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Original investigations

Genetic analysis of carbamyl phosphate synthetase I deficiency

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Summary. Carbamyl phosphate synthetase I deficiency (CPSD) is an autosomal recessive disorder of ureagenesis characterized by hyperammonemic coma in the neonatal period. To study the genetic basis of CPSD we have performed a molecular analysis of the CPS I genes in CPSD patients from six unrelated families. Using a cDNA probe for the human CPS I gene and restriction endonuclease mapping techniques, we observed no abnormality in the number or size of the hybridizing DNA fragments from the seven affected individuals examined. These findings suggest that no gross alteration affected the CPS I genes. We did detect a frequent restriction fragment length polymorphism (RFLP) at the CPS I locus which we employed as a linkage marker. Our results suggest the polymorphic CPS I restriction fragments cosegregate with the CPSD phenotype, and that linkage disequilibrium exists between the CPSI RFLPs studied and the affected alleles. The RFLPs described may enable prenatal detection of CPSD in families where the coupling phases between CPSD alleles and RFLPs can be determined.

Introduction

Mitochondrial carbamyl phosphate synthetase (CPS I) catalyzes the first step in the urea cycle, the synthesis of carbamyl phosphate from bicarbonate, ATP, and ammonium. Deficiencies of the urea cycle enzymes are often associated with neonatal hyperammonemia, impaired mental and physical development, and protein intolerance. Two distinct clinical patterns resulting from CPS I deficiency (CPSD) have been described. Complete or virtually complete deficiency of CPS I, if untreated, results in a lethal hyperammonemia manifest in the first week of life (Gelehrter and Snodgrass 1974). A less severe, delayed onset form has been observed in individuals exhibiting a partial deficiency (Arashima and Matsuda 1972). Both types of CPSD have an autosomal recessive mode of inheritance (McReynolds et al. 1981). Prompt treatment with protein restriction, amino acid supplementation, and activation of alternative pathways of waste nitrogen excretion can prolong survival and improve the clinical course of the disorder (Brusilow et al. 1982). Prenatal detection of CPSD is currently limited to in utero liver biopsy because the enzyme is not expressed in amniocytes (Shih 1976).

In order to study the genetic basis of CPSD and to provide a linkage marker for prenatal diagnosis of the disorder, we have performed a molecular analysis of nuclear DNA from six families affected with the disorder. Utilizing a cDNA probe for the CPS I gene and restriction endonuclease mapping, we were unable to detect a deletion or rearrangement of the CPS I gene in these families. However, we found a frequent Bgl I restriction fragment length polymorphism (RFLP), enabling linkage analysis. Our results suggest cosegregation of the CPSD phenotype with CPS I alleles in informative pedigrees. Use of this polymorphism should help in the isolation of abnormal CPS I alleles for structural characterization as well as enable prenatal diagnosis of CPSD in at risk couples where the linkage phases can be determined.

Materials and methods

Patients. Seven patients previously diagnosed as having CPSD from six unrelated families were studied. The criteria used for diagnosis were two or more of the following: hyperammonemic coma, a previous sibling with CPSD, a plasma citrulline level of zero to trace, normal urinary orotate, and deficient CPS I activity on liver biopsy. The affected children in Families 1 (Applegarth et al. 1979) and 2–5 had the neonatal form of CPSD, while the child in Family 6 had the late onset type of CPSD.

Restriction endonuclease analysis. High molecular weight DNA was isolated from peripheral leukocytes or from fibroblasts (Kunkel et al. 1977). Approximately 5 µg of DNA from each individual were digested overnight with various restriction endonucleases, using the reaction conditions suggested by the manufacturer. The resulting DNA fragments were separated by electrophoresis in agarose gels, transferred to nitrocellulose, and hybridized to human CPS I sequences contained in a 1200 bp insert of the clone pCPSh2 (Southern 1975; Scott et al. 1979; Adcock and O'Brien 1984). This insert was radiolabeled with ^{32}P-dATP and ^{32}P-dCTP (Schachat and Hogness 1973). Washing of filters and autoradiography were formed as previously described (Scott et al. 1979).

Linkage analysis. The linkage analysis was performed using the method of maximum likelihood (Morton 1955) and the computer program LIPED (Ott 1974) which was used to calculate the odds in favor of linkage between CPS I RFLP and CPSD, versus nonlinkage.

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Results

Restriction endonuclease mapping of the CPS I gene in CPSD individuals. To determine if any large deletions, insertions, or rearrangements of the CPS I genes could be detected, we selected three restriction enzymes (Eco RI, Hind III, and Pst I) that gave multiple hybridizing fragments when DNA was digested with each and hybridized to the CPS I cDNA probe. Autoradiogram patterns obtained after these digestions did not demonstrate abnormalities in the number or size of hybridizing DNA fragments from the affected individuals as compared to normal controls (Fig. 1).

