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Linkage of Type II and Type III Cystinuria to 19q13.1: Codominant Inheritance of Two Cystinuric Alleles at 19q13.1 Produces an Extreme Stone-Forming Phenotype

Marshall L. Stoller,[1] Jeremy E. Bruce,[2] Carol A. Bruce,[2] Tatiana Foroud,[3] Sandra C. Kirkwood,[3] and Peter J. Stambrook[2]*

[1]*Department of Urology, University of California, San Francisco, California*
[2]*Department of Cell Biology, Neurobiology, and Anatomy, University of Cincinnati College of Medicine, Cincinnati, Ohio*
[3]*Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, Indiana*

Cystinuria, a renal tubule disease affecting urinary cystine excretion with or without kidney stone formation, previously was mapped to chromosome region 2p.21. Mutations in the gene *SLC3A1* or NBAT, the reported candidate gene for cystinuria at 2p.21, have been demonstrated in individuals with the autosomal recessive Type I cystinuria phenotype. Recently, the Type III cystinuria phenotype was mapped to chromosome region 19q13.1. Here we report a kindred of 39 persons in two families of cystinurics, Types II and III, that support linkage to 19q13.1 and exclude 2p.21. Based on a dominant model of inheritance, two-point analysis of the entire pedigree produced a maximum lod score (Z_{max}) of 3.82 at marker D19S425. Multipoint analysis yielded a lod score of 4.96 at this marker, and a resultant lod score of 5.90 using a codominant model of inheritance. Furthermore, a candidate gene interval of 8.9 cM, flanked by markers D19S225 and D19S223, was obtained using multipoint and haplotype analyses. Thus, this kindred demonstrates the linkage of Type II cystinuria to 19q13.1 and confirms the linkage of Type III cystinuria at 19q13.1 while excluding the marker D19S225 that was previously included in the critical interval. Am. J. Med. Genet. 86:134–139, 1999.

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*Correspondence to: Dr. Peter J. Stambrook, Department of Cell Biology, Neurobiology, and Anatomy University of Cincinnati, College of Medicine, P.O. Box 670521, Cincinnati, Ohio 45267-0521. E-mail: Peter.Stambrook@uc.edu

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INTRODUCTION

Cystinuria is a common inherited disorder that affects up to 1 in 7,000 persons worldwide [Levy et al., 1972; Segal and Thier, 1995] and as many as 1 in 2,500 persons in some populations in Europe and Israel [Weinberger et al., 1974; McKusick, 1998]. Cystinuria is caused by a defect in renal proximal tubule cystine transport and is characterized clinically by recurrent cystine urinary calculi, with frequent obstructive upper tract uropathy, with or without infection, and nephropathic crystallization. Normally, individuals excrete approximately 1.6 liters of urine per day with a cystine excretion rate of less than 30 mg/24 hr. Cystinurics may excrete all or a portion of their filtered cystine into the urine, in some cases at a rate exceeding the glomerular filtration rate [Webber et al., 1960], resulting in a urine cystine excretion rate of 600 to 1400 mg/24 hr.

Traditionally, cystinurics have been classified into three types based on the uptake of cystine, lysine, and arginine by biopsied jejunal mucosa. Type I cystinuria, an autosomal recessive trait, is the most common type (70%) [Harris et al., 1955], with patients exhibiting an inability to transport any of the three amino acids. The less-common Type II (10%) and Type III (20%) exhibit semi-dominant inheritance [Harris et al., 1955]. Type II heterozygotes excrete moderate to severe amounts of cystine and dibasic amino acids: they demonstrate excretion rates that range from greater than 250 mg/24 hr to 1400 mg/24 hr. In contrast, Type III heterozygotes excrete mildly elevated amounts that range from 100 mg/24 hr to 300 mg/24 hr [Goodyer et al., 1993]. Clinical discrimination between Type II and Type III is made by repeat 24-hr quantitative urinary

cystine analysis. Although Type I homozygotes have been commonly described as the most-severely affected, all three types of cystinuria may lead to clinical symptoms.

