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REVIEW ARTICLE

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Molecular basis of phenotypic variation in patients with argininemia

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Abstract Argininemia is an autosomal recessive disorder caused by a deficiency in the liver-type arginase enzyme. Clinical manifestations include progressive spastic diplegia and mental retardation. While the quality of life can severely deteriorate in most such patients, some do show remarkable improvement in neurological symptoms while on controlled diets. We examined the thesis that differences in clinical responses to dietary treatment are based on molecular heterogeneity in mutant arginase alleles. Genomic DNAs from 11 patients with argininemia were examined using the polymerase chain reaction, cloning, and sequencing. Nine mutations representing 21/22 mutant alleles were identified in 11 patients with argininemia, and four of these mutations were expressed in vitro to determine the severity of enzymatic defects. We found that these mutations accounted for 64% of the mutant alleles in our patients. Based on findings in vitro expression tests, the mutations can be considered either severe or moderate. Patients with at least one moderate mutant allele responded well to dietary treatment; concentrations of plasma arginine were controlled within 300 μ*M*. In contrast, patients with two severely mutated alleles did not respond to dietary treatment and plasma arginine was over 400 μ*M*. Argininemia is heterogeneous at the molecular level. The degree of clinical improvement during dietary treatment is reflected in the concentration of arginine in plasma, as a measure of metabolic control. Plasma arginine levels during treatment is reflected in the concentration of arginine in plasma, as a measure of metabolic control. Plasma arginine levels during treatment correlated with types of molecular defects in the arginase genes.

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Introduction

Argininemia (McKusick 20780) is an autosomal recessive disorder caused by a deficiency in the liver-type arginase enzyme (EC 3.5.3.1). Clinical manifestations of argininemia are progressive loss of psychomotor functions, spastic tetraplegia (more severe in lower limbs), hyperactivity of deep-tendon reflexes, seizures, and failure to thrive (Brusilow and Horwich 1989).

Clinical features of urea-cycle diseases other than argininemia are generally related to multiple episodes of hpyerammonemia. However, the concentration of ammonium in the plasma is only slightly elevated in most patients with argininemia. Neurological symptoms such as a marked degree of spastic diplegia have not been described as a regular feature in hyperammonemias associated with other defects in the urea cycle. These neurological manifestations can be progressive even when concentrations of plasma ammonium are within normal levels. This means that hyperammonemia is not the sole factor related to neurological damage in these patients. Elevated concentrations of plasma arginine or its five catabolites seem to be directly linked to the neurological symptoms seen in these patients (Marescau et al. 1990).

Human liver-type arginase catalyzes the hydrolysis of arginine at the last step in urea synthesis, and urea and ornithine are produced. This enzyme consists of 322 amino acid residues with a molecular mass of 34,732 daltons (Haraguchi et al. 1987). The arginase gene is 11.5 kb long, includes eight exons (Takiguchi et al. 1988), and has been assigned to chromosome band 6q23 (Sparkes et al. 1986).

We searched for possible correlations between genotypes and differences in clinical responses to dietary treatment in 11 patients with argininemia. Differences in the degree of clinical improvement during dietary treatment may relate to the nature of the mutations.

Materials and methods

Patients

Clinical manifestations in the 11 patients studied before and during dietary treatment are summarized in Table 1. These patients who were followed from 1 to 21 years included three related Puerto Ricans (patients 1–3; Snyderman et al. 1977, 1979), two unrelated white French Canadians (patients 4–5; Lambert et al. 1991), one Pakistani (patient 6; Brockstedt et al. 1990), one African American (patient 7; Scheuerle et al. 1993), and four unrelated Japanese (patients 8–11; Haraguchi et al. 1990; Uchino et al. 1992). The enzymatic activity of arginase proteins in erythrocytes from all patients was 0%–5% of that of normal arginase protein.

All patients were prescribed the dietary treatment proposed by Snyderman et al. (1977); while patients 1 and 2 showed clinical improvement, this diet provided a limited amount of protein in the form of essential amino acids and arginine intake was limited to less than 400 mg a day. Sodium benzoate was also given to patients 4, 5, 10, and 11 and patient 7 was given sodium phenylbutyrate. During this treatment, plasma ammonium was almost completely controlled in all but patient 10.

