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Molecular analysis of human argininosuccinate lyase: Mutant characterization and alternative splicing of the coding region

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ABSTRACT **Argininosuccinic acid lyase (ASAL) deficiency is a clinically heterogeneous autosomal recessive urea cycle disorder. We previously established by complementation analysis that 28 ASAL-deficient patients have heterogeneous mutations in a single gene. To prove that the ASAL structural gene is the affected locus, we sequenced polymerase chain reaction-amplified ASAL cDNA of a representative mutant from the single complementation group. Fibroblast strain 944 (≈1% of residual ASAL activity), from a late-onset patient who was the product of a consanguineous mating, had only a single base-pair change in the coding region, a C-283 → T transition at a CpG dinucleotide in exon 3. This substitution converts Arg-95 to Cys (R95C), occurs in a stretch of 13 residues that is identical in yeast and human ASAL, and was present in both of the patient's alleles but not in 14 other mutant or 10 normal alleles. Expression in COS cells demonstrated that the R95C mutation produces normal amounts of ASAL mRNA but little protein and <1% ASAL activity. We observed that amplified cDNA from mutant 944 and normal cells (liver, keratinocytes, lymphoblasts, and fibroblasts) contained, in addition to the expected 5′ 513-base-pair band, a prominent 318-base-pair ASAL band formed by the splicing of exon 2 from the transcript. The short transcript maintains the ASAL reading frame but removes Lys-51, a residue that may be essential for catalysis, since it binds the argininosuccinate substrate. We conclude (*i*) that the identification of the R95C mutation in strain 944 demonstrates that virtually all ASAL deficiency results from defects in the ASAL structural gene and (*ii*) that minor alternative splicing of the coding region occurs at the ASAL locus.**

Argininosuccinic acid lyase (ASAL; EC 4.3.2.1.) deficiency, or argininosuccinic aciduria, is an autosomal recessive inborn error of the urea cycle characterized by significant clinical and genetic heterogeneity (1, 2). The biochemical basis of the clinical heterogeneity is unclear; there is only a partial correlation between the clinical phenotype and residual ASAL activity in cultured fibroblasts (2) and other tissues (1). Extensive genetic heterogeneity has been established in the mutant population by complementation analysis, but all of the complementation is intragenic (i.e., all 28 mutants studied are affected at a single locus) (2). Since intragenic complementation is due to interactions between the mutant allelic subunits of a homomultimeric protein and because ASAL is a homotetramer, the data are consistent with the ASAL structural gene being the affected locus (2).

Additional evidence suggests that the ASAL structural gene is the site of the mutations in this condition. Thus, although cross-reacting material (CRM) of the approximate size of the normal monomer (≈50 kDa) is detectable in almost every mutant (3), the quantity of the monomer is highly variable and does not correspond to the amount of residual enzyme activity. In addition, the ASAL polypeptide in the majority of mutants appears to be unstable, since significant CRM is present in one to five bands smaller than the 50-kDa monomer (3). In agreement with the fact that most mutants are CRM-positive, the great majority (25/28) produce ASAL mRNA of normal size and abundance, and none have gross rearrangements or deletions of the ASAL gene (4). We interpret these data as indicating that the mutations are single base-pair substitutions, small deletions, or rearrangements in the gene encoding the ASAL polypeptide.

The human ASAL gene, covering ≈25 kilobases (kb) (5), has been demonstrated to have intron/exon borders and exon sizes that are identical (W. E. O'Brien, personal communication) to the rat gene, which has 16 exons (6). A 1565-base-pair (bp) cDNA containing the complete human ASAL coding region has been isolated and is predicted to encode a 463-residue polypeptide of 51.6 kDa (5), whose translation is initiated in exon 1.

To prove definitively that the ASAL structural gene is the affected locus, we have used the polymerase chain reaction (PCR) to amplify ASAL cDNA from the fibroblasts of affected patients. We report here the identification of an ASAL structural gene mutation in a patient with a late-onset form of the disease, establishing this gene as the affected locus in this classic aminoacidopathy. In addition, we provide evidence that minor differential splicing of the ASAL transcript occurs in normal tissues. Unexpectedly, the exon that is removed contains a critical component of the catalytic part of the protein, the putative argininosuccinate binding site.

