Mapping a new suggestive gene locus for autosomal dominant nephrolithiasis to chromosome 9q33.2–q34.2 by total genome search for linkage

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Abstract

Background. Nephrolithiasis is a complex, multifactorial disease resulting from genetic and environmental interaction. The pathogenesis of nephrolithiasis is far from being understood. So far, no gene locus for autosomal dominant nephrolithiasis only has been described. We here identified a new suggestive gene locus for autosomal dominant nephrolithiasis by a genome-wide search for linkage in a Spanish kindred with nephrolithiasis.

Methods. Clinical data, blood and urine samples of 18 individuals from a Spanish kindred with nephrolithiasis were collected. We performed a genome-wide search for linkage using 380 polymorphic microsatellite markers.

Results. Nephrolithiasis segregated in this Spanish kindred in a pattern compatible with autosomal dominant inheritance. The total genome search yielded the highest two-point LOD score of \( Z_{\text{max}} = 1.99 \) (\( \theta = 0 \)) for marker \( D9S159 \) on chromosome 9q33.2–q34.2. Multipoint analysis of 24 polymorphic markers used for further fine mapping resulted in a LOD score of \( Z_{\text{max}} = 2.7 \) (\( \theta = 0 \)) for markers \( D9S1881-D9S164 \), thereby identifying a new gene locus for autosomal dominant nephrolithiasis (NPL1). Two recombination events define \( D9S1850 \) as the centromeric flanking marker and \( D9S1818 \) as the telomeric flanking marker, restricting the NPL1 locus to a 14 Mb interval.

Conclusion. We here identified a new suggestive gene locus (NPL1) for autosomal dominant nephrolithiasis. It is localized on chromosome 9q33.2–q34.2. The identification of the responsible gene will provide new insights into the molecular basis of nephrolithiasis.

Keywords: haplotype analysis; nephrolithiasis; recombination

Introduction

Nephrolithiasis is a common condition, which affects 5–9% of the European population [1]. It is a multifactorial disease, resulting from interactions of genetic and environmental effects. A variety of environmental factors contribute to stone formation: dietary habits, life-style, climate and socio-economic status [2,3]. Stone-forming disease may also be associated with hypercalciuria, hyperphosphaturia, hyperoxaluria, hypocitraturia, hyperuricosuria, cystinuria, persistent high or low urinary pH and low urine volume [4]. In nephrolithiasis, mostly polygenic inheritance is assumed, especially in nephrolithiasis with hypercalciuria [5]. Genetic influence may alter dietary absorption (hypercalciuric and hyperoxaluric states), renal tubular reabsorption [Dent’s disease, familial hypomagnesaemia with hypercalciuria and nephrocalcinosis (FHHNC) and cystinuria] or the endogenous production of stone-forming materials [Lesch–Nyhan syndrome (LNS), xanthinuria type 1 and primary hyperoxaluria (PH)]. Most of these monogenic disorders result in an accumulation of a stone-forming...
salt, which may begin crystallization due to an abnormally high urinary concentration. Stone formation is inhibited by substances that prevent crystallization (e.g. citrate, glycosaminoglycans, and renal proteins such as nephrocalcin, uromodulin and uropontin) and thereby balance the crystallization promoters [6–8]. However, the pathophysiological mechanism of nephrolithiasis and the genetic contribution is still incompletely understood. In genetic forms of nephrolithiasis, different pathomechanisms are described as causing stone formation: (i) aberrant trafficking of proteins to mitochondria, rather than to peroxisomes (PHs); (ii) defective degradation of reabsorbed proteins in endosomes (Dent’s disease); (iii) impaired function of tubular transporter proteins (FHHNC and nephrolithiasis with hypophosphatemia); and (iv) defective metabolic enzymes (Kelley–Seegmiller syndrome). We here identify a new suggestive autosomal dominant locus for nephrolithiasis (NPLI) on chromosome 9q33.2–q34.2 by a total genome search for linkage.

