A novel mutation in ARG1 gene is responsible for arginase deficiency in an Asian family

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ABSTRACT

إن نقص إنزيم الأرجينيز هو مرض وراثي يؤثر على استقلاب الحامض الأميني أرجنين وينتقل من جيل لآخر بصفه وراثية متنحية. الأرجينيز هو الإنزيم الأخير في حلقه اليوريا الاستقلابية وهو المسئول عن تحويل الأرجنتين إلى يوريا وأورنيثين. أن أعراض هذا المرض تظهر في مرحلة الطفولة على شكل تخلف في التطور الذهني، إعاقات حركيه، تخلف في النمو وارتفاع دوري في تركيز الأمونيا بالدم بين فتره وأخرى. يوجد هناك على الأقل 19 طفرة في جين (ARG1) مسببه لهذا المرض مما يدل على تنوعه بين المرضى من الناحية الجزيئية. قمنا بعرض تقرير عن طفرة جديدة (c.93delG) في هذا الجين موجودة في 3 أطفال مصابين بهذا المرض من عائله واحده من أصل باكستاني ومقيمة بدولة الإمارات العربية المتحدة. أن الطفرة الجديدة ناتجة عن ضياع نيوكليوتايد واحد من هذا الجين (p.T30fsX44) و لهذا تؤدي إلى عدم إنتاج إنزيم أرجينيز الذي يعمل بشكل فعال بالخلايا المعنية وبالتالي نستنتج أن هذه الطفرة هي المسبب لهذا المرض في الأفراد المصابين من هذه العائلة.

Argininemia is a rare autosomal recessive metabolic disorder caused by a deficiency in the arginase enzyme, which is the final enzyme in the urea cycle and responsible for the hydrolysis of arginine to urea and ornithine. The disease becomes symptomatic during childhood and is characterized by progressive spastic quadriplegia, progressive mental impairment, growth retardation, and periodic episodes of hyperammonemia. At least 19 distinct mutations in the ARG1 gene have been identified indicating the molecular heterogeneity of this condition. We report a homozygous novel mutation (c.93 delG) in the ARG1 gene from 3 affected children of a Pakistani family living in the United Arab Emirates. The mutation is expected to lead to a frame shift after the thirtieth residue and a stop codon at residue 44 (p.T30fsX14). Therefore, this mutation is expected to result in complete loss-of-function of the arginase enzyme and therefore is the mostly likely cause of argininemia in this family.

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Argininemia is a rare autosomal recessive disorder caused by deficiency in the cytosol liver type arginase AI enzyme (L-arginine urea-hydrolase; E.C. 3.5.3.1). This condition is also called hyperargininemia, arginase deficiency, or ARG1 deficiency. The disease becomes symptomatic during childhood and is characterized by progressive spastic quadriplegia, mental retardation, exaggerated deep tendon reflexes, and episodes of hyperammonemia.[1] The urea cycle is a series of 6 reactions necessary to rid the body of the nitrogen generated by the metabolism, primarily of amino acids, from diet or released as the result of endogenous catabolism. Arginase is the final enzyme in the urea cycle and is responsible for the hydrolysis of arginine to urea and ornithine in the liver. If this step of the urea cycle cannot be performed, ammonia accumulates in the blood and body tissues leading to brain damage and/or death due to its high toxicity. In addition, the arginase enzyme is responsible for regenerating the urea cycle by generating ornithine. The arginase AI gene (ARG1) is located on chromosome 6q23 and consists of 8 exons encoding a protein of 322 amino acids with an estimated molecular mass of 34,732 Daltons.[1] At least 19 distinct disease causing mutations (**Table 1**) in ARG1 have been identified so far in different human populations indicating that argininemia is heterogeneous at the molecular level.[2-11] Comparison of the coding sequences of the arginase gene from humans, rats, and a number of lower organisms, reveals high amino acid conservation in several regions of the protein with significant divergence in others.[7] Significantly, most of the missense mutations

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causing the disease in humans are affecting highly conserved residues leading to loss of enzymatic activity and potential perturbation of the native structure of the protein.[1] The diagnostic symptoms of argininemia include elevated arginine levels in blood, urine, and cerebrospinal fluid. This condition may present as a neurological disorder (spastic paraplegia) without significant hyperammonemia, and the diagnosis can be missed if plasma amino acid analysis is not performed. Red cells are low in arginase activity. The enzymatic properties of arginase are identical in fetal and adult red cells providing a diagnostic testing method to be used for prenatal testing via cordocentesis.

Case Report. ***Description and clinical features of patients.*** The index patient, a girl, was diagnosed with arginase deficiency at the age of 7 years. The first symptom was an episode of inappropriate behavior at the age of 6 and a half years. This episode lasted for around 2 days, and was followed by 2 similar episodes. Six months later, during one of these metabolic crises, she developed gait problems. Nine months after the symptoms had started, she was investigated at Great Ormond Street Hospital in London. Her serum ammonia turned out to be high at 168 μmol/L (10-40 μmol/L). An amino acid profile showed a strongly raised arginine of 836 micromol/L (normal 40-120) with a moderately raised glutamine of 861 micromol/L (normal 480-800), which led to the diagnosis of arginase deficiency. At diagnosis, she had abnormal liver function tests, with an alanine transferase (ALT) of 283 U/L (normal 10-35) and a prothrombin time of 15.5 seconds (control 9.9-12.5). She was started on a low protein diet as well as sodium benzoate and sodium phenylbutyrate. Since treatment was started, there have been no major metabolic crises. Her ammonia, arginine, and glutamine have remained under control and her liver function tests have been normal throughout. She has residual neurological abnormalities, mainly spastic diplegia with an awkward gait, despite a lengthening of the Achilles tendons. She attends a normal school, but her performance is low average. The parents, who are second cousins originally from Pakistan, have since produced 2 boys with the same disease. They were started on treatment from the beginning of life and are now 4 and 3 years old. They have not had any metabolic crisis, and their metabolic parameters are well controlled. Their mental and motor development are completely normal.