MATERIALS AND METHODS

Cell Strains. Fibroblasts were obtained and cultured as described (2, 3). Lymphoblasts were cultured in α-minimal medium and supplemented with 15%–20% (vol/vol) fetal bovine serum without antibiotics.

Vectors. The plasmid Bluescript (Stratagene) was used as a cloning and sequencing vector. The plasmid p91023(B) is an expression vector containing the adenovirus late promoter and the simian virus 40 polyadenylylation signal (7). The p91023/ASAL plasmid, constructed by standard techniques (8), contained a full-length ASAL cDNA (5) with 114 bp of untranslated 5′ sequence and 46 bp of untranslated 3′ sequence. The mutation was cloned into this construct using a *Mlu* I–*Oxa*nI cassette. pSV2Apap (9) is an expression vector containing the full-length placental alkaline phosphatase cDNA and was used as a control in the transfection experiments. SC-12 is an M13 phage with a 1.5-kb fragment containing exons 2–5 of the ASAL genomic DNA (a gift from W. E. O'Brien, Baylor College of Medicine).

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Abbreviations: ASAL, argininosuccinate lyase; PCR, polymerase chain reaction; nt, nucleotide(s).

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Oligonucleotides. The oligonucleotide primers for PCR were based on the published ASAL cDNA sequence (5, 10); we designated the initial nucleotide of the translated sequence as the first nucleotide. The complete coding sequence was amplified in three overlapping fragments using paired primers, A–F (Fig. 1). Primers A, C, and E corresponded to nucleotides (nt) −27 to −3, nt 400 to 424, and nt 909 to 930, respectively. Primers B, D, and F were complementary to nt 445 to 468, nt 943 to 967, and nt 1398 to 1422, respectively. Each oligonucleotide had a 9-bp sequence containing a *Bam*HI restriction site at its 5′ end. A genomic PCR was performed using oligonucleotide G corresponding to the 3′ end of intron B [5′-CATGCTGCTACCCACTACAG-3′ (W. E. O'Brien, personal communication)] and primer H with complementary bases 324 to 348 of the cDNA sequence. The 27-bp oligonucleotides used for Southern blot experiments corresponded to nt 13 to 39 and nt 256 to 274, of the cDNA sequence.

Northern and Southern Blot Analysis. Total RNA was prepared from stationary-phase skin fibroblasts by cell lysis in guanidinium thiocyanate and centrifugation through a cushion of 5.7 M CsCl (11). RNA (20 μg) was electrophoresed as described in Fourney *et al.* (12). RNA was transferred to Hybond-C nitrocellulose (Amersham), hybridized, and washed as suggested by Maniatis *et al.* (8) with the following modifications: The prehybridization buffer contained 5× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0), 1× Denhardt's solution (0.02% Ficoll 400/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin), 20 mM sodium phosphate (pH 6.6), 1% SDS, and 50% (vol/vol) formamide (Fluka). Hybridization buffer also contained 10% (wt/vol) dextran sulfate. The filters were hybridized overnight at 42°C. Autoradiography was done using Kodak XAR film at −70°C for 1–7 days with DuPont Cronex Lightning Plus intensifying screens. DNA was digested as recommended by the enzyme manufacturer, electrophoresed through 0.7% agarose, transferred to Hybond-N, and hybridized as described above. When using oligonucleotide probes, prehybridization and hybridization were done as above but washes were done at room temperature and 55°C in 2× SSC. Nondenaturing acrylamide gel electrophoresis was done as described by Maniatis *et al.* (8). Molecular mass markers were from BRL.

Reverse Transcription. Total RNA (20 μg) was reverse-transcribed using the protocol and enzyme of the manufacturer (RPN.1256; Amersham) with the following modifications. Oligo(dT) or 100 ng of an oligonucleotide complementary to the cDNA residues 445–468 were used to prime reverse transcription. The total RNA (7.5 μl) was denatured by incubation in 10 mM methylmercury hydroxide, followed by inactivation of the methylmercury hydroxide with 18.6 mM 2-mercaptoethanol. Reverse transcription was done in the Amersham buffer for 1 hr at 42°C, in a total volume of 20 μl. The entire reverse transcription reaction mixture was then used directly as the substrate in the PCR.