The primary defect in Type I cystinurics likely involves the sodium-independent $b^{0,+}$-like amino acid exchanger designated NBAT (Neutral and $b^{0,+}$-like Amino Acid Transporter), which is responsible for cystine transport in the S3 segment of the nephron and in the jejunum [Mora et al., 1996]. NBAT is a Type-II glycoprotein with an intracellular N-terminus and a single transmembrane domain that is 685 residues in length and exhibits significant homology to another amino acid transporter designated 4F2hc. Although NBAT transports arginine, lysine, ornithine, and cystine in exchange for cytosolic neutral amino acids, 4F2hc initiates transport of arginine, lysine, ornithine, and histidine, but not cystine. Examination of the Solute Carrier, family 3: amino acids (*SLC3A1*), which encodes NBAT, has identified mutations in individuals with Type I cystinuria [Calonge et al., 1994; Calonge et al., 1995; Bisceglia et al., 1996; Gasparini et al., 1995; Pras et al., 1995].

Although NBAT is responsible for cystine transport in the S3 segment, the bulk of renal cystine transport occurs in the S1-S2 segment of the nephron. S1-S2 segment transport is mediated by a high-capacity, low-affinity system that is sodium-dependent and unshared with dibasic amino acid transporters [Silbernagl, 1988; Segal et al., 1977; Foreman et al., 1980; McNamara et al., 1981]. A defective S1-S2 transport mechanism is a likely cause of Type II/Type III cystinuria. The cystine that is taken up in the S1-S2 segment is normally reduced to cysteine in the tubular cells of the nephron and transported basolaterally along the nephron via the peritubular capillary bed. Recently the gene for Type III cystinuria was mapped to 19q13.1 [Bisceglia et al., 1997]. Here we confirm that both Type II and Type III cystinuria map to chromosome 19q13.1, and show that offspring with both Type II and Type III alleles exhibit an extreme stone-forming phenotype. Furthermore, we present evidence that the gene located at 19q13.1 is a cystinuria gene and that phenotypic differences between Type II and Type III cystinuria are likely due to allelism at this locus.

MATERIALS AND METHODS

Patients, Quantitative Urinalysis, and DNA Samples

Individuals from a large kindred with multiple confirmed cystinurics were examined at a large family reunion while consuming the same foods and beverages for an entire weekend. Twenty-four hour urine samples were obtained from 39 individuals (20 female, 19 male) age 4–87 years (Fig. 1). Twenty-four-hour urine collections included total volume excreted, quantitative urinary cystine, uric acid, calcium, creatinine, sodium, chloride, and potassium. Cystine measurements were obtained by ion-exchange chromatography (Mission Pharmacal, Dallas, TX) to classify relatives into one of the three phenotypic groups. Venipuncture was performed on 33 individuals and DNA was extracted using salt precipitation [Miller et al., 1988; Opelz, 1992]. Informed consent was obtained from all participating family members.

Genotyping

Eight markers at 19q13.1 and six markers at 2p21 were selected for genotyping in order to confirm or exclude previously reported cystinuric loci. Primers for the microsatellite markers were obtained from either Research Genetics Inc. (Huntsville, AL) or the University of Cincinnati DNA Core Lab. All PCR reactions were performed in 50 μL aliquots containing 50 ng DNA, 2.0 mM $MgCl_2$ (Promega) 100 μM dNTP mix (Sequenase), and 25 pmoles of each primer. After an initial denaturation for 5 min at 96°C and subsequent addition of 0.5 units Taq Polymerase (Promega), reactions were carried out for 35 cycles (94°C for 40 sec, 55°C for 30 sec, and 72°C for 30 sec) followed by a final extension at 72°C for 2 min. For markers D19S225, D19S915, D19S900, and D19S881 annealing temperatures of 53°C, 50°C, 58°C, and 56°C were used, respectively. Amplified products were pooled, and electrophoresed on an ABI Prism 377 DNA sequencer. Fragment length was determined using the ABI Prism Genescan 2.1 software program. The results of the Genescan analysis were reviewed and interpreted by two independent observers. Any discrepancies were resolved by reexamination of the gels.

