GENETIC RENAL DISEASE / ORIGINAL ARTICLE

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Renal polyamine excretion, tubular amino acid reabsorption and molecular genetics in cystinuria

Abstract Cystinuria is an autosomal recessive disorder of the tubular and intestinal resorption of cystine, ornithine, lysine and arginine leading to nephrolithiasis. Three cystinuria types can be distinguished by the mode of inheritance (true recessive or intermediate) and by the pattern of the intestinal amino acid transport. In the present study phenotypes were assessed by the urinary excretion of amino acids related to creatinine, the percentage tubular amino acid reabsorption and the urinary excretion of polyamines as a possible indicator of the intestinal transport defect. However, our thorough phenotyping did not reveal more than two cystinuria types. Genotypes were examined in linkage analyses and single-strand conformation polymorphism-based mutation identification. The SLC3A1 mutations M467T and T216M were disease causing in our homozygous patients of type I cystinuria. We can show the association of type I cystinuria with SLC3A1 and of non-type I cystinuria with a yet unidentified gene on chromosome 19q13.1. Our phenotype and genotype analyses provide evidence for only two types of cystinuria in the investigated patient cohort.

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Introduction

Classic cystinuria is a heritable disorder of amino acid transport in epithelial cells of the renal proximal tubule and of the intestinum. It is characterized by urinary hyperexcretion of cystine and the dibasic amino acids lysine, ornithine and arginine. Due to its poor solubility, cystine precipitates to form urinary tract calculi with subsequent obstruction, infection and possibly renal insufficiency.

In family studies two modes of inheritance can be observed. Cystinuria may be transmitted either as a true recessive (type I) or as an intermediate trait (non-type I = type II and III) [1]. In the latter state heterozygotes excrete the affected urinary amino acids in larger quantities than normal but less than in the homozygous state. Double heterozygotes with alleles of both the true recessive and the intermediate type excrete cystine and dibasic amino acids in amounts as high as homozygotes [2]. Rosenberg et al. [1, 3] observed differences in the intestinal amino acid resorption, which led to the differentiation between type II and III. Up to now two cystinuria loci have been published: SLC3A1 (2p16.3) [4, 5] and a locus on chromosome 19q with a $Zmax$ of 13.11 for D19S225 [6, 7].

Previous studies focussed either on cystinuria phenotyping or on genetic analyses. The aim of our study was to describe the different cystinuria phenotypes and their genetic correlates as precisely as possible by the use of minimally invasive methods. We investigated 17 cystinuria-families with altogether 117 probands. The renal transport defect was assessed by urinary amino acid excretion and percentage tubular amino acid reabsorption ($\%T_{aa}$). To estimate the intestinal transport defect, the urinary excretion of the polyamines cadaverine, putrescine, spermidine and spermine was determined as a pos-

sible marker. We analyzed the corresponding genotypes by haplotyping the marker loci D2S119 and D2S177 linked with SLC3A1 in 80 probands (10 families). In those families in which an association of the SLC3A1 markers with the cystinuria phenotype was not excluded (the two families with type I cystinuria and both double heterozygous families), single-strand conformation polymorphism (SSCP)-based mutation analysis of this gene was performed. A search for the possible locus of non-type I cystinuria was then initiated in the remaining six families (54 persons).

Methods

Probands

We examined 117 probands from 17 families with cystinuric children, after obtaining informed consent. Eleven cystinuric children were identified in a neuroblastoma-screening program that had been extended for the determination of cystinuria [8]. These patients have not so far shown any stone formation. In addition, 6 cystinuric patients, treated for recurrent stone formation at the Children's Hospital of the Medical School Hannover were investigated along with their families. All probands had normal glomerular filtration.

Laboratory techniques for the study of phenotypes

To avoid bacterial breakdown of amino acids, urine samples were passed through a sterile filter (pore size: 0.2 μm) and immediately stored at –20°C. Blood samples were deproteinized within 30 min after collection to prevent cystine from binding to plasma protein. Amino acids were determined by ion exchange chromatography on a Biotronic amino acid analyzer LC 5000 [2]. Polyamine analysis was performed on a modified Biotronic amino acid analyzer LC 2000, using a K^+-gradient instead of a Li^+-gradient and fluorescence detection with o-phthaldialdehyde instead of photometry with ninhydrin. Using this method polyamines could be detected in urine samples at concentrations as low as 0.01 μmol/l. Creatinine was determined by a kinetic Jaffé reaction. Urinary amino acids and polyamines were corrected per gram of creatinine. The determination of %T_{aa}, originally performed with inulin as marker of glomerular filtration rate [9], was modified to creatinine and based on spot urine samples [10]. This method required simultaneously collected blood and urine samples. The time gap between venipuncture and void never exceeded 30 min. The %T_{aa} was calculated by the following equation:

$$\%T_{aa}=(1-C_{aa}/C_{crea})\times100=(1-(U_{aa}\times P_{crea})/(P_{aa}\times U_{crea}))\times100.$$

where C_{aa}/C_{crea} = renal clearance of amino acids/creatinine, and $U_{aa}/P_{crea}/P_{aa}/U_{crea}$ = concentrations of amino acids and creatinine in urine an plasma.