PCR Amplification and cDNA Cloning. The PCR was carried out for 30 cycles in the Cetus buffer (13), with the primers at a concentration of 1 μM, under the following conditions: denaturation for 30 sec at 94°C, renaturation for 30 sec at 55°C, extension for 2 min at 72°C. After amplification, the products were electrophoresed through a 0.7% agarose gel and visualized with ethidium bromide. Genomic PCR used 0.3–0.5 μg of genomic DNA as the substrate, with primers G and H. The PCR products were chromatographed on a Sephadex (G 50-150) (Sigma) spin column (8). One-half of the products were then digested overnight with *Bam*HI and electrophoresed on 0.7% low-melting-temperature agarose. The band corresponding to the amplified cDNA was cut out of the gel and ligated into the *Bam*HI-digested sequencing vector Bluescript, using T4 DNA ligase (BRL) (25°C and 18 hr).

Quantitative PCR was performed using the method of Chelly *et al.* (14), with the following modifications. Reverse transcriptions [primed with oligo(dT) or ASAL-specific oligonucleotide B] and PCRs were performed as described using ^{32}P-end-labeled oligonucleotide primers, A and B. Samples (5 μl) of each reaction mixture were taken every three cycles of the PCR, starting with cycle 15. Samples were electrophoresed on a 5% native acrylamide gel, at 20 W for ≈5 hr. The film was dried and exposed for various times. The density of the bands was determined on a WYSE/Molecular Dynamics computing densitometer (model 300A) and analyzed as described in Chelly *et al.* (14).

Expression of Mutant ASAL cDNA. Transfection of the expression vectors was performed by the method of Chen and Okayama (15). Approximately 10^6 COS-1 cells were plated in a 100-mm Petri dish. The p91023(B) vector (20 μg), alone or with either a normal or mutant (R95C) full-length lyase insert (20 μg), and 15 μg of pSV2Apap were transfected by calcium phosphate coprecipitation and harvested 72 hr later. After transfection, cells were used to prepare RNA and crude cell lysates for protein and enzyme assays.

Cell lysis, protein electrophoresis, and immunoblot analysis were done as described (3). Assays of placental alkaline phosphatase were done as described by Henthorn *et al.* (9). Protein was measured by the method of Lowry *et al.* (16) using bovine serum albumin as a standard. ASAL enzyme activity was assayed as described (17) and normalized for protein and placental alkaline phosphatase activity.

Other Methods. Isolated restriction fragments were radiolabeled to a specific activity $>10^8$ cpm/μg by using random oligonucleotide primers (18). Radiolabeling of oligonucleotides was done by the phosphate exchange method as described in Maniatis *et al.* (8). Single-stranded DNA was prepared from Bluescript clones by the method of Vieira and Messing (19) and sequenced by the dideoxynucleotide method, using T7 or internal primers and Sequenase (20).

RESULTS

Mutant Cell Strain. We chose to analyze mutant cell strain 944 for two reasons. (*i*) The patient was the product of a consanguineous mating and, therefore, was likely to be homozygous for a single mutant allele. (*ii*) RNA blot analysis demonstrated ASAL mRNA of normal size and abundance, and Southern blots showed no change in band intensity or restriction enzyme pattern (data not shown), consistent with the presence of a small mutation not affecting transcription.

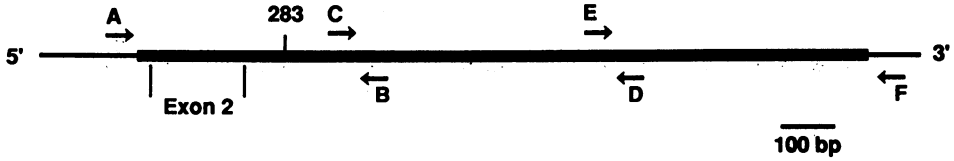

FIG. 1. ASAL cDNA with the location of the paired primers (A and B, C and D, E and F) used for amplification. A first-strand cDNA was reverse-transcribed and then amplified using the above primers (see text). The position of the C-283 → T transition and the size and placement of exon 2 are shown.

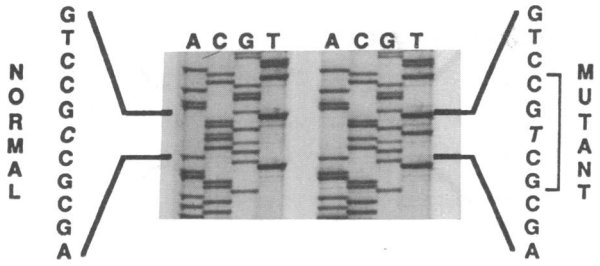

FIG. 2. Partial sequence of the cloned ASAL cDNA of a control fibroblast and of mutant 944. The control sequence (nt 278–288) in the region of the mutation (to the left) corresponds to that of mutant 944 (to the right), except at bp 283 (in italics). The substitution changes the normal codon 95 CGC (arginine) to TGC (cysteine) and creates a *Bbv* I site (square bracket).

