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Research Article

Identification of ARG1 Mutations in Patients with Arginase 1 Deficiency

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Abstract:

Introduction: Argininemia is an autosomal recessive uncommon metabolic condition, caused by mutations in arginase enzyme. Variable clinical symptoms of argininemia might bring about a delayed diagnosis. In order to prove an argininemia condition, a genetic test result is needed. This study aims to describe two cases of argininemia homozygote mutations.

Materials and Methods: Whole exome sequencing (WES) was utilized to detect disease causing variants. To prove adverse impacts of a novel variant and in silico analysis, PROVEAN web server is chosen. The effects of the novel mutation on the enzyme's structure are shown in Chimera software using normal and mutated structures derived from SWISS model web server.

Result: WES revealed two cases of autosomal recessive hyperargininemia. In one of the patients, a homozygous missense mutation of c.491G>A was detected, which is a novel ARG1 variant (p.Trp164Ter). Bioinformatics databases and the variant's protein structure proved its deleterious effect on the enzyme's function. The other patient was affected by a reported mutation of c.703G>A (p.G235R).

Conclusion: The presence of various types of neurological and metabolic disorders with the same clinical findings with argininemia might lead to a lack of early diagnosis for beginning efficacious treatments. WES provides these patients with the opportunity to become diagnosed and receive therapies as early as possible.

Keyword: Arginase 1 deficiency, ARG1, Whole exome sequencing (WES).

Introduction

The number of live births affected by arginase 1 deficiency has been estimated between 1 in 300,000 to two million births [1]. Hyperargininemia is an uncommon autosomal recessive (AR) disorder linked to urea cycle reactions. Arginase regulates the final step of the urea cycle, which involves removing nitrogen from the amino acid arginine and converting it to urea for removal from the body. This process also produces ornithine, a molecule required for the urea cycle to restart [2].

The indications of arginase-1 deficiency are distinct from those consistent with other urea cycle diseases. During the first six to twelve months of life, most newborns with arginase-1 deficiency show no clinical symptoms. Besides, only a small percentage of infants with arginase-1 deficiency develop severe hyperammonemia or hyperammonemia coma, which are common in the other urea cycle abnormalities. However, almost all of the affected children have developmental delays, and many of them also experience seizures [1].

ARG1 with eight exons codes for arginase 1, spanning a 15-kb genomic area on chromosome 6q23 [2]. Arginase 1 hydrolyses arginine to generate urea, and a defective arginase ends up in hyperargininemia condition. High arginine levels in plasma, mild hyperammonemia appearing as spastic paraparesis, and increasing neurological manifestations are some of the signs of arginase 1 deficiency [1]. Patients with arginase deficiency can follow the guidelines outlined in Urea Cycle Disorders (reduction of protein intake and administration of nitrogen-scavenging medicines). Maintaining plasma arginine concentrations close to the normal level (54–134 nmol/mL) should be the target in their

process of check-up [3].

Argininemia is considered as a curable infantile disease, in which the sooner diagnosis occurs, the better outcomes patients can expect [3]. With the rapid growth of next-generation sequencing (NGS) methods, such as whole genome sequencing (WGS) and whole exome sequencing (WES) methods, early detection of metabolic diseases has become a possible reality. They are dramatically increasing our understanding of the genetic mechanism of a dozen of human disorders [4]. This study purposes to detect variants responsible for arginase 1 deficiency in our patients and separate disease-causing alterations in the genome from benign and common variants according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

Materials and Methods

Participants

The patients had gradually increasing gait abnormalities, showing developmental delays. Their familial histories were normal, with no significant disorders. The first patient was referred to the Beski Genetic centre by age six and used orthopedic shoes because of flat feet. An increased ammonia and plasma arginine have been shown in his blood tests and HPLC analysis. Urine organic acid analysis revealed a mild elevation of Uracil and Orotic acid, suggesting a urea cycle disorder in him. The second patient was a girl aged five with the age of disease onset at two years old. Her symptoms included increased deep tendon reflex of the lower limbs, progressive spastic limbs, speech problems, and mild atrophy and dilated CSF in MRI. She has gradually lost her speech and walking abilities. Both patients had normal consanguineous parents, showing an autosomal recessive inheritance pattern. Patients and their parents were provided with informed consent forms to sign.

