Identification of a New Locus for Medullary Cystic Disease, on Chromosome 16p12

Francesco Scolari,[1] Daniela Puzzer,[4] Antonio Amoroso,[4] Gianluca Caridi,[5] Gian Marco Ghiggeri,[5] Rosario Maiorca,[1] Paolo Aridon,[2] Maurizio De Fusco,[2] Andrea Ballabio,[2,3] and Giorgio Casari[2]

[1]Division and Chair of Nephrology, Spedali Civili and University of Brescia, Brescia, [2]Telethon Institute of Genetics and Medicine and [3]Universita Vita-Salute, San Raffaele Biomedical Science Park, Milan, [4]Genetics Service, Istituto per l'Infanzia Burlo Garofolo and University of Trieste, Trieste, and [5]Laboratory of Nephrology, G. Gaslini Institute, Genoa, Italy

Summary

Autosomal dominant medullary cystic disease (ADMCKD) is an interstitial nephropathy that has morphologic and clinical features similar to autosomal recessive nephronophthisis. The typical renal dysfunction associated with ADMCKD results mainly from a defect in urinary concentration ability, although results of urinalysis are normal. Recently, a locus on chromosome 1 was associated with ADMCKD, in DNA from two large Cypriot families, and genetic heterogeneity was inferred. We describe the genomewide linkage mapping of a new locus for medullary cystic disease, ADMCKD2, on chromosome 16p12 in a four-generation Italian pedigree. The family with ADMCKD2 fulfills the typical diagnostic criteria of ADMCKD, complicated by hyperuricemia and gouty arthritis. Marker D16S3036 shows a maximum two-point LOD score of 3.68, and the defined critical region spans 10.5 cM, between D16S500 and SCNN1B1–2. Candidate genes included in the critical region are discussed.

Introduction

Autosomal dominant medullary cystic disease (ADMCKD; MIM 174000) is a renal disorder characterized by structural defects in the renal tubules, which lead to a reduction of urine concentrating ability and a decrease in sodium conservation. The disease progresses toward renal failure, which generally requires dialysis or transplantation in patients aged <50 years. The disease is, however, heterogeneous in several clinical aspects, including age at onset, rate of progression, and clinical appearance. This variability contributes to the diagnosis of ADMCKD being either rare or confused with other clinical entities. ADMCKD is usually diagnosed in the third or fourth decade of life; however, cases of children with the disease, with slower progression toward renal failure, are not uncommon (Gardner 1971). Renal symptoms are mainly defects in urinary concentration ability with normal results of urinalysis.

The presence of small cysts in renal medulla is a central feature of the disease, although the cysts may be absent in the early stages. Discovery of these medullary cysts may also be confounded by the presence of cysts acquired as a result of chronic renal failure, in some patients. In the most frequent presentation of ADMCKD, which is characterized by tubulointerstitial fibrosis and thickening of the tubular basement membrane, renal pathologic findings are not specific and are similar to those found in other pathological entities such as juvenile-onset nephronophthisis (NPH; MIM 256100). The major difference between ADMCKD and NPH is the relative inheritance pattern, which is recessive in the latter, with typical onset of renal symptoms in early childhood and rapid evolution toward renal failure in the second decade of life (Hildebrandt et al. 1992; Neumann et al. 1997). Recent advances in the molecular genetics of NPH have helped differentiate between the two entities (Hildebrandt et al. 1997; Saunier et al. 1997; Christodoulou et al. 1998; Fuchshuber et al. 1998; Scolari et al. 1998; Stavrou et al. 1998).

A clear-cut definition for diagnosis of ADMCKD was needed for further differentiation among ADMCKD phenotypes. This led us to define several criteria for the diagnosis of ADMCKD (Scolari et al. 1998; see Methods), on the basis of family inheritance and clinical and pathological characteristics, including associated symptoms such as hyperuricemia. Recently, genetic linkage of ADMCKD to the ADMCKD1 locus was established on chromosome 1 (Christodoulou et al. 1998). In the pre-

sent article, we report genomewide linkage analysis of DNA from a large family and identification of a new locus associated with ADMCKD, thus showing the genetic heterogeneity of this clinically homogeneous renal disorder.