The residual activity of mutant 944 was ≈1%, and small amounts of the ≈50-kDa ASAL monomer were shown to be present in fibroblasts by immunoblot analysis (3). Cell strain 944 belongs to the single complementation group defined for ASAL deficiency and complements two other mutants (2).

Identification of the Molecular Defect in Mutant 944. To determine the sequence of the ASAL coding region in strain 944, total RNA was reverse-transcribed and the single-stranded ASAL cDNA was amplified in three overlapping ≈500-bp fragments (Fig. 1) and cloned. Sequencing of six clones of each fragment identified a single substitution, a C → T transition at nt 283 in exon 3 (Fig. 2). This transition, in the first nucleotide of codon 95, changes the residue from an arginine to a cysteine (R95C). No other differences from the normal cDNA sequence (1395 bp) (5, 10) were found in strain 944. Notably, the R95C substitution occurs in a 13-amino acid sequence, corresponding to residues 83–96 in the human ASAL protein, which is completely conserved in the yeast and human proteins (10).

To confirm that the nucleotide change found in the cloned PCR material was present in both alleles of mutant 944, genomic PCR products were analyzed with the restriction enzyme *Bbv* I, since the transition at nt 283 creates a new *Bbv* I site [GCTGC(N)$_8$] (21). A genomic fragment was amplified that contained all of exon 3, exon 4, and the intervening sequences. In the DNA of mutant 944, this ≈570-bp fragment (the exact size is unknown because the intron has not been sequenced) was predicted to contain two *Bbv* I restriction sites, one at the putative site of mutation (85 bp from the 5′ end of the genomic fragment) and a second in exon 4 (41 bp from the 3′ end of the fragment), which served as a positive control for digestion by the enzyme. The digestion of amplified DNA (Fig. 3) from controls (lanes 9–11), eight other ASAL-deficient mutants (lanes 1–8), and the cloned normal genomic fragment SC-12 (lane 14) generated the expected ≈530-bp fragment. In contrast, the DNA of mutant 944 (lane 12) gave a smaller fragment of ≈450 bp, consistent with digestion at the normal site and also at the new mutant *Bbv* I site. The new *Bbv* I site created by the mutation was not detected in PCR products from 14 unrelated mutant or 10 normal alleles examined. The complete absence of the ≈530-bp digestion product in the amplified 944 genomic DNA indicated that this patient was homozygous for the R95C substitution, consistent with the consanguineous parentage. *Bbv* I digestion of amplified 944 cDNA from a second independent reverse transcription and amplification also demonstrated that 944 was homozygous for the mutation (data not shown).

Transient Expression Assay. Transient expression studies were done to confirm that the R95C mutation conferred a loss of ASAL activity. After construction of the mutant cDNA/p91023(B) vector, the entire mutant cDNA was sequenced in the plasmid to establish that the C → T transition at bp 283 was the only substitution. COS-1 cells were then transfected with the empty p91023(B) vector, and the vector with the mutant or normal cDNAs. Fig. 4*A* is a histogram of the ASAL enzymatic activity of cells transfected with the vector alone (mean ± SD of four dishes, 67 ± 11 cpm per μg per hr), with the cDNA containing the R95C mutation (84 ± 8 cpm per μg per hr; $n = 4$), or with the normal cDNA (1416 ± 169 cpm per μg per hr; $n = 4$). A Northern blot of total RNA from transfected cells probed with ASAL (Fig. 4*B*) and catalase (band 3) as an internal control (Fig. 4*C*) demonstrated that a large amount of ASAL mRNA was generated from both the control and mutant ASAL cDNAs. The major ASAL transcript (band 1) is larger than normal (band 2) because of the addition to the ASAL cDNA of stabilizing vector sequences (7). Immunoblot analysis demonstrated ASAL protein of the expected size in cells transfected with the normal cDNA (Fig. 4*D*). In transfectants of the mutant cDNA vector, or the empty vector, only a trace of ASAL protein was detectable on long exposure of the autoradiogram, consistent with the enzymatic activities found.