Statistical analysis was done using multivariant analysis and least significant difference tests. A P value less than 0.01 was considered to be significant. To investigate intra-individual variation, eight of our probands were examined in repeated measurements. In addition, probands with identical cystinuria genotypes were compared with each other.

Linkage analysis to SLC3A1

Out of the 17 cystinuria families, 10 were suitable for genetic studies including linkage analysis. In addition, one double heterozygous family was suitable for mutation analysis in the SLC3A1 gene, but not informative in linkage analysis. Phenotypes were as-

sessed as described above. Patients in two of the families showed the true recessive cystinuria phenotype (type I), two exhibited the intermediate phenotype (non-type I) and in two other families the cystinuria patients seemed to be double heterozygous for both phenotypes. In the five remaining families, the tubular reabsorption values of cystine and the dibasic amino acids suggested heterozygosity for the intermediate phenotype. High molecular weight DNA was extracted from blood leukocytes according to standard protocols.

The microsatellite markers D2S177 and D2S119, flanking the SLC3A1 gene on either side, were amplified by polymerase chain reaction (PCR) using published primers [4] and genomic DNA as template; 1 μl of PCR product was mixed with 2 μl of formamide stopmix (see next section) and 5 μl H_2O and electrophoresed on denaturing 6% polyacrylamide/8 M urea gels at 1800 V and 50°C. The gels were fixed and silver stained according to standard protocols.

Alleles were defined by length within each family and linkage to the SLC3A1 gene was assessed by visual inspection.

Search for new mutations

Subsequently, we searched for mutations in those patients whose families did not contradict linkage to the SLC3A1 gene (homozygotes of type I cystinuria and double heterozygotes). SLC3A1 exons were amplified from genomic DNA using intronic primers published by Purroy et al. [11]. Exons 1 and 4 could not be amplified, although different PCR conditions, nested PCR and exonic primers were tested. The mutation analysis was based on SSCP analysis; 4 μl of PCR product were mixed with 6 μl SSCP-stopmix containing 95% formamide, 20 mM EDTA and 0.1% of both bromophenol blue and xylene cyanol. These samples were denatured at 95°C for 3 min, chilled at –20°C and then loaded on a nondenaturing 6% polyacrylamide gel containing 5% glycerol and 0.8× TBE. Electrophoresis was performed in 0.8× TBE buffer at 10 W overnight (10–12 h) or at 35 W for 4–5 h in a cold room. Bands were visualized by silver staining. Samples with aberrant migration of single strands were sequenced using the Sequenase 2.0 PCR product sequencing kit (Amersham/USB) and [α-^{33}P]dATP for radiolabelling, according to the manufacturers' protocol.

Linkage analysis in intermediate cystinuria families

We searched for homozygous markers in a patient affected with non-type I cystinuria, assuming homozygosity by descent of the expected mutant allele, as the patient's parents were first cousins. Six additional families were scanned for linkage to the chromosome 19-positive region, using the markers D19S414, D19S416, D19S225, D19S220, D19S412 and D19S418. The investigations were performed at the German Microsatellite Center in Berlin [12, 13].

Results

Classification of probands

In this study 117 probands from 17 families with cystinuric children were investigated. They were classified according to their renal amino acid excretion and the mode of inheritance in their families. We could distinguish two characteristic patterns of renal amino acid hyperexcretion differing from the normal state: (a) moderately increased excretion rates of cystine and lysine, and (b) distinctly increased excretion rates of cystine, ornithine, lysine and arginine.

The first pattern can usually be observed in non-type I heterozygotes, while the second is characteristic of type I

