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WHOLE EXOME SEQUENCING MAKE A DEFINITIVE DIAGNOSIS OF A VIETNAMESE PATIENT WITH A LATE ONSET UREA CYCLE DISORDER

Ngoc-Lan Nguyen[1,2], Ngoc Khanh Nguyen[3], Chi Dung Vu[3], Nguyen Thi Thu Huong[1,2], Nguyen Huy Hoang[1,2,✉]

[1]*Graduate University of Science and Technology, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*
[2]*Institute of Genome Research, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*
[3]*Center for Rare Diseases and Newborn Screening, Department of Endocrinology, Metabolism and Genetics, Vietnam National Children's Hospital, 18/879 La Thanh, Dong Da, Hanoi, Vietnam*

✉To whom correspondence should be addressed. E-mail: nhhoang@igr.ac.vn

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SUMMARY

Our report describes a female presenting with vomiting, fever, coma and right hemiplegia at 26 months of age. Biochemical tests revealed hyperammonemia, hyperlactatemia, elevated glutamine level, elevated transaminase and disorder of prothrombin time. She was priory diagnosed with urea cycle disorders (UCDs). UCDs are caused by mutations in eight genes that regulate the synthesis of enzymes and cofactors involved in urea metabolism. Singleton whole exome sequencing was applied to screen causative variants in these genes in the patient at 6 years of age. The result showed one heterozygous stop loss mutation c.1065A>G in the *OTC* gene as a potential disease causing in the patient. The mutation c.1065A>G leads to alternation of stop codon to tryptophan, resulting in elongation of fourteen amino acids in ornithine transcarbamylase (OTC) protein (p.Ter355TrpextTer14). Sanger sequencing in the family revealed the mutation c.1065A>G was not present in healthy parents and brother. Therefore, this mutation is considered as a de novo mutation in the patient. The mutation c.1065A>G was conferred to pathogenic according to the standards and guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology with 1 strong (PS2), 3 moderate (PM2, PM4 and PM5) and 1 support criteria (PP2). Although OTC deficiency is an X-linked recessive inheritance, approximately 15% of females carrying heterozygous variants showed the late onset OTC deficiency. Therefore, in combination of clinical presentations, laboratory findings and molecular genetic analyses, we made a definitive diagnosis of the patient with late onset OTC deficiency, a disorder of UCDs.

Keywords: *c.1065A>G, de novo mutation, OTC, p.Ter355TrpextTer14, urea cycle disorder, Vietnamese patient*

INTRODUCTION

The urea cycle is a biochemical process that occurs in the liver, the kidneys, and small intestinal enterocyte in which the wastes from protein metabolism are converted to water-soluble urea, which is a less toxic substance for excretion. The urea cycle starts within the mitochondria with biotransformation of NH_3 to carbamoyl phosphate by the

carbamoylphosphate synthetase I (CPS1). Ornithine transcarbamylase (OTC) catalyzes the conversion of carbamoyl phosphate to citrulline in the presence of ornithine. Citrulline is released to the cytosol and condensed with an aspartate to form argininosuccinate under catalyzation of argininosuccinic acid synthetase (ASS1). Argininosuccinic acid lyase (ASL) catalyzes the cleavage of argininosuccinate to form fumaric acid and arginine. Arginine is rapidly hydrolyzed to urea and ornithine by the arginase (ARG1). Urea is excreted and ornithine returns to mitochondria by the catalyzation of the ornithine translocase (ORNT1 or SLC25A15). In addition, the N-Acetyl glutamate synthase (NAGS) which catalyzes the conversion of acetyl-CoA and glutamate to N-acetyl-L-glutamate, an obligate cofactor for carbamoylphosphate synthetase I, and citrin (SLC25A13) which is an aspartate/glutamate carrier, are also involved in the urea cycle. The urea cycle disorders (UCDs) are inherited deficiencies in one of the eight enzymes (Ah Mew *et al.,* 2017). The urea cycle disorder is a rare disease with an estimated incidence of one in 35,000 (Stone *et al.,* 2020). The severity of disease depends on the type of enzyme deficiency and the level of the enzyme inactivation (Ah New *et al.*, 2017). For example, severe deficiency or loss of activity of CPS1, OTC, ASS, ASL and NAGS lead to serious illness. In milder forms causing by partial enzyme deficiency, symptoms may be triggered by illness or stress at any time of life, leading to increase plasma ammonia levels. If the urea cycle disorders are not detected early and treated promptly, patients may lead to serious complications, even life-threatening (Endo *et al.,* 2004).

Table 1. Molecular genetics in urea cycle disorders.