Alternative Splicing of Exon 2 in Human ASAL mRNA. During our examination of ASAL transcripts in normal and mutant cells, we observed that the PCR products from the 5′ third of the cDNA (between primers A and B in Fig. 1) of both control fibroblasts and mutant 944 had, in addition to the expected band of 513 bp, an additional prominent band of 318 bp. To determine the origin of this smaller band, we probed Southern blots of the amplified product with oligonucleotides

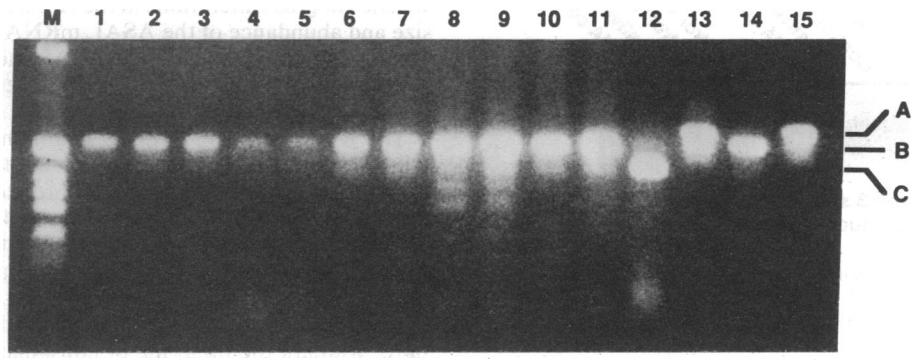

FIG. 3. *Bbv* I digestion of PCR products amplified from genomic DNA, including exons 3 and 4 and the intervening sequences. One-tenth of the PCR reaction mixture was digested with *Bbv* I and electrophoresed on a 0.7% agarose gel. The amplified DNA before digestion by *Bbv* I, from a cloned fragment of control genomic DNA (lane 15) and from mutant 944 (lane 13), is 570 bp (band A). *Bbv* I digestion of the 570-bp fragment generates a 530-bp band (band B) from eight ASAL mutants (lanes 1–8) other than strain 944, three control DNA samples (lanes 9–11), and a cloned genomic control fragment (lane 14). *Bbv* I digestion of the 570-bp band from mutant 944 (lane 12) produces only the 450-bp band (band C) predicted by cleavage at the predicted mutation. The molecular size markers are the 1-kb ladder from BRL (lane M).

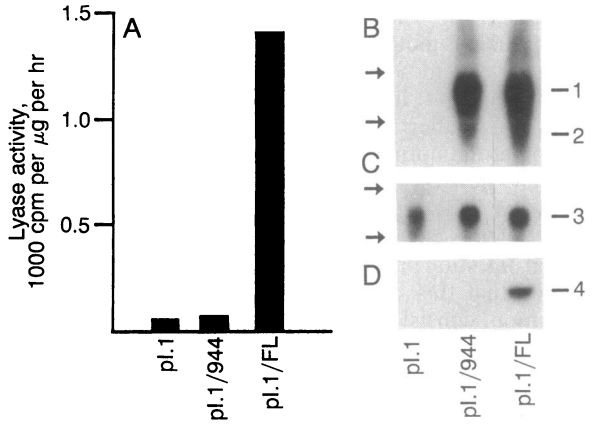

FIG. 4. Transient expression of control and mutant 944 ASAL cDNAs, analyzed by enzyme activity, RNA blots, and immunoblots. (*A*) Histogram of the ASAL enzyme activities of COS-1 cells transfected with the p91023(B) vector alone (pl.1), the vector with the cDNA containing the C-283 → T mutation (pl.1/944), and the vector with the normal cDNA (pl.1/FL). (*B*) Northern blot of total RNA from the three transfections probed with the ASAL cDNA and exposed for 18 hr. Arrows indicate the positions of the 18S and 28S RNAs. (*C*) The same Northern blot as in *B*, probed with catalase (band 3), as an internal control. (*D*) Immunoblot probed with ASAL antisera demonstrating the expected ≈50-kDa band of the monomer.

to specific exons and cloned and sequenced the 318-bp product. An oligonucleotide complementary to exon 2 (195 bp) hybridized to the 513-bp fragment of the PCR-amplified cDNA but not to the 318-bp band (Fig. 5). In contrast, an oligonucleotide complementary to exon 3 hybridized to both the 513- and 318-bp bands suggesting that the 318-bp band did not contain exon 2 (Fig. 5). The sequence of the short cDNA demonstrated that exon 2 had been spliced out of it (Fig. 6). We have detected this short transcript in every tissue and cell strain we have examined to date, including normal human liver, lymphoblasts, keratinocytes, and nine fibroblast cell strains (two normal and seven mutant). Notably, no other significant evidence for alternate splicing was observed in any of the PCR products of the ASAL cDNA in any cell or tissue examined.