Fig. 1 Urinary amino acid excretion expressed as µmol/g creatinine of 117 probands in 17 families. *I/I* type I homozygotes (*n*=3), *II/II* type II or III homozygotes (*n*=3), *DH* double heterozygotes (*n*=3), *n/II* type II or III heterozygotes (*n*=48), *n/n* normals (*n*=60)
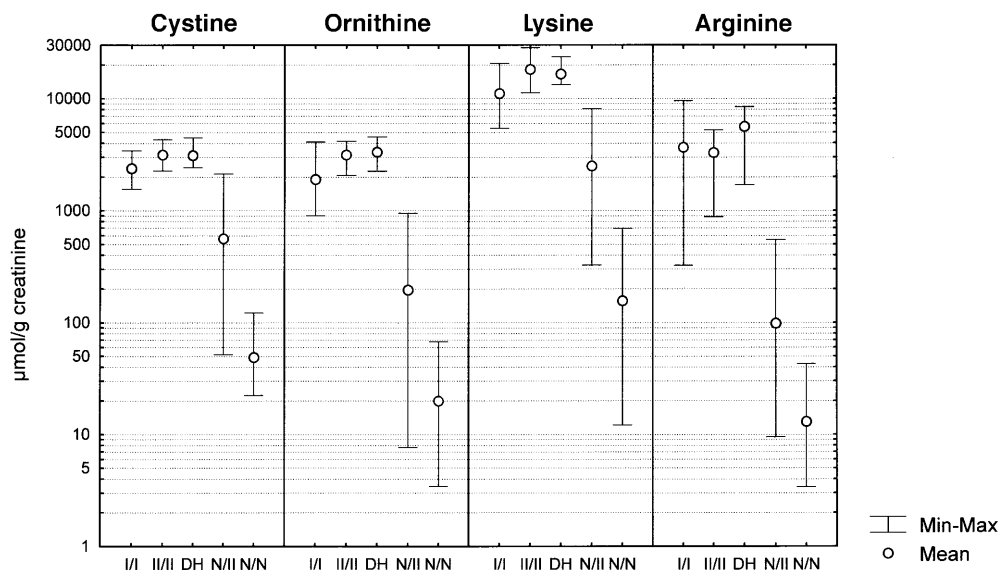
homozygotes, non-type I homozygotes and double heterozygotes. Type I heterozygotes show normal amino acid excretion. We determined the mode of inheritance in our cystinuria families, comparing the amino acid excretion patterns of relatives. Accordingly, we assigned our probands to the following groups:

1. Type I homozygotes (3 probands in 2 families)
2. Non-type I homozygotes (3 probands in 3 families)
3. Double heterozygotes (3 probands in 2 families)
4. Type I heterozygotes (7 probands in 4 families)
5. Non-type I heterozygotes (48 probands in 10 families) and
6. No carriers of cystinuria genes (53 probands in all 17 families).

The urinary excretion and the percentage tubular reabsorption of cystine, ornithine, lysine and arginine, and the urinary excretion of the polyamines putrescine and cadaverine was compared between these groups. The data of group 5 was added to group 6, as they show no differences.

Amino acid excretion

Two pathological and one normal group could be distinguished by the amino acid excretion expressed as µmol/g creatinine alone (Fig. 1). The first group, type I homozygotes (I/I) and non-type I homozygotes (II/II), as well as double heterozygotes (DH) excreted the affected amino acids in similar amounts with very high excretion rates of cystine (1565–4494 µmol/g creatinine), ornithine (901–4569 µmol/g creatinine), lysine (5427–28530 µmol/g creatinine) and arginine (325–8439 µmol/g creatinine).

The second pathological group, non-type I heterozygotes (N/II), showed moderately increased excretion rates of cystine (52–2129 µmol/g creatinine) and lysine (327–8153 µmol/g creatinine), and sometimes

of ornithine (8–953 µmol/g creatinine) and arginine (9–550 µmol/g creatinine) as well.

The control group, consisting of seven obligate type I heterozygotes and 53 probands who were not carriers of a cystinuria gene (N/N), showed normal excretion rates of cystine (22–122 µmol/g creatinine), ornithine (3–67 µmol/g creatinine), lysine (12–694 µmol/g creatinine) and arginine (3–43 µmol/g creatinine).

The differences between the mean values of the three groups, described above, were significant at $P<0.01$ (multivariant analysis and LSD test). Within these three groups no further differentiation was possible.

Eight probands were investigated in repeated measurements. Their excretion rates varied intra-individually over a wide range. The largest variations were observed in one non-type I heterozygote, who had a cystine excretion rate of 156 µmol/g creatinine on one day and 643 µmol/g creatinine on another day (ornithine 19/219 µmol/g ceratinine; lysine 689/3888 µmol/g ceratinine; arginine 42/100 µmol/g ceratinine). The following fluctuations of cystine excretion rates (µmol/g creatinine) were observed in the other patients: 1566/1775 (type I homozygote); 3062/4319 (non-type I homozygote); 2818/4494, 2422/2987 (double heterozygotes); 46/60 (type I heterozygote); 191/285, 52/95 (non-type I heterozygotes). Similar differences were observed between relatives with identical cystinuria genotypes.

