Molecular Genetics and Metabolism Reports 26 (2021) 100706

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

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

Short Communication

A deep intronic variant is a common cause of OTC deficiency in individuals with previously negative genetic testing

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ARTICLE INFO

Keywords:
Urea cycle disorder
Genetic testing
Intronic variant
Ornithine transcarbamylase deficiency
OTC c.540+265G>A

ABSTRACT

Pathogenic variants in non-coding regions of genes encoding enzymes or transporters of the urea cycle can lead to urea cycle disorders (UCDs). However, not all commercially available testing platforms interrogate these regions. Here, we used a gene panel based on massively parallel sequencing (MPS) in 10 individuals with clinical or pedigree-based evidence of a proximal UCD but without a molecular confirmation of the diagnosis. We identified causal variant(s) in 5 of 10 individuals, including in 3 of 7 individuals in whom prior molecular testing was unrevealing. We show that a deep-intronic pathogenic variant in *OTC*, c.540+265G>A, is an important cause of ornithine transcarbamylase (OTC) deficiency.

1. Introduction

Urea cycle disorders (UCDs) are inborn errors of metabolism characterized by impaired ureagenesis and an inability to dispose waste-nitrogen, resulting in accumulation of ammonia. The proximal UCDs, which include carbamoyl phosphate synthetase 1 deficiency (CPS1D, MIM# 237300), *N*-acetylglutamate synthase deficiency (NAGSD, MIM# 237310), and ornithine transcarbamylase deficiency (OTCD, MIM# 311250), are characterized by low levels of plasma citrulline and risk of life-threatening episodes of hyperammonemia. Although elevated orotic acid levels in the plasma or urine can be useful to distinguish OTCD from NAGSD and CPS1D, genetic testing is frequently necessary to make an accurate diagnosis and provide appropriate genetic counseling for families.

OTCD is the most common proximal UCD. Based on studies conducted by the Urea Cycle Disorders Consortium and data from newborn screening programs, the calculated prevalence of OTCD has been estimated to be 1 in 56,500 births [1]; the range for the estimated prevalence is between 1 in 14,000 and 1 in 77,000 births [2,3]. More than 400 different pathogenic variants in *OTC* have been described to cause OTCD; these include missense, nonsense, frameshift, indel, and splice site variants, as well as copy number variants in the genomic region of X chromosome harboring *OTC* [4,5].

However, nearly one-fifth of individuals in whom a diagnosis of OTCD is made based on reduced enzyme activity do not have an identifiable pathogenic variant in the 10 exons or exon-intron boundaries of *OTC* [6]; including assays to interrogate for copy-number variants can improve diagnostic yield to nearly 90% [5,7]. In individuals without identifiable pathogenic variants in the coding regions of *OTC*, causal variants may be located in the regulatory regions, such as promoters or enhancers, or deep intronic regions (*i.e.*, beyond ~20–30 base-pairs from the exon-intron boundary) [8–10].

Thus, in this study, we explored the utility of a massively-parallel-sequencing (MPS) based gene panel for molecular diagnosis of a proximal UCD in ten individuals with a clinical or biochemical diagnosis of a proximal UCD but without prior molecular confirmation. This gene panel interrogates coding regions, exon-intron boundaries, and recurrently observed deep intronic pathogenic variants in genes that encode enzymes and transporters of the urea cycle. Using this approach, we were able to provide a molecular diagnosis in 5 out of the 10 individuals and demonstrate that a deep intronic variant, c.540 + 265G > A in *OTC* is a common cause of OTCD in individuals with previously negative genetic testing.

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https://doi.org/10.1016/j.ymgmr.2020.100706
Received 27 December 2020; Accepted 28 December 2020

2. Methods

2.1. Study participants

This study was approved by the Institutional Review Board of Baylor College of Medicine, Houston, TX. Informed consent was obtained from all participants. Inclusion criteria were: age 2 months to 64 years, diagnosis of a proximal UCD based on the presence of one of the following criteria: documented hyperammonemic episode with plasma ammonia over 100 μmol/L, documented low plasma citrulline, or presence of family history. Exclusion criteria were: individuals with diagnosis of distal UCD or transporter defect; individuals with exome or genome sequencing completed; individuals with diagnosis of mitochondrial disorder, fatty acid oxidation disorder, organic acidemia, or other inborn error(s) of metabolism.

