Clinical Biochemistry 50 (2017) 686–689

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Identification of three novel mutations in fourteen patients with citrullinemia type 1

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ARTICLE INFO

Article history:
Received 29 September 2016
Received in revised form 15 January 2017
Accepted 24 January 2017
Available online 27 January 2017

ABSTRACT

Objectives: Citrullinemia type 1 (CTLN1) is an autosomal recessive genetic disorder caused by mutations in the *argininosuccinate synthetase 1* (*ASS1*) gene, which encodes for the argininosuccinate synthetase enzyme. Here, we report genetic and clinical characterizations of 14 patients with citrullinemia type 1.

Design & methods: The study group consisted of 14 patients (4 females, 10 males) diagnosed with citrullinemia type 1 from three centers in Turkey. Age of onset, clinical presentation, initial citrulline and ammonia levels, family history and molecular genetic analysis were retrospectively evaluated.

Results: The mean age of the cohort and the mean age at the time of diagnosis were 48.3 ± 36.5 months (min: 12 days, max: 10 years) and 11.6 ± 26.2 months (min: 3 days, max: 8 years), respectively. In four patients, a homozygous p.Gly390Arg pathogenic variant was detected. All patients homozygous for p.Gly390Arg were diagnosed during the newborn period with the clinical presentation of classical citrullinemia. In each two patients, homozygous p.Arg86His, c.773 + 49C>T and p.Gly362Val pathogenic variants were detected. Clinical presentation was compatible with the mild form of the disease in patients homozygous for c.773 + 49C>T and for Gly362Val. Novel compound heterozygous genotypes (p.Ala164Pro/p.Gly390Arg; p.Leu290Pro/p.Gly390Arg; p.Thr389Pro/p.Gly390Arg) were identified in five patients. Of these, three siblings with CTLN1 were diagnosed with the compound heterozygous genotype p.Ala164Pro/p.Gly390Arg at the age of 4 days, 5 days and 2 years, respectively. The other two patients with novel compound heterozygous genotypes (p.Leu290Pro/p.Gly390Arg; p.Thr389Pro/p.Gly390Arg) were identified in the first month of life as neonatal onset form and were born to non-consanguineous parents.

Conclusion: In our study, consistent with the literature, a correlation was found between homozygous p.Gly390Arg mutation and the classic neonatal onset form. Mild citrullinemia was detected in patients with c.773 + 49C>T or p.Gly362Val pathogenic variants. This study adds to our understanding of the molecular genetic background of patients with CTLN1, and allows to infer on the correlation between the genotype and phenotype of the disease.

1. Introduction

Citrullinemia type 1 (CTLN1) (MIM# 215700) is an autosomal recessive genetic disorder caused by mutations in the *argininosuccinate synthetase 1* (*ASS1*) gene, which encodes the argininosuccinate synthetase enzyme [1]. In most patients, disease onset occurs during the early neonatal period with hyperammonemia and neurologic manifestations. In some patients, symptomatic hyperammonemia develops during childhood, adulthood or during and shortly after pregnancy, referred to as the late-onset form [2,3]. Additionally, symptomatic patients with elevated plasma citrulline levels are termed as mild citrullinemia cases [4–6].

Argininosuccinate synthetase 1 (ASS1) gene is located on chromosome 9q34.1 and consists of 16 exons (of which only 14 are coding with start of translation on exon 3) encoding 412 amino acids. The pathogenic variants p.Gly390Arg, p.Arg363Trp, and p.Gly14Ser have been identified with the severe phenotype [4,5,7–9]. In contrast, p.Tyr190Asp, p.Trp179Arg, p.Val263Met and p.Val269Met were already described in patients with late-onset form of the disease [6,9]. Although certain pathogenic variants are associated with specific clinical manifestations, there is still no exact correlation between the genotype and

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http://dx.doi.org/10.1016/j.clinbiochem.2017.01.011
0009-9120/

phenotype. Herein, we present 14 citrullinemia patients (9 of them with the neonatal form, 5 of them with the late-onset form) with 8 different genotypes comprising 8 different pathogenic variants, including the novel variants p.Ala164Pro, p.Leu290Pro and p.Thr389Pro. We aimed to evaluate the genotype-phenotype correlation in our patient cohort and, hereby, to extend our knowledge of citrullinemia type I phenotypes.

2. Methods

2.1. Patients

Fourteen patients from 12 unrelated families were included in the study from three pediatric metabolic units in Turkey (Fig. 1). All patients were diagnosed based on clinical findings and/or elevated plasma citrulline (reference range: 10–50 μmol/l) levels. Age of onset, clinical presentation, initial plasma citrulline, arginine and blood ammonia levels, family history and molecular genetic analysis were retrospectively evaluated. Residual enzyme activity and effect of the mutation on protein structure and function were not analyzed.

The study was approved by the Ethics Committee of Dokuz Eylul University Hospital (Ethics Committee approval no.: 2016/23-04).

2.2. Molecular genetic analysis

Based on the suggestive clinical and biochemical situation, we performed straightforward mutation analysis at the *ASS1* locus as described [5]. In brief, DNA from EDTA blood was used for sequencing of all *ASS1* coding exons (exons 3 to 16) as well as the close adjacent intronic sequences. Primer sequences were as in [5] with only little adaptation e.g. to cover the known change c.773 + 49C>T. Found mutations were confirmed in the parents of the patient whenever respective samples were available. Mutation analysis was done after parental consent into the genetic studies was obtained.