Subjects and Methods

Subjects and Diagnostic Criteria

The four-generation Italian family, which included 10 members (4 men and 6 women) affected by ADMCKD and diagnosed as indicated below, is depicted in figure 1. Four affected members (III-3, III-6, III-9, and IV-6) were given a clinical and histological diagnosis of ADMCKD; three members (II-3, III-4, and IV-1) had only a clinical diagnosis of ADMCKD; and three deceased relatives (I-1, II-1, and II-4) had a diagnosis of chronic nephritis listed on their death certificates. Five affected family members (II-3, III-3, III-6, III-9, and IV-6) had hyperuricemia (that required specific treatment with allopurinol) associated with ADMCKD; in two subjects (II-3 and III-9), hyperuricemia was complicated

by gouty arthritis. The age at diagnosis of ADMCKD ranged from 16 to 54 years (mean age, 31.5 years). Renal cysts were documented by sonogram in one subject (II-3). Twelve available members (III-1, III-2, III-5, III-7, III-8, III-10, III-11, III-12, IV-2, IV-3, IV-4, and IV-5) were screened and proved to be negative for the disease (see table 1).

Diagnostic criteria were as follows: (1) autosomal dominant inheritance; (2) defective urine concentration with polyuria, isosthenuria, and relatively normal urinalysis results; (3) normal- or small-sized kidneys with occasional small medullary cysts; and (4) renal histologic findings characterized by tubular-interstitial fibrosis with infiltrates, tubular atrophy, and thickening of the tubular basement membrane with periodic acid Schiff–positive material. Available family members were considered healthy if they had results showing normal renal function, negative renal sonography, negative urinalysis, and urinary osmolality >800 mOsm/l, after overnight dehydration. In subjects with chronic renal insufficiency, hyperuricemia was defined as an elevation of plasma urate levels disproportionate to the degree of renal in-
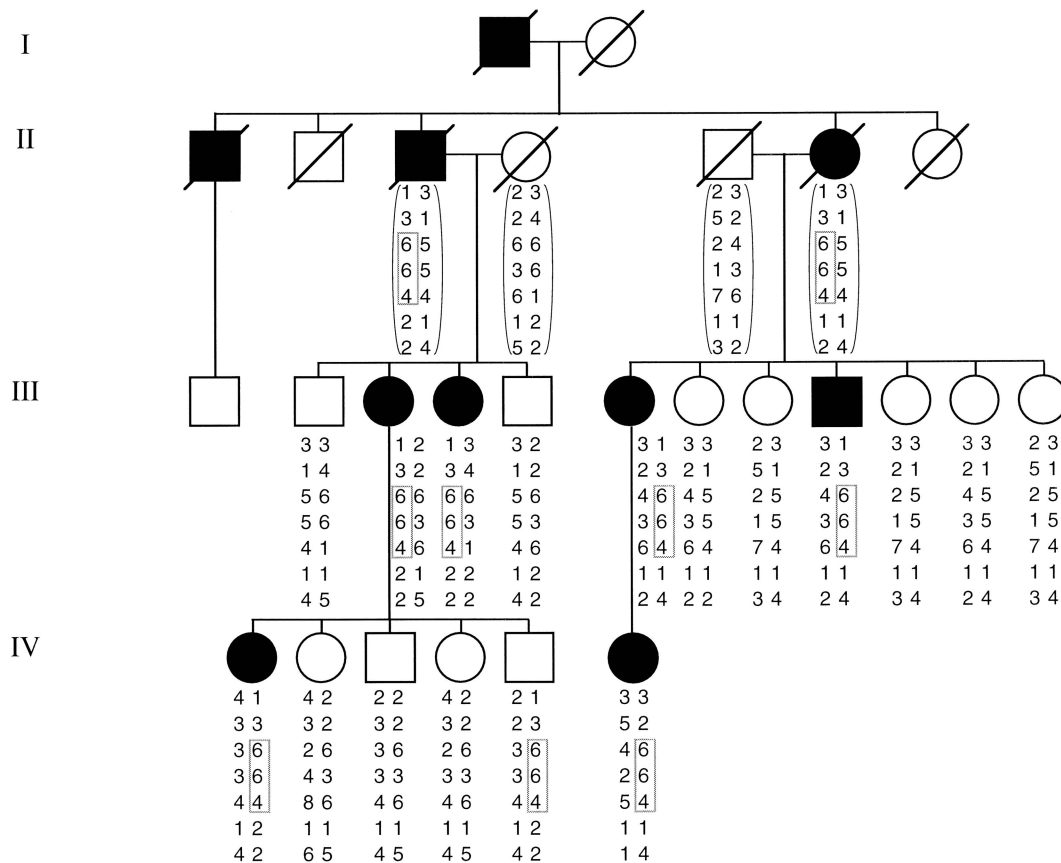

Figure 1 Pedigree of the family with ADMCKD2. Haplotypes for markers D16S405, D16S500, D16S3017, and markers D16S3036, D16S3041, SCNN1B1–2, and D16S420, are shown from top to bottom. The disease-associated haplotype is boxed. Second-generation inferred haplotypes are shown between brackets.