A good response to dietary treatment was observed in patients 1–4. The intelligence quotients (IQs) of patients 1, 2, and 4 increased more than 20 points and the severity of spastic diplegia or hyperactivity of deep-tendon reflexes was markedly reduced. Patient 3 who reached age 18 years in 1994 developed normally and neurological symptoms were nil. Patients 5–7 showed an intermediate response to treatment. Their IQs increased about 20 points and although there was a slow residual impairment. Patients 8–11 responded poorly. Neurological conditions of patients 8 and 10 deteriorated rapidly and mental retardation was severe. Neurological findings in patients 9 and 11 were severe even before treatment was begun and neurological factors remained arrested even from early infancy.

Plasma arginine was controlled within 300 μ*M* in patients 1–7 and was elevated to over 400 μ*M* in patients 8–11.

Methods

The methods used in this study have been described elsewhere (Haraguchi et al. 1990; Uchino et al. 1992). Mutagenesis for the missense mutation of I11T (see Results for abbreviations) was done according to the method of Matsuura et al. (1994), using the polymerase chain reaction (Saiki et al. 1988). The mutant complementary DNA with I11T was confirmed to have a normal sequence, except for its own mutation, as determined by the dideoxynucleotide chain reaction termination method (Sanger et al. 1977) (data not shown).

Protein characterization

Normal and mutant human liver arginase proteins were expressed in *Escherichia coli* strain JM83, according to Ikemoto et al. (1990). The proteins were recovered from the *E. coli* by sonication and separated on SDS–10% polyacrylamide gels. The immunoblot analysis was according to Towbin et al. (1979), using the anti-human liver arginase antibody to detect human arginase. Proteins were determined by the method of Lowry et al. (1951), with BSA as a standard. The activity of arginase was measured by the method of Schimke (1961).

Results

Identification of mutation and expression of mutant arginase protein

Molecular analyses of arginase mutations in the four Japanese patients have been reported elsewhere (Haraguchi et al. 1990; Uchino et al. 1992). Previously identified mutations included the nonsense mutation of W122X, missense mutation of G235R, and three deletions (77del1A, 262–265del1AAGA, and 842del1C), which led

Table 1 Clinical manifestations of 11 patients with argininemia (Tx, Treatment; IQ, intelligence quotient; DQ developmental quotient; mo, month(s) old; do, days old; yo, years old; WISC, Wechsler Intelligence Scale for Children)

Patient	Consanguinity	Ethnicity	Onset of Food intolerance (mo)	Age of Onset[a] (mo)	Age of Tx (mo)	Plasma arginine (μ*M*)		Mental evaluation (tested by WISC)		Motor changes during Tx
						Before Tx	During Tx	Before Tx	During Tx	
1	–	Puerto Rican	6	< 30	43	538– 570	250–300	IQ 44	IQ 73	Well improved
2	–	Pureto Rican	3	3	48	508– 540	250–300	DQ 61	IQ 83	Well improved
3	–	Puerto Rican	–	–	Birth	228[b]	216–270	Normal	Normal	Normal
4	+	French Canadian	4	15	90	895	89–241	IQ 52	IQ 74	Well improved
5	–	French Canadian	6	10	31	505	43–105	Retarded	Improved	Slowly improved
6	+	Pakistani	–	30	46	907	50–300	Retarded	Improved	Improved
7	–	African American	108	60	108	304– 334	104–311	Retarded	IQ 62	Partly improved
8	–	Japanese	–	< 9	9	683	383	DQ 85	IQ < 10	Deteriorated
9	+	Japanese	< 21 do	< 3	25 yo	778	686	IQ < 10	IQ < 10	Unimproved
10	+	Japanese	> 43	13	55	605	350–600	IQ 80	IQ < 10	Deteriorated
11	–	Japanese	18 do	< 1	50	341[c]–1049	432–757	IQ < 10	IQ < 10	Unimproved

