Localization, by Linkage Analysis, of the Cystinuria Type III Gene to Chromosome 19q13.1

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Summary

Cystinuria is an autosomal recessive aminoaciduria in which three urinary phenotypes (I, II, and III) have been described. An amino acid transporter gene, SLC3A1 (formerly rBAT), was found to be responsible for this disorder. Mutational and linkage analysis demonstrated the presence of genetic heterogeneity in which the SLC3A1 gene is responsible for type I cystinuria but not for type II or type III. In this study, we report the identification of the cystinuria type III locus on the long arm of chromosome 19 (19q13.1), obtained after a genomewide search. Pairwise linkage analysis in a series of type III or type II families previously excluded from linkage to the cystinuria type I locus (SLC3A1 gene) revealed a significant maximum LOD score (Z_{max}) of 13.11 at a maximum recombination fraction (θ_{max}) of .00, with marker D19S225. Multipoint linkage analysis performed with the use of additional markers from the region placed the cystinuria type III locus between D19S414 and D19S220. Preliminary data on type II families also seem to place the disease locus for this rare type of cystinuria at 19q13.1 (significant Z_{max} = 3.11 at θ_{max} of .00, with marker D19S225).

Introduction

Cystinuria is a heritable disorder (MIM 220100) of amino acid transport, which is transmitted as an autosomal recessive trait (McKusick 1990). The disease, one of the most common genetic disorders, with an overall prevalence of 1/7,000 (Segal and Thier 1995), is due to the defective transport of cystine and dibasic amino acids through the epithelial cells of the renal tubule and the intestinal tract (Rosenberg et al. 1965). Cystine has a low solubility, and its precipitation results in the formation of calculi in the urinary tract, leading to obstruction, infections, and, ultimately, renal insufficiency (Segal and Thier 1995). Three biochemical types of classic cystinuria have been described elsewhere (Rosenberg et al. 1966*a*). Type I heterozygotes show normal aminoaciduria, whereas type II and type III heterozygotes have high or moderate hyperexcretion of cystine and dibasic amino acids, respectively. In contrast to type I and type II homozygotes, type III homozygotes show a nearly normal increase in cystine plasma levels after oral cystine administration. These different types were thought to be due to allelism of the same gene (Rosenberg et al. 1966*b*), although the involvement of distinct genetic loci for type I and type III cystinuria has been suggested (Goodyer et al. 1993).

A human kidney cDNA, named "rBAT" (the human gene is designated "SLC3A1"), which elicits the transport of cystine, dibasic amino acids, and some neutral amino acids via a $b^{0,+}$-like transport system in *Xenopus* oocytes, was isolated (Bertran et al. 1993; Lee et al. 1993) and was considered a good candidate for the disease: rBAT is responsible for reabsorption of cystine and dibasic amino acids, in the S3 segment of the nephron and the small intestine, through a tertiary active-transport mechanism by the heteroexchange with neutral amino acids (Furriols et al. 1993; Pickel et al. 1993; Chillarón et al. 1996; Mora et al. 1996). Mutational and functional analysis demonstrated that mutations in the SLC3A1 gene cause cystinuria (Calonge et al. 1994). At present, nearly 25 mutations in the SLC3A1 gene (including missense mutations, stop codons, splicing-site mutations, insertions, and deletions) have been found in patients with cystinuria from different origins (Calonge et al. 1994; Gasparini et al. 1995; Miyamoto et al. 1995; Pras et al. 1995; Bisceglia et al. 1996; Horsford et al. 1996). The SLC3A1 gene was localized to

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the short arm of chromosome 2 (2p16.3-21) (Yan et al. 1994; Calonge et al. 1995*a*). Linkage analysis that used microsatellite markers of this region confirmed the SLC3A1 gene as a locus for cystinuria (Pras et al. 1994; Calonge et al. 1995*b*).

Recently, linkage analysis and genotype-phenotype correlation data demonstrated the presence of genetic heterogeneity and suggested that only type I cystinuria was due to mutations in the SLC3A1 gene, whereas another locus (or loci) should be responsible for type II and type III cystinuria (Calonge et al. 1995*b*; Gasparini et al. 1995). In this study, we report the mapping of a gene for the cystinuria type III locus to chromosome 19q13.1. Moreover, preliminary data also suggest that the rare cystinuria type II might be due to a defect at this locus.