A polymorphic Bgl I restriction site at the CPS I locus. To determine if CPSD was genetically linked to the CPS I gene, we needed an RFLP near the CPS I gene that could be employed as a linkage marker. To detect such an RFLP, DNA from the parents of the affected individuals was digested with

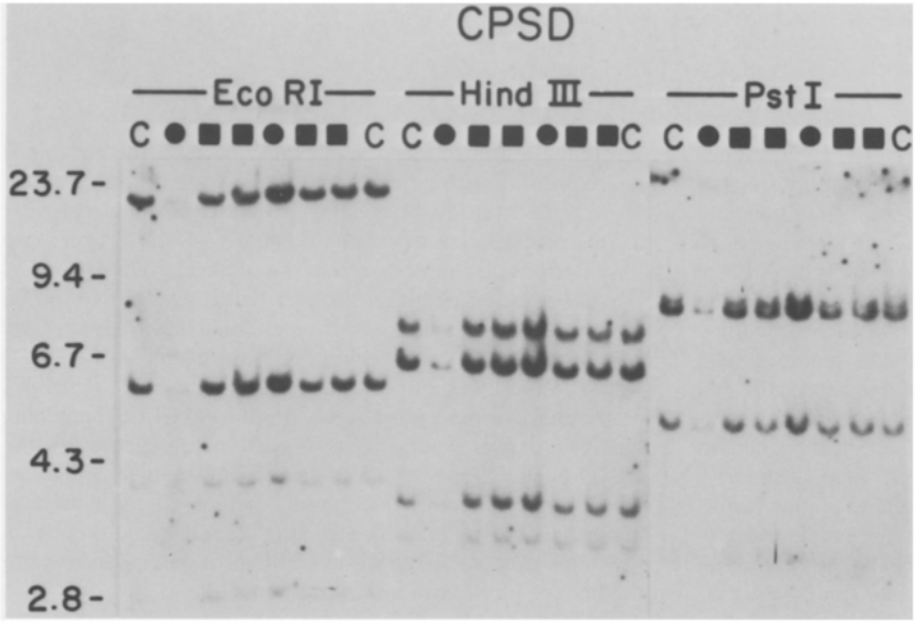

Fig. 1. Autoradiogram patterns of DNAs from control (C) and from affected individuals *(solid symbols)* in six CPSD families. DNAs were digested with the restriction endonucleases Eco RI, Hind III, or Pst I and were then hybridized to the CPS I cDNA probe. Fragment sizes in kilobase pairs (kb) are shown on the left. Note: The very light patterns seen in the first affected individual in each panel were demonstrated, on other radiograms, to be normal and were due to underloading of DNA