Table 1

Clinical, Biochemical, and Histological Findings of ADMCKD2 Family

Subject	Diagnosis	Age at Diagnosis_Age at Screening (years)	Morning Urinary Osmolarity (mOsm H$_2$O/kg)	Renal Ultrasound	Renal Histology	Serum Creatinine/ Uric Acid (mg/dl)
II-3	ADMCKD	54_	300	Small kidneys with cysts	ND	2/12.0
III-1	NO	_44	860	Negative	ND	.8/4.7
III-2	NO	_43	900	Negative	ND	.9/ND
III-3	ADMCKD	18_	300	Small kidneys w/o cysts	TIN	1.5/9.6
III-4	ADMCKD	25_	330	Small kidneys w/o cysts	ND	1.6/5.9
III-5	NO	_36	861	Negative	ND	.8/ND
III-6	ADMCKD	30_	315	Small kidneys w/o cysts	TIN	1.3/9.2
III-7	NO	_45	900	Negative	ND	.9/4.5
III-8	NO	_44	895	Negative	ND	.7/4.2
III-9	ADMCKD	29_	295	Small kidneys w/o cysts	TIN	1.7/12
III-10	NO	_38	910	Negative	ND	.97/2.8
III-11	NO	_37	805	Negative	ND	.8/ND
III-12	NO	_35	900	Negative	ND	.9/3.1
IV-1	ADMCKD	16_	310	Small kidneys w/o cysts	ND	1.6/5.5
IV-2	NO	_23	1054	Negative	ND	.7/2.8
IV-3	NO	_21	1183	Negative	ND	.8/
IV-4	NO	_22	857	Negative	ND	.7/2.3
IV-5	NO	_10	812	Negative	ND	.6/ND
IV-6	ADMCKD	18_	330	Normal kidneys w/o cysts	TIN	1.5/9.5

NOTE.—NO = normal phenotype, w/o = without, ND = not done, and TIN = Tubulo-interstitial nephritis.

sufficiency in subjects with chronic renal insufficiency (Murray and Goldberg 1975).

Linkage Analysis

After informed consent, a 10-ml venous blood sample was obtained from six affecteds and seven healthy relatives. DNA was extracted from peripheral blood lymphocytes as described elsewhere (Sambrook et al. 1989). A genomewide microsatellite screening was done with 358 highly polymorphic fluorescent markers (Gyapay et al. 1994; Dib et al. 1996). This set of markers, which are formatted and properly fluorescence-labeled to allow efficient multiplexing on an automated sequencer, covers all the autosomal chromosomes at an average reciprocal distance of ~10 cM.

The DNA from six affected members was subjected to genomewide screening, and this preliminary set of linkage data was analyzed with the MLINK program of LINKAGE (Lathrop et al. 1984). Selected chromosomal regions were further investigated with additional markers mapping telomeric and centromeric to the candidate loci. For linkage calculations, ADMCKD was modeled as an autosomal dominant trait with two liability classes for age-dependent penetrance: 0.5 for age <15 years and 0.9 for age >15 years. The mutation rate was assumed to be 10^{-5}. Recombination frequencies (θ) were considered to be equal between male and female subjects. Marker alleles were assumed to be equifrequent in the preliminary screening, whereas published allelic frequencies were used for candidate loci calculations. Mul-

tipoint linkage analysis was done by LINKMAP (Lathrop et al. 1984), with the same parameters.

Results

The loci responsible for autosomal recessive NPH1 (Hildebrandt et al. 1997) and the ADMCKD1 form (Christodoulou et al. 1998) were excluded from linkage with the family in our previous study (Scolari et al. 1998; G. Casari, unpublished material). We therefore considered this pedigree for genomewide linkage mapping. We tested the probability of detecting significant linkage by simulation analysis of 400 replicate pedigrees by using SLINK (Weeks et al. 1990), considering the phenotype with a frequency of 1×10^{-5}. The maximum pairwise LOD score obtained from 400 simulated pedigrees was 4.01 at $\theta = .02$. A genomewide search was performed on all affected pedigree members. The selected loci suggestive of linkage (LOD score >1.0) were further characterized by testing all pedigree members' DNA and by locally increasing marker density.