Patients, Material, and Methods

Patients and DNA Samples

The patients presented with typical cystinuria symptoms. Urinary excretion values of cystine and dibasic amino acids (lysine, arginine, and ornithine) were determined by quantitative ion-exchange chromatography or by reverse-phase high-pressure liquid chromatography. Amino acid–content measurements were corrected per gram of creatinine. The patients and their relatives were typed according to the classification by Rosenberg et al. (1966*a*, 1966*b*), as described elsewhere (Calonge et al. 1995*b*). In brief, obligate carriers showing urinary excretion values higher than those in the range for type I heterozygotes, for at least one amino acid and for the sum of urinary cystine plus the dibasic amino acids, were classified as non–type I heterozygotes. Among them, it was possible to distinguish between type II and type III heterozygotes: type II heterozygotes showed urinary excretion values higher than those in the range for type III heterozygotes (Calonge et al. 1995*b*), for at least one amino acid and for the sum of urinary cystine plus the dibasic amino acids. Peripheral blood was obtained from all subjects, and DNA was isolated from blood leukocytes according to standard methods.

Two-Step Search for Linkage

For the genomewide search, the ABI PRISM Linkage Mapping Set (Perkin-Elmer) was used. It is characterized by >375 markers that define a 10-cM-resolution human-index map. PCR reactions using fluorescently labeled primers were run under the conditions suggested by the supplier. An aliquot of PCR reaction was run in an ABI PRISM 373 DNA sequencer (Perkin-Elmer), and the results were processed by GENESCAN software. Allele assignation was carried out by use of Genotyper™ software. To facilitate the genome search, screening was limited to the affected sibs of a subset of four families (F3, F5, F42, and F48; fig. 1). One of these families was the result of a mating between first cousins; thus, allele homozygosity was sought in the three affected children. In the sibs of the remaining families, identity by descent was expected. Regions showing allele sharing, within each sib group, were further analyzed by the inclusion of more members of the family and by the typing of more microsatellites of the region. To saturate the chromosome 19–positive region, additional pairs of fluorescently labeled primers were either (*a*) from the Oswell Linkage Mapping Set, in the case of marker D19S225 (Reed et al. 1994), or (*b*) were specifically synthesized, in the case of markers D19S882, D19S409, and D19S425 (Dib et al. 1996).

Statistical Analysis

Statistical analysis was performed on the basis of an autosomal disease with complete penetrance, in which type II or type III carriers showed a partially dominant mode of inheritance whereas type I carriers were considered to be completely recessive. The disease-gene frequency was set at .012, and all marker alleles were considered to be equally frequent. Loops of consanguinity were broken. Two-point linkage analysis was performed using the MLINK program, version 5.1, from the LINKAGE computer package (Ott 1992). Values for the maximum LOD score (Z_{max}) were calculated with the ILINK program from the same computer package. The ~95% confidence limits for the maximum recombination fraction (θ_{max}) at Z_{max} were calculated by the 1-LOD-down method (Ott 1992). Multipoint linkage analysis was carried out by use of the LINKMAP program. Alleles were downcoded without loss of informativeness, to reduce computing time. Recombination frequencies were transformed to map distances by Kosambi's formula, and multipoint linkage analysis assumed no interference or no sex differences in recombination frequencies (Kosambi 1994). The HOMOG program was used to test for nonallelic heterogeneity, by use of pairwise LOD scores between D19S225 and the disease locus.

Results

The study was performed on Italian families excluded from linkage with the SLC3A1 gene, which is located on chromosome 2p16 (Pras et al. 1994; Calonge et al. 1995*a*). Thus, families F5, F7, F25, F28, and F42 (fig. 1) previously were excluded elsewhere (Calonge et al. 1995*b*), and families F41, F48, F58, F62, and F78 showed recombinations (not shown) for the SLC3A1 intragenic markers described elsewhere (Gasparini et al. 1995; Bisceglia et al. 1996). The informativeness of markers in the 2p16 type I–cystinuria region of family F3 (fig. 1) was not enough to exclude family F3 from this locus; no SLC3A1 mutations were found in the two