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cols from 5 ml of peripheral blood collected in EDTA tubes

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Whole-Exome Sequencing

DNA was extracted from Whole Blood samples of two patients and their parents using GeneAll DNA extraction kit (GeneAll, Seoul, Korea). Parental samples were collected to determine inherited mutations. Exon sequences were prepared by SureSelect human All exon Enrichment V7 array. Whole-exome sequencing (WES) was conducted for patients by paired-end sequencing on NovaSeq 6000 instrument (Macrogen Europe, Amsterdam, The Netherlands) with the coverage of more than 20X for 90% of the target region, resulting in ~6 Gb of raw data.

The BurrowsWheeler alignment (version 0.7.5a,18) was utilized to align cleaned reads to the human reference genome (hg19). Duplicate reads were detected by Picard (version 2.25) (http://broadinstitute.github.io/picard); Genome Analysis ToolKit (GATK), version 2.4–9 (ref. 19), was used for realignment of insertions and deletions. Variants were called with both GATK and Samtools programs, which were combined and annotated with various databases consequently (dbSNP, 1000 Genomes Project, ClinVar). The annotation analysis of detected variants was performed by ANNOVAR software.

In order to recognize Indels and single nucleotide variants (SNVs), we took advantage of both probability and quality based algorithms. For prioritization of gene/variant, a minimum depth of 30X and minor allele frequency of 1% were selected as the cut off. Synonymous, heterozygote frameshift, splice site, and missense variants were filtered in the WES annotation data of patients according to the predicted AR inheritance from the pedigree analysis.

Sanger Sequencing

Sanger sequencing was carried out on two affected patients and their healthy parents to validate WES findings and co-segregation of the pathogenic variants within the families. Sequencher (Gene Codes) program was chosen for the interpretation and analysing of sequencing results.

In silico analysis

For the prediction of p.Trp164Ter mutation impacts on Arg1 protein structure and stability, online programs were applied, including PROVEAN [5], I-Mutant software [6], and SNAP 2 (https://rostlab.org/services/snap2web). Furthermore, SWISS model server and Chimera software visualized alterations on the tri-dimensional structure of Arg1 protein [7, 8].

Results

Two homozygous variants were discovered through filtering synonymous, non-coding, and common variants in WES annotated data. Sanger sequencing validated homozygote status of discovered mutations in patients and confirmed their heterozygous states in the healthy parents, which are consistent with AR inheritance pattern according to genetic pedigrees of families (Table 1 and Figure 1).

Table 1. Results derived from whole exome and sanger sequencing.

Sequencing results								
	Age	**Gender**	**Chief Complaint**	**WES Variant**	**Zygosity**	**Sanger Sequencing**	**Inheritance**	**ACMG Classification**
First Patient	6	Male	Microcephaly, developmental delay	(NM_000045.4) c.491G>A (p.Trp164Ter)	HOM	Confirmed	AR	Pathogenic

Second Patient	5	Female	progressive spastic limbs, speech problems	(NM_000045) c. 703G>A (p.Gly235Arg)	HOM	Confirmed	AR	Pathogenic

HOM: homozygote, AR: autosomal recessive, ACMG: The American College of Medical Genetics and Genomics. (ACMG)

One of the patients was affected by a novel nonsense mutation c.491G>A (p.W164X) in the ARG1 gene, which could explain his clinical manifestations related to the urea cycle. The conversion was predicted pathogenic by a variety of mutation bioinformatic servers, such as PROVEAN and SNAP 2. In addition, this variant wasn't recorded on any human mutation databases, such as gnomAD and dbSNP. Sanger sequencing validated a complete co-segregation of this pathogenic variant. The novel mutation resulted in replacing a highly conserved aromatic amino acid of Tryptophan with a stop codon (Figure 1), changing the enzyme's length, structure, and ability for binding to substrates.