2.2. Genetic testing

Each participant had their blood drawn at a facility close to their place of residence which was then shipped to the testing facility. Testing was performed by the Clinical Laboratory Improvement Amendments-and College of American Pathologists-accredited laboratory at Baylor Genetics, Houston, TX, USA. The samples were interrogated using a panel which includes *ARG1* (NM_000045.3), *ASL* (NM_000048.3), *ASS1* (NM_000050.4), *CPS1* (NM_001875.4), *NAGS* (NM_153006.2), *OTC* (NM_000531.5), *SLC25A13* (NM_014251.2), and *SLC25A15* (NM_014252.3). The exonic regions of these genes were enriched using a capture-based method. The average coverage for the targeted bases is >100×; more than 95% targeted bases are covered at a depth > 20× and more than 85% are covered at a depth > 40×. Regions with known low coverage or genomic regions with sequence similarity to pseudogenes were interrogated by multiplex massively parallel sequencing or Sanger sequencing. Twenty base pairs from all exon-intron boundaries were interrogated. The extent and depth of intronic coverage varies; however, known deep intronic pathogenic variants such as c.540 + 265G > A, c.867 + 1126A > G, and c.1005 + 1091C > G in *OTC* were well covered by the panel. The panel is periodically updated to increase the diagnostic accuracy. The amplified products were sequenced using the Illumina HiSeq platform. Nucleotide numbering was based on the corresponding GenBank accession number; nucleotide 1 corresponds to the A of the start codon ATG. The transcripts listed here are used throughout this report unless otherwise noted.

3. Results

The characteristics of participants, clinical phenotype, laboratory evaluation, and previous testing results are summarized in Table 1.

Participants 1, 2 and 7 were found to have a deep intronic variant c.540 + 265G > A in *OTC*, which is 265 bp downstream of exon 5. This variant has been previously reported as a pathogenic variant in two individuals with OTCD [9,10]. Prior clinical testing in participants 1 and 2 and in the affected male infant of participant 7 had been reported as normal. Participants 3, 5, and 6 had no detectable pathogenic variants. This was concordant with prior sequencing of *OTC* for each of these three participants.

Participants 4 and 8 had not had any prior molecular testing and received specific diagnoses through this study. Participant 4 had a c.67C > T variant in *OTC* (p.R23*) that results in a premature stop codon. Participant 8 had two pathogenic variants in *CPS1*: c.1201G > C (p.G401R) [11] and c.2883_2895del13 (p.Y962Sfs*11) [12]. Although the phase of these variants was not discernible from this testing alone, given the clinical history of marked hyperammonemia, this individual was diagnosed with CPS1D. Interestingly, this patient also had mild orotic aciduria (2.55 g/L, upper limit of normal being 1.07 g/L), which is more typical of OTCD and not CPS1D.

Participant 9 had a son affected by OTCD with a confirmed hemizygous exon 2 deletion of *OTC*. Prior MPS of *OTC* which included targeted analysis for this familial variant was negative. The testing in this study was also reported as negative. However, an assessment of read counts over exon 2 demonstrated a decrease in the number of reads that could be consistent with mosaicism. However, this aberration did not reach the threshold for reporting.

4. Discussion

In this study, we describe the results of applying an MPS-based gene panel for the molecular diagnosis of proximal UCD. We made a specific diagnosis in 5 of 10 participants, including 3 of 7 participants with prior negative molecular testing.

Three participants with a phenotype consistent with OTCD had negative results on prior sequencing of *OTC*. However, in this study, they were found to harbor an intronic variant in *OTC*, c.540 + 265G > A. This deep intronic variant was previously identified by Ogino et al. (2007) [9] in a male child who died at 1 year of age from neurologic injury secondary to hyperammonemia. This variant was determined to create a new splice acceptor site, resulting in a 135-nucleotide insertion in the mature mRNA between exons 5 and 6. Previously, Caldovic et al. systematically catalogued variants in *OTC* and reported on genotype-phenotype correlations. In their report, only 53 of 417 pathogenic *OTC* variants were intronic [5]. Among these, only 3 were deep intronic variants that were more than 10 bp from the exon-intron boundary (c.1005 + 1091C > G, c.867 + 1126A > G, and c.540 + 265G > A).

Approximately 20% of participants with a clinical diagnosis of a proximal UCD do not have an identifiable pathogenic variant in the coding regions or exon-intron boundaries [6]. Jang et al. established that perhaps a quarter of remaining diagnoses are attributable to promoter or enhancer variants in *OTC* [8], and several studies support the role of intronic and splice-site variants [13–16]. The results of this study suggest c.540 + 265G > A is a common non-coding causal variant, especially in individuals in whom sequence variants or copy number variants have not been detected in coding regions. Our results support that all clinically-available OTC sequencing-based testing platforms should incorporate this deep intronic variant.

This study also illustrates some areas for improvement of panel-based testing. Participant 8 was diagnosed with CPS1D based on detection of two pathogenic variants in *CPS1*; however, this individual also had a mild orotic aciduria which is more typical of OTCD. Phasing of the variants could be of significant importance in such scenarios. When parental testing is not feasible, sequencing platforms with longer read lengths could utilize polymorphisms to phase variants [17]. Another area for improvement is copy-number variant identification. Participant 9 had a son with an intragenic deletion of *OTC*. She herself had a reduced number of reads in the region, but this did not meet threshold to be called as a deletion, illustrating the need for pipelines optimized to this task. Importantly, it is likely that more deep intronic and other non-coding causal variants causing UCDs will be uncovered; clinical diagnostic platforms need to be updated to interrogate these new variants.