[a] Onset age of neurological symptoms: a tip-toe gait, delay in development, mental retardation

[b] Patient 3 was diagnosed at birth. The concentration of plasma arginine before treatment (228 μ*M*) was about ten times higher than that of normal plasma arginine at 18 h of age (Snyderman et al. 1979)

[c] This plasma arginine (341 μ*M*) was measured 4 days after introducing intravenous drip infusion and fasting. Plasma arginine increased to 1049 μ*M* after reintroduction of protein feeding

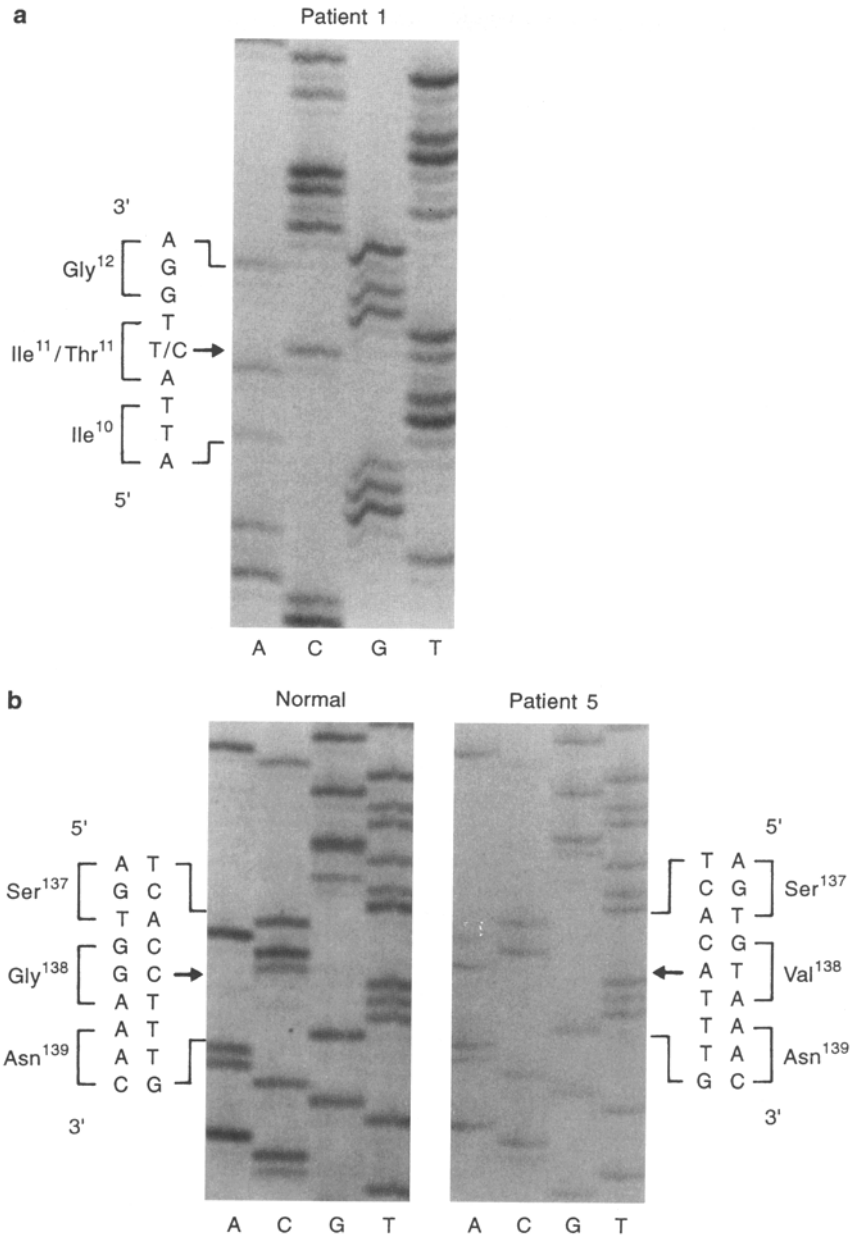
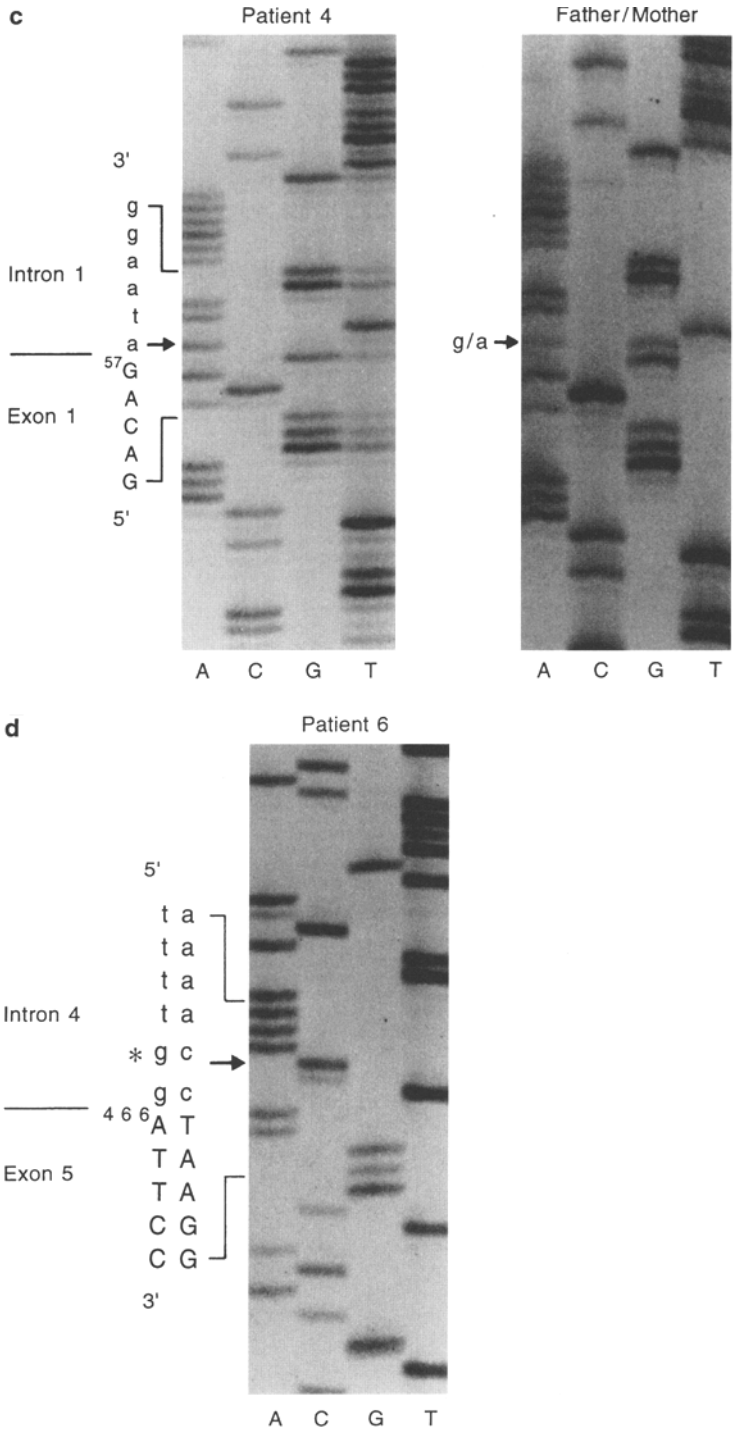