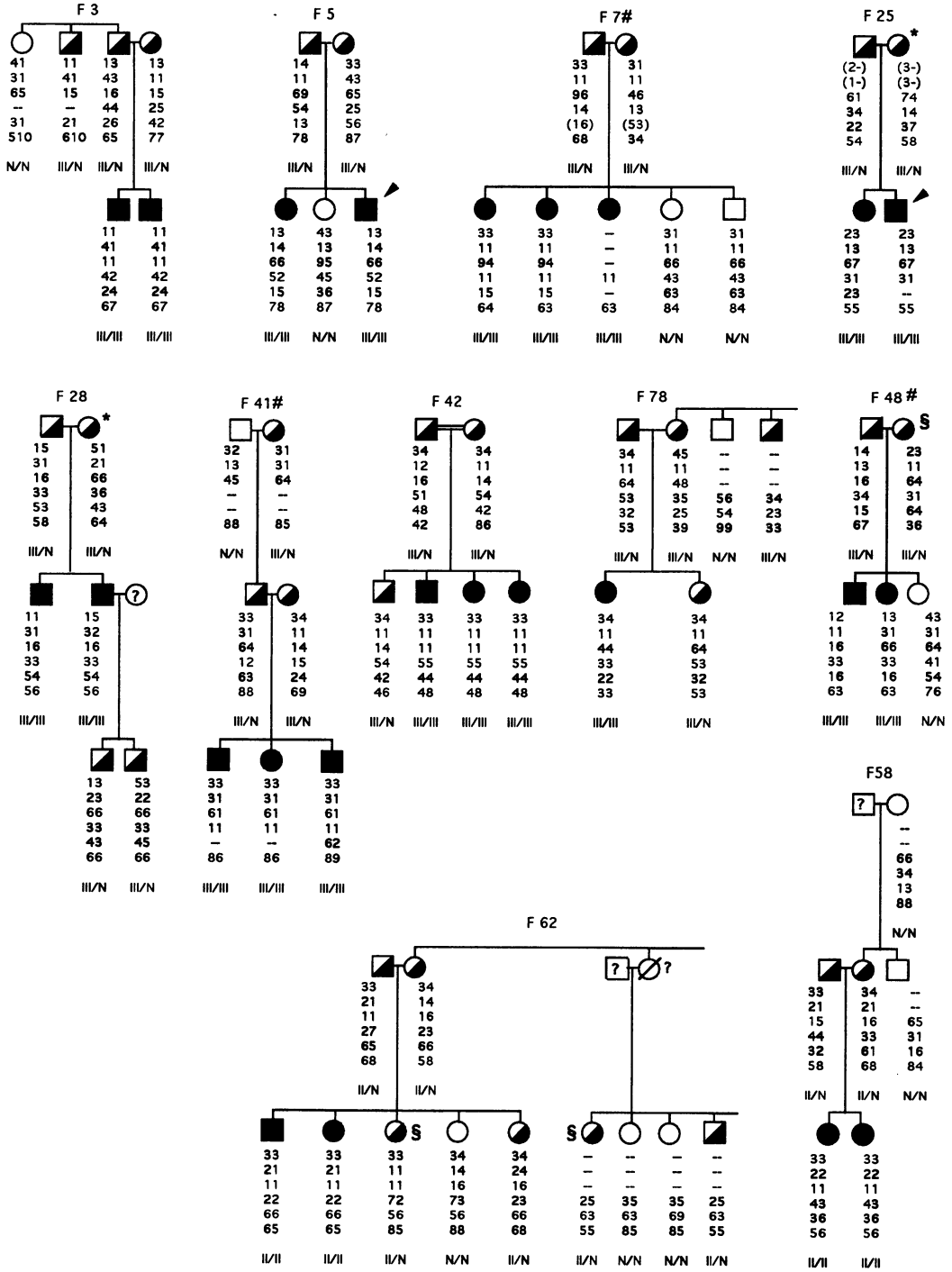

Figure 1 Cystinuria families analyzed. Families F3, F5, F7, F25, F28, F41, F42, F78, and F48 transmit type III cystinuria, and families F58 and F62 transmit type II cystinuria. Squares denote males; circles denote females; diagonal lines denote deceased individuals; and double lines denote consanguinity. Unblackened symbols denote normal individuals (N/N) with a silent urinary phenotype (i.e., noncarriers). Blackened symbols denote the homozygous state (i.e., type III/III or type II/II), and half-blackened symbols denote cystinuria carriers with a urinary phenotype (i.e., cystinuria type III heterozygotes [III/N] or type II heterozygotes [II/N]). Question marks (?) denote individuals with unclassified phenotypes. Two obligate heterozygotes with urinary phenotypes between that of type III heterozygotes and that of type I heterozygotes are indicated by an asterisk (*). Three heterozygotes with urinary phenotypes between that of type III heterozygotes and that of type II heterozygotes are indicated by a section symbol (§). Two affected individuals without a history of cystine stones are indicated by an arrow. Numbers under the individual symbols represent the haplotypes given by the following markers: D19S882 (top number), D19S409, D219S414, D19S225, D19S425, and D19S220 (bottom number). A dash indicates nonanalyzed alleles. Alleles within parentheses are inferred. A number symbol (#) beside the family number indicates the recombinant families, which define the centromeric (family F48) and telomeric (families F7 and F41) limits.