Methods

Patient recruitment

Two researchers (M.T.F.W. and I. Z.) travelled from Germany to Tenerife, Spain and visited the patients individually to obtain a first-hand updated history. Blood samples, 24h urine samples and clinical data of the kindred with nephrolithiasis were ascertained from 18 individuals of a Spanish kindred, in which nephrolithiasis segregated in all affected individuals (Figure 1). Informed consent was obtained from patients, their parents and siblings. The kindred originates from La Gomera, one of the Canary Islands. In the 1920s, the whole family emigrated to Venezuela. After 50 years, the entire family returned to Tenerife. Diagnosis was established by their internist or urologist physicians in Venezuela. Urinary biochemical ratios (calcium/creatinine, phosphate/creatinine and magnesium/creatinine) were evaluated according to the reference values given in Matos et al. [9]. The study was approved by the ethics committee of the Albert-Ludwigs-University Freiburg, Germany.

Haplotype analysis

Genomic DNA was isolated by standard methods. Haplotype analysis was performed in 18 individuals (including 10 affected individuals and inferred in an additional three individuals) (Figure 1). A total of 380 microsatellite markers from the Genethon final linkage map [10] were used at an average distance of 11cM. For further fine mapping on chromosome 9q33.2–q34.2, 22 additional microsatellite markers were used, with an average spacing of 1.5cM within a 22cM interval (14 Mb). The order and distance for the microsatellite markers were obtained from the deCode database [11]. In cases of missing information for a marker, we referred to the Genethon database [10]. Order and sex-averaged distances (given in cM in parentheses) between these markers (Figure 1) from centromeric to q telomeric are as follows: D9S275 (2.82), D9S1850 (4.14), D9S1881 (6.01), D9S1825 (0.15), D9S1789 (0), D9S266 (0), D9S1829 (0.54), D9S1798 (0.44), D9S1821 (1.01), D9S1819 (0.09), D9S904 (0.22), D9S918 (0), D9S1827 (0), D9S260 (0), D9S752 (2.02), D9S1795 (0), D9S159 (0.5), D9S1831 (1.6), D9S179 (3.35), D9S2157 (0.38), D9S164 (3.5), D9S1818 (7.31), D9S1826 (6.3) and D9S1838. Fluorescently labelled polymerase chain reaction (PCR) products were detected by a MegaBace-1000 semi-automated genotyping analysis system (Amersham, Uppsala, Sweden). Data were analysed by Genetic Profiler Software, version 1.1 (Amersham, Uppsala, Sweden). Two-point LOD score calculations were performed by the LINKAGE program package, with the help of the LINKRUN computer program (T. F. Wienker, unpublished). The nephrolithiasis phenotype exhibited an age-related penetrance; therefore, an ‘affecteds-only’ strategy was applied for LOD score analysis, using an autosomal dominant model. Since an affecteds-only strategy was used, penetrance was set at 100%. For haplotyping and computation of multipoint LOD scores, the program SIMWALK was used. Allele frequencies were calculated according to the Fondation Jean Dausset-CEPH database.

Results

Clinical data

In this Spanish kindred, 18 individuals were available for genetic analysis and in three additional individuals we were able to infer haplotypes (Figure 1). In 10 living individuals, the diagnosis of nephrolithiasis was made. The clinical and laboratory findings of the affected patients, as far as available, are shown in Tables 1 and 2. Dietary habits did not vary greatly between affected and unaffected individuals. In all 10 affected individuals, at least one episode of stone passage was documented, with a maximum of eight episodes in individual III-9. Individual III-9 was initially diagnosed with primary hyperparathyroidism but, after removal of the parathyroid adenoma, she still had three episodes of nephrolithiasis in the presence of normal parathyroid hormone (PTH) values, demonstrating that her nephrolithiasis was independent of her primary hyperparathyroidism. Age of onset ranged from 11 to 35 years (median 23 years). In four individuals with repeated episodes of nephrolithiasis, surgical intervention was necessary. In individual II-2, percutaneous nephrolithotomy was performed at the age of 38 years. In individual II-4, percutaneous nephrolithotomy was done at the age of 54 years and extracorporeal shock-wave lithotripsy (ESWL) at the age of 60 years. Individual III-1 underwent ESWL at the age of 31 years. In individual III-9, percutaneous nephrolithotomy was done twice and ESWL four times between the ages of 33 and 39 years. Stone composition was reckoned by the patients to consist of calcium oxalate in four affected individuals and of urate in one affected individual. In the remaining five patients, no data were available on stone chemistry. Paresthesia, muscle weakness and flank pain were described in three patients (III-1, III-3 and III-9); and macrohematuria (III-9) and microhematuria (III-1) were each reported
Fig. 1. Results from haplotype analysis of the 24 microsatellite markers at the NPL1 locus in 21 (18 living and three inferred) individuals from the Spanish nephrolithiasis kindred. Markers are shown on the left from centromeric to q-terminal (top to bottom). Circles denote females, squares denote males. Filled symbols denote affected individuals. Inferred haplotypes are shown in parentheses. Differently shaded bars symbolize haplotypes. Paternal haplotypes are drawn to the left, maternal haplotypes to the right. All 11 individuals with nephrolithiasis (filled symbols) carry the black shaded haplotype. There seems to be incomplete penetrance, since individuals III-5 and IV-2 have no clinical evidence of nephrolithiasis so far, although they carry the black shaded haplotype. Note the recombination events in individuals III-9 and III-1, which identify markers D9S1850 and D9S1818 (underlined) as the centromeric and telomeric flanking markers (underlined) of the NPL1 locus, respectively. They delimit the 14 Mb critical interval for NPL1.
in one individual. Taking all available laboratory data into consideration, no strong common explanation for nephrolithiasis was found. No significant hypercalciuria was documented. Hypomagnesemia was diagnosed in II-6, III-1 and III-9. Mild hypermagnesuria was only found in II-6. Interestingly, in most affected individuals, FE Mg was elevated. Affected individuals showed an FE Mg ranging between 4.96 and 11.66% (median 7.75%). In contrast, unaffected individuals showed an FE Mg ranging between 1.3 and 7.45% (median 4.67%). However, in four of seven analysed unaffected individuals, FE Mg was also elevated (>5%). Uric acid, serum creatinine and PTH were only elevated in individual II-4, due to renal insufficiency and gout (age of onset 76 years). In all other individuals, serum phosphate, serum uric acid, alkaline phosphatase, urinary magnesium and urinary uric acid were normal.

