12.5)
Total number alive	**281 (75.5%)**	**110 (77.5%)**	**83 (70.3%)**	**17 (70.8%)**
Permission to contact	**185 (65.8%)**	**81 (73.6%)**	**63 (75.9%)**	**11 (64.7%)**
No permission to contact	96	29	20	6
Reason:				
proband refused	31	13	7	1
contact unknown	8	3	0	1
other*	57	13	13	4
Interviewed	**182 (98.3%)**	**79 (97.5%)**	**63 (100%)**	**11 (100%)**
Not interviewed	3	2	0	0
Reason:				
relative refused	2	1	0	0
other	1	1	0	0

* 'other' reason was usually 'relative residing overseas' or 'not English speaking'

Appendix 10c. Response rates of siblings by patients' urinary Ca^{2+} and K^+ excretion status.

	Normal Ca^{2+}/ Normal K^+	**Normal Ca^{2+}/ Low K^+**	**High Ca^{2+}/ Normal K^+**	**High Ca^{2+}/ Low K^+**
Overall total number	**404**	**145**	**122**	**34**
Deceased	5	2	2	0
Mean age at death (SD)	24.1 (18.5)	23.5 (0.7)	38.5 (12.0)	N/A
Total number alive	**399 (98.8%)**	**143 (98.6%)**	**120 (98.4%)**	**34 (100%)**
Permission to contact	**277 (69.4%)†**	**79 (55.2%)**	**92 (76.7%)†**	**24 (70.6%)**
No permission to contact	122	64	28	10
Reason:				
proband refused	50	19	14	2
contact unknown	7	4	0	0
other*	65	41	14	8
Interviewed	**272 (98.2%)**	**78 (98.7%)**	**91 (98.9%)**	**24 (100%)**
Not interviewed	5	1	1	0
Reason:				
relative refused	3	0	1	0
other	2	1	0	0

* 'other' reason was usually 'relative residing overseas' or 'not English speaking';
† significantly higher than normal Ca^{2+}/Low K^+.

Appendix 10d. Response rates of spouses by patients' urinary Ca^{2+} and K^+ excretion status.

	Normal Ca^{2+}/ Normal K^+	**Normal Ca^{2+}/ Low K^+**	**High Ca^{2+}/ Normal K^+**	**High Ca^{2+}/ Low K^+**
Overall total number	**133**	**41**	**36**	**9**
Deceased	0	0	0	0
Mean age at death (SD)	N/A	N/A	N/A	N/A
Total number alive	**133 (100%)**	**41 (100%)**	**36 (100%)**	**9 (100%)**
Permission to contact	**117 (88.0%)**	**36 (87.8%)**	**33 (91.7%)**	**9 (100%)**
No permission to contact	16	5	3	0
Reason:				
proband refused	9	2	1	0
contact unknown	0	0	0	0
other	7	3	2	0
Interviewed	**115 (98.3%)**	**35 (97.2%)**	**32 (97.0%)**	**9 (100%)**
Not interviewed	2	1	1	0
Reason:				
relative refused	0	0	1	0
other	2	1	0	0

Appendix 11. Characteristics of parents, siblings and spouses of kidney stone patients (n=333 patients) presented separately by patients' urinary Ca^{2+} and UA excretion status.

	Urinary Ca^{2+} Status				Urinary UA Status			
	Normal Ca^{2+}		High Ca^{2+}		Normal UA		High UA	
	n	%	n	%	n	%	n	%
Parents	**N = 271**		**N = 74**		**N = 284**		**N = 61**	
Mean age, years	271	64.1 (9.4)	74	66.2 (9.9)	284	63.6 (9.6)	61	69.0 (7.9)***
Male gender	111	41.0	33	44.6	119	41.9	25	41.0
Current smoker	45	16.7	8	10.8	47	16.6	6	9.8
Mean BMI (kg/m^2)	269	27.3 (4.8)	74	26.4 (3.7)	282	27.3 (4.7)	61	26.3 (4.1)
Obesity	69	26.0	13	17.6	70	25.2	12	19.7
Weight gain	46	17.5	13	17.6	50	18.1	9	14.8
Kidney stone disease	42	15.7	18	24.7	47	16.8	13	21.7
Treated hypertension	121	44.7	36	48.7	125	44.0	32	52.5
Treated diabetes	36	13.3	9	12.2	37	13.1	8	13.1
Siblings	**N = 362**		**N = 115**		**N = 359**		**N = 118**	
Mean age, years	362	41.3 (9.7)	115	43.7 (8.5)*	359	40.8 (9.6)	118	45.3 (8.2)***
Male gender	179	49.5	46	40.0	167	46.5	58	49.2
Current smoker	85	23.5	29	25.2	96	26.7	18	15.3*
Mean BMI (kg/m^2)	356	26.6 (4.8)	115	26.8 (5.4)	354	26.4 (4.8)	117	27.4 (5.6)
Obesity	74	20.9	23	20.0	71	20.1	26	22.2
Weight gain	98	28.1	37	32.5	104	30.0	31	26.7
Kidney stone disease	51	14.2	28	24.4*	52	14.6	27	22.9*
Treated hypertension	37	10.2	25	21.7**	33	9.2	29	24.6***
Treated diabetes	9	2.5	6	5.2	5	1.4	10	8.5***
Spouses	**N = 153**		**N = 40**		**N = 146**		**N = 47**	
Mean age, years	153	40.6 (7.7)	40	42.1 (7.4)	146	40.2 (7.7)	47	43.1 (7.0)*
Male gender	73	47.7	17	42.5	77	52.7	13	27.7**
Current smoker	32	21.1	10	25.0	32	22.1	10	21.3
Mean BMI (kg/m^2)	153	25.8 (4.9)	40	26.4 (4.5)	146	26.0 (4.5)	47	25.7 (5.6)
Obesity	27	17.8	9	22.5	30	20.7	6	12.8
Weight gain	42	28.2	9	22.5	42	29.2	9	20.0
Kidney stone disease	4	2.6	1	2.5	4	2.7	1	2.1
Treated hypertension	8	5.2	5	12.5	10	6.9	3	6.4
Treated diabetes	3	2.0	1	2.5	2	1.4	2	4.3