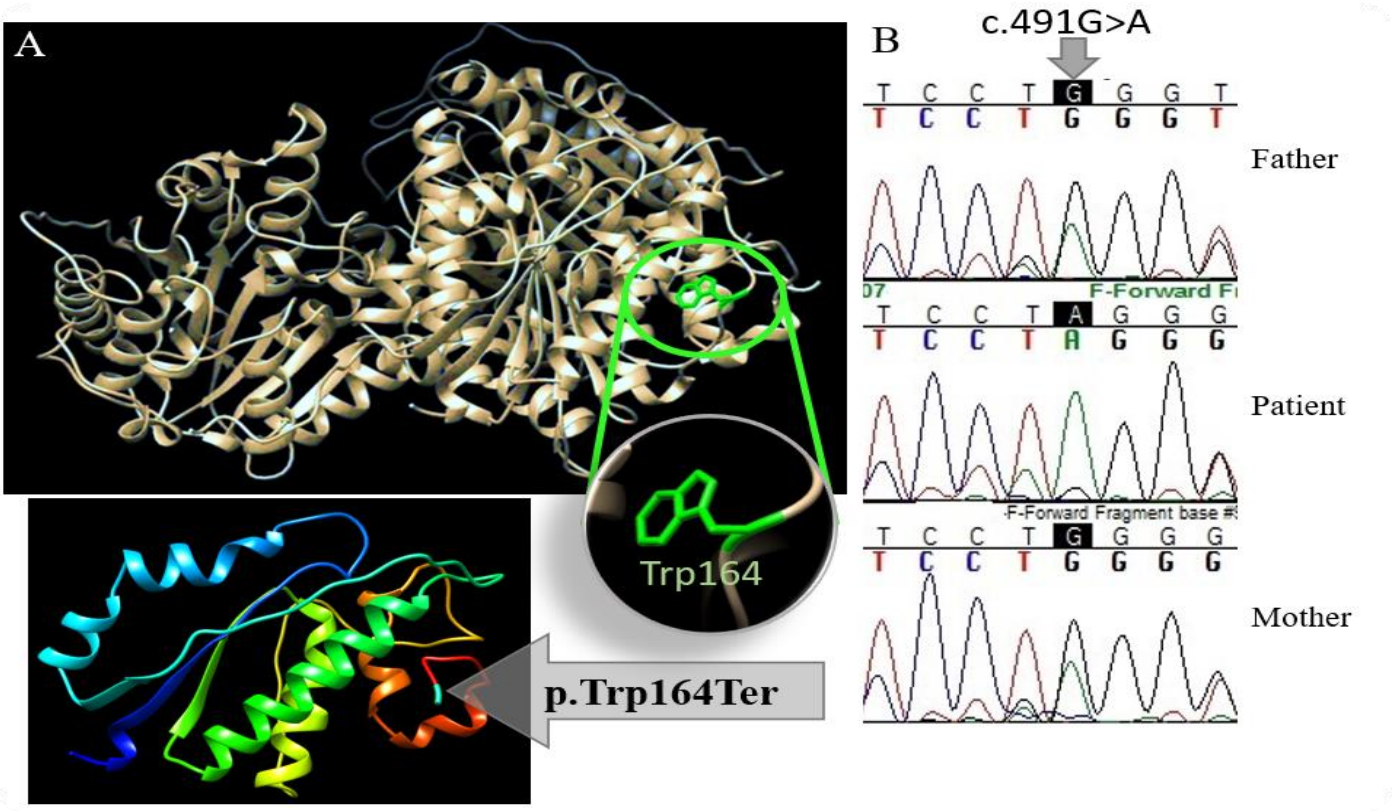

Figure 1. A. The discovered novel mutation (c.491G>A, p. Trp164Ter) visualization by Chimera software revealed its significant impact on the enzyme's structure. Wild type amino acid has shown in green which is changed to a stop codon shown in cyan. B. Sanger sequencing validated its segregation through the family.

