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Mutation Spectrum and Genotype–Phenotype Correlation in a Cohort of Argentine Patients with Ornithine Transcarbamylase Deficiency: A Single-Center Experience

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Abstract

X-linked ornithine transcarbamylase deficiency (OTCD) is the most common urea cycle disorder. Hemizygous males with complete deficiency manifest neonatal acute hyperammonemia, while those with partial deficiency have a late presentation. The symptomatology of heterozygotes depends on the inactivation pattern of X chromosome. Hyperammonemic episodes can cause neurological damage and are potentially fatal. Here, we match clinical, biochemical, and molecular findings with bioinformatics analyses to report genotype–phenotype correlations in 14 Argentine patients with OTCD from 11 unrelated families: 4 hemizygotes with neonatal onset (complete *OTC* gene deletion, 533C > T, c.540+1G > A, c.697delG); 4 hemizygotes with late onset (c.216+1G > A, c.386G > A, c.622G > A, c.829C > T); and 6 symptomatic heterozygotes (complete *OTC* gene deletion, c.533C > T, c.452T > G, c.540+1G > A, dupE1-9/delE10). Three of these mutations were previously unreported: c.540+1G > A, c.697delG, and dup1-9/del10. Our data highlight the relevance of combining molecular and bioinformatics analyses for accurate diagnosis and outcome prediction in suspected patients with OTCD and the importance of carrier testing for effective genetic counseling.

Keywords

urea cycle disorders, ornithine transcarbamylase deficiency, OTC deficiency, OTC mutations, hyperammonemia

Introduction

Ornithine transcarbamylase (OTC; EC 2.1.3.3) catalyzes the formation of citrulline and inorganic phosphate from carbamyl phosphate and ornithine in the urea cycle.[1] The human *OTC* gene is located on the short arm of the X chromosome (Xp11.4; https://www.omim.org/entry/300461). The OTC enzyme is expressed almost exclusively in the liver and intestinal mucosa, but full activity of the urea cycle occurs only in the liver.

Ornithine transcarbamylase deficiency (OTCD; MIM#311250), an X-linked disease, is the most frequent hereditary defect of ureagenesis with an estimated incidence of 1:56 500 newborns per year in the United States.[2] Hemizygotes with complete OTCD present acute hyperammonemia in the first week of life, while males with partial deficiency have a late presentation, sometimes in adulthood.[3] The severity of OTCD symptoms in women depends mainly on the pattern of X inactivation in the liver, and it ranges from asymptomatic to almost as severe as in affected males.[4]

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In general, the clinical signs and symptoms are caused by the toxic effects of hyperammonemia and glutamine in central nervous system. Patients may present lethargy, vomiting, neurological, and behavioral abnormalities and in the most severe cases, cerebral edema, coma, and death. Biochemical abnormalities include hyperammonemia, increased plasma glutamine and decreased or absent citrulline, and increased urinary orotic acid, although these last 2 findings are not always manifested or detected. Carrier identification is very important as it allows timely genetic counseling, thus minimizing the risk of potentially fatal hyperammonemic crisis.[5]

Therapeutic approaches to urea cycle defects, including OTCD, rely on ammonia scavengers (sodium benzoate and sodium phenylbutyrate) in combination with nutritional therapy (low-protein diet, arginine, or citrulline supplementation). Meanwhile, hemodialysis or peritoneal dialysis is used in cases of severe hyperammonemia (>500 μmol/L in neonates and children; >200 μmol/L in adults). In some patients, liver transplantation is also considered.[6]

To date, a total of 504 disease-causing mutations in the *OTC* gene have been reported in the literature[7] or registered in the Human Gene Mutation Database (www.hgmd.org). Mutations that block the enzyme's catalytic action are invariably associated with acute neonatal hyperammonemia, while those that allow residual activity may manifest over a wide age range, from the neonatal period to late adulthood. Phenotypic heterogeneity is observed for mutations that preserve residual activity of the enzyme, even among members of the same family; the cause of this is still not well understood and seems to be due to a combination of genetic factors and environmental variables such as infections or other causes of catabolic stress. Bioinformatic analysis of mutations using in silico strategies is used in OTCD and other genetic diseases to predict severity.