patients of this family after the analysis of the complete coding region of the gene (data not shown). To facilitate the genome search, the screening initially was limited to a subset of four sib groups containing at least two affected members each (families F3, F5, F42, and F48; fig. 1). The search was performed using the ABI PRISM Linkage Mapping Set, which comprises >375 markers selected from the linkage map generated by Généthon (Gyapay et al. 1994). The markers are organized into 28 different panels. After analysis of 16 different panels corresponding to chromosomes 1, 2, 7, 8, 12, 13, and 15–22, we detected only a few loci for which the three affected siblings of a consanguineous family were homozygous, and the affected individuals of the remaining four couples shared identical alleles. The alleles of these loci, named "bingo loci," then were typed in the corresponding parents. This step allowed us to discard some false-positive loci resulting from noninformativeness of the parents themselves. At the end, one locus remained on 19q (D19S414). After this step, five additional microsatellite markers of chromosome 19 (D19S882, D19S409, D19S225, D19S425, and D19S220) were chosen and analyzed, in nine type III and in two type II families (fig. 1), including those previously used for the genome rapid scanning. The order of and the distances between the above-mentioned markers, as deduced from published maps (Dib et al. 1996), is cen–D19S882–D19S409–2 cM–D19S414–3 cM–D19S225–3 cM–D19S425–3 cM–D19S220–tel.

Pairwise linkage analysis, carried out on the whole sample, showed a Z_{max} of 13.11 and 10.27, with markers D19S225 and D19S425, respectively, at a recombination frequency of .00 (table 1). Positive but lower LOD scores also were obtained, with markers D19S882, D19S409, D19S414, and D19S220, for recombination frequencies >.00 (table 1). When type II families (see fig. 1) were analyzed separately, positive and significant LOD scores were obtained (Z_{max} = 3.11 at θ_{max} of .00, with marker D19S225). The analysis of the recombinants places the centromeric limit of the region at marker D19S414, whereas the telomeric limit is defined by marker D19S220.

The map-specific Z_{max} obtained with multipoint analysis is shown in figure 2. The Z_{max} occurred in the region between markers D19S225 and D19S425, indicating that the most likely position for the gene is between these two markers, which are separated by ~3 cM. Genetic heterogeneity was tested using the HOMOG program, which indicated the presence of genetic homogeneity (α = 1).

Discussion

For many years, cystinuria was considered a genetically homogeneous disorder, and the three different biochemical types (I, II, and III) were thought to be due to allelism of the same gene (Rosenberg et al. 1966*b*). Recently, genotype-phenotype correlation data, combined with linkage results, provided strong evidence for the presence of genetic heterogeneity in cystinuria (Calonge et al. 1995*b*; Gasparini et al. 1995; Horsford et al. 1996), for which the SLC3A1 gene would be responsible only for type I cystinuria, not for type II and type III. Genetic heterogeneity now has been definitely proved by the localization of an additional cystinuria locus on the long arm of chromosome 19q13.1. The genomewide search performed on a large series of type III families and on two type II families led to the identification of highly significant LOD scores, with the markers D19S225 and D19S425. Pairwise LOD scores >3.00 also were obtained when the remaining four markers were used. Analysis of recombinants and multipoint data indicates that the most likely location of this new cystinuria gene is between the markers D19S225 and D19S425, which were found to be ~3 cM apart. The centromeric limit of this region is placed at D19S414, whereas the telomeric region is at D19S220. The presence of genetic homogeneity seems to indicate that both type II and type III cystinuria map within this region. Nevertheless, we should consider that, despite the significant linkage, the number of type II families included

