Gene 533 (2014) 240–245

Contents lists available at ScienceDirect

Gene

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

Novel complex Re-Arrangement of *ARG1* commonly shared by unrelated patients with Hyperargininemia

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ARTICLE INFO

Article history:
Accepted 23 September 2013
Available online 5 October 2013

Keywords:
Hyperargininemia
Arginase deficiency
ARG1
Complex re-arrangement

ABSTRACT

Background: Hyperargininemia is a very rare progressive neurometabolic disorder caused by deficiency of hepatic cytosolic arginase I, resulting from mutations in the *ARG1* gene. Until now, some mutations were reported worldwide and none of them were of Southeast Asian origins. Furthermore, most reported mutations were point mutations and a few others deletions or insertions.
Objective: This study aims at identifying the disease-causing mutation in the *ARG1* gene of Malaysian patients with hyperargininemia.
Methodology: We employed a series of PCR amplifications and direct sequencing in order to identify the mutation. We subsequently used quantitative real-time PCR to determine the copy number of the exons flanking the mutation. We blasted our sequencing data with that of the reference sequence in the NCBI in order to obtain positional insights of the mutation.
Results: We found a novel complex re-arrangement involving insertion, inversion and gross deletion of *ARG1* (designated g.insIVS1+1899GTTTTATCAT;g.invIVS1+1933_+1953;g.delIVS1+1954_IVS2+914;c.del116_188;p.Pro20SerfsX4) commonly shared by 5 patients with hyperargininemia, each originating from different family. None of the affected families share known relationship with each other, although four of the five patients were known to have first-cousin consanguineous parents.
Conclusion: This is the first report of complex re-arrangement in the *ARG1*. Further analyses showing that the patients have shared the same geographic origin within the northeastern part of Malaysia prompted us to suggest a simple molecular screening of hyperargininemia within related ethnicities using a long-range PCR.

1. Introduction

Arginase I is the final enzyme in the urea cycle that catalyzes the hydrolysis of arginine to urea and ornithine. Arginase I is the product of *ARG1*, of which mutations have been described to cause deficiency of the enzyme. *ARG1* is located in the long arm of chromosome 6 (6q23) and is made up of eight exons that code for 322 amino acids (Iyer et al., 1998).

Hepatic Arginase I Deficiency causes hyperargininemia (OMIM # 207800), a rare inborn errors of urea cycle which is inherited in an autosomal recessive manner with the incidence of only 1 in 2,000,000 (Scaglia and Lee, 2006). Unlike the other inborn errors of urea cycle which usually present with an acute hyperammonemic crisis, hyperargininemia usually has a more chronic presentation in later childhood with neurologic features such as delayed developmental milestones, spastic diplegia, learning difficulties, and epilepsy (Iyer et al., 1998; Lee et al., 2011). The extreme spasticity observed in this disorder is not found in other urea cycle defects and the frequent epileptic symptom is seldom related to episodes of hyperammonemia (Scaglia and Lee, 2006). Some researchers have suggested that an accumulation of guanidino compounds that could interfere with GABAergic transmission is the possible pathogenic mechanism (Lee et al., 2011). Guanidino compounds have been shown to inhibit the cerebral cortical sodium-potassium adenosine triphosphatase (ATPase) of rats at concentrations comparable with those seen in affected humans (Scaglia et al., 2004). The ATPase is

Abbreviations: *ARG1*, Arginase 1 Gene; ATPase, Adenosine Triphosphatase; DNA, DeoxyriboNucleic Acid; dNTP, deoxyribuNucleotide TriPhosphate; GABA, Gamma-AminoButyric Acid; NCBI, National Center for Biotechnology Information; NHLBI, National Heart, Lung and Blood Institute; OMIM, Online Mendelian Inheritance in Man; PCR, Polymerase Chain Reaction; qPCR, quantitative real-time PCR; RefSeq, Reference Sequence; Sdn. Bhd, Sendirian Berhad (Co. Ltd. in Malaysian).

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http://dx.doi.org/10.1016/j.gene.2013.09.081

essential to maintenance of the electrochemical gradient of neurons, and its inhibition may also be involved in the pathogenesis of the seizure disorder associated with this disease (Mori et al., 1990).

The Human Gene Mutation Database (Stenson, 2009) reported to date at least 37 mutations have been identified in *ARG1* gene among different populations worldwide. Reported *ARG1* mutations were mostly missense, with only very few cases involving deletion and/or insertion without reported complex re-arrangement (Stenson, 2009). In addition, there has been no documented evidence of a common *ARG1* mutation shared by patients within the same or related ethnicities, thus making it difficult for simple molecular diagnostic screening (Carvalho et al., 2012; Tsang et al., 2012; Vockley et al., 1996). Here we reported a novel complex re-arrangement mutation of *ARG1* shared by 5 unrelated patients with hyperargininemia and suggested a simple molecular diagnostic screening.

2. Methods

2.1. Patients and DNA Extraction

Six patients were referred to our laboratory in Human Genome Centre Universiti Sains Malaysia for molecular genetic analyses of *ARG1* gene, from Hospital Kuala Lumpur and Hospital Universiti Sains Malaysia. All patients were of Malay ethnicity. Information on parental origins was obtained through interview. Family pedigree of each patient was analysed for three generations. Diagnosis of hyperargininemia was established based on clinical features and laboratory analysis. Informed consent was taken prior to blood taking. Genomic DNA was extracted from whole blood using commercially available kit (QIAamp® DNA Mini Blood Extraction).