In the current study, we matched clinical data with molecular, biochemical, and bioinformatic analyses to perform a genotype–phenotype correlation analysis of OTC mutations found in patients with OTCD from Argentina diagnosed at a single center (CEMECO, Children's Hospital of Córdoba).

Patients and Methods

We performed a descriptive, comparative, and correlational study of patients diagnosed with OTCD in CEMECO, Children's Hospital of the Santísima Trinidad de Córdoba, between 1990 and 2017.

Biochemical analyses included determination of amino acids[8] and urinary orotic acid[9] by high-performance liquid chromatography and measurement of plasma ammonia by spectrophotometry (Ammonium Kit, Randox Laboratories LTD, United Kingdom).

The *OTC* gene mutations were identified using polymerase chain reaction amplification, classical sequencing (Sanger), and multiplex ligation-dependent probe amplification.[10,11] Mutations were identified by comparison with the GenBank reference sequence for human *OTC* (GenBank entries: NG_008471.1, NP 000522.3, NM 000531.5, NC 000023.11).

Missense mutations were analyzed using different computational algorithms: CLUSTALW2 (http://www.clustal.org/clustal2/), SIFT (http://blocks.fhcrc.org/sift/SIFT.html), Polyphen2(http://genetics.bwh.harvard.edu/pph/), PoPMuSiC (http://babylone.ulb.ac.be/popmusic/), and SIFT Indel (http://siftdna.org/www/SIFT_indels2.html).

The study was approved by the Institutional Committee on Ethics of Research in Children and Adult Health. Informed consent was obtained prior to collection of biological samples.

Results and Discussion

A total of 14 patients with OTCD belonging to 11 unrelated families in Argentina were identified (Table 1). The phenotypic distribution was 28.5% (4/14) hemizygotes with neonatal-onset disease, 28.6% (4/14) hemizygotes with late-onset disease, and 42.8% (6/14) symptomatic heterozygous females. The age of onset was between 48 hours and 10 years (mean: 29.9 months).

Clinical manifestations varied between cases, with patients presenting 2 or more of the following signs and symptoms either in the acute or in the chronic presentation: drowsiness, lethargy, encephalopathy, convulsions, vomiting, behavior alterations, irritability, developmental delay, recurrent dysfunction of the liver or cholestasis, and coma. Compared to other population groups, our patients differed in their phenotypic distribution due to a greater number of symptomatic heterozygotes.[7,12,13] Regarding disease progression, all 4 OTCD males with the neonatal form died, while 3 of 4 males with late onset survived. Neonatal forms were not diagnosed in symptomatic females. Among these, and due to the severity of the hyperammonemic crises, 2 of 6 patients died and another one fell into a vegetative state. Previous reports have shown a survival of 80% to 90% in symptomatic heterozygotes and a generally favorable prognosis.[14-16] In those patients who survived the hyperammonemic crisis, therapy consisted of low-protein diet, treatment with ammonia scavengers, and essential amino acid supplementation according to international recommendations and patient characteristics.

Biochemical findings (pooled data) for our patients with OTCD at disease onset included mean plasma ammonia: 547 μmol/L (range: 70-1500 μmol/L, normal range: <50 μmol/L), plasma glutamine: 800 μmol/L (range: 500-1300 μmol/L; normal range: 450-750 μmol/L), citrulline, and arginine within normal or decreased ranges and urinary orotic acid excretion in 13 of 14 cases.

Definitive diagnosis was obtained through genetic analysis, which revealed 10 different *OTC* gene mutations in the patient series, 3 of them previously unreported (Table 1). The identified mutations were uniquely found in each family, except for patients 9 and 10, who shared the same mutation although there was no known relationship between them.

