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Experimental research

Clinical and molecular analysis of isovaleric acidemia patients in the United Arab Emirates reveals remarkable phenotypes and four novel mutations in the *IVD* gene

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ABSTRACT

Isovaleric acidemia (IVA) is an autosomal recessive inborn error of leucine metabolism caused by deficiency of mitochondrial isovaleryl-CoA dehydrogenase (IVD). Accumulation of isovaleryl-CoA derivatives to toxic levels results in clinical symptoms of the disease. Here, we investigate the clinical and molecular features of Arab patients with IVA. Patients from five unrelated families were evaluated clinically and for defects in the *IVD* gene. Four novel mutations (p.F382fs, p.R392H, p.R395Q and p.E408K) have been identified with p.R395Q occurring in two families. In addition, molecular modeling of the identified missense mutations predicted their damaging effects on the protein and computational analysis of the p.F382fs mutation predicted the disruption of a 3′ splicing site resulting in inactive or unstable gene product. Furthermore, we found an unusual case of a 17 years old female homozygous for the p.R392H mutation with no clinical symptoms. Our results illustrate a heterogeneous mutation spectrum and clinical presentation in the relatively small UAE population.

1. Introduction

Isovaleric acidemia (IVA, OMIM ID: #243500) is an autosomal recessive inborn error of leucine metabolism that can cause significant morbidity and mortality. It is caused by a deficiency in isovaleryl-CoA dehydrogenase (IVD; EC ID: 1.3.99.10), a mitochondrial matrix enzyme that catalyzes the oxidation of isovaleryl-CoA to 3-methylcrotonyl-CoA in the leucine degradation pathway [1–3]. Deficiency of this enzyme leads to an accumulation of isovaleryl-CoA and its toxic metabolites. Patients with IVA typically present during the neonatal period with acute encephalopathy, vomiting, dehydration, severe metabolic acidosis and a disturbed mental status. The characteristic odor of “sweaty feet” may present during the acute attacks. Since the implementation of expanded newborn screening by Tandem Mass Spectrometry in many countries, IVA can be diagnosed pre-symptomatically [4]. It has been found that IVA is caused by mutations in the *IVD* gene which is encoding for the isovaleryl-CoA dehydrogenase [5,6]. This enzyme is a member of the acyl-CoA dehydrogenase (ACAD) family of enzymes, all of which share significant sequences and employ a similar enzyme mechanism for the α,β-dehydrogenation of acyl-CoA substrates [7]. The IVD enzyme is encoded by the nuclear gene which is located on chromosome 15q14-15 and consists of 12 exons spanning 15 kb of genomic DNA [8]. The precursor peptides encoded by this gene are synthesized in the cytoplasm, transported into the mitochondria and processed to homotetramers, with each monomer containing a noncovalently but tightly bound flavin adenine dinucleotide

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molecule [9]. To date, fewer than 40 mutations causing this rare condition have been reported worldwide and most have been point mutations or single-nucleotide insertions/deletions [10,11]. A significant proportion of the mutant alleles leads to abnormal splicing of the IVD RNA and subsequently complete lack of the IVD protein [6]. However, a phenotype/genotype correlation has not been established. Our study reports the clinical features of patients with IVA from the UAE and establishes the molecular basis of IVA in those patients. In addition, we carried out molecular modeling of isovaleryl-CoA dehydrogenase domains harboring the missense mutations found in our patients and "*in silico*" splicing site prediction for the seven nucleotide deletion to elucidate their pathogenicity.

2. Materials and methods

2.1. Subjects

Five IVA patients (three males and two females) from five unrelated families from UAE were recruited for this study (Fig. 1). In all the cases studied, consanguinity was evident as illustrated in the pedigree. This project was approved by the Al-Ain Medical Human Research Ethics Committee-protocol number 10/09.

2.2. Biochemical analysis

Biochemical diagnosis was based on the detection of isovalerylglycine and other metabolites in urine as well as isovaleryl carnitine in plasma and blood spots. After obtaining informed consent from the parents, blood samples were collected from the patients and their family members. Genomic DNA was isolated from peripheral blood leukocytes using the Wizard genomic DNA purification kit according to the manufacturer's instruction (Promega, Madison, WI, USA).