2.2. Polymerase Chain Reaction and Direct DNA Sequencing

Polymerase Chain Reaction was performed using GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The primers for each exon of *ARG1* gene were synthesized according to sequences as described previously (Cardoso et al., 1999; Grody et al., 1992; Uchino et al., 1992).

In order to clarify amplification failure of *ARG1* exon 2, we employed long-range PCR amplification using two of the primers described previously; forward primer of 5'-TGACTGACTGGAGAGCTCAAG-3' (Uchino et al., 1992) and reverse primer of 5'-ACACAGTTATTCAACAAGACC-3' (Cardoso et al., 1999). The long-range PCR condition was 2 min at 94 °C for initial denaturation, followed by 12 sec at 94 °C, 30 sec at 60 °C and 15 min at 68 °C for 30 cycles and a final extension done at 68 °C for 7 min. Amplifications were performed in 30 μL volume containing 1 μg of genomic DNA, 1X Buffer S, 100 μM dNTPs, 1.1 μM of each primer and 2 U MaxTaq polymerase (Vivantis Technology, USA) (Fig. 1).

We subsequently designed a series of primers (refer to Fig. 2(B)) in order to identify the breakpoint deletion based on the *ARG1* genomic sequence provided in the NCBI Nucleotide Database (accession no. GI_163954920; RefSeq NG_007086.2; Primers were synthesized by First Base Laboratories Sdn. Bhd., Malaysia). Direct DNA Sequencing was done to identify the mutations, using ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

2.3. Real-Time qPCR

In order to provide information on homozygosity of the deletion, we performed real-time quantitative PCR for identifying the copy number of exons 1 and 3 which are flanking the deleted region. The real-time quantitative PCR was performed according to previously described method (Tran et al., 2008) using LightCycler® 1.5 (Roche Diagnostics, Mannheim, Germany) and Human β-actin as reference gene. Each experiment used 3 serial template dilutions and was done twice (duplicate experiments).

2.4. Bioinformatics Analysis

We used Basic Local Alignment Search Tool (BLAST®) provided by the NCBI Nucleotide Database, 2013 (http://blast.ncbi.nlm.nih.gov/Blast.cgi), in order to obtain positional insights into the deleted segment.

3. Results

3.1. Patients Features

Table 1 summarizes the phenotypes, genotypes and other information of the patients. All patients showed neurological deficits as well as striking elevation of plasma arginine level. Almost all showed delayed developmental delay, while one of the patients showed acute infantile hyperammonemia. All were known to have parental origin from Kelantan, one state in the north-eastern part of Peninsular Malaysia. All but one patient were born from first-cousin consanguineous marriage. Three-generation pedigree analyses of all the patients showed no relationship between any families.

3.2. Molecular Genetic Analyses

We initially tried to amplify each of the 8 exons in the *ARG1*of all the 6 patients. In one of the patients [patient ID 002(2)] we successfully amplified all of the 8 exons. Upon direct DNA sequencing, we identified a nonsense mutation in *ARG1* exon 8 (Table 1). However, we experienced consistent failure in amplifying exon 2 of the gene for the other 5 patients. We investigated this further by performing long-range PCR amplification encompassing exon 1 to exon 3, where the amplification worked well. Our long-range PCR identified a reduction of approximately 2.5 kilobases in the PCR product of the amplified region, which was consistently found in all 5 patients and suggesting a segmental genomic loss between exon 1 and exon 3 (Fig. 1).

We subsequently performed a series of PCR amplifications and direct sequencing to identify the breakpoint of the deletion (Fig. 2A). Upon

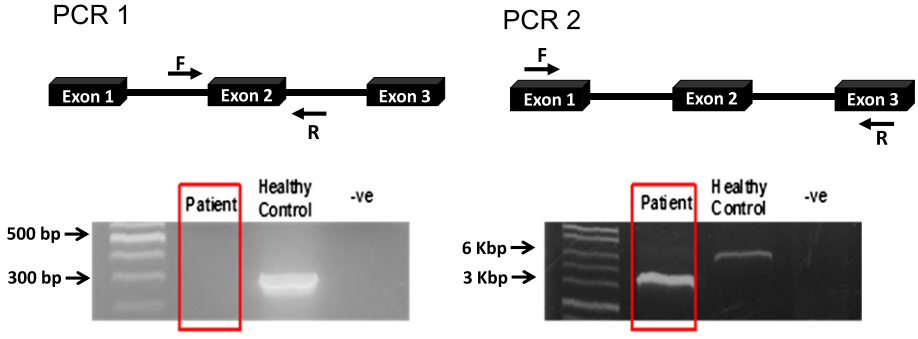

Fig. 1. PCR 1 shows the amplification failure of exon 2, which was subsequently clarified by PCR 2 that employed long-range primers. PCR 2 shows a notable size reduction in a region between exon 1 and exon 3 of the patient. Amplification in PCR 2 encompassed exon 1, intron 1, exon 2, intron 2 and exon 3.