The complete deletion of the *OTC* gene identified in family I results in total loss of OTC protein function in the hemizygous patient. The clinical presentation in the female carriers of this family was, as expected, extremely variable, given the X-linked inheritance of OTCD.[4]

Table 1. *OTC* Gene Mutations, Clinical Presentation, and Outcomes Identified in 14 Argentine Patients with OTCD.

Family	Patient	Gender	Presentation	Neurological Involvement	Nucleotide Change	Protein/Mutation Effect	Clinical Outcome/Remarks
I	1	Female	Late	Yes	–	Complete deletion	Decompensation during pregnancy and death
I	2	Female	Late	Yes	–	Complete deletion	Stable
I	3	Male	Neonatal	Yes	–	Complete deletion	12 son, death in neonatal period
II	4	Male	Late	No	c.216+1G>A	Splice site errors	Stable
III	5	Male	Late	Yes	c.386G>A	p.Arg129His /splicing defects	Stable
IV	6	Female	Late	Yes	c.452T>G	p.Leu151Arg	Stable
V	7	Female	Late	Yes	c.533C>T	p.Thr178Met	Persistent vegetative state
V	8	Male	Neonatal	Yes	c.533C>T	p.Thr178Met	Death in neonatal period
VI	9	Female	Late	Yes	c.540+1G>A[a]	Splice site errors	Stable
VII	10	Male	Neonatal	Yes	c.540+1G>A[a]	Splice site errors	Death at 6 m, 2 brothers died in neonatal period
VIII	11	Male	Late	–	c.622G>A	p.Ala208Thr	Stable
IX	12	Male	Neonatal	Yes	c.697delG[a]	p.Leu232Leufs*14	Death in neonatal period
X	13	Male	Late	Yes	c.829C>T	p.Arg277Trp	Death upon first disease crisis (10 years old)
XI	14	Female	Late	Yes	–	Exons 1-9 duplication; exon 10 deletion[a,b]	Death at 2 years old

[a]Novel mutation.
[b]De novo mutation.

The novel mutation c.697delG (family IX) results in a change in the reading frame, producing a premature stop codon at amino acid 14, thereby truncating the protein in the position 245, near its carboxyl terminus. Bioinformatic analysis by SIFT Indel (SIFT 4G Annotator version 2.3) suggests that the transcript is therefore exposed to nonsense-mediated decay, which makes this mutation equivalent to complete gene deletion because the messenger RNA (mRNA) is not translated. The program assigns a classification of 12 to this change, score obtained according to several factors such as the fraction of conserved bases of DNA, resulting amino acids, the maximum relative indel location, and its distance to the union with the exon, which predicts a highly harmful outcome (confidence of 0.858).[17]

The chromosomal rearrangement dup1-9/del10 described in patient 14 has not been reported. It entails a duplication in heterozygosis of the region between exons 1 and 9 plus a deletion of the contiguous region that includes exon 10 until the end of the gene, extending further into a region of the adjacent gene *TSPAN7*, whose disruption is associated with X-linked mental retardation. This alteration was demonstrated to be spontaneous. Even with specific treatment for OTCD, this girl had frequent decompensations due to infectious processes and developed a severe crisis that led to cerebral edema, cardiorespiratory arrest, and death.

Five previously described single-base substitutions in the *OTC* gene (c.386G>A; c.452T> G; c.533C>T; c.622G>A; c.829C>T)[18] were also found in our patients (Table 1). The first is located on the last nucleotide of exon 4 and was classified initially as missense (p.Arg129His), although subsequent studies revealed that it partially affected the splicing process[19]; this mutation, identified in our study in a male with late disease onset, had been reported in both males and symptomatic females with severe or mild presentations. The other changes, all missense mutations, were analyzed using 3 computational algorithms (SIFT, Polyphen, and PoPMuSiC). Given the difficulty of assaying OTC enzymatic activity in patients, in silico studies are considered the most feasible and reliable way to analyze the severity of the mutations. After analyzing all the in silico results (Table 2), the c.533C>T mutation can be considered the most damaging of the series due to its strict conservation in all the analyzed species and the scores obtained in all the computational analyses. Moreover, it correlates precisely with the clinical manifestation of our patient, a seriously affected symptomatic carrier with a sibling that died perinatally.