Furthermore, a previously reported pathogenic variant was discovered in the second patient, recorded at the first time in 1992 by Uchino and colleagues [9]. This mutation changes Glycine amino acid to an Arginine, a positively charged amino acid at physiological pH, resulting in altered protein features.

Discussion

There are different clinical symptoms in arginase 1 deficiency in comparison with other urea cycle diseases. The children display no clinical signs in the early year of childhood and progressively develop disease manifestations, including increasing spastic, physical retardation, intellectual disability, and epilepsy [5,6]. The same symptoms can be seen in various neurological conditions such as cerebellar ataxia, spastic paraplegia, and degenerative disorders; therefore, it might not be easily separable from other human diseases [7,8]. Some patients reveal nonspecific signs in early childhood, like protein intolerance and physical retardation [9]. Our cases had typical clinical symptoms of progressive spastic paraparesis, unsteadily walking, and spastic diplegia. Argininemia is a potentially treatable disease of urea cycle disorders, especially in its early stages. Diagnostic basis mainly includes typical clinical manifestation and molecular genetic testing.

In the current study, we described two patients from consanguine parents with proved argininemia disorder. Their clinical manifestations and biochemical tests proposed a urea cycle disorder. After performing WES and filtering common and synonymous variants, we found their responsible mutations, confirming by Sanger sequencing in patients and their parents. One of them carried a novel mutation in ARG1 gene resulting in the replacement of Tryptophan amino acid with a stop codon (c.491G>A, p.Trp164Ter), and

the other is affected by a previously reported variant (c.703G>A, p.Gly235Arg), both of which are pathogenic in the homozygote state. Furthermore, the mutations aren't listed in variant databases for healthy individuals.

Although the consequence effects of the novel mutation have not yet been explored by functional studies and through in vitro experiments, we studied probable effects of the novel mutation by bioinformatics databases and a computational model, proving its pathogenicity. Using SWISS model website and Chimera software, we visualized its deleterious impacts and highly conservative nature. The mutation (p.Trp164Ter) is located in exon five of ARG1 gene, and its pathogenic effect is in line with studies describing nonsynonymous mutations in these conserved regions [10]. The replacement of a G base with an A base causes the conversion of Tryptophan amino acid codon (TGG) to a termination codon (TAG), which is the main reason for creating a defective arginase enzyme with a shorter length. As a matter of fact, three main binding site regions place after the mutation site, so the enzyme would lose all of its binding sites to substrates.

The other mutation, c.703G>A (rs104893948), can also lead to the production of a dysfunctional enzyme. This mutation is located in exon seven of ARG1 gene and was reported in a Japanese patient by Uchino and his colleagues for the first time. It reduces the enzyme's relative activity to less than 0.1% of a normal arginase enzyme [11]. So far, this mutation has been seen in other patients with progressive spastic tetraplegia and mental and physical retardation [10].

Some limitations of our cases and our study are as follows: Patients were referred to the centre after some years of starting their pathological symptoms; as a result, they didn't receive appropriate medical managements in previous years. Functional studies haven't been performed for the discovered novel mutation to determine its destructive effects practically. Sequencing methods have some drawbacks as well. For example, after filtering the raw data, some potential mutations might be sequencing artifacts, which increases the number of confirmation tests and their prices.

As a treatable disease, early diagnosis of argininemia is of paramount importance to improve treatment outcomes. Although some biochemical and spectrometry tests can assess arginine levels in children, their results might be misleading in some cases [12]. In contrast, WES is capable of discovering ARG1 and other genes mutations even on the first day of a newborn life. Both considered patients in our study received proper treatments after the diagnosis of their disorder. Further investigation to prove the pathogenicity of the novel mutation found in this study is suggested.

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