Disease	Gene	Locus	Exons	OMIM	Inheritance
Carbamylphosphate synthetase I deficiency	*CPS1*	2q34	38	608307 or 237300	AR
Ornithine transcarbamylase deficiency	*OTC*	Xp11.4	10	300461 or 311250	XLR
Argininosuccinate synthetase deficiency	*ASS1*	9q34.11	16	603470 or 215700	AR
Argininosuccinate lyase deficiency	*ASL*	7q11.21	17	608310 or 207900	AR
Arginase deficiency	*ARGI*	6q23.2	8	608313 or 207800	AR
N-Acetyl glutamate synthase deficiency	*NAGS*	17q21.31	7	608300 or 237310	AR
Ornithine translocase deficiency	*SLC25A15*	13q14.11	7	603861 or 238970	AR
Citrin deficiency	*SLC25A13*	7q21.3	18	603859 or 605814 or 603471	AR

AR, autosomal recessive; XLR, X-linked recessive.

The genetic etiology of UCDs is due to mutations in one of the genes that regulate the synthesis of enzymes and cofactors involved in urea metabolism (Table 1). All most of the genes are autosomal recessive inheritance, except for *OTC*, an X-linked recessive inheritance (Table 1). Mutations in the *OTC* gene have been found as the most common event in UCDs cases, follows by the occurrence of mutations in *ASS1* and *ASL* genes (Martín-Hernández *et al.,* 2014; Nettesheim *et al.,* 2017; Silvera-Ruiz *et al.,* 2019). Mutations in the *CPS1*, *ARG1*, *NAGS*, *SLC25A15* and *SLC25A13* are rare in UCDs cases.

Due to eight genes involved in UCDs and their multiple coding regions, researchers have turned to apply the next generation sequencing to detect variants in UCDs cases (Choi *et al.*, 2017; Zhao *et al.*, 2019; Lin e*t al.*, 2019; Yan *et al.*, 2019). Here, we report a female patient with a prior diagnosis of urea cycle disorders. Whole exome sequencing revealed the patient carries a stop loss mutation in the *OTC* gene. A genotype-phenotype correlation is discussed.

MATERIALS AND METHODS

The patient was diagnosed with urea cycle disorders at the Vietnam National Children's Hospital. Written informed consent was obtained from the patient's parents prior to sample collection and analysis. This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Institute of Genome Research (No. 16/QD-NCHG, Institute of Genome Research Institutional Review Board, Hanoi, Vietnam).

Genomic DNA samples were extracted from blood using QIAamp DNA Blood Mini Kit (Qiagen, Germany). The DNA library and exome library were prepared with an Agilent SureSelect Target Enrichment Kit (Agilent Technologies, CA, USA) and an Agilent SureSelect Human All exon v6 Kit (Agilent Technologies, CA, USA), respectively, according to the manufactures' protocols. Exome library was sequenced on an Illumina NovaSeq 6000 platform (Illumina, CA, USA). Paired-end sequences were mapped to the GRCh37/hg19 reference human genome using the Burrows–Wheeler Aligner (BWA) bwa-0.7.12. Duplicates were removed by using the Picard MarkDuplicates picard-tools-1.1130. Variant calling was performed using Genome Analysis Toolkit (GATK) v3.4.0. Variant annotation was generated by SnpEff v4.1g.

Variants with minor allele frequency >0.01 were excluded by comparing with 1,000 Genome Database (http://browser.1000genomes.org). Identified variants were interpreted based on information found in the Exome Variant Server (https://evs.gs.washington.edu/EVS/), dbSNP 142, the Human Genome Mutation Database (http://www.hgmd.cf.ac.uk/ac/ index.php) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar). The candidate variant was viewed using the Integrative Genomic Viewer (IGV) version 2.6.3 (http://software.broadinstitute.org/software/igv/home).

The pathogenicity of the candidate variant was classified according to the recommendations of the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (Richards *et al.*, 2015).

Sanger sequencing was applied to confirm the mutation in the patient and check co-segregation in the patient' parents and sibling. The primer set for amplification of DNA sequence containing variant was designed using Primer blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast). PCR conditions used for the amplification were, 95^{o}C for 12 min followed by 35 cycles at 95^{o}C for 45 s, 58^{o}C for 45 s, and 72^{o}C for 45 s, and final extension at 72^{o}C for 8 min. Amplified PCR product (605 bp) was checked on 1.5% agarose gel. Sanger sequencing was performed using ABI Big Dye Terminator on 3100 Genetic Analyser (Applied Biosystems, USA). Target sequence was compared with the *OTC* gene sequence from the NCBI database (NM_000531.6).