**Total genome scan for linkage**

The mode of inheritance for nephrolithiasis in this kindred is clearly autosomal dominant, with at least two incidents of male-to-male transmission, which excludes X-linked inheritance (Figure 1). Thus, all autosomal recessive or X-linked forms of nephrolithiasis were excluded. In the initial total genome search for linkage, only one locus exhibited full co-segregation with the nephrolithiasis phenotype. This locus was positioned at microsatellite markers D9S1825 and D9S2157 on chromosome 9q33.2–q34.2, yielding an initial suggestive maximum two-point LOD score of $Z_{\text{max}} = 1.65$ ($\theta = 0$) for marker D9S2157. Further fine mapping with an additional 22 microsatellite markers confirmed this locus with a suggestive two-point LOD score $Z_{\text{max}} = 1.99$ at marker D9S159 (Table 3). Haplotype analysis showed clear evidence that the disease allele co-segregated with all affected subjects.
Multipoint analysis of the 24 microsatellite markers resulted in a maximum LOD score of 2.7 for markers D9S1881–D9S164 at physical position 122 255 469–131 632 180 (Figure 2). A recombination event in III-9 defined marker D9S1850 as proximally flanking, and a recombination in III-1 identified marker D9S1818 as distally flanking the critical genetic region within a 14 Mb (22 cM) interval on chromosome 9q33.1–q34.3 (Figure 1).

Discussion

We here describe a new suggestive locus for autosomal dominant nephrolithiasis (*NPL1*). Using an ‘affecteds-only’ approach, a suggestive LOD score was found on chromosome 9q33.2–q34.2. This critical chromosomal region extends over 22 cM, which corresponds to 14 Mb. Approximately 170 genes are located within this region. There are no linkage data.
from animal models for nephrolithiasis that would show synteny to this locus.

Except an association of nephrolithiasis in some affected individuals with reduced tubular reabsorption for magnesium, which was also found in some unaffected individuals, we could not detect a consistent biochemical association with the nephrolithiasis phenotype. Therefore, we chose the strongest criterion for the affected status: documentation of passage of a urinary concrement. The two individuals that carry the ‘affected’ haplotype (III-5 and IV-2) are at risk to develop nephrolithiasis (III-5 is 37 years of age and IV-2 is 23 years of age). The lack of a strong common reason for nephrolithiasis in this kindred may be explained by the possibility that we did not find a locus responsible for promoting stone formation by accumulation of a lithogen substance, but by a loss of function of a stone formation inhibitor. Different urinary inhibitors such as urinary glycosaminoglycans (GAGs), like mucin, osteopontin, fibronectin, bikunin, nephrocalcin, inter-


References


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Conflict of interest statement. None declared.

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