Fig. 1 a–d Determination of four novel mutations by the dideoxynucleotide chain termination method (Sanger et al. 1977). Nucleotide number and amino acid residues are indicated, beginning with the first residue of ATG and the initiator methionine of arginase complementary DNA, respectively. **a** The *arrow* indicates a transition from thymine (T) to cytosine (C) at nucleotide position 32 in the sense strand nucleotide sequence in the allele from patient 1, leading to a missense mutation from isoleucine (Ile) to threonine (Thr) at amino acid residue 11 (abbreviated as I11T). The missense mutation of I11T was also found in alleles from patients 2 and 3. **b** The *arrow* indicates adenine (A) at nucleotide position 413 in the allele from patient 5 and C at the same position in an allele from a normal person. This substitution of C to A in the anti-sense strand nucleotide sequence results in the transversion of guanine (G) to T in the sense strand in the allele from patient 5, leading to the missense mutation from glycine (Gly) to valine (Val) at amino acid residue 138 (abbreviated as G138V). **c** The transition of G to A just one base after G at nucleotide position 57 in a donor site of intron 1 (57 + 1G→A) was detected in the allele from patient 4. Patient 4 is homozygous for the mutant allele and her parents are heterozygous for the mutant allele and the wild-type allele. Patient 5 had the same splicing mutation in one allele. **d** The substitution of T to C was found at two bases before T at nucleotide position 466 in an acceptor site of intron 4 of the anti-sense strand in the allele from patient 6, leading to the transition of A to G in the sense strand (abbreviated as 466 – 2A→G)

