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Brief communication

Clinical and mutational investigations of tyrosinemia type II in Northern Tunisia: Identification and structural characterization of two novel TAT mutations

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Abstract

Tyrosinemia type II or Richner–Hanhart Syndrome (RHS) is an autosomal recessive disorder characterized by keratitis, palmoplantar keratosis, mental retardation, and elevated blood tyrosine levels. The disease is due to a deficiency of hepatic cytosolic tyrosine aminotransferase (TATc), an enzyme involved in the tyrosine catabolic pathway. Because of the high rate of consanguinity this disorder seems to be relatively common among the Arab and Mediterranean populations. RHS is characterized by inter and intrafamilial phenotypic variability. A large spectrum of mutations within *TATc* gene has been shown to be responsible for RHS. In the present study, we report the clinical features and the molecular investigation of RHS in three unrelated consanguineous Tunisian families including 7 patients with confirmed biochemical diagnosis of tyrosinemia type II. Mutation analyses were performed and two novel missense mutations were identified (C151Y) and (L273P) within exon 5 and exon 8, respectively. The 3D-structural characterization of these mutations provides evidence of defective folding of the mutant proteins, and likely alteration of the enzymatic activity. Phenotype variability was observed even among individuals sharing the same pathogenic mutation.

Keywords: Tyrosinemia type II; Amino acidemias; Structural models; Mutation screening; Haplotype analysis; Clinical heterogeneity

Introduction

Tyrosinemia type II, (MIM 276600), also referred to as Richner–Hanhart Syndrome (RHS), is a recessive autosomal genodermatosis described independently by Richner [1] and Hanhart [2] as a distinct clinical syndrome. In 1973, Goldsmith et al. [3] pointed out the similarity between the tyrosinemia type II and the oculo-cutaneous syndrome. Because of the high rate of consanguinity, this disorder seems to be relatively common among the Arab and Mediterranean populations [4,5,8]. However, no data is available on the disease prevalence. The disorder shows a wide range of clinical presentations affecting skin (in 80% of reported cases) and eye (in 75% of

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reported cases), associated to mental retardation (in over of 60% of reported cases) [6]. Skin manifestations of the disease usually begin after the first year of life, but may begin as early as one month of age and consist of progressive painful, non-pruritic, and hyperkeratotic plaques on soles and palms, often associated with hyperhidrosis [7]. The eye symptoms typically occur before skin lesions develop, include photophobia, redness lacrimia, and pain. The mental retardation is variably associated with RHS and ranges from severe retardation, associated with microcephaly and other organ abnormalities in some cases, to slight decrease in intelligence. There is no relationship between age of diagnosis and mental retardation. However, the degree of mental retardation may be related to the level of plasma tyrosine [4]. The presenting complaints and the disease manifestations may be confined exclusively to the skin [9,10] or to the eyes [11,12]. RHS is an inborn error of metabolism due to a deficiency in hepatic cytosolic aminotransferase (TATc; L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5), an enzyme that catalyses the transamination reaction converting tyrosine to *p*-hydroxyphenylpyruvate. The absence or the significant diminution of the activity of TAT results in tyrosinemia, tyrosinuria, and increased level of urinary tyrosine metabolites, namely *p*-hydroxyphenylacetate, *p*-hydroxyphenylpyruvate, *p*-hydroxyphenyllactate, and *N*-acetyl tyrosine [13]. An elevated plasma and/or urine tyrosine level accompanying typical clinical signs and symptoms is generally sufficient for diagnosis [12]. Several studies showed that tyrosine restricted and phenylalanine restricted diet provide rapid resolution of oculocutaneous signs and symptoms in RHS and may prevent mental retardation [12,14].

The *TAT* coding gene has been assigned to human chromosome 16(q22.1–q22.3) [15–17]. A large spectrum of mutations has been shown to be responsible for RHS [18,5]. The R433Q and R433W substitutions identified in the *TAT* gene were shown to cause, essentially, a complete loss of TAT enzymatic activity when expressed as recombinant proteins in *Escherichia coli* [5]. Two polymorphisms have also been identified (P15S, S103S) [5]. Despite RHS cases being most frequently reported among Arab and Mediterranean populations, little is currently known about the molecular genetics of the disease in geographic areas outside the United States and Europe. In the present study, we report the clinical features and the molecular investigation of RHS in three unrelated consanguineous Tunisian families including patients with confirmed biochemical diagnosis of tyrosinemia type II. Two novel homozygous missense mutations C151Y and L273P in the *TAT* gene have been identified. The 3D-structural characterization of these mutations provides evidence of defective folding of the mutant proteins, and likely alteration of the enzymatic activity.

Materials and methods

Patients

Three unrelated consanguineous families (family 1, 2 and 3), with a total of 7 patients and 21 unaffected family members, were investigated. All patients were interviewed with a questionnaire including a pedigree drawing to identify possible cases of RHS among their relatives. Available members underwent ocular and dermatological examination. The clinical and biochemical data of tyrosinemia type II patients were reviewed retrospectively. All patients were diagnosed on the basis of clinical symptoms of RHS, the diagnosis of RHS was confirmed biochemically with high tyrosine levels in plasma and/or urine.

Most patients are offsprings of consanguineous intermarriage between first-degree cousins. No relationship between parents of patients VI-1 and VI-2 for family RHS-2 could be established, although these parents had the same surname and originated from the same city. All samples were collected after informed consent from all the individuals participating in this study or their parents for affected children.

Histological examination

Skin biopsies were performed for at least one patient in each explored family. Specimens were taken from the margins of the extending lesions of soles. The samples were fixed in 10% formaldehyde solution and paraffin embedded. Four to five micrometer sections were processed and stained with H–E (hematoxylin–eosin).

Genotyping and mutation analysis

Genomic DNA was isolated from whole blood leucocytes from healthy subjects, patients and their parents when possible, according to standard procedures. Haplotype and mutational analysis were performed as described in Charfeddine et al. [19]. Three polymorphic microsatellites markers encompassing the *TAT* locus: D16S515–D16S3067–D16S3026 were selected according to the Genethon mapping panel and the genetic maps available on NCBI and Ensembl genome browsers (www.ncbi.nlm.nih.gov) and (www.ensembl.org). Microsatellite markers were selected based on their percentage of heterozygosity (>70%) and closeness to the *TAT* gene. Intronic oligonucleotide primers flanking the *TAT* coding exons were designed and mutation screening was performed by direct sequencing of corresponding PCR products. Primer sequences are available upon request. Putative mutations were confirmed by sequencing of both strands. Specific PCR was used to rule out their occurrence in at least 100 control chromosomes.

Structural analysis

Since no three-dimensional structure of TAT is available, we predicted a structure by comparative modelling based on homologous proteins and proteins with similar sequence and related function. A BLAST [20] search of the PDB database [21] to identify proteins with know three-dimensional structure and significant sequence similarity to TAT resulted in 14 possible structural templates for modelling. Of these, we used the structures of the 11 proteins with known aminotransferase activity. We then prepared a multiple sequence alignment between the TAT sequence, the template sequences, and other similar sequences found in the non-redundant sequence data base with the program T-COFFEE [22]. Structural models of the wild-type protein and the mutants were then constructed with the program MODELLER [23]. All sequence alignment and modelling was performed in a semi-automated way using a home-written computer program (BisKit, http://sourceforge.net/projects/biskit). The structures were displayed and analysed using VMD [24].

Results

Clinical data

Family RHS-1

In 1991, patients V-1, V-2 and V-3 belonging to the large consanguineous family RHS-1 from Northern Tunisia presented to the Dermatology Department for an evaluation