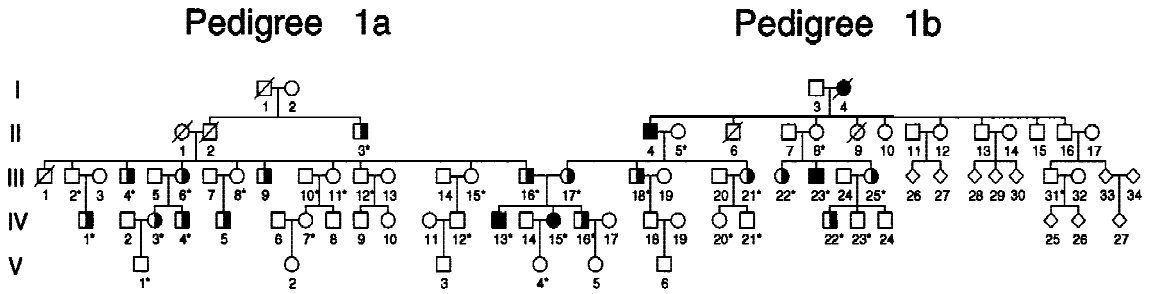

Fig. 1. Family Pedigree (Pedigrees 1a and 1b) Generations are indicated to the left of the pedigree. Numerical assignments to each individual are noted below each symbol. An **asterisk** next to the assigned number indicates an individual whose DNA was collected for the study. Stone-forming individuals are shaded entirely in black. Half-shaded figures represent individuals with sub-lithogenic cystinuria.

Linkage Analysis

Analysis of the pedigree in Figure 1 indicated autosomal dominant transmission of cystinuria. Several unaffected parents (Individuals II-1, II-8, III-2, and III-8) have affected children, implying reduced penetrance. Because it was not possible to determine disease status for children under 10 years, they were not included in the analyses. Analyses were performed using both branches of the family simultaneously. However, due to possible heterogeneity, each branch of the family (Pedigree 1a and Pedigree 1b, Fig. 1) was also analyzed separately. Individuals IV-13, 15, and 16, common to both pedigrees, were included in both series of analyses. Individual IV-16, whose disease clinically resembles that of Pedigree 1a, was considered affected in the analyses of Pedigree 1a and unaffected in the analyses of Pedigree 1b.

The kindred was analyzed for linkage to chromosomes 2 and 19 using a dominant model of inheritance with 70% penetrance for both males and females, an estimate based on the observed number of nonpenetrant obligate gene carriers in the pedigree, and a disease allele frequency of 0.001. A second series of linkage analyses were performed using a codominant model of disease inheritance that may more accurately reflect the disease in this family. Individuals with elevated cystine levels and/or stones were assumed to have one deleterious allele whereas Individuals IV-13 and IV-15, with the most-severe disease in the family, were assumed to have inherited two deleterious alleles, consistent with a codominant model of inheritance. Penetrance was fixed at 70% for the heterozygous genotype and 100% for the homozygous genotype with a disease allele frequency of 0.001. Population frequencies of the marker alleles were estimated with the USERM13 subroutine of the MENDEL suite of programs [Boehnke, 1991]. Two-point lod scores were calculated using the program MLINK as implemented in the FASTLINK package, version 3 [Schaffer et al., 1994; Cottingham et al., 1993; Lathrop and Lalouel, 1984]. Multipoint linkage analyses were performed with the program VITESSE [O'Connell and Weeks, 1995] using the same two disease models. Due to the complexity of the pedigree, multipoint linkage analysis for the dominant model was performed with the pedigree split into Pedigree 1a and Pedigree 1b with Individuals IV-13, IV-15, and IV-16 assigned to Pedigree 1a and all considered affected. Multipoint analysis for the codominant model, however, was performed in the full pedigree. Order and distances between markers were determined by using the Genethon map (www.genethon.fr/html/map).

RESULTS

Phenotype Classification

Phenotypes were classified based on quantitative urinary cystine levels obtained from the 24-hr collec-

TABLE I. Two-Point Linkage Results for Chromosome 19 Using the Dominant Model of Disease Inheritance