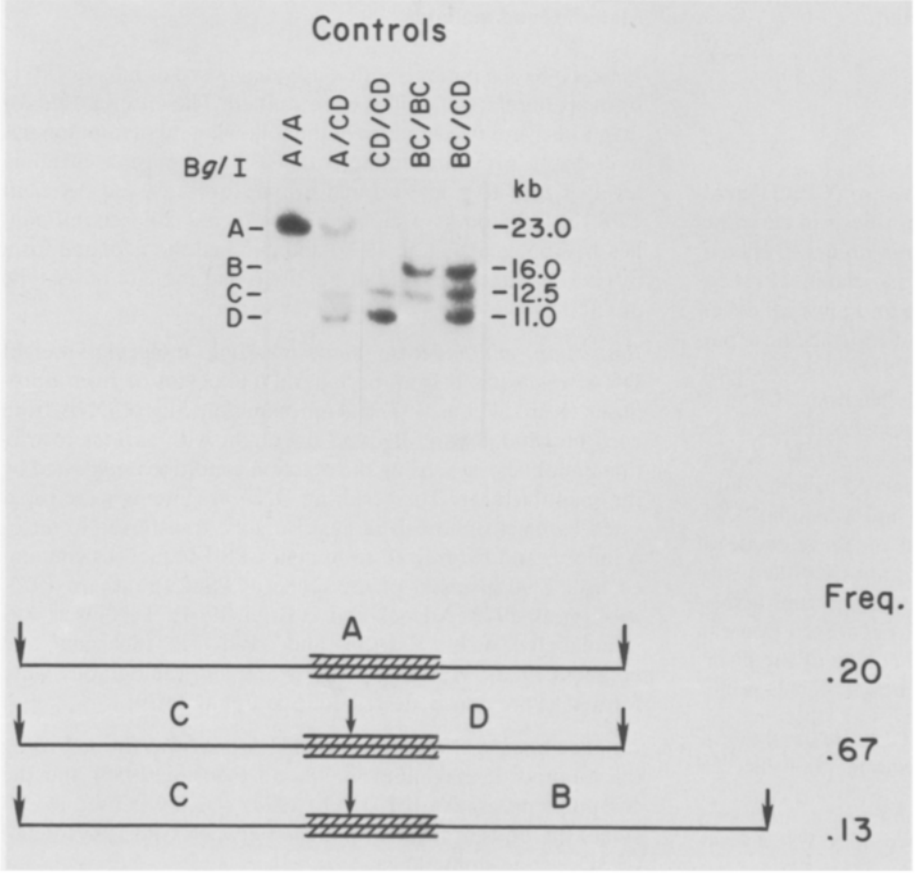

Fig. 2. Autoradiogram patterns of DNA from five control individuals after digestion with Bgl I restriction endonuclease and hybridization to the CPS I cDNA probe. The RFLP patterns are indicated above each lane and the fragment sizes in kb are shown on the right. A model of the Bgl I cleavage sites needed to produce the observed RFLPs is shown along with the frequency of each pattern (64 control alleles examined)

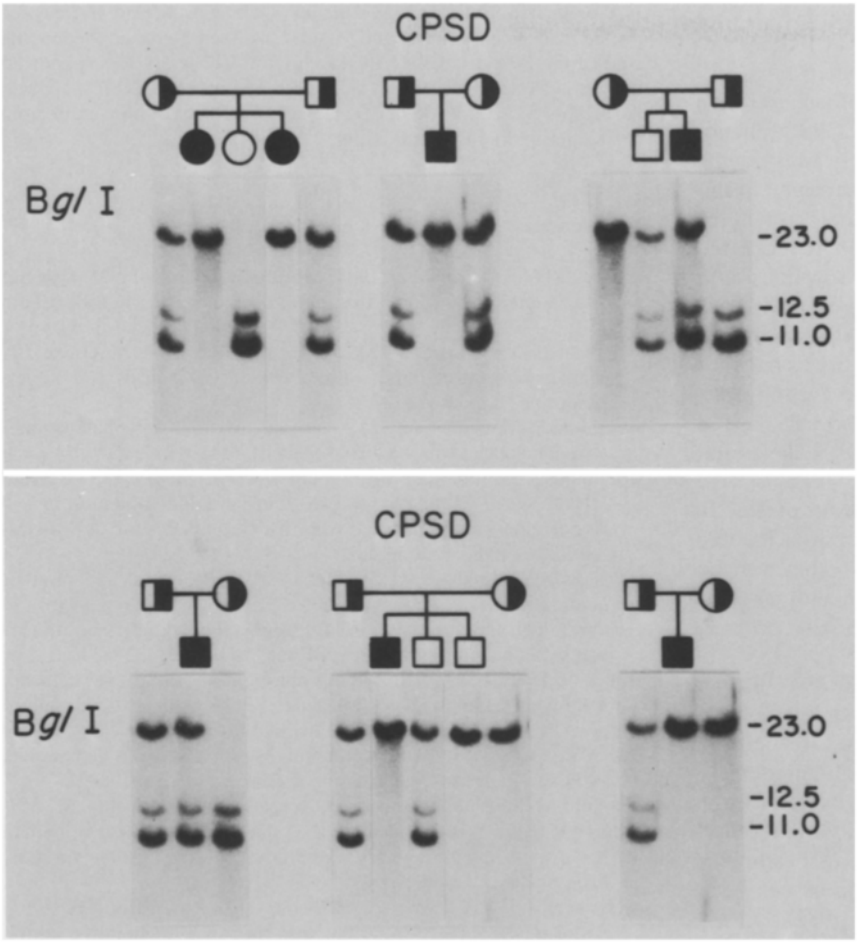

Fig. 3. Autoradiogram patterns of DNA from six CPSD families after digestion with Bgl I restriction endonuclease and hybridization to the CPS I cDNA probe. The fragment sizes in kb are shown on the right

each of 12 randomly selected restriction endonucleases: Bam HI, Bgl I, Bgl II, Eco RI, Hinc II, Hind III, Msp I, Pvu II, Sst I, Taq I, Xba I, and Xho I. Only after digestion with Bgl I did we observe a polymorphic variation in the autoradiogram patterns. Five patterns were detected in controls (Fig. 2). The following results strongly suggest that these patterns are the result of a Bgl I restriction site polymorphism: (i) identical patterns are obtained using a ten-fold excess of enzyme; (ii) segregation patterns observed in six CPSD families and six control families are completely consistent with Mendelian inheritance; and (iii) constant cleavage patterns with other restriction endonucleases exclude large insertions or deletions near the CPS I gene. The frequency of the various allelic fragments containing CPS I sequences in control alleles (64 examined) and a model of the relative locations of the two Bgl I polymorphic restriction sites needed to produce the fragments are shown in Fig. 2.