Tubular amino acid reabsorption (%T$_{aa}$)

Again, only two pathological and one normal group could be distinguished by the tubular amino acid reabsorption (Fig. 2). In the first group, homozygotes (I/I + II/II) and double heterozygotes (DH), tubular reabsorption was severely reduced for cystine (–87.8–81.0%), ornithine (17.6–92.0%), lysine (–9.1–79.5%) and arginine (–32.2–98.0%). In the second, non-type I heterozygotes

Fig. 2 Tubular amino acid reabsorption ($\%T_{aa}$) of 117 probands in 17 families. *I/I* type I homozygotes (*n*=3), *II/II* type II or III homozygotes (*n*=3), *DH* double heterozygotes (*n*=3), *n/II* type II or III heterozygotes (*n*=48), *n/n* normals (*n*=60)
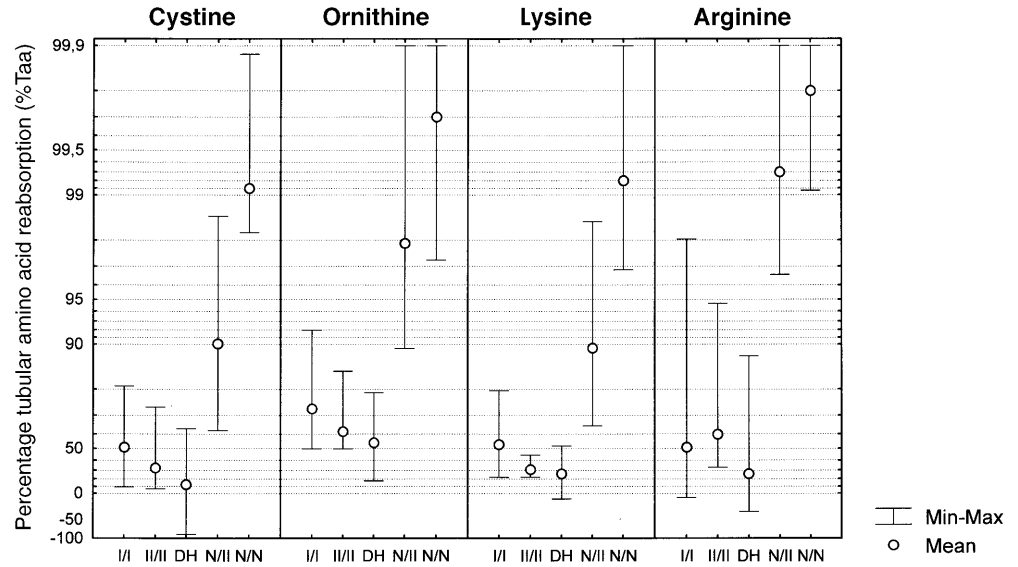
Fig. 3 Patterns of tubular amino acid reabsorption in cystinuria. Mean values of 60 probands with normal tubular amino acid reabsorption are compared with the individual amino acid reabsorption patterns of 4 cystinuric patients
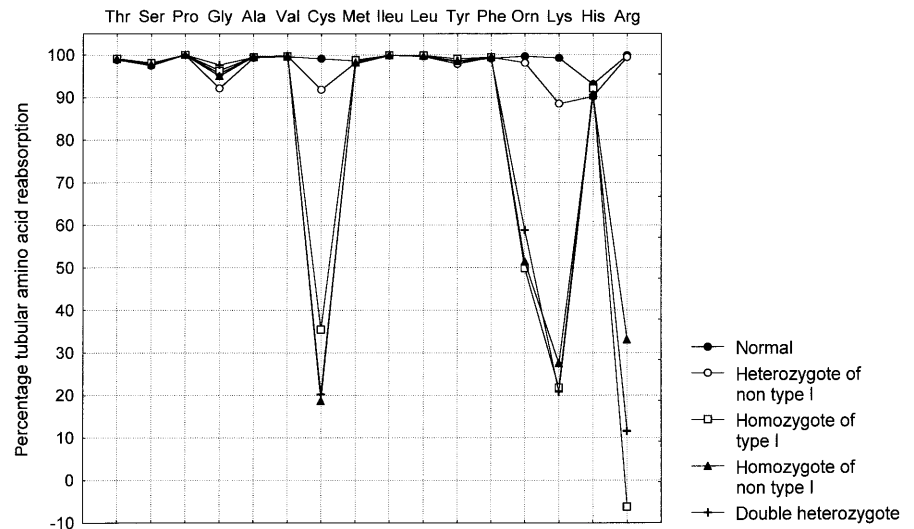
(N/II), $\%T_{aa}$ was moderately reduced for cystine (62.1–98.6%) and lysine (64.6–98.5%), while ornithine and arginine were reabsorbed normally in most cases (96.6–99.9%). The third group, type I heterozygotes and the controls (N/N), removed cystine and dibasic amino acids nearly completely from the tubular lumen (96.8–99.9%).

The differences between the mean values of these three groups were significant with *P*<0.01 (multivariant analysis and LSD-test). When compared to the amino acid excretion, these groups were differentiated more clearly by the percentage tubular reabsorption. Nevertheless, further subgroups of cystinuria could also not be distinguished by this method.