Locus	Θ (cM)	0.00	0.01	0.05	0.10	0.20	0.30	0.40	Z_{max}	Θ
D19S915	0.0									
Pedigree 1a		−0.46	1.01	1.56	1.65	1.44	1.00	0.45	1.65	0.096
Pedigree 1b		−1.50	−0.34	0.19	0.31	0.26	0.12	0.01	0.032	0.121
Full Pedigree		−2.70	−0.06	1.11	1.42	1.33	0.90	0.36	1.45	0.129
D19S225	8.2									
Pedigree 1a		−1.17	−0.11	0.55	0.78	0.80	0.57	0.22	0.83	0.152
Pedigree 1b		0.65	0.63	0.58	0.51	0.38	0.25	0.12	0.65	0.000
Full Pedigree		−1.09	−0.04	0.59	0.79	0.78	0.55	0.23	0.83	0.142
D19S425	5.0									
Pedigree 1a		2.99	2.96	2.80	2.57	2.01	1.34	0.60	2.99	0.000
Pedigree 1b		1.96	1.92	1.78	1.58	1.16	0.71	0.28	1.96	0.000
Full Pedigree		3.82	3.77	3.54	3.20	2.40	1.49	0.58	3.82	0.000
D19S422	1.6									
Pedigree 1a		2.71	2.68	2.53	2.30	1.76	1.11	0.41	2.71	0.000
Pedigree 1b		0.29	0.28	0.24	0.20	0.12	0.05	0.01	0.29	0.000
Full Pedigree		2.43	2.39	2.24	2.01	1.48	0.88	0.29	2.43	0.000
D19S881	1.1									
Pedigree 1a		2.35	2.33	2.20	2.01	1.53	0.94	0.31	2.35	0.000
Pedigree 1b		1.66	1.64	1.55	1.42	1.07	0.68	0.30	1.66	0.000
Full Pedigree		2.89	2.86	2.72	2.47	1.84	1.08	0.36	2.89	0.000
D19S223	1.2									
Pedigree 1a		2.10	2.09	2.02	1.89	1.50	1.00	0.42	2.10	0.000
Pedigree 1b		−1.91	−1.88	−1.41	−0.96	−0.50	−0.28	−0.13	0.36	0.838
Full Pedigree		−0.39	−0.36	0.08	0.43	0.60	0.44	0.15	0.61	0.188
D19S913	2.3									
Pedigree 1a		0.90	0.93	0.96	0.93	0.75	0.48	0.17	0.96	0.05
Pedigree 1b		−1.61	−0.57	0.01	0.19	0.24	0.15	0.05	0.25	0.171
Full Pedigree		−0.72	0.35	0.97	1.12	0.99	0.64	0.22	1.12	0.115
D19S900	0.7									
Pedigree 1a		2.46	2.43	2.30	2.10	1.60	1.00	0.36	2.46	0.000
Pedigree 1b		−1.15	−1.04	−0.34	0.04	0.27	0.27	0.16	0.29	0.242
Full Pedigree		0.19	0.29	0.92	1.19	1.11	0.74	0.28	1.22	0.129

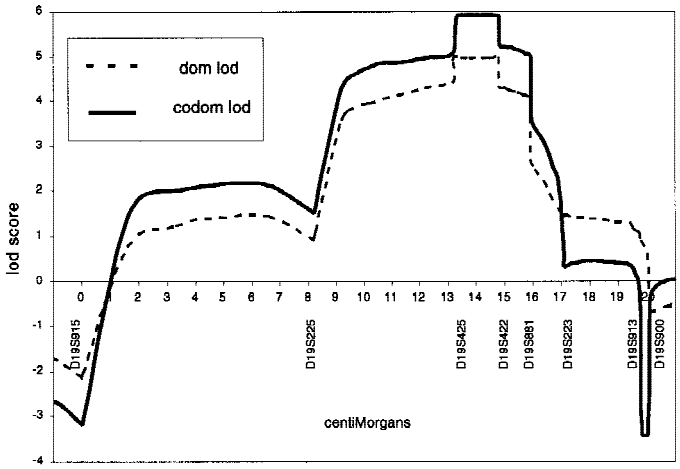

Fig. 2. Multipoint linkage analysis of eight markers on chromosome 19. Using a codominant model analysis, the maximum lod score was 5.90 at marker D19S425. A dominant model analysis also shows a maximum lod score at marker D19S425, but with a slightly lower value of 4.96. D19S915 was arbitrarily assigned map distance 0.0.