Table 1

Pairwise LOD Scores between Type III and Type II Cystinuria and Chromosome 19

	LOD Score at Recombination Frequency of								
Markers	.00	.01	.05	.10	.20	.30	.40	Z_{max}	θ_{max}
D19S882	$-\infty$	3.52	4.79	4.65	3.42	1.88	.55	4.82	.06
D19S409	$-\infty$	3.27	4.00	3.76	2.66	1.42	.43	4.00	.05
D19S414	$-\infty$	9.37	9.00	7.94	5.51	3.07	1.02	9.41	.02
D19S225	13.11	12.81	11.56	9.99	6.80	3.73	1.17	13.11	.00
D19S425	10.27	10.05	9.15	7.95	5.46	3.02	1.01	10.27	.00
D19S220	$-\infty$	7.53	8.28	7.60	5.37	2.96	.95	8.31	.04

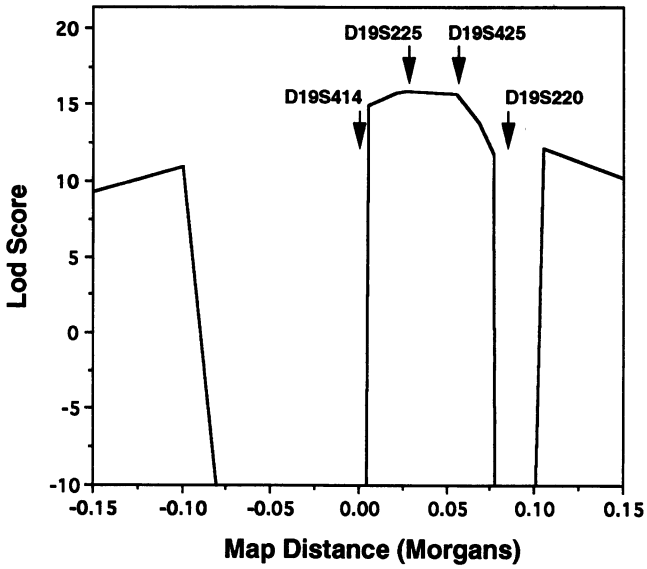

Figure 2 Multipoint linkage for four chromosome 19q13.1 markers spanning the type III–cystinuria candidate region. Genetic distances between the markers were obtained from the most recent Généthon genetic map (Dib et al. 1996). The maximum multipoint LOD score, obtained when all 11 families (type III and type II) were combined, was 15.90, with the D19S225 marker. Map distance 0.00 is placed at the D19S414 marker.

in the present article is too small to allow us to obtain a definitive assignment. In addition, it will be necessary to characterize further the type II phenotype by testing the intestinal transport defect of these type II patients.

The obvious candidate genes for type III cystinuria are those corresponding to a sodium-dependent low-affinity cystine and dibasic–amino acid transporter expressed in the S1 and S2 segments of the proximal tubule and/or to a functionally associated subunit of an oligomeric rBAT transporter (Wang and Tate 1995; Palacín et al. 1996). The region marked by D19S225 and D19S414 is contained in an already available YAC contig (Garcia et al. 1995). No clear candidate genes have been identified in this region. Among the several genes mapped in this region, the sodium-channel beta-1 subunit gene (SCN1B) (Makita et al. 1994) could be envisaged as a candidate for the disease, because of the suggested sodium dependence of the amino acid–transport defect in cystinuric patients (Segal and Thier 1995). Recently, a cDNA with an associated amino acid–transport activity resembling that of the brush-border sodium-dependent neutral amino acid transporter B^0 has been cloned (Kekuda et al. 1996). The characteristics of system B^0 (i.e., not acting on dibasic amino acids), as well as its localization in chromosome 19q13.3 (i.e., more telomeric than the type III cystinuria region) (Jones et al. 1994), weaken this gene's candidacy for cystinuria. In this scenario, cDNA selection and exon trapping techniques will be extremely useful in the isolation of expressed sequences from this area and in the construction of a transcriptional map of the type III–cystinuria chromosomal region.

In conclusion, in this study we have described a new cystinuria locus that should allow the identification of the cystinuria type III and type II gene(s). This will be extremely useful to the understanding of the biochemistry of cystine reabsorption and will provide benefits for genetic counseling and for the prevention of this common disease. In addition, the methodology used, combining homozygosity mapping with identity-by-descent mapping, further increase the feasibility of a rapid scanning of the whole human genome by use of commercially available mapping sets.

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