RESULTS

Clinical presentation

The UD01 patient is the second child of healthy unrelated Vietnamese parents with an unremarkable family history. She was born at 36 weeks of gestation with a birth weight of 2.3 kg and had normal development. At 26-month-old, she presented with vomiting for 3 days, then fever, convulsion and coma and was admitted at the province hospital. She was diagnosed and managed as encephalopathy at the province hospital for 7 days, but her consciousness was worsened, therefore, she was transferred to the Vietnam National Children's Hospital. On admission, she was fever, coma, and right hemiplegia. The biochemical investigation revealed hyperammonemia (414 µg/dL; normal: <50 µg/dL), hyperlactatemia (5.8 mmol/L; normal: 1.1-2.3 mmol/L), elevated blood glutamine (840 µmol/L; normal: 530±81 µmol/L), elevated alanine aminotransaminase (ALT 140 UI/L; normal: 7-59 UI/L) and aspartate aminotransaminase (AST 327 UI/L; normal: 10-40 UI/L), elevated urinary orotic and uracil acid levels, normal levels of lysine and phenylalanine, and disorder of prothrombin time with international normalized ratio of 3.03. She was diagnosed with urea cycle disorders and managed to stop feeding; infused glucose (10 mg/kg/min), L-carnitine (100 mg/kg/day) and L-arginine (500 mg/kg/day) for 8 hours, however her consciousness did not improve and hemofiltration was performed. After 48 hours of management, she was alert absolutely and blood ammonia level became normal (89 µg/dL).

At the age of six, she was a development delay with DQ of 75% and genetic study was performed after the informed consent was obtained from her parents. Since 26-month-old to six-year-old, she had 8 recurrent episodes of hyperammonemia. Her elder brother was 7-year-old with unremarkable history.

Whole exome sequencing

Whole exome sequencing generated 55,118,778 reads with 8,181 Mbp (Table 2). By using BWA to map the reads to the human reference genome (GRCh37/hg19), 99.8% of total reads could be mapped accurately. Number of de-duplicated reads were 49,633,339, of which 79.6% were aligned to the target regions with a mean of 80.8x sequencing depth. We observed that 98.2%, 94.1% and 86.6% of the bases in target regions has a depth of coverage or greater than 10x, 20x and 30x, respectively. These data reflected the reliability of sequencing data and could be used for further analysis.

Table 2. Summary of the whole exome sequencing data.

	Parameters
Total reads	55,118,778
Total read bases (bp)	8,181,468,554
Target regions (bp)	60,456,963
Average throughput depth of target regions (X)	135.3
Initial mappable reads (mapped to human genome) and %	55,028,506 (99.8%)
Non-redundant reads and %	49,633,339 (90.1%)
On-target reads and %	39,516,444 (79.6%)
On-target bases (bp)	4,888,533,411
Mean depth of target regions (X)	80.8
% Coverage of target regions (> 10X)	98.2
% Coverage of target regions (> 20X)	94.1
% Coverage of target regions (> 30X)	86.6
Number of variants	108,276

Variant calling resulted 108,276 variants in the UD01 patient (Table 2). Forty-nine variants involved in 8 known related disease genes have been detected (Table 3). All these variants have mapping quality ≥ 60.0, indicating they have a high likelihood of being true. Among them, eight variants showed the minor allele frequency < 0.01, of which, 5 intron variants were eliminated. Three remained heterozygous variants included c.1048A>G in the *CPS1* gene, c.1065A>G in the *OTC* gene and c.656-5C>A in the *ASL* gene. Two variants c.1048A>G in the *CPS1* gene and c.656-5C>A in the *ASL* gene were reported as benign variants in the ClinVar database (Table 3). Only variant c.1065A>G in the *OTC* gene was selected as a disease causing (Table 3). The variant c.1065A>G had a read depth of 54, in which, the normal allele A accounted for 21 and the mutant allele G accounted for 33 (Fig. 1).

Table 3. Variants in known genes associated with urea cycle disorders.