According to previous reports and our own experience, mutations that completely abrogate the processing of OTC mRNA or the protein's catalytic activity usually manifest with acute neonatal-onset hyperammonemia. That would be the case of the novel splicing mutation c.540+1G>A, to which we attribute the severe neonatal form observed in patient 10 and his siblings, who died at birth. Interestingly, the same mutation was also identified in patient 9, a seemingly unrelated heterozygous female with late disease onset. On the other hand, patient 4 (a male with a late form) also has a splice-site mutation (c.216+1G>A) but in this case it affects the genesis and processing of mature mRNA, in agreement with the original description of this mutation.[18] Individuals with sequence variants that allow residual OTC activity may develop hyperammonemia at any time in life, while others remain asymptomatic. In patients with late-onset OTCD, acute hyperammonemia can be triggered by factors such as fasting status, infections, invasive medical procedures, or other environmental

Table 2. Bioinformatic Analysis of Missense Mutations in the *OTC* Gene.

		Polyphen[a]		SIFT[b]		PoPMuSiC[c]			
Patient	Mutation	Score	Prediction	Score	Prediction	AccSte (%)	ΔΔG (kcal/mol)	Residual Preservation[d]	Remarks
6	c.452T>G p.Leu151Arg	0.995	Probably damaging	0	Affects protein function	1.55	2.29	Preserved in 10 species	Symptomatic heterozygous female
7/8	c.533C>T p.Thr178Met	0.999	Probably damaging	0	Affects protein function	1.97	0.14	Preserved in 11 species	Symptomatic heterozygous female/male, early onset
10	c.622G>A p.Ala208Thr	0.996	Probably damaging	0	Affects protein function	1.69	0.24	Preserved in 7 species	Male, late onset
11	c.829C>T p.Arg277Trp	0.997	Probably damaging	0	Affects protein function	1.95	1.34	Preserved in 11 species	Male, late onset

[a]*Polymorphism Phenotyping*: approach based on sequence and protein structure, assigns a score of 0 to benign variations, 0.5 for possibly damaging, and 1.0 for damaging.
[b]*Sorting Intolerant from Tolerant*: approach based on evolution, the result of the analysis is a binary classification of 1 "tolerated" and 0 "not tolerated".
[c]*Prediction of Protein Mutant Stability Changes:* based on thermodynamic stability, characterized by free energy of folding ΔG, and the solubility of variants. AccSte %: percentage of accessibility to the solvent of the mutated protein compared to the normal one; ΔΔG: Relative folding free energy variation expressed in kcal/mol.
[d]According to amino acid sequence alignments using CLUSTALW2, the alignment for *OTC* gene was performed in 11 orthologous sequences.

triggers resulting in increased protein catabolism and ammonia production.[7]

In conclusion, we discussed the clinical presentations, biochemical findings, disease outcomes, and mutation spectrum of a cohort of patients with OTCD from Argentina evaluated at a single center. Genotype identification allowed exact diagnosis and highlighted the importance of carrier testing for adequate genetic counseling. Among the 10 genetic defects described in our patient series, 3 are, to the best of our knowledge, novel and had not been reported until now. The identified mutations were validated with bioinformatic tools correlating genotype–phenotype, which allowed us to accurately predict, in most cases, disease presentation and progression.

As the most common genetic defect within the spectrum of urea cycle disorders, suspicion of OTCD, early confirmatory diagnosis, and prompt initiation of treatment are essential to avoid hyperammonemic crises that could cause irreversible neurological damage and even death.

Declaration of Conflicting Interests

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