* $p < .05$; ** $p < .01$; *** $p < .001$.

Appendix 12. Characteristics of parents, siblings and spouses of kidney stone patients by Ca^{2+} and K^+ excretion status. The four urinary groups are mutually exclusive.

	Normal Ca^{2+} Excretion				High Ca^{2+} Excretion				
	Normal K^+		Low K^+		Normal K^+		Low K^+		
	n	%	n	%	n	%	n	%	p-value
Parents	**N = 182**		**N = 79**		**N = 63**		**N = 11**		
Mean age, years	182	65.6 (9.0)	79	59.8 (9.5)	63	66.7 (9.6)	11	63.0 (12)	<0.001
Male gender	72	39.6	34	43.0	28	44.4	5	45.5	0.884
Current smoker	28	15.5	17	21.5	7	11.1	1	9.1	0.340
Mean BMI (kg/m^2)	179	27.1 (4.7)	78	27.9 (5.2)	63	26.3 (3.7)	11	27.5 (4.2)	0.207
Obesity	42	23.5	24	30.8	10	15.6	3	27.3	0.209
Weight gain	33	18.8	13	16.7	9	14.1	4	36.4	0.340
Kidney stone disease	30	16.8	11	14.1	12	18.8	6	60.0	0.005
Treated hypertension	83	45.6	32	40.5	29	46.0	7	63.6	0.463
Treated diabetes	22	12.2	11	13.9	9	14.1	1	9.1	0.944
Siblings	**N = 272**		**N = 78**		**N = 91**		**N = 24**		
Mean age, years	272	42.2 (9.3)	78	38.4 (10.9)	91	43.9 (8.2)	24	42.8 (9.4)	0.002
Male gender	135	49.6	39	50.0	36	39.6	10	41.7	0.346
Current smoker	61	22.4	23	29.5	24	26.4	5	20.8	0.563
Mean BMI (kg/m^2)	265	26.3 (4.4)	78	27.5 (6.1)	91	25.7 (4.0)	24	31.2 (7.6)	<0.001
Obesity	47	17.7	24	30.8	11	12.1	12	50.0	0.001
Weight gain	64	24.6	31	40.3	23	25.6	14	58.3	<0.001
Kidney stone disease	39	14.4	11	14.1	22	24.2	6	25.0	0.104
Treated hypertension	30	11.0	7	9.0	15	16.5	10	41.7	<0.001
Treated diabetes	9	3.3	0	0	2	2.2	4	16.7	0.001
Spouses	**N = 115**		**N = 35**		**N = 32**		**N = 9**		
Mean age, years	115	41.5 (7.3)	35	38.3 (8.4)	32	41.8 (6.8)	9	43.0 (10)	0.120
Male gender	53	46.1	17	48.6	13	40.6	4	44.4	0.916
Current smoker	23	20.0	8	23.5	8	25.0	2	25.0	0.915
Mean BMI (kg/m^2)	114	25.8 (4.8)	35	25.8 (5.4)	32	26.9 (4.7)	8	24.5 (3.2)	0.563
Obesity	20	17.5	7	20.0	8	25.0	1	12.5	0.766
Weight gain	27	23.9	14	42.4	9	28.1	0	0	0.060
Kidney stone disease	3	2.6	1	2.9	1	3.1	0	0	0.969
Treated hypertension	7	6.1	1	2.9	5	15.6	0	0	0.143
Treated diabetes	3	2.6	0	0	1	3.1	1	12.5	0.123

Ca^{2+}, calcium; K^+, potassium; BMI, body mass index.