to premature termination at codons 31, 132, and 289, respectively.

In the present study, genes from seven non-Japanese patients with argininemia were examined and four novel mutations were detected (Fig. 1). A base substitution of T to C at nucleotide position 32 in exon 1 resulted in replacement of isoleucine for threonine at codon 11 (abbreviated as I11T). Another base substitution of G to T at nucleotide position 413 in exon 4 led to replacement of glycine for valine at codon 138 (abbreviated as G138V).

Fig. 1 c–d



The other two mutations were base changes at splicing junctions, i.e., subsitution of G to A at a donor site of intron 1 (abbreviated as 57 + 1G→A) and substitution of A to G at an acceptor site of intron 4 (abbreviated as 466 – 2A→G): both substitutions violate the GT/AG rule (Shapiro and Sneapathy 1987). As no patient was subjected to liver biospy, mRNAs were not available.

Genotypes of the 11 patients are given in Table 2. The missense mutation of G235R was detected in one allele from each of the three related Puerto Ricans (patients 1–3), the Pakistani (patient 6), the African American (patient 7), and one Japanese (patient 8). Another unrelated Japanese (patient 9) was homozygous for this mutation. The French Canadian (patient 4) was homozygous for the mutation of 57 + 1G→A. This mutation was found in one allele from another unrelated French Canadian (patient 5). The missense mutation of G138V found in the other allele from patient 5 was also detected in another Japanese patient (data not shown).

The mutant arginase protein with I11T was expressed in *Escherichia coli,* and enzyme activity and electro-

Table 2 Relation of genotype to clinical response during dietary treatment (A question mark (?) denotes an undetermined mutation. Mutations other than those previously noted in the other allele from patient 7 have not been detected.)

Patient	Genotype	Clinical response
1	I11T/G235R	Good
2	I11T/G235R	
3	I11T/G235R	
4	57 + 1G → A/57 + 1G → A	
5	57 + 1 G → A/G138V	
6	466 – 2A →G/G235 R	
7	? /G235R	
8	W122X/G235R	Poor
9	G235R/G235R	
10	842de1C/842de1C	
11	77de1A/262–265de1AAGA	

phoretic mobility were analyzed. The mutant protein with I11T was located at the same position as wild-type arginase protein (Fig. 2). The potential of the mutant protein with I11T to convert arginine to urea was 10%–18% (the mean: 12%), of that of normal arginase protein. The mutant arginase proteins previously analyzed (W122X, G235R, 842de1C; Uchino et al. 1992) had less than 1% of the control activity in vitro (Table 3). These four mutant arginase alleles accounted for 64% of all of the mutant alleles in these 11 patients with argininemia.

Relation of genotype to clinical response during dietary treatment

Correlations between genotypes and the clinical response to dietary treatment are summarized in Table 2.

Patients 1–3 are compound heterozygotes for the mutant allele with I11T and the mutant allele with G235R. Results of the expression test (Table 3) and the nature of the clinical manifestations (Table 1) indicated that the mutant allele with I11T facilitated remarkable clinical improvement in these patients on dietary treatment for argininemia. The degree of the metabolic defect induced by the missense mutation of I11T is considered to be "moderate".