In repeated examinations we observed wide fluctuations of the $\%T_{aa}$, similar to those of the renal amino acid excretion. The tubular reabsorption of cystine for example deviated from day to day as follows: 77.7%/81.0% (type I homozygote); 6.9%/30.7% (non-

type I homozygote); –87.8%/20.2%, 38.4%/63.2% (double heterozygotes); 98.7%/99.0% (type I heterozygote); 89.6%/97.3%, 94.5%/96.3%, 98.0%/98.1% (non-type I heterozygotes). Again, similar differences were observed between relatives with identical cystinuria genotypes.

Cystinuria could be distinguished easily from other hyperaminoacidurias by its characteristic pattern of tubular amino acid reabsorption. In homozygotes and double heterozygotes the $\%T_{aa}$ of cystine, lysine, ornithine and arginine was distinctly reduced, while the other amino acids were reabsorbed in normal proportions (Fig. 3). The reabsorption patterns were very similar in type I homozygotes, non-type I homozygotes and double heterozygotes and, thus, could not contribute to a further differentiation between these groups. Non-type I heterozygotes had a nearby normal reabsorption of arginine and ornithine and an only moderately reduced reabsorption of cystine and lysine, and therefore could be clearly distinguished from homozygotes and double heterozygotes.

Fig. 4 Urinary polyamine excretion (in μmol per gram creatinine) of 113 probands in 16 families. *I/I* type I homozygotes (*n*=3), *II/II* type II or III homozygotes (*n*=3), *DH* double heterozygotes (*n*=3), *n/II* type II or III-heterozygotes (*n*=46), *n/n* normals (*n*=58)
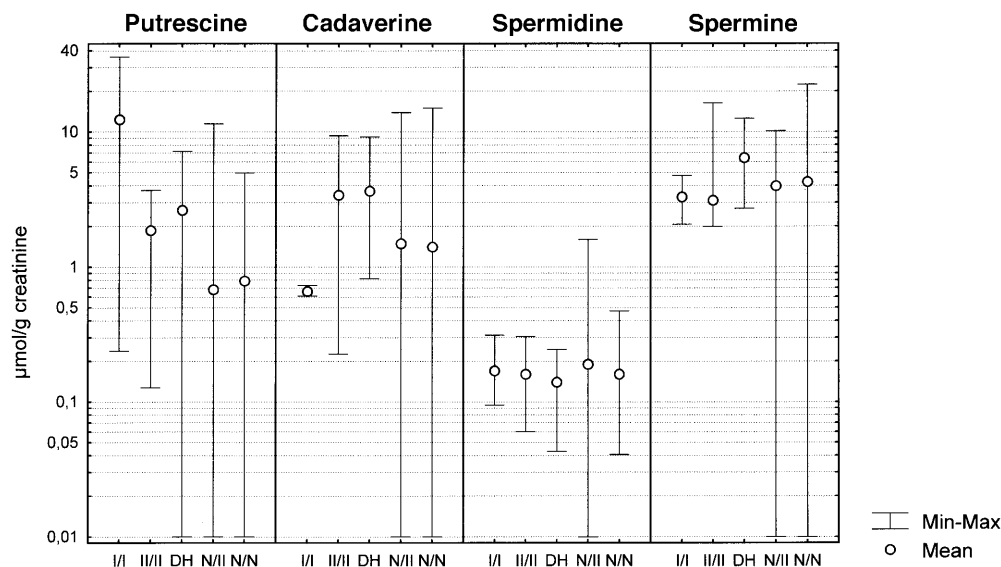
Polyamine excretion

We investigated the urinary excretion of the polyamines putrescine, cadaverine, spermidine and spermine as possible markers of the impaired intestinal transport of dibasic amino acids. The urinary excretion of putrescine and cadaverine appeared to be moderately higher in homozygotes (I/I + II/II) and double heterozygotes (DH) than in non-type I heterozygotes (N/II) and controls (N/N) (Fig. 4). However, no significant differences between any of these groups could be found.

Mutations and polymorphisms in the SLC3A1 gene

We found mutations of the SLC3A1 gene in four out of six alleles from patients of type I cystinuria (two families with homozygous siblings and two double heterozygous families). In one family of German descent, a patient was homozygous for the M467T mutation in exon 8 of the SLC3A1 gene [5]. Two siblings of a Greek family were shown to be homozygous for mutation T216M, a mutation in exon 3 [14].

To determine carriers of these mutations, all members of the respective families were tested by *Nla*III digestion of PCR products, as T216M results in the gain and M467T in the loss of a restriction site for this enzyme.

In one of the double heterozygous families, the affected child was heterozygous for a hitherto unreported deletion of three thymidines in the polypyrimidine tract within the branch/acceptor splice site of intron 9. To determine whether this represented a frequent polymorphism, an analysis of 120 normal chromosomes was performed, expecting a 139-bp or, if the three thymidines were deleted, a 136-bp product and seperating the PCR products on a 10% polyacrylamide gel. The deletion could not be detected in any of the control samples. However, segregation analysis within the family showed that the patient's father was a carrier of the 3T deletion, whereas his sister was not, both being heterozygous carriers of non-type I cystinuria. We conclude that the 3T allele did not segregate with either phenotype and, therefore, is most probably a neutral intronic variant. In the other double heterozygous family we were not able to identify cystinuria-specific changes in the SLC3A1 sequence.