Appendix 13. Disease aggregation in relatives by patients' urinary abnormality using GEE regression vs. Cox regression.

	Patients' urinary group					
	High Ca^{2+} excretion †		High uric acid excretion †		High Ca^{2+} + low K^{+} excretion ‡	
	GEE regression	**Cox regression**	**GEE regression**	**Cox regression**	**GEE regression**	**Cox regression**
	OR (95% C.I.) §	HR (95% C.I.) §	OR (95% C.I.) §	HR (95% C.I.) §	OR (95% C.I.) §	HR (95% C.I.) §
Hypertension						
1st degree relatives	1.8 (1.1-2.8)*	1.1 (0.7-1.6)	1.4 (0.9-2.3)	1.0 (0.7-1.5)	3.3 (1.5-7.4)**	1.1 (0.7-1.7)
Parents	1.1 (0.7-2.0)	0.8 (0.5-1.3)	1.0 (0.6-1.8)	0.8 (0.5-1.3)	1.9 (0.6-6.1)	1.0 (0.5-1.7)
Siblings	2.9 (1.4-6.2)**	1.9 (1.04-3.4)*	1.9 (0.9-3.9)	0.6 (0.3-1.2)	5.3 (2.1-13.4)***	2.1 (0.96-4.6)
Spouses	1.8 (0.4-7.3)	0.5 (0.1-4.5)	0.5 (0.1-3.7)	0.8 (0.2-3.5)	0 cases	0 cases
Diabetes						
1st degree relatives	1.6 (0.7-3.5)	1.2 (0.6-2.4)	1.4 (0.7-2.7)	0.7 (0.3-1.5)	2.7 (0.7-10.5)	1.6 (0.6-4.6)
Parents	1.2 (0.5-2.9)	1.2 (0.6-2.6)	0.9 (0.4-2.2)	0.4 (0.1-1.2)	1.0 (0.1-8.7)	1.4 (0.3-7.4)
Siblings	2.1 (0.7-6.3)	0.7 (0.1-4.5)	2.8 (0.8-9.8)	0.4 (0.1-2.0)	4.1 (1.1-15.6)*	4.5 (0.4-52.0)
Spouses	1.2 (0.1-9.6)	sparse data	2.1 (0.3-14.3)	sparse data	sparse data	sparse data

Abbreviations: Ca^{2+} , calcium; K^{+} , potassium; OR , odds ratio; HR , hazard ratio; GEE , Generalized Estimating Equation; 95% C.I. , 95 percent confidence interval.

* $p < .05$; ** $p < .01$; *** $p < .001$;

† , reference group is patients with normal levels of the urinary marker in question.

‡ , reference group is patients with normal urinary Ca^{2+} and K^{+}.

§ , adjusted for patients' age, gender, body mass index, antihypertensive medication use or personal history status, and relative age (also adjusted for other potential confounders based on a 10% change in the point estimate; see manuscripts 1 and 2).

Appendix 14. Crude and adjusted odds ratios and 95% confidence intervals of kidney stone disease in populations of Asian ancestry compared to Europeans.

Ethnic origin	No. of KSD pts.	Pop. size in Greater Toronto Area	Prevalence Rate of KSD (%)	Unadjusted prevalence odds ratio (95% CI)	Age-adjusted prevalence odds ratio (95% CI)	Age- and gender adjusted prevalence odds ratio (95% CI)
European	736	1 416125	0.052	†1.00	†1.00	†1.00
African	50	156 222	0.032	0.62 (0.46–0.82)**	0.69 (0.52–0.91)*	0.70 (0.53–0.94)*
East Asian	49	254 367	0.019	0.37 (0.28–0.50)***	0.38 (0.28–0.51)***	0.38 (0.29–0.51)***
Latin American	35	43 969	0.080	1.53 (1.09–2.15)*	1.66 (1.18–2.33)**	1.67 (1.19–2.35)**
South Asian	128	247 551	0.052	1.00 (0.83–1.20)	1.08 (0.89–1.30)	1.07 (0.88–1.29)
West Indian	43	36 706	0.117	2.25 (1.66–3.07)***	2.38 (1.75–3.23)***	2.41 (1.77–3.27)***
Filipino	45	72 565	0.062	1.19 (0.88–1.61)	1.21 (0.90–1.64)	1.27 (0.94–1.72)
Southeast Asian	14	30 568	0.045	0.88 (0.52–1.50)	0.96 (0.57–1.63)	0.96 (0.56–1.62)
West Asian	36	30 788	0.117	2.25 (1.61–3.14)***	2.45 (1.75–3.42)***	2.40 (1.71–3.35)***
Arabic	41	22 855	0.179	3.45 (2.52–4.73)***	3.76 (2.74–5.15)***	3.65 (2.66–5.00)***

†, Europeans are the reference group for comparisons; 95% C.I., 95 percent confidence interval;
* p<.05; ** p<.01; ***p<.001.