Cosegregation of CPS I alleles and CPSD phenotype. In five of the six families, at least one of the parents was heterozygous for an RFLP containing CPS I sequences. In Family 1 (Fig. 3, *top left*) the mother and father were heterozygous for the A and CD CPS I fragments (Figs. 2 and 3). Both affected children inherited the A allele from each parent while the unaffected child inherited the CD allele of each parent. Thus in this family it appears that the defective CPS I allele of both parents is in phase with the 23 kb fragment containing the A allele. In Family 2 (Fig. 3, *top center*) the same patterns as those of Family 1 are seen. The affected individual is homozygous for the A allele while the unaffected child is homozygous for the CD allele (data not shown). Family 3 (Fig. 3, *top right*) is uninformative in our analysis, since the mother and father are oppositely homozygous (A/A and CD/CD, respectively) for CPS I alleles and both children are heterozygous for the A and CD fragments.

In the remaining three families (Fig. 3, *bottom*), the father is heterozygous for the A and CD fragments. In these families we are unable to distinguish between the mothers' alleles. For this reason only when the paternal allele not found in the affected child is present in subsequent children could one suspect that child is not affected. In the families studied the CPS I alleles inherited by affected individuals were A (10/12) and CD (2/12) (Fig. 3).

Discussion

We have taken advantage of a cloned cDNA fragment homologous to the CPS I gene and restriction endonuclease mapping techniques to explore the molecular basis of CPSD in six affected families. We were unable to demonstrate a large deletion, insertion, or rearrangement of the CPS I gene in either neonatal or late onset types of CPSD. We did, however, detect RFLPs containing CPS I sequences after Bgl I digestion. These were used to mark individual alleles at this locus, and thus follow their segregation within the six CPSD pedigrees. Our results suggest but do not prove that in the

families studied the CPS I RFLPs cosegregate with the CPSD phenotype. From our data the odds in favor of linkage between the CPS I RFLPs and the CPSD alleles are 6.3:1.

Two factors have limited the use of our approach to prove cosegregation between CPS I alleles and the CPSD phenotype. First, CPS I activity can be assayed only by studying hepatic tissue. Since liver biopsy cannot be performed in unaffected sibs, we were unable to characterize them as heterozygous or homozygous normal. This limitation reduced the number of informative matings, resulting in the low odds ratio for the calculated likelihood of linkage between the CPS I alleles and CPSD. Second, although five of six families were informative in our analysis, study of only two RFLPs may not allow determination of allelic assortment within a given pedigree. Additional RFLPs within or flanking the CPS I gene could aid in the characterization of abnormal CPS I alleles and prenatal detection of CPSD.

While we have not characterized the alterations present in the CPS I alleles of affected individuals, our approach could assist in the isolation of affected alleles since they can be distinguished from normal alleles and from one another. Our results suggest that the defects at the CPS I gene that occur in CPSD may be subtle (i.e., point mutations).

Heterogeneity in CPSD is suggested by the two distinct clinical patterns of the disorder: the lethal hyperammonemia manifest only days after birth and the less severe delayed onset form. Our results are also consistent with heterogeneity in the molecular defects at the CPS I locus. As can be seen in Fig. 3, the alleles of different affected individuals are not linked to the same CPS I RFLP in all families. The association of different RFLP patterns with different mutations has been well described in the thalassemias (Orkin et al. 1982).

Interestingly, the frequencies of CPS I alleles observed in affected individuals (A—0.83 and CD—0.17) are different from those seen in controls (A—0.20 and CD—0.67) (χ^2 = 16.15, $P<0.001$). These differences in distribution suggest linkage disequilibrium exists between the CPS I alleles of the CPSD individuals and the RFLPs studied. Such linkage disequilibrium has been shown previously between the β^S allele and an RFLP (Kan and Dozy 1978).

The utility of RFLPs in prenatal diagnosis by linkage analysis has been clearly demonstrated for the hemoglobinopathies (Kan and Dozy 1978; Boehm et al. 1983). Such an approach does not require knowledge of the exact defects underlying the disorder, only a determination of the linkage phases between affected alleles and the RFLPs used as markers. Thus the genotype of the fetus can be inferred in families where the linkage phases of both parents' alleles are known, and the disorder can be potentially excluded if the linkage phases of only one parent's alleles are known. Using the Bgl I derived RFLPs in an analogous way, our results suggest that if linkage is proven the fetal CPS I phenotype could be predicted in about 50% of families at risk for CPSD.

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