We finally found a conservative change of isoleucine 618 to methionine [15] in two of the families. A mismatch primer was designed to create an *Msl*I restriction site, with *Msl*I recognizing the ATG but not the ATA codon. A total of 118 normal chromosomes were tested and a frequency of the methionine allele of 0.26 in the German population was determined.

Linkage analyses

We could not exclude a linkage of SLC3A1 to the true recessive phenotype (type I) in all families with homozygous siblings, whereas no linkage was evident in intermediate (non-type I) cystinuria families.

Homozygosity mapping of a possible further cystinuria locus revealed chromosome 19q as a highly probable candidate region of non-type I cystinuria (Fig. 5, family II), [4, 6, 7]. The markers D19S414, D19S416, D19S425, D19S220, D19S412 and D19S418 were typed in six additional families to narrow the region of the possible gene location. D19S414 could be excluded as a candidate locus by a recombination in family IX (Fig. 5). In family VII (Fig. 5) the allele of non-type I cystinuria segregated with the chromosome 19 markers, assuming a recombination between the markers D19S416 and D19S425 in one proband.

Discussion

Previous studies of cystinuria focussed either on phenotype definitions [1, 3, 9, 16–19], pathophysiological ex-
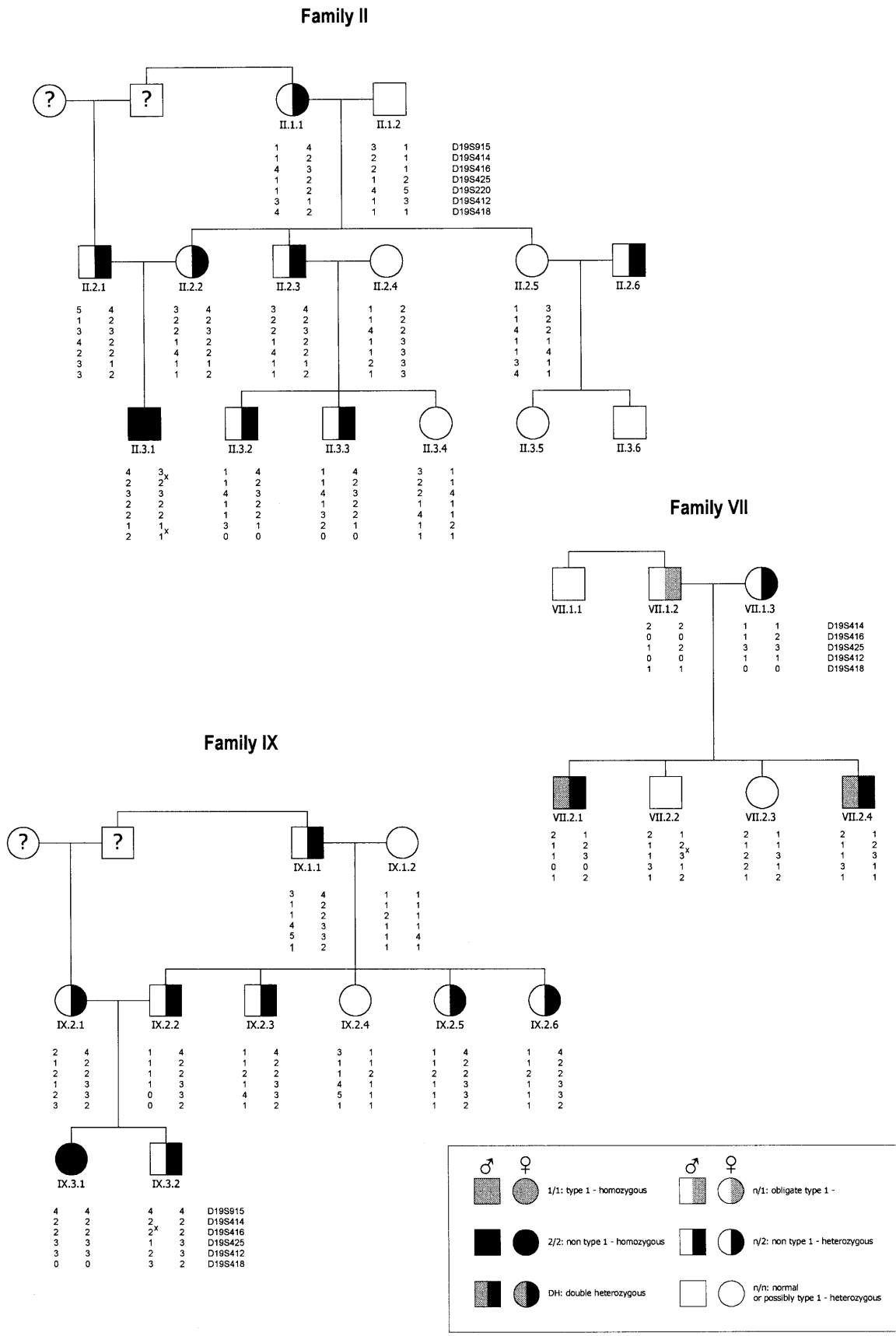
Fig. 5 Microsatellite markers of the non-type I cystinuria locus. Possible recombinations are marked by a *cross*