5. Conclusion

The role of non-coding variants in OTCD and other proximal UCDs is well established, but traditional molecular testing does not routinely detect these variants. We show that the deep intronic variant c.540 + 265G > A in *OTC* is a common cause of OTCD in individuals with previous negative molecular testing. The results indicate the importance of updating genetic testing as new technologies and analytic methods become available.

Disclosures

E.S. is an employee of Baylor Genetics, an organization which offers

Table 1
Clinical characteristics of participants and molecular testing results.

ID	Sex	Age at enrollment	Ethnicity	Age at onset	Diagnosis of proximal UCD based on	Max recorded ammonia in μM*	Min recorded citrulline in μM (LLN)*	Orotic aciduria	Co-morbidities	Most recent medications	Family History	Prior negative testing	Diagnosis	Variant(s) found by gene panel used in this study
1	F	17y	West Indian	3y	HA episode (>1)	250	24 (16)	N/A	Seizures	NaPB, citrulline	−	*OTC* sequencing, *OTC* MLPA, aCGH	OTCD	*OTC* c.540 + 265G > A
2	F	51y	Caucasian	N/A	Low citrulline, no HA	N/A	N/A	N/A	TS, ASD, ID	GBP, citrulline	N/A	*OTC*, *NAGS*, *CPS1* sequencing, *OTC* aCGH	OTCD	*OTC* c.540 + 265G > A
3	F	5y	Caucasian	4 m	HA episode (>1)	107	N/A	N/A	Seizures	N/A	N/A	*OTC* sequencing	N/A	None
4	F	32y	Caucasian	N/A	HA episode (>1)	123	2 (6)	N/A	Anxiety	GBP, citrulline	−	None	OTCD	*OTC* c.67C > T(p.R23*)
5	F	54y	Native American, Hispanic	N/A	Affected son (participant 6)	N/A	6 (6)	N/A	T2DM	GBP, citrulline	+	*OTC* sequencing	N/A	None
6	M	26y	Native American, Hispanic	9y	HA episode (>1)	451	7 (6)	+	Kidney stone osteopenia, ASD	GBP, citrulline	N/A	*OTC* sequencing	N/A	None
7	F	41y	Caucasian	31y	Affected son and daughter	96	7 (16)	+	Hyperthyroidism	Citrulline	+	*OTC* sequencing in affected son	OTCD	*OTC* c.540 + 265G > A
8	F	41y	Native American, Hispanic	26y	HA episode (>1)	480	9.4 (6)	+	None	GBP, citrulline	−	None	CPS1D	*CPS1* c.1201G > C (p.6401R) / c.2883_2895del13 (p.Y962Sfs*11)
9	F	35y	Hispanic	34y	Affected son with exon 2 deletion in *OTC*	N/A	N/A	N/A	None	N/A	+	*OTC* sequencing and deletion/duplication	N/A	None
11	F	1y	Caucasian	1y	Low citrulline	91	6 (9)	N/A	Growth failure	N/A	N/A	58 gene panel by MPS that included *NAGS*, *CPS1*, *OTC*	N/A	None

Abbreviations: ASD, Autism Spectrum Disorder; aCGH, array Comparative Genomic Hybridization; CPS1D, Carbamoyl Phosphate Synthetase I Deficiency; GBP, Glycerol-phenylbutyrate; HA, Hyperammonemia; ID, Intellectual Disability; LLN, Lower Limit Normal; MLPA, Multiplex Ligation-dependent Probe Amplification; MPS, Massively Parallel Sequencing; NAGS, *N*-acetylglutamate Synthase; N/A, Not Available; NaPB, Sodium Phenylbutyrate; OTCD, Ornithine Transcarbamylase Deficiency; T2DM, Type 2 Diabetes Mellitus; TS, Tourette syndrome. Transcripts used: *CPS1* (NM_001875.4), *OTC* (NM_000531.5).

* The maximum recorded ammonia and the minimum recorded citrulline are based on records that were available for review and may not represent the maximum or minimum levels during the participant's lifetime.

commercial genetic testing. The remaining authors have no disclosures.

Acknowledgements

This study was funded by the National Urea Cycle Disorders Foundation and Recordati Rare Diseases, Inc. The project was supported in part by the Clinical Translational Core of the Baylor College of Medicine Intellectual and Developmental Disabilities Center (P50HD103555) from the Eunice Kennedy Shriver National Institute of Child Health and Human Development. The content is solely the responsibility of the authors and does not necessarily represent the official views of NIH. LCB is supported by a Burroughs Wellcome Career Award for Medical Scientists.

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