No.	Gene	MQ	Zygosity	Effect	Exon	HGVS.c	HGVS.p	Allele Frequency	ClinVar
1	*CPS1*	60.0	HET	intron	1/38	c.3+12G>A	.	0.480831	Benign
2	*CPS1*	60.0	HET	insertion	2/39	c.15_16insTTC	p.Ile5_Lys6insPhe	0.477037	.
3	*CPS1*	60.0	HET	intron	7/38	c.640-183_640-182delAC	.	0.223642	.
4	***CPS1***	**60.0**	**HET**	**missense**	**11/39**	**c.1048A>G**	**p.Thr350Ala**	**.**	**Benign**
5	*CPS1*	60.0	HET	synonymous	11/39	c.1050C>T	p.Thr350Thr	0.591254	Benign
6	*CPS1*	60.0	HET	intron	19/38	c.2211-15G>T	.	0.136581	Benign
7	*CPS1*	60.0	HOM	intron	27/38	c.3355-79C>T	.	1.000000	.
8	*CPS1*	60.0	HET	intron	28/38	c.3423-29A>T	.	0.422724	.
9	*CPS1*	60.3	HOM	intron	30/38	c.3577-21delT	.	0.833067	.
10	*CPS1*	60.2	HOM	intron	33/38	c.3946-19delT	.	.	.
11	*CPS1*	62.6	HOM	intron	34/38	c.4021-110_4021-109insTG	.	.	.
12	*CPS1*	60.0	HOM	intron	35/38	c.4119+62A>G	.	0.951278	.
13	*CPS1*	60.0	HET	missense	37/39	c.4235C>A	p.Thr1412Asn	0.288538	Benign
14	*ASS1*	60.0	HET	intron	1/15	c.-67-1157A>G	.	0.864018	.
15	*ASS1*	60.0	HET	intron	1/15	c.-67-187T>C	.	0.799521	.
16	*ASS1*	60.0	HOM	intron	2/15	c.-5-172T>C	.	0.948682	.
17	*ASS1*	60.0	HET	intron	3/15	c.105+115G>A	.	0.579473	.
18	*ASS1*	60.0	HOM	intron	9/15	c.597+18A>G	.	0.792332	Benign
19	*ASS1*	60.0	HOM	intron	9/15	c.597+81A>G	.	0.92512	.
20	*ASS1*	60.0	HOM	intron	9/15	c.597+178G>A	.	0.606629	.
21	*ASS1*	60.0	HET	intron	11/15	c.773+184G>T	.	0.209864	.
22	*ASS1*	60.0	HET	intron	12/15	c.839-88A>T	.	.	.

23	*ASS1*	60.0	HET	intron	15/15	c.1194-210A>G	.	0.147165	.
24	*OTC*	60.0	HOM	intron	5/9	c.540+134G>A	.	0.632053	.
25	*OTC*	60.0	HOM	intron	5/9	c.541-63G>A	.	0.596556	.
26	*OTC*	61.1	HOM	intron	9/9	c.1005+125_1005+126insT	.	0.635762	.
27	***OTC***	*60.0*	***HET***	***Stop loss***	***10/10***	***c.1065A>G***	***p.Ter355TrpextTer14***	***.***	***.***
28	*ASL*	60.0	HET	intron	2/16	c.12+105C>T	.	0.531949	.
29	*ASL*	60.0	HET	intron	2/16	c.12+227C>G	.	0.528554	.
30	*ASL*	60.0	HET	intron	8/16	c.602+123T>C	.	0.75	.
31	*ASL*	60.1	HET	intron	8/16	c.603-90_603-89delGT	.	.	.
32	*ASL*	60.0	HET	intron	9/16	c.655+124A>G	.	0.676917	.
33	*ASL*	60.0	HET	intron	9/16	c.656-102T>C	.	0.750998	.
34	***ASL***	**60.0**	**HET**	**Splice site**	**9/16**	**c.656-5C>A**	**.**	**0.00179712**	**Benign**
35	*ASL*	60.0	HET	intron	13/16	c.978+30C>T	.	0.75	.
36	*ASL*	60.0	HET	intron	13/16	c.978+63C>T	.	0.576877	.
37	*ASL*	60.0	HET	intron	14/16	c.1063-57G>C	.	0.578275	.
38	*ARG1*	60.0	HOM	intron	7/7	c.826+73_826+74insA	.	0.979233	.
39	*NAGS*	60.0	HOM	intron	3/6	c.916-57T>C	.	0.947085	.
40	*NAGS*	60.0	HOM	intron	6/6	c.1451+9T>C	.	0.960264	.
41	*SLC25A13*	60.0	HOM	synonymous	12/18	c.1197A>G	p.Leu399Leu	0.409145	.
42	*SLC25A13*	60.0	HOM	intron	10/17	c.1022-84G>A	.	0.402756	.
43	*SLC25A13*	60.0	HET	intron	10/17	c.1021+123T>C	.	0.007987	.
44	*SLC25A13*	60.0	HOM	intron	9/17	c.933+162G>A	.	0.403355	.
45	*SLC25A13*	60.0	HOM	intron	9/17	c.933+131C>A	.	0.983027	.
46	*SLC25A13*	60.0	HOM	intron	8/17	c.848+28delT	.	.	.
47	*SLC25A15*	60.0	HET	missense	6/7	c.760A>T	p.Ile254Leu	0.322684	Benign
48	*SLC25A15*	60.0	HET	intron	6/6	c.781+75T>C	.	0.402556	.
49	*SLC25A15*	60.0	HET	intron	6/6	c.782-164A>G	.	0.511981	.