The chromosome 16p locus, preliminarily identified by the fluorescent markers D16S407 and D16S405 (LOD scores of 1.36 and 2.10 at $\theta = .1$ and .05, respectively), was confirmed with flanking markers. As reported in table 2, marker D16S3036 achieves a maximum value of 3.68 with no recombination within the disease locus. A 10.5-cM critical region between markers D16S500 and SCNN1B1–2 (a polymorphic dinucleotide repeat intragenic to the beta subunit of the epithelial

Table 2

Two-Point LOD Score Values for 16p13–12 Markers

	LOD Score at $\theta =$						
Marker	.00	.01	.05	.10	.20	.30	.40
D16S423	−5.98	−1.43	−.54	−.14	.15	.15	.06
D16S407	−3.29	.69	1.27	1.36	1.15	.73	.24
D16S3114	.00	.02	.09	.13	.16	.14	.09
D16S405	−2.28	1.66	2.10	2.06	1.64	1.02	.34
D16S500	−1.91	2.02	2.43	2.35	1.87	1.20	.47
D16S3017	2.71	2.66	2.45	2.19	1.64	1.04	.41
D16S3036	3.69	3.62	3.36	3.01	2.27	1.46	.59
D16S3041	1.32	1.30	1.21	1.08	.83	.56	.28
SCNN1B1-2	−2.97	−.30	.29	.45	.43	.26	.07
D16S420	−4.38	−.38	.35	.62	.71	.51	.19

sodium channel; Shimkets et al. 1994) was defined by recombinant pedigree members. A common haplotype (6_6_4) for markers D16S3017, D16S3036, and D16S3041 is shared by all affected individuals (fig. 1); individual IV-5 is a carrier of the disease-associated haplotype but does not show the ADMCKD phenotype, probably because of his young age (10 years), which is much less than the age at onset among the other affected family members (aged 16–20 years; table 1). Data from subject III-4 was observed to be a double recombinant for marker D16S3036 (or for markers D16S3017 and D16S3036, because D16S3017 is not informative) in the unaffected maternal haplotype.

The multipoint linkage graph (fig. 2) shows a multipoint LOD score of 4.18 around D16S3036, thus identifying a new locus for ADMCKD that we named "ADMCKD2." Several partially characterized transcripts (GeneMap98), and some known genes, map to this critical region, including a phosphodiesterase gene (Bolger et al. 1993), the multidrug resistance-associated protein (Cole et al. 1992), the alanine aminotransferase (Sohocki et al. 1997), the ubiquinol-cytochrome c reductase complex core protein 2 precursor (Hosokawa et al. 1989), the major Yo paraneoplastic antigen (Fathallah Shaykh et al. 1991), the mu-crystallin (Kim et al. 1992), and the uromodulin/Tamm-Horsfall glycoprotein (Pennica et al. 1987). The genes coding for the beta and gamma subunits of the kidney epithelial sodium channel (SCNN1B and SCNN1G; Voilley et al. 1995) are both localized to a 400-kb area in 16p12 at the centromeric boundary of the critical region and have been excluded from linkage to ADMCKD2 by the intragenic marker SCNN1B1–2.

Discussion

ADMCKD has been considered a rare disease, on occasion, because of a paucity of symptoms at early stages and aspecific clinical and pathological features once renal failure has developed. Furthermore, because of several clinical and pathological similarities, ADMCKD has

been easily confused with juvenile NPH. Advances in the molecular genetics of renal cyst diseases have contributed to a definite differentiation between these two clinical entities. Recently, linkage analysis and positional cloning approaches to recessive juvenile NPH have contributed to the identification of a candidate gene mapping to chromosome 2q13, which is deleted in >65% of patients with NPH1 (Hildebrandt et al. 1997).

An autosomal dominant form of ADMCKD has been mapped to chromosome 1q21 in DNA from members of two large Cypriot families affected with renal cysts, hyperuricemia, and gout (Stavrou et al. 1998). No obvious candidate genes have been proposed. The identification of a common haplotype in both families with ADMCKD is probably due to a founder effect, frequently observed in small island communities. In the present article, we show evidence of linkage of ADMCKD2 associated with chromosome 16p12 in DNA from a large Italian family. In addition to the typical renal cysts, affected members show hyperuricemia and gout. These symptoms are also found in the Cypriot families and seem to identify a more homogeneous subgroup of ADMCKD. Despite symptom and clinical homogeneity, the data in the present article show genetic heterogeneity.