tions. Members of Pedigree 1a exhibit a Type III heterozygous phenotype with an average cystine excretion level of 108 mg/gr creatinine (range = 16–268). Individuals from Pedigree 1b exhibit the Type II heterozygous phenotype with an average cystine excretion of 220 mg/gr creatinine (range = 117–753). Two individuals from Pedigree 1b form cystine renal calculi: Individual II-4 excreted 226 mg cystine/gr creatinine; Individual III-23 excreted 397 mg cystine/gr creatinine. Both individuals reported a long history of multiple bouts of renal colic that required urologic care for more than 30 years. Individual II-4 was not available for venipuncture and subsequent genotyping, although he did complete a 24-hr urine. Two of the children from the union of Individuals III-16 and III-17 are severely affected and form multiple renal calculi. Individuals IV-13 and IV-15 excrete over 500 mg cystine/gr creatinine per day; age of first stone formation was 5 years. The third child from this union, IV-16, has an intermediate phenotype without stone formation, similiar to individuals in Pedigree 1a (Fig. 1).

Linkage to Chromosome 19, and Exclusion of Chromosome 2

Multipoint analyses excluded linkage to chromosome 2 using the dominant model of inheritance in each branch of the family (Fig. 1, Pedigrees 1a and 1b) when analyzed separately (lod < –2.0). Multipoint analysis also excluded linkage to chromosome 2 (lod < –2.0), using the codominant model of inheritance in the full pedigree. Significant evidence was found for linkage to chromosome 19 using the dominant model of disease inheritance. The maximum two-point lod score in the combined pedigree was 3.82 with the marker D19S425 (θ_{max} = 0.00). Separate analysis of each branch of the family also supported linkage to chromosome 19 with a maximum lod score of 2.99 at D19S425 in Pedigree 1a and a maximum lod score of 1.96 with the same marker in Pedigree 1b (Table I). Multipoint analysis, using the dominant model in the two pedigrees, resulted in a combined maximum lod score of 4.96 at marker D19S425 (Fig. 2). Similarly, using the codominant model of disease inheritance, the maximum two-point lod score was 4.78 with marker D19S425 (θ_{max} = 0.00) (Table II). Multipoint analysis using this model resulted in a maximum lod score of 5.90 at marker D19S425 (Fig. 2). All lod-score results were stable across order-of-magnitude changes in disease allele frequency estimate as well as changes in the penetrance function.

Haplotype analysis of the affected individuals in the full pedigree was used to identify several recombinants that established the minimum candidate interval (Fig. 3). Individual IV-3 exhibited a recombination with the marker D19S225 delineating the critical proximal region. Individuals III-25 and IV-22 both manifested a recombination with marker D19S223. Together, these recombinants established a minimum candidate inter-

TABLE II. Two-Point Linkage Results for Chromosome 19 Using the Co-dominant Model of Disease Inheritance

Locus	Θ (cM)	0.00	0.01	0.05	0.10	0.20	0.30	0.40	Z_{max}	Θ
D19S915	0.0	–6.38	0.58	1.68	1.87	1.58	1.00	0.39	1.87	0.102
D19S225	8.2	–1.32	0.21	0.86	1.04	0.98	0.68	0.29	1.06	0.129
D19S425	5.0	4.78	4.71	4.41	3.99	3.02	1.93	0.81	4.78	0.000
D19S422	1.6	2.68	2.65	2.48	2.24	1.67	1.02	0.36	2.68	0.000
D19S881	1.1	3.99	3.95	3.72	3.37	2.53	1.54	0.56	3.99	0.000
D19S223	1.2	–inf	–1.00	0.18	0.61	0.78	0.58	0.22	0.79	0.184
D19S913	2.3	–1.48	0.21	0.87	1.04	0.95	0.62	0.22	1.05	0.122
D19S900	0.7	–3.17	0.69	1.80	2.01	1.74	1.12	0.46	2.01	0.106