MQ, mapping quality. HET, heterozygous; HOM, homozygous.

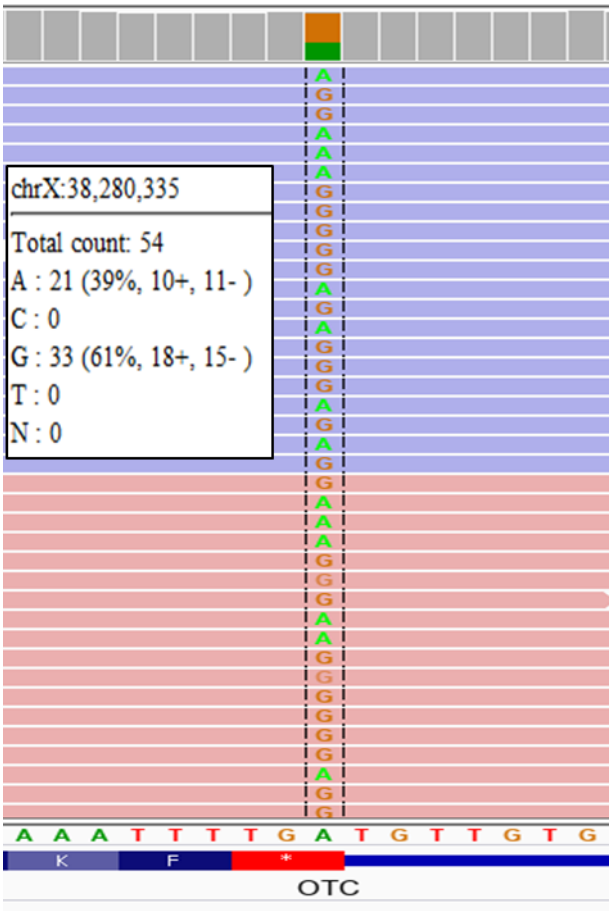

Figure 1. Integrative Genomic Viewer (IGV) illustrated the detection of the heterozygous variant c.1065A>G in the *OTC* gene using whole exome sequencing.

Sanger sequencing in the patient's family

Sanger sequencing revealed a heterozygous A>G at the c.1065 in exon 10 of the *OTC* gene in the patient (Fig. 2). This mutation causes a substitution of stop codon (TGA) to tryptophan (TGG) at position 355 (p.Ter355Trp) in the amino acid sequence (Fig. 2A). The next available stop codon obtains 42 nucleotides downstream of the original stop codon and elongation of fourteen amino acids in the C terminal region of OTC protein. Clinically unaffected family members were also screened and showed the wild type genotype (Fig. 2B). These results suggest that the heterozygous missense mutation c.1065A>G (p.Ter355TrpextTer14) in the patient is a de novo mutation.

Pathogenicity interpretation

The allele frequency of c.1065A>G variant was not found in the 1,000 Genome Database (http://browser.1000genomes.org) or 6,500 Exome database. No clinical information of this variant in ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar). This variant has been reported as a disease mutation in the Human Gene Mutation Database under an accession HGMD ID CM973235. The standards and guidelines of ACMG consist 5-tier variant classification, including pathogenic, likely pathogenic, variants of uncertain significance, likely benign and benign. Classification of a variant based on multiple lines of evidence, such as evidence from population, computational and predictive data, functional data, segregation data,

de novo data, allelic data and other database. Each pathogenic criterion is weighted as very strong (PVS1), strong (PS1-4), moderate (PM1-6) and supporting (PP1-5) (Richards *et al.*, 2015). The variant c.1065A>G evaluated containing 1 strong (PS2), 3 moderate (PM2, PM4 and PM5) and 1 support criteria (PP2) (Table 4). Therefore, c.1065A>G was conferred to a pathogenic variant according to the ACMG rules.

Taking together, clinical and biochemical results supported a diagnosis of the patient with UCDs, meanwhile, genetic molecular analyses indicated the patient carries a pathogenic variant in the *OTC* gene, leading a definitive diagnosis of patient with OTC deficiency-a disorder of UCDs.