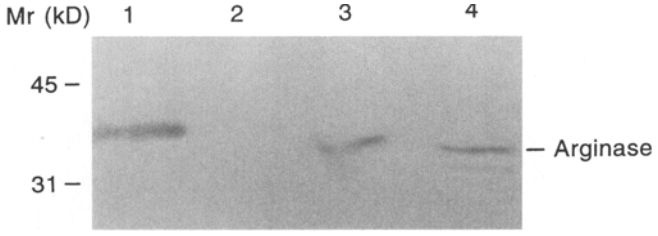


Fig. 2 Immunoblot analyses for normal arginase protein and mutant arginase protein with I11T expressed in *Escherichia coli*. Purified human arginase *(lane 1)*, proteins from *E. coli* culture expressing vector plasmid *(lane 2)*, normal recombinant arginase expressed in *E. coli (lane 3)*, and mutant protein with I11T expressed in *E. coli (lane 4)* were analyzed. For each condition 10 μl of the sonicated supernatant (10 μg for each protein), 0.2 μg of purified human arginase, and a molecular marker were subjected to electrophoresis on 10%-SDS gels. The proteins were stained with anti-human arginase antibody. Mutant protein with I11T *(lane 4)* was detected at the same position as normal arginase

Table 3 Biochemical characterization of four mutant arginase proteins (A plus sign (+) denotes presence and a minus sign (–) denotes absence.)

Mutant protein	Immunoblotting	Activity (% of control)
I11T	+	10–18
G235R	+	< 1
W122X	–	< 1
842de1C	+	< 1

Patient 8 is a compound heterozygote for W122X/G235R and patients 9 and 10 are homozygous for the allele with G235R and for the allele with 842de1C, respectively. Based on results of expression tests (Table 3) and clinical manifestations (Table 1), the mutant alleles with G235R, W122X, and 842de1C correlated with neurological deterioration in patients 8–10. The degree of metabolic defect induced by these mutations was considered to be "severe", as was that associated with the two deletions (77de1A and 262–5de1AAGA) in patient 11.

Discussion

The clinical phenotype of argininemia is relatively heterogeneous. In many cases, episodes of vomiting or irritability are present throughout infancy and a tip-toe gait or delay in development in toddlers can appear gradually. These neurological manifestations are progressive and the quality of life deteriorates. However, as some patients show a good response to dietary treatment, we searched for a possible relationship between the clinical response to dietary treatment and gene mutations. Based on our findings, the variation in clinical responses of these patients can be explained in terms of the nature of the mutation(s) in the arginase gene.

Lower concentrations of plasma arginine in these patients were linked to the degree of improvement in neurological factors (Tables 1, 2).

The poor response to treatment of some patients may be due to late initiation of treatment once deterioration becomes evident, poor compliance, and ingestion of a low protein diet instead of a mixture of essential amino acids. However, the relation between mutation and the degree of residual enzyme activity does not support this notion.

In cases of phenylketonuria (PKU; Okano et al. 1991), patients who are heterozygous for a mild and severe mutant allele show only mild manifestations of the disease when the predicted phenylalanine hydroxylase activity is at least 15%. In addition, patients who are homozygous or compound heterozygous for severe mutant alleles manifest features of classical PKU. In argininemia, patients

1–3 were compound heterozygous for moderate and severe mutant alleles, and showed a good response to dietary treatment. Patients 8–10 had two severe mutant alleles and showed a poor response to the treatment. Our results suggest that patients who carry at least one moderate mutant allele will likely experience significant improvement of clinical manifestations while on the proper diet. Such patients accounted for approximately 30% of the subjects in our study.

Since patient 3 had been treated from birth and developed normally, greater improvement in patients 1, 2, and 4–7 may have been forthcoming if dietary treatment had been initiated earlier. However, excluding patients 3 and 9, the average period from onset of the disease to diagnosis was about 35 months. Like most diseases, early diagnosis and early treatment reap greater benefits. On the other hand, patients with two severe mutant alleles may do poorly despite rigorous dietary control and such patients may be candidates for gene therapy or liver transplantation.

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