The 10.5-cM critical region of ADMCKD2 is dense with transcripts (see Results), and the considerable size of this genomic area renders a positional cloning approach difficult. Nonetheless, the localization of the uromodulin (also known as "Tamm-Horsfall protein") gene
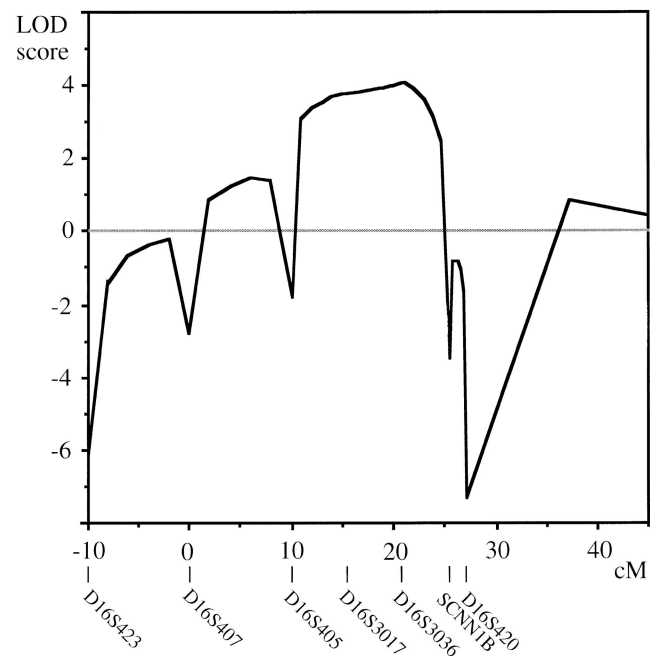

Figure 2 Multipoint linkage graph of the region surrounding the ADMCKD2 locus.

to the ADMCKD2 critical region (Pook et al. 1993) is interesting. Uromodulin is expressed mainly in the kidney, where it is localized to the epithelial cells of the thick ascending limb (TAL) of the Henle loops (Allen and Tischer 1976), and has been functionally associated with the water nonpermeability of the TAL (Sikri et al. 1978), a function that is altered in ADMCKD. Furthermore, abnormal peritubular and interstitial deposits of uromodulin have been considered characteristic of NPH and tubulointerstitial inflammatory disease (Resnick et al. 1978; Zanger et al. 1978). However, DNA from additional pedigrees will need to be analyzed to refine the linkage mapping of the ADMCKD2 locus and to suitably narrow the candidate region for a positional cloning approach. The identification of the new ADMCKD2 locus and evidence of genetic heterogeneity, notwithstanding the shared common phenotype, will further contribute to the understanding of cystic disease pathogenesis.

Acknowledgments

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GeneMap98, http://www.ncbi.nlm.nih.gov/genemap98/ (a new gene map of the human genome)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for ADMCKD [MIM 174000] and NPH [MIM 256100])

References

Allen F, Tischer CC (1976) Morphology of the ascending thick limb of Henle. Kidney Int 9:8–22

Bolger G, Michaeli T, Martins T, St. John T, Steiner B, Rodgers L, Riggs M, et al (1993) A family of human phosphodiesterases homologous to the dunce learning and memory gene product of Drosophila melanogaster are potential targets for antidepressant drugs. Mol Cell Biol 13:6558–6571

Christodoulou K, Tsingis M, Stavrou C, Eleftheriou A, Papavlou P, Patsalis PC, Ioannou P, et al (1998) Chromosome 1 localization of a gene for autosomal dominant medullary cystic kidney disease. Hum Mol Genet 7:905–911

Cole SP, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, et al (1992) Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science 258:1650–1654

Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, et al (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Fathallah Shaykh H, Wolf S, Wong E, Posner JB, Furneaux HM (1991) Cloning of a leucine-zipper protein recognized by the sera of patients with antibody-associated paraneoplastic cerebellar degeneration. Proc Natl Acad Sci USA 88:3451–3454

Fuchshuber A, Deltas CC, Berthold S, Stavrou C, Vollmer M, Burton C, Feest T, et al (1998) Autosomal dominant medullary cystic kidney disease: evidence of gene locus heterogeneity. Nephrol Dial Transplant 13:1955–1957