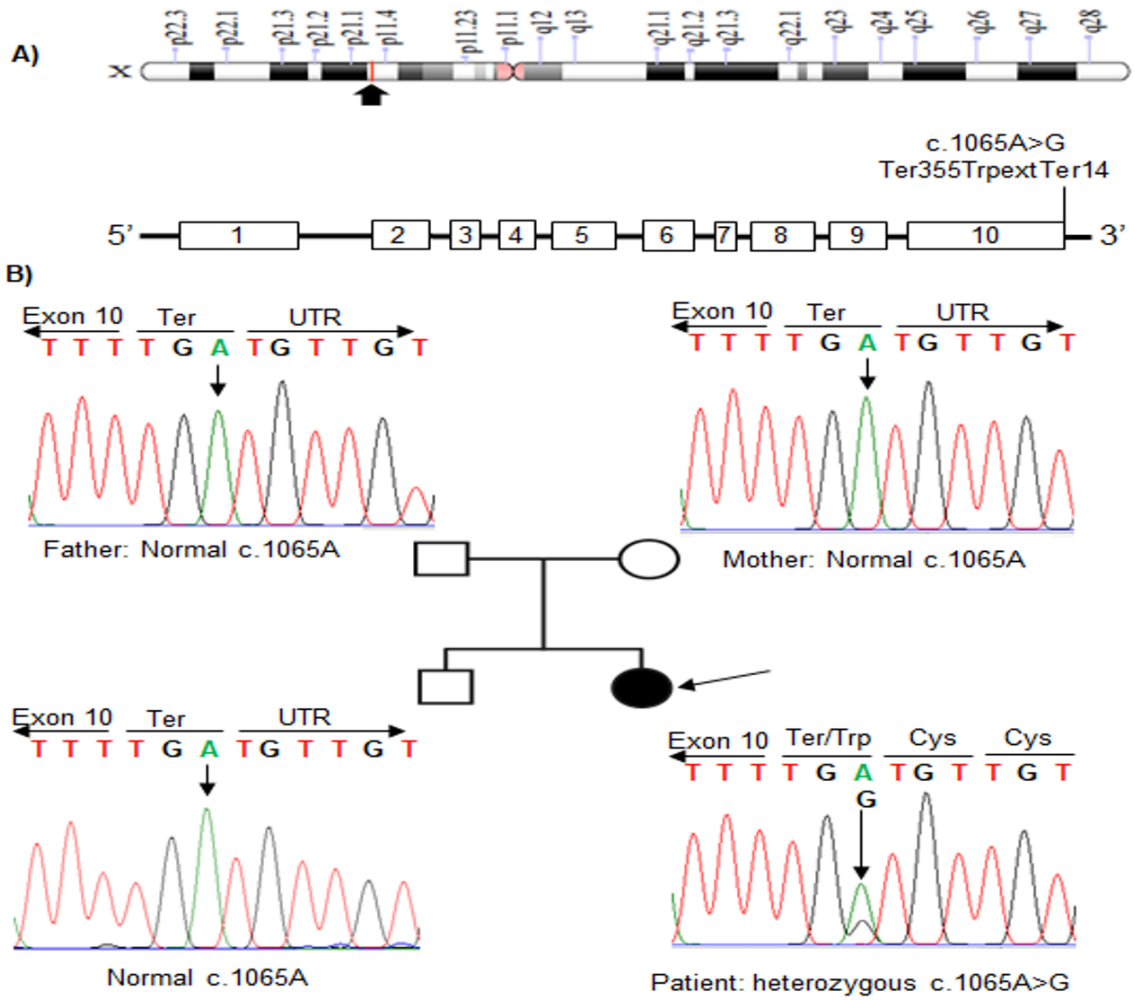

Figure 2. The mutation c.1065A>G in the *OTC* gene in the patient's family. Squares, males; circles, females; arrow, patient. (**A**) The location of the *OTC* gene on the X chromosome and c.1065A>G in the *OTC* gene. (**B**) Patient shown a heterozygous stop loss mutation c.1065A>G in exon 10. The non-stop mutation caused the replacement of termination codon to tryptophan and could lead to the elongation of fourteen amino acids in the C terminal region of OTC protein. The parents and brother were asymptomatic without mutation, indicating that the mutation is de novo in the patient.

Table 4. Classification of c.1065A>G (p.Ter355TrpextTer14) as a pathogenic variant according to American College of Medical Genetics and Genomics recommendations.

Evidence of pathogenicity	Category	Annotation
1 Strong	PS2	De novo in the patient with the disease and no family history.
3 Moderates	PM2	Absent in 1,000 Genomes Project, Exome Variant Project, Genome Aggregation Database
	PM4	Stop loss variant
	PM5	Previous study: c.1065A>T (p.Ter355CysextTer14) is pathogenic This study: c.1065A>G (p.Ter355TrpextTer14)
1 Support	PP2	Missense variant in a gene that has low rate benign missense variation and in which missense variants are a common mechanism of disease.