To examine the relative abundance of the short and full-length PCR products obtained with oligonucleotide primers A and B, we used the quantitative PCR method of Chelly *et al.*

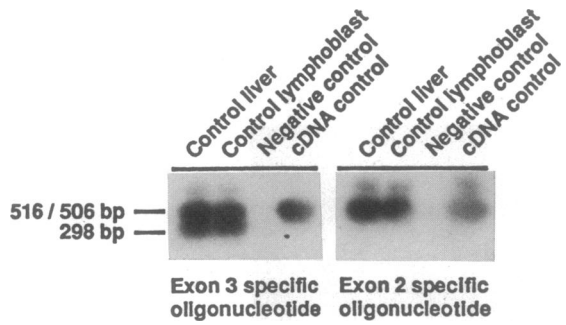

FIG. 5. Hybridization of total PCR products of the 5′ one-third of ASAL cDNA (primers A and B in Fig. 1) to exon-specific oligonucleotides. The negative control was RNA carried through the reaction but without the addition of reverse transcriptase. The positive control was the cloned cDNA amplified under the same conditions as the other samples. One-tenth of the PCR reaction mixture was electrophoresed through 0.7% agarose and transferred to Hybond. The blot was probed with an oligonucleotide complementary to exon 2, stripped, and reprobed with an oligonucleotide complementary to exon 3, as indicated. Molecular mass markers from the BRL 1-kb ladder are indicated on the left.

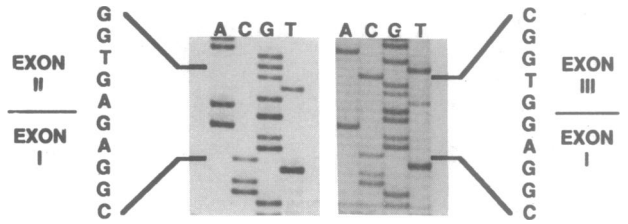

FIG. 6. Partial sequence of the ASAL cDNA from the 318-bp transcript. The sequence of the control cDNA on the left demonstrates the junction of exon 1 and exon 2. On the right, the sequence of the shortened transcript shows a perfect splice between exons 1 and 3, without any exon 2 sequence.

(14). Analysis of sequentially sampled PCR reactions in the linear phase of amplification demonstrated that the 300-bp band was 15% of the amplified cDNA made from control strain 2294, using oligo B to prime the reverse transcription. When oligo(dT) was the primer for reverse transcription, the 300-bp band from strains 2294 and 1254 was 25% and 14% of the cDNA, respectively (data not shown). The slopes of the amplification curves of the 513-bp and 318-bp bands were indistinguishable in all three experiments, providing no evidence for preferential amplification of the shorter cDNA.

The removal of the second exon from the transcript maintains the ASAL open reading frame. The putative protein translated from the short transcript would not have the 65 amino acids encoded by exon 2 (residues 5–69 inclusive) and would have a predicted mass of 44.6 kDa. Remarkably, Lys-51, which has been suggested to be the argininosuccinate binding site of the enzyme (22), is removed by this splicing event. The 318-bp fragment from mutant 944 (but not from normal cells) was found to have the codon 95 C → T transition, as determined by *Bbv* I restriction analysis of total PCR products (not shown).

DISCUSSION

The identification of a mutation in the ASAL structural gene in mutant 944 establishes that the great majority of patients with argininosuccinic aciduria, if not all, have mutations at this locus, since we have previously demonstrated that 28 ASAL-deficient cell strains, including 944, have mutations in the same gene. The single substitution changed codon 95 from arginine to cysteine (R95C). Restriction enzyme analysis demonstrated that the patient is homozygous for the mutation as predicted from the consanguinity of the parents.