DNA analysis. Informed consent was taken from the parents for the molecular analysis, genomic DNA was extracted from blood using Qiagen Flexi Gene kit following the manufacturer's standard protocol instructions. Oligonucleotides were designed using Primer3[12] to amplify the 8 coding exons and splice sites. All the exons (1-8) of the ARG1 gene were then amplified by polymerase chain reaction (PCR) according to the

Table 1 - All the reported mutations in ARG1 gene from arginase deficiency patients.

Mutation	Type	Reference
c.93 delG (V44X)	Small Deletion	This study
I11T	Missense	5
R21X	Nonsense	9
K75X	Nonsense	7
W122X	Nonsense	4
D128G	Missense	6
G138V	Missense	5
H141L	Missense	6
G235R	Missense	7
G235R	Missense	4
T290S	Missense	10
R291X	Nonsense	3
IVS+1(G-A)	Splice Site	5
IVS-2(A-G)	Splice Site	5
GGGTG^GAAGAaGGCCCTACAG- 25(codon)	Small Deletion	2
GGCACAA^GTCaagaAGAACGGAAG - 87(codon) (262-265 or 263-266 in exon 3)	Small Deletion	2
TCACACT^GATatcAACACTCCAC- 128(codon)	Small Deletion	7
GAACCCA^TCCcTGGGGAAGAC - 281(codon)	Small Deletion	4
712(codon), 237(nucleotide) GGACTG^GACCggaccggaccCATCTTTCAC	Insertion	9
10753 bp, intr. 1-poly A site	Large deletion	8

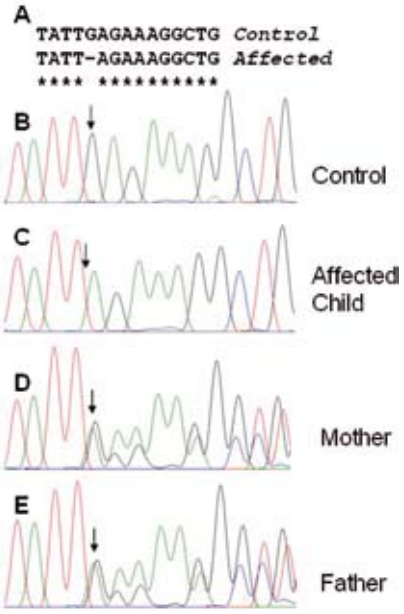

Figure 1 - Identification of the c.93 delG mutation in the Asian family showing **A**) ClustalW alignment showing single base pair deletion. **B**) The chromatogram of a normal person as control, and **C**) for the affected child showing a base pair deletion leading to a frame shift mutation (c.93delG). **D & E**) are the chromatogram of parents showing the heterozygous nature of their alleles.

following protocol: After the initial denaturation step (5 minutes at 94°C), DNA samples were amplified for 30 cycles using an annealing temperature of 58°C for one minute, extension at 72°C for 1.30 minutes with final extension cycle at 72°C for 8 minutes. Amplification was performed in 50µl reaction volume using 50ng of genomic DNA. The products were analyzed on 1.8% agarose then purified using the EXO-SAP-IT protocol (USB Inc., Cleveland, Ohio, USA). Purified PCR products were sequenced using an ABI gene Analyzer (Macrogen Inc., Seoul, South Korea). Among the 8 exons examined, a homozygous single base deletion was found on exon 2 of all 3 affected children (**Figure 1**). Both the parents were heterozygous in that position. The single base deletion is found in the c.93 delG, that would lead to a reading frame shift after amino acid 30 and create a new stop codon at residue 44 (p.V44X). It is expected that this mutation will lead to complete loss-of-function of the arginase enzyme, and therefore it is mostly likely that this mutation is the cause of argininemia in this family.

Discussion. Our findings add a novel mutation to the list of argininemia causing mutations (**Table 1**). Arginase deficiency has been reported in Middle Eastern populations including in Palestine[8] and Saudi Arabia.[3] The identification of the new mutation reported here indicates heterogeneity of this rare condition in this population. In addition, identification of the molecular basis of genetic diseases is crucial for prevention of the occurrence of new cases of potential fatal diseases. This is especially true for genetic disorders prevalent in Arab and other Middle Eastern countries.[13] The high prevalence of consanguineous marriages in those populations had led to the high prevalence of recessive disorders in those populations. Identification of the exact molecular cause of recessive disorders will allow geneticists and genetic counselors to provide effective approaches to prevention in high-risk families. The information obtained can be used to identify the carriers within members of the extended families. In addition, a prenatal diagnosis approach has been shown to be successful in the cases of argininemia.[8,11]

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