2.3. Mutation analysis

All 12 coding regions and their flanking intron sequences of the *IVD* gene were amplified by polymerase chain reaction (PCR) using appropriate primers. The mutation analysis was carried out at the Genetic Diagnostic Network Laboratory and at the Disease Prevention through genetic testing laboratory [12]. Potential novel mutations were defined by exclusion from the Human Gene Mutation Database [13] and the previously reported mutations on PubMed [14]. In addition, pathogenicity of novel variants was evaluated using the commonly available online prediction softwares such as Polyphen, SIFT and Mutation Taster which predict that these variations are 'probably damaging', 'not tolerated' and 'disease causing', respectively.

2.4. Molecular modeling of isovaleryl-CoA dehydrogenase mutations

The program Deep-View version 3.5.1 [15] was used to model the structural impact of the three missense mutations (p.R392H, p.R395Q and p.E408K) on isovaleryl-CoA dehydrogenase. The isovaleryl-CoA PDB template chain A (PDB ID: 1ivh) [16] was loaded into this program. The appropriate Arg392, Arg395 and the Glu408 residue were mutated to His, Gln and Lys respectively using the "mutation tool" within the Deep-View program. The best rotamer of each mutated residue was selected. The three-dimensional model structure of IVD was visualized with PYMOL [17].

2.5. In silico prediction of cryptic splicing site mutation in IVD gene

To explore the consequence of the heterozygous deletion 1136_1138+4delTTGGTGA affecting exon11 and the donor splicing site of *IVD* gene. *In silico* prediction, was performed using the Scan program provided by the laboratory of Human Molecular Genetics and Genomic Disorders of the University of Western Ontario [18]. The Delila software package was used to scan genomic DNA sequence with weight matrices for sites with positive Ri values in bits of information. The effect of this deletion was predicted from Ri values of each natural and variant primary and secondary splice site based on the criteria reported by Schneider 1997 [19]. The Make-Walker package was used to generate a graphic display of the analysis [19–21].

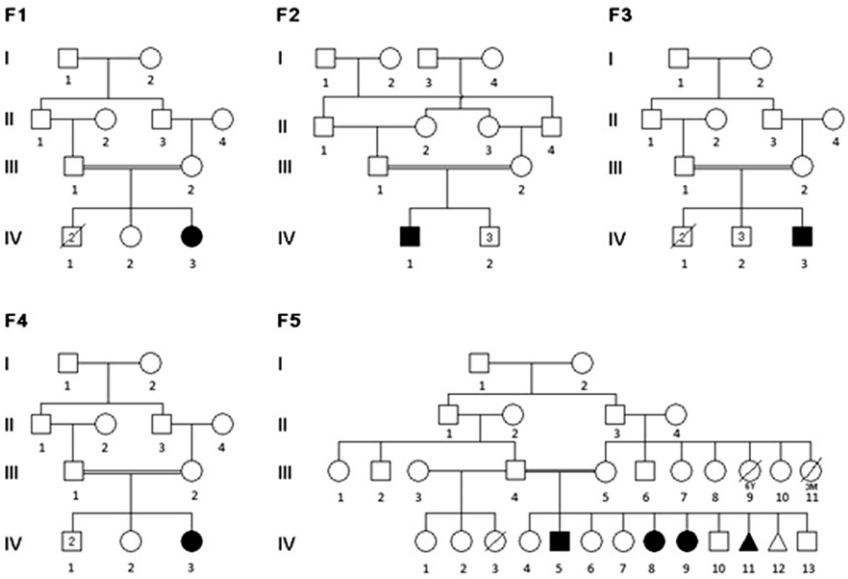

Fig. 1. Pedigrees of five unrelated families from UAE with isovaleric acidemia. F1-5 represent family ID from 1 to 5; *circles and squares* denote females and males respectively; *triangles* indicate abortion; *filled symbols* represent affected members; *symbols with diagonal lines* symbolize deceased individuals; *double lines* denote consanguineous marriage; *Y*: years; *M*: month; *roman numbers* indicate the first generation until their offspring; *arabic numbers* represent available individuals.