aminations [20–25] or molecular analysis of the genetic basis [26–30]. In the present study, both cystinuria phenotypes and their genetic correlates have been described as precisely as possible using minimally invasive methods.

Although the urinary excretion of amino acids in cystinurics has been examined in many studies, it has not been finally resolved whether two or three different cystinuria phenotypes can be distinguished by this parameter. Harris et al. [31] described two different cystinuria phenotypes that could be defined by the urinary excretion of cystine and dibasic amino acids in family studies. Heterozygotes excreted these amino acids either in normal or in moderately increased amounts. Accordingly, Harris differentiated between recessive cystinuria type I and an intermediate type II. Rosenberg et al. [1], on the other hand, described three phenotypes (I, II and III) on the basis of different intestinal amino acid transport patterns. These observations, however, were not confirmed by any other study.

In the present study, urinary excretion rates of cystine, ornithine, lysine and arginine were determined in 60 normals, 48 non-type I heterozygotes and 9 homozygotes and double heterozygotes. By family studies we were able to differentiate between the recessive and the intermediate type described by Harris et al. However, our data clearly demonstrate that the intermediate type of cystinuria (non-type I) cannot be divided into two subgroups by the urinary amino acid excretion.

To demonstrate the possible pitfalls of classifications for type I, II and III when based on the urinary amino acid excretion only, we compared the results of our present study to the reference intervals for the sum of urinary cystine, ornithine, lysine and arginine published by Goodyer in 1993 [18]. Our data of 48 non-type I heterozygotes show large variations over a wide range. In repeated measurements, urinary excretion rates varied from day to day to such an extent that, according to Goodyer's classification, the same proband would have been assigned to type II on one day and to type III on another. Similar differences occurred between relatives with identical cystinuria genotypes. Concerning the urinary amino acid excretion, we found no basis for the differentiation of two distinct intermediately inherited cystinuria phenotypes.

We showed $\%T_{aa}$ to be the most sensitive parameter of the tubular amino acid transport in vivo [32]. Up to now, the $\%T_{aa}$ has only been examined in a few cases of cystinuria [33]. Compared to the urinary amino acid excretion, normals and non-type I heterozygotes can be distinguished more clearly by the $\%T_{aa}$ value. The differentiation between homozygotes or double heterozygotes and non-type I heterozygotes, however, has not been improved. As described above, we observed large variations of the tubular reabsorption of cystine, ornithine, lysine and arginine in repeated measurements of the same cystinuric patients as well as in relatives with identical genotypes. Under these circumstances, deviations in the $\%T_{aa}$ could not be interpreted as different cystinuria phenotypes. This was also supported by our molecular genetic analysis.

We observed an association of the homozygous M467T mutation with a mild cystinuria phenotype in one German patient, resembling more the intermediate heterozygous form (non-type I) than the true recessive homozygous form (type I). Purroy et al. [11], however, presented more seriously affected M467T homozygous siblings of a Spanish family. M467T represents 26% of all Spanish and Italian cystinuria type I chromosomes [34]. Apart from the mutant SLC3A1 protein, other factors appear to be involved in the transport processes. These and perhaps inter-individual differences in the expression of SLC3A1 might be reflected by the observed phenotype discrepancies concerning the M467T mutation.

Homozygosity for the mutation T216M was found in two siblings of a Greek family affected with type I cystinuria. Again, we observed differences in phenotypical expression of the same genotype. Whereas one proband shows tubular secretion of arginine, his sister has minimal, but positive reabsorption values.

We were not able to identify SLC3A1 mutations in any of the presumed double heterozygous families. The 3T deletion in the branch/acceptor splice site preceding exon 10 was present in a single family and could not be found in 120 normal chromosomes. One might expect some relation to the cystinuria phenotype, as severe reductions of the poly-T tract are shown to affect the mRNA splicing of several genes. By investigating the segregation of the deletion within the family, we could, however, exclude a disease-causing effect on the cystinuria phenotype. We conclude that the 3T allele, as it does not segregate with either phenotype, is probably a neutral intronic variant.