The presence of a point mutation in the ASAL coding region is consistent with previous studies of this mutant: the absence of gross alterations in the ASAL gene (4), the normal size and abundance of the ASAL mRNA (4), the normal size but reduced abundance of the ASAL monomer (3), and the fact that this mutant is able to complement positively the ASAL deficiency of two other mutants (2).

The R95C mutation is not a common substitution at the ASAL locus; it was not observed in a total of 24 other alleles from five normal and eight other unrelated mutant cell lines. The low frequency of this mutation (0/14 in unrelated alleles) is consistent with the prediction (23) that for a rare recessive lethal disease, without a heterozygote advantage, each patient is likely to have unique mutations. True homozygotes will be uncommon, apart from patients like the one described here, who are the offspring of consanguineous matings.

The R95C ASAL mutation would be expected to be disruptive for two reasons. (*i*) The substitution replaces a bulky cationic residue, arginine, with a more compact neutral amino acid, cysteine. (*ii*) The mutation affects a highly conserved region of the protein. Yeast and human ASAL have a high degree of amino acid conservation (51% identity

or 71% if conservative changes are included) (10). The identical amino acids are not randomly distributed but are clustered in seven regions from 6 to 18 residues long. The R95C substitution occurs in a 13-amino acid stretch that is identical in both the yeast and human enzymes, suggesting that this region is critical for subunit interaction, catalysis, or stability and is intolerant of change. Our observation that the introduction of the R95C mutation into the wild-type cDNA causes a virtually complete loss of catalytic function demonstrates that the C-283 → T substitution is not a benign polymorphism. The ability of the R95C allele to participate in intragenic complementation suggests that a critical disruption of subunit interaction is not the primary effect of this mutation. Rather, the pronounced effect of the mutation on the abundance of the protein in the transient expression studies indicates that the mutation decreases the stability of the enzyme, consistent with its effect in fibroblasts (3).

Unexpectedly, we obtained evidence that the second exon of the ASAL gene is spliced out of the ASAL mRNA in a variety of tissues as well as cultured fibroblast and lymphoblast strains. No other evidence for differential splicing was ever observed. Our preliminary quantitative studies suggest that this widely distributed alternate transcript may constitute ≈15% of the ASAL mRNA. Although this finding requires corroboration by methods that are not PCR-dependent, the short transcript appears to be a consistent gene product and not simply an artifact reflecting the ability of the PCR to amplify the cDNAs of rare aberrant splice events. The identification of the R95C mutation in the short transcript of mutant 944, but not controls, establishes that this mRNA is transcribed from the ASAL structural gene, rather than related sequences present on chromosome 22 (5, 10). The only other evidence for heterogeneity in ASAL transcripts is variation in the 3′ polyadenylylation site in cDNA clones from liver (5).

The biological significance of the transcript from which exon 2 has been removed is unclear. A distinct possibility is that it has no function. It is of interest that alternate splicing of the second exon occurs in the transcripts of two other enzymes of nitrogen waste metabolism, ornithine aminotransferase (24), and argininosuccinate synthetase (25), although in both of these genes, exon 2 is noncoding. On the other hand, differential splicing is a well-established mechanism for generating protein isoforms that may have different functions (26, 27). The alternate splicing of ASAL that occurs in the translated sequence and does not change the reading frame is of particular significance because the spliced exon contains Lys-51, which participates in the binding of argininosuccinate (22). Thus, an ASAL homotetramer composed of exon-2-less subunits may not have catalytic activity toward argininosuccinate. These subunits, if they are ever present in significant quantity, might be capable of associating with the 50-kDa monomers to prevent formation of the enzymatically active homotetramer, thereby regulating ASAL activity. However, previous immunoblot studies of normal human fibroblasts (3) did not demonstrate an ASAL-related polypeptide of the size (44.6 kDa) predicted by the cDNA without exon 2, although such a protein may not be recognized by ASAL antisera.

A third possibility is that the short transcript may have a physiological role unrelated to the enzymatic activity of ASAL. Unexpected diversity of function for the protein product of the ASAL locus has been demonstrated in other species. Piatagorsky *et al.* (28) demonstrated that the δ-crystallins in chicken and duck are argininosuccinate lyase; however, no evidence for alternate splicing of the ASAL transcript has been presented in these or other species. We have established that alternate splicing occurs at this locus. Although the function, if any, of the shortened transcript remains to be defined, it appears that the ASAL locus produces more than a single gene product.

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