Gardner KD Jr (1971) Evolution of clinical signs in adult-onset cystic disease of the renal medulla. Ann Intern Med 74:47–54

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, et al (1994) The 1993–94 Généthon human genetic linkage map. Nat Genet 7:246–339

Hildebrandt F, Otto E, Rensing C, Nothwang HG, Vollmer M, Adolphs J, Hanusch H, et al (1997) A novel gene encoding an SH3 domain protein is mutated in nephronophthisis type 1. Nat Genet 17:149–153

Hildebrandt F, Waldherr R, Kutt R, Brandis M (1992) The nephronophthisis complex: clinical and genetic aspects. Clin Invest 70:802–808

Hosokawa Y, Suzuki H, Toda H, Nishikimi M, Ozawa T (1989) Complementary DNA encoding core protein II of human mitochondrial cytochrome bc1 complex: substantial diversity in deduced primary structure from its yeast counterpart. J Biol Chem 264:13483–13488

Kim RY, Gasser R, Wistow GJ (1992) mu-crystallin is a mammalian homologue of agrobacterium ornithine cyclodeaminase and is expressed in human retina. Proc Natl Acad Sci USA 89:9292–9296

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Murray T, Goldberg M (1975) Chronic interstitial nephritis: etiologic factors. Ann Intern Med 82:453

Neumann HP, Zauner I, Strahm B, Bender BU, Schollmeyer P, Blum U, Rohrbach R, et al (1997) Late occurrence of cysts in autosomal dominant medullary cystic kidney disease. Nephrol Dial Transplant 12:1242–1246

Pennica D, Kohr WJ, Kuang WJ, Glaister D, Aggarwal BB, Chen EY, Goeddel DV (1987) Identification of human uromodulin as the Tamm-Horsfall urinary glycoprotein. Science 236:83–88

Pook MA, Jeremiah S, Scheinman SJ, Povey S, Thakker RV (1993) Localization of the Tamm-Horsfall glycoprotein (uromodulin) gene to chromosome 16p12.3–16p13.11. Ann Hum Genet 57:285–290

Resnick JS, Sisson S, Vernier RL (1978) Tamm-Horsfall protein abnormal localization in renal disease. Lab Invest 38:550–555

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Saunier S, Calado J, Heilig R, Silbermann F, Benessy F, Morin G, Konrad M, et al (1997) A novel gene that encodes a protein with a putative src homology 3 domain is a candidate gene for familial juvenile nephronophthisis. Hum Mol Genet 6:2317–2323

Scolari F, Ghiggeri GM, Casari G, Amoroso A, Puzzer D, Caridi G, Valzorio B, et al (1998) Autosomal dominant medullary cystic disease: a disorder with variable clinical pictures and absence of linkage with the NPH1 locus. Nephrol Dial Transplant 13:2536–2546

Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, Schambelan M, Gill JRJ, et al (1994) Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. Cell 79:407–414.

Sikri KL, Foster CL, Bloomfield FJ, Marshall RD (1978) Colocalization by immunofluorescence and by light- and electron-microscopic immunoperoxidase techniques of Tamm-Horsfall glycoprotein in adult hamster kidney. Biochem J 181:525–532

Sohocki MM, Sullivan LS, Harrison WR, Sodergren EJ, Elder FF, Weinstock G, Tanase S, et al (1997) Human glutamate pyruvate transaminase (GPT): localization to 8q24.3, cDNA and genomic sequences, and polymorphic sites. Genomics 40:247–252

Stavrou C, Pierides A, Zouvani I, Kyriacou K, Antignac C, Neophytou P, Christodoulou K, et al (1998) Medullary cystic kidney disease with hyperuricemia and gout in a large Cypriot family: no allelism with nephronophthisis type 1. Am J Med Genet 77:149–154

Voilley N, Bassilana F, Mignon C, Merscher S, Mattei MG, Carle GF, Lazdunski M, et al (1995) Cloning, chromosomal localization, and physical linkage of the beta and gamma subunits (SCNN1B and SCNN1G) of the human epithelial amiloride-sensitive sodium channel. Genomics 28:560–565

Weeks DE, Ott J, Lathrop GM (1990) SLINK: a general simulation program for linkage analysis. Am J Hum Genet Suppl 47:A204

Zanger RA, Cotran RS, Hoyer JR (1978) Pathologic localization of Tamm-Horsfall protein in interstitial deposit in renal disease. Lab Invest 38:52–57