Results from our linkage analyses were consistent with the possibility that type I cystinuria in all our informative families is linked to SLC3A1, whereas non-type I cystinuria is linked to the chromosome 19 locus. In proband II.3.1 (Fig. 5) a double recombination between the markers D19S915 and D19S416 and, on the other hand, D19S220 and D19S418 is assumed. These markers span a region of about 26 cM. In two other families recombinations were observed: in family IX D19S414 and in family VII D19S416 were shown to mark the centromeric border of the possible gene location (Fig. 5). Comparing our results with those of previous studies [6, 7], the most probable gene location of non-type I cystinuria lies between D19S416 and D19S425, a region spanning about 1.5 cM. The isolation of one or more cystinuria genes in the 19q13.1 region and genotyping, as well as a careful phenotyping of cystinuric patients (including their intestinal amino acid resorption) will help to finally resolve the question about the number of different cystinuria types.

As a promising marker of the intestinal resorption of dibasic amino acids we have tested the urinary excretion of the polyamines putrescine, cadaverine, spermidine and spermine. Elevated amounts of putrescine and ca-

daverine in the urine of cystinuric patients were first observed by Udranszky and Baumann in 1889 [35]. Milne et al. [36] explained this phenomenon with the impaired intestinal uptake of dibasic amino acids in the gut of cystinuric patients.

The use of polyamines as markers for particular diseases is controversial because of their low specifity and sensitivity. The amount of polyamines formed by bacterial decarboxylation of dibasic amino acids in the gut depends on the amount of protein uptake. However, polyamines do not solely originate from bacterial decarboxylation of dibasic amino acids, they are also formed in the human body and can be found in all tissues [37]. Embryonic, neoplastic and other rapidly growing tissues contain polyamines in particularly high concentrations [37]. Elevated amounts were observed in the urine of patients with malignancies [38], liver cirrhosis [39] or ARDS [40]. Some authors interpret the urinary hyperexcretion of polyamines as a marker of catabolic metabolism in general [41, 42].

Up to now only few data about the urinary polyamine excretion in cystinuric patients have been published [35, 36, 43, 44]. Kohne and Bremer [44] determined systematically the di- and polyamines in the urine. Healthy controls of different age as well as patients suffering from cystinuria, intestinal malabsorption, enteritis or vitamin D-deficiency rickets were investigated. Their results suggested a dependence of the urinary excretion of the polyamines cadaverine and putrescine on the intestinal amino acid resorption.

In the present study we were able to measure urinary polyamines in concentrations as low as 0.01 µmol/l using fluorescence detection. With this highly sensitive method, putrescine, cadaverine, spermidine and spermine were found in the urine of most of our probands, including the healthy controls. The urinary excretion of putrescine and cadaverine was moderately higher in homozygotes and double heterozygotes than in non-type I heterozygotes and controls, but in multivariant analysis the mean value differences between these groups turned out to be not significant. Spermidine and spermine were excreted in similar amounts by cystinurics and controls. Thus, the urinary excretion of putrescine, cadaverine, spermidine and spermine turned out to be of little value for the assessment of the intestinal amino acid transport defect associated with cystinuria. To verify the phenotype definition of Rosenberg et al. [1], the resorption defect would have to be investigated by more subtle and specific means. Suitable methods for this purpose may require invasive diagnostic techniques, like endoscopy for double lumen perfusion or jejunal mucosa biopsies.

With regard to heterozygotes, statistical analysis and family studies indicate the distinction of two different phenotypes. The genetic analysis of our patients confirmed this differentiation. Considering the great variability in cystinuria phenotype expression, we found no basis for the differentiation of more than two genetically distinct cystinuria types.

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LITERATURE ABSTRACT

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Predictors of urinary tract infection at the first prenatal visit

We identified maternal demographic, behavioral, and medical history factors that predict bacteriuria (that is, symptomatic and asymptomatic urinary tract infection) at prenatal care initiation. We applied logistic regression modeling to data from all prenatal care recipients who delivered during 1990–1993 and resided in selected North Carolina counties ($n = 8037$), omitting those with diabetes mellitus, human immunodeficiency virus, or structural urologic abnormalities. The two strongest predictors of bacteriuria at prenatal care initiation were an antepartum urinary tract infection prior to prenatal care initiation [for whites, adjusted prevalence odds ratio (POR) = 2.5, 95% CI 0.6–9.8; for blacks, POR = 8.8, 95% CI 3.8–20.3] and a pre-pregnancy history of urinary infection (POR = 2.1, 95% CI 1.4–3.2). For white women only, education beyond high school and age greater than or equal to 30 years were inversely associated (POR less than or equal to 0.6). Sickle cell hemoglobin nearly doubled the prevalence odds for bacteriuria among African-Americans (POR = 1.9, 95% CI 1.0–3.5), whereas African-Americans with normal hemoglobin had reduced prevalence odds compared with whites (POR = 0.6, 95% CI 0.4–0.9). This study suggests predictors not considered before, including race controlling for sickle cell disease or trait and antepartum urinary tract infections prior to prenatal care.