DISCUSSION

WES can detect single nucleotide variants, small deletions/insertions and canonical splicing variants in numerous genes. WES can provide a genetic diagnosis in the patients with monogenic disorders as well as genetic heterogeneous diseases with one single test (Haer-Wigman *et al.*, 2017). In addition, bioinformatics re-analysis with available WES data is sufficient when a new gene panel is implemented or using as an in-house reference data (Jalkh *et al.*, 2019; Salfati *et al.*, 2019). In this study, WES disclosed a heterozygous mutation c.1065A>G in exon 10 of the *OTC* gene in a patient priory diagnosed with urea cycle disorders. This mutation was not detected in 30 available whole exome sequencing data of ethnically match patients who are not related to a urea cycle disorder. Sanger sequencing revealed the mutation in the heterozygous state in the patient. However, it was noted that WES has limitations such as the possible missing information on large deletion, inversion, copy number variations, duplications or deep intronic regions.

Mutations in the *OTC* gene are known to cause OTC deficiency (OTCD, OMIM#311250) which is the most common error in the urea cycle disorders (Ah Mew *et al.*, 2017). The mutation c.1065A>G leads to the failing in the termination process in the translation stop codons. This stop loss mutation would create an open reading frame of 369 amino acids compared with 355 amino acids in the wild type OTC protein. Only a 2-year and 10-month-old male with late onset OTCD has been reported to associate with stop loss mutation in the *OTC* gene (Storkanova *et al.*, 2013). Particularly, the mutation was change of A to T (c.1065A>T), leading to a substitution of stop codon by cysteine (p.Ter355CysextTer14). As suggested by Hediger *et al.* (2018), our patient is also classified as late onset of OTC deficiency because she presented initial symptoms at 26 months of age. Such results indicate that stop loss mutations in the *OTC* gene lead to late onset OTC deficiency. Hamby *et al.* (2011) also noted that alternations in translation termination codons from 0-49 nucleotides downstream could not have much change in the clinical symptoms due to stable mRNAs which lead to translation of near normal protein. The stop loss mutation in the *OTC* gene only resulted an extra 42 nucleotides in the 3'-UTR, therefore, resulting a

late phenotype of OTC deficiency. The future studies of the elongated OTC protein on its localization, stability and functions which help fulfill the picture of the UCDs. The mutation c.1065A>G is de novo mutation in the patient that is in agreement with the finding of a high frequency of de novo mutations in the female patients with OTCD in the previous studies (Climent *et al.*, 1999; Tuchman *et al.*, 2002; Brassier *et al.*, 2015). The mutation c.1065A>G is classified as a pathogenic variant according the American College of Medical Genetics and Genomic recommendations. Therefore, this mutation is considered as causative mutation in the patient.

UCDs result in an excess ammonia, which acts as a neurotoxin, leading major symptoms like vomiting, lethargy and coma (Gropman *et al.*, 2007). Such symptoms were manifested in our patient. In addition, the patient carried atypical symptoms of UCDs like acute liver injury and right hemiplegia. Classification of acute liver injury in the patient is hepatocellular injury and liver dysfunction due to ALT>250 IU/L and INR>2, as described by Gallagher *et al.,* (2014). The ALT level in the patient (327 UI/L), who carries heterozygous amino acid replacement, is classified as severe. This genotype-phenotype is consistent with the finding of Caldovic *et al.,* (2015). However, the INR of our patient was 3.45, which is higher than mildly and moderately elevated INR present in female subjects with a late onset OTC deficiency in the study of Caldovic *et al.,* (2015). The right hemiplegia is typical of stroke-like attacks, therefore, we screened putative pathogenic variants in 13 genes, including *ADA2*, *ATP1A2*, *ATP1A3*, *CACNA1A*, *COL4A1*, *COL4A2*, *GLA*, *NOTCH3*, *OTC*, *POLG*, *SCN1A*, *SCN5A*, and *SLC2A1* genes, which have been listed in Hemiplegia/Stroke NGS panel of Fulgent (https://www.fulgentgenetics.com/hemiplegia-stroke). We only obtained a pathogenic potential heterozygous mutation c.1065A>G in the *OTC* gene. Our result supports the suggestion of Grauw *et al.* (1990), in which the urea cycle defects should be considered a metabolic cause of stroke-like attacks in the differential diagnosis of acute hemiplegia in childhood.