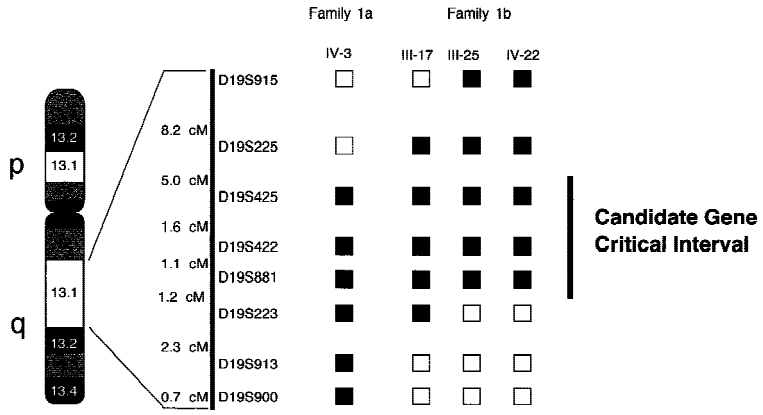

Fig. 3. Delineation of critical interval. Schematic of chromosome 19 depicting the microsatellite markers amplified and subsequently analyzed for linkage. **Open squares** represent markers that have been excluded from the critical interval by observed recombinations, whereas **filled squares** represent consistent inheritance of marker associated with the disease haplotype. The critical interval for our candidate gene is between markers D19S225 and D19S223. The distance of this interval is 8.9 cM.

val of 8.9 cM, flanked by the markers D19S225 and D19S223.

DISCUSSION

The gene for Type III cystinuria has been assigned to the long arm of chromosome 19 (19q13.1) by Bisceglia et al. [1997] who analyzed 9 families; however, linkage of Type II cystinuria to this region was considered tentative by the authors because only two small Type II pedigrees were analyzed. We report on 2 large kindreds, in which heterozygotes secrete either high (Type II) or intermediate levels (Type III) of cystine. In both kindreds, the gene for elevated cystine excretion maps to chromosome 19q13.1 with a multipoint lod score of 4.96 at the marker D19S425 using a dominant model of inheritance. When a codominant model was employed, the lod score increased to 5.90. Bisceglia et al. obtained their maximum lod score (3.11) at D19S225. However, we identified a recombination with this marker in our kindred.

The inheritance of these marker alleles is consistent with the phenotypes based on quantitative urinary excretion of cystine per gram of creatinine, autosomal dominant inheritance, and incomplete penetrance. Type II and Type III cystinuria have different clinical presentations. The Type III family in this study suffered no adverse sequelae from their disease, and were unaware that they excreted abnormal amounts of urinary cystine. In contrast, two heterozygote individuals in the Type II family required multiple treatments to manage their stone disease.

Based on observations of the observed number of obligate gene carriers in the pedigree, the Type III cystinuria kindred (Pedigree 1a) is characterized by 90–100% penetrance for cystinuria but zero risk for urinary stone disease, whereas the Type II cystinuria kindred (Pedigree 1b) demonstrates 40–50% penetrance for cystinuria and 16-25% risk for forming stones. The excretion phenotype is additive: the members of the pedigree who are descendants of Individuals III-16 and III-17 excrete cystine at a mean rate of 302 (±229) mg/gr Cr per 24 hr (SD) whereas the Type III (Pedigree 1a) and Type II (Pedigree 1b) families excrete cystine at a rate of 108 (±86.6) mg/gr Cr per 24 hr and 220 (±204) mg/gr Cr per 24 hr, respectively.

Individuals IV-13 and IV-15 both have a more-severe phenotype than other affected individuals in either branch of the pedigree. Following haplotype analysis, each was discovered to have inherited a copy of the disease haplotype from each branch of the pedigree. In contrast, their less severely affected sibling, Individual IV-16, only inherited a disease haplotype from his father, a member of Pedigree 1a. Although the Type II and Type III cystinuria kindreds present differently, they both link to chromosome 19q13.1 and likely represent allelic variants of the same gene. The genotype–phenotype correlation of Individual IV-16 supports our hypothesis of allelic variation at chromosome 19q13.1. His disease haplotype is consistent with that inherited in Pedigree 1a, and his clinical presentation is stone-free, like that of Pedigree 1a.

This study definitively establishes the genetic basis of Type II cystinuria and provides additional insight into the extreme clinical phenotype of individuals who inherit two codominant alleles of the cystinuria gene(s) at 19q13.1. Cloning of the Type II/Type III cystinuria gene(s) should elucidate the physiological defect as well as the molecular mechanisms underlying the dominant form of this disease. More importantly, the results of this study will facilitate genetic counseling and help direct appropriate management of those patients at risk for recurrent cystine nephrolithiasis.

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