Reference sequence AY034076.1 from GenBank was used with "+ 1" corresponding to the A of the ATG translation initiation codon for cDNA numbering.

3. Results

We evaluated 14 patients (4 females, 10 males) with citrullinemia type 1. The mean age of the group and the mean age at the time of diagnosis were 48.3 ± 36.5 months (min: 12 days, max: 10 years) and 11.6 ± 26.2 months (min: 3 days, max: 8 years), respectively.

Three patients (P10a, P10b, P10c) were siblings. Ten patients had a consanguineous background. Neonatal, late-onset and mild citrullinemia were detected in 9, 2 and 3 patients, respectively. Out of 9 patients with neonatal onset citrullinemia, 4 died in the first month of life due to hyperammonemic encephalopathy. In 6 patients, further relatives diagnosed with citrullinemia were identified following the diagnosis in the index-patient. Among these, 4 had the neonatal form (Table 1). Furthermore, the initial plasma citrulline levels in all patients with the neonatal form and mild citrullinemia were above 1200 μmol/l and below 600 μmol/l, respectively.

In all patients, pathogenic variants of the *ASS1* gene were identified on both alleles and were confirmed, whenever possible, in genomic DNA of the parents. Patients were affected by eight different genotypes and in total eight different pathogenic variants were detected. The most common variant was p.Gly390Arg, which was identified on at least one allele in 8 of 12 independent patients (Table 1). In all patients (P1, P2, P3, P4) homozygous for p.Gly390Arg, the clinical manifestation was consistent with the neonatal form of citrullinemia. Two of them (P1, P4) were admitted with encephalopathy and died on the 12th and 17th day of life, respectively. Two patients (P5, P6) homozygous for p.Gly362Val presented with less pronounced elevation of plasma citrulline. Both patients had parental consanguinity and were admitted to the clinic without specific symptoms at the age of 15 days and 2 years, respectively. Hyperammonemia was never documented and no clinical findings were observed. Additionally, in the family history of P5, other

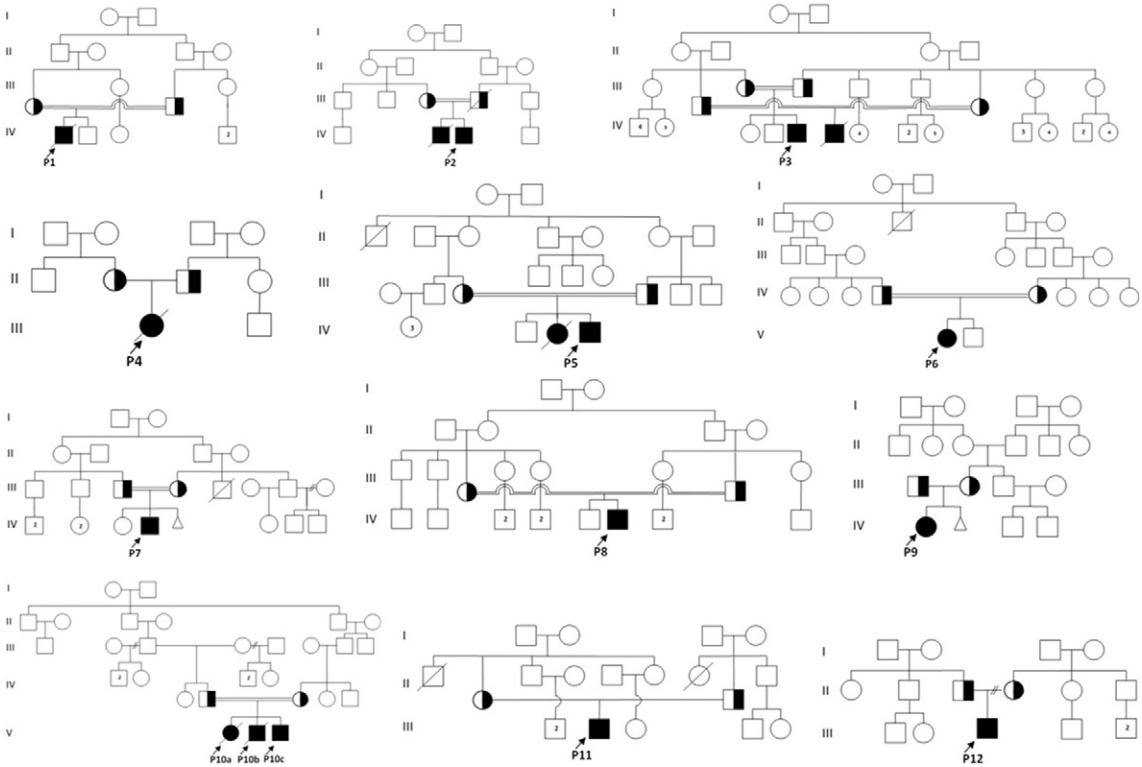

Fig. 1. The pedigree of patients with citrullinemia type 1.