OTCD is an X-linked genetic condition, therefore, heterozygous females carry a normal allele and an abnormal allele. However, lyonization causes random inactivation of one of the two alleles and a mosaic pattern of expression in females, leading approximately 15% of heterozygous females showed the mild late-onset phenotypes of OCTD (Batshaw *et al.*, 1986). The patient in this study falls into this group. The symptoms of carrier females of OTCD may happen at any time in life. Indeed, after the first admission, the patient had 8 recurrent episodes of hyperammonemia. Therefore, the patient should be considered at risk for hyperammonemia episodes and need proper management and counseling.

CONCLUSION

In this study, WES technique led to the detection a causative mutation for OTC deficiency which is one of genetic factor underlining UCDs. The obtained result also expands the spectrum of phenotypes and genotypes in heterozygous female carriers with OTCD which are essential for productive genetic testing as well as genetic counseling.

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GIẢI TRÌNH TỰ TOÀN BỘ VÙNG MÃ HÓA CHẨN ĐOÁN XÁC ĐỊNH MỘT BỆNH NHÂN VIỆT NAM VỚI RỐI LOẠN CHU TRÌNH CHUYỂN HÓA URÊ KHỞI PHÁT MUỘN

Nguyễn Ngọc Lan[1,2], Nguyễn Ngọc Khánh[3], Vũ Chí Dũng[3], Nguyễn Thị Thu Hường[1,2], Nguyễn Huy Hoàng[1,2]

[1]*Học viện Khoa học và Công nghệ, Viện Hàn lâm Khoa học và Công nghệ Việt Nam*
[2]*Viện Nghiên cứu hệ gen, Viện Hàn lâm Khoa học và Công nghệ Việt Nam*
[3]*Trung tâm Sàng lọc Sơ sinh và Quản lý Bệnh hiếm, Khoa Nội tiết-Chuyển hóa-Di truyền, Bệnh viện Nhi Trung ương*

TÓM TẮT

Nghiên cứu của chúng tôi miêu tả về một trẻ nữ, nhập viện lần đầu lúc 26 tháng tuổi với các triệu chứng lâm sàng như nôn mửa, sốt, hôn mê và liệt bên phải. Kết quả xét nghiệm sinh hóa cho thấy bệnh nhân có nồng độ amoniac và lactate cao, tăng glutamine, tăng transaminase và rối loạn đông máu. Bệnh nhân được chẩn đoán sơ bộ mắc rối loạn chu trình chuyển hóa urê. Nhóm rối loạn này gây ra bởi đột biến trên tám gen điều hòa sinh tổng hợp các enzyme và đồng yếu tố trong chuyển hóa urê. Giải trình tự toàn bộ vùng mã hóa được áp dụng để sàng lọc các biến thể gây bệnh trong tám gen trên khi bệnh nhân được 6 tuổi. Kết quả phát hiện một đột biến mất bộ ba kết thúc c.1065A>G ở trạng thái dị hợp tử trên gen *OTC* là biến thể tiềm năng gây bệnh ở bệnh nhân. Đột biến c.1065A>G làm thay thế bộ ba kết thúc thành tryptophan, dẫn đến kéo dài thêm 14 amino acid trên protein ornithine transcarbamylase (OTC). Kết quả giải trình tự đoạn gen *OTC* chứa đột biến c.1065A>G bằng phương pháp Sanger trong gia đình bệnh nhân cho thấy đột biến này là đột biến mới phát sinh do không xuất

hiện ở bố mẹ và anh trai bệnh nhân. Đột biến c.1065A>G được đánh giá là gây bệnh theo hướng dẫn của Hiệp hội Bệnh học Phân tử và Hiệp hội Di truyền Y học Hoa Kỳ với 1 tiêu chí gây bệnh mức độ mạnh (PS2), 3 tiêu chí gây bệnh mức độ thường (PM2, PM4 and PM5) và 1 tiêu chí gây bệnh mức hỗ trợ (PP2). Bệnh thiếu hụt enzyme OTC là bệnh di truyền lặn trên nhiễm sắc thể X, tuy nhiên khoảng 15% phụ nữ mang biến thể dạng dị hợp tử có biểu hiện bệnh ở thể khởi phát muộn. Do dó, kết hợp kết quả lâm sàng với sinh hóa và di truyền, bệnh nhân được chuẩn đoán xác đinh là mặc bệnh thiếu hụt enzyme OTC, một loại bệnh trong rối loạn chu trình chuyển hóa urê.

Keywords: *c.1065A>G, đột biến mới phát sinh, OTC, p.Ter355TrpextTer14, rối loạn chu trình chuyển hóa u rê, bệnh nhân Việt Nam*