Comprehensive Mutation Scanning in the Ornathine Transcarbamylase Gene using Hi-Res Melting™ with LCGreen® Plus

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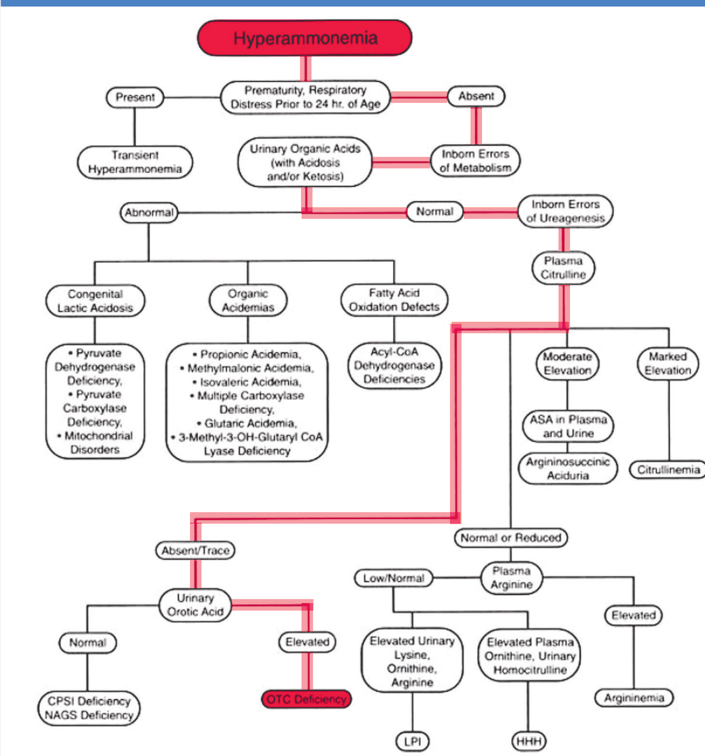
Introduction

Ornithine transcarbabmylase (OTC, OMIM #311250) deficiency is an X-linked (Xp21.1) inborn error of metabolism involving the urea cycle. OTC deficiency is the most common urea cycle defect (UCD). The algorithm for differential diagnosis of UCD's is complex and labor intensive (Figure 1). Early identification and treatment is critical for a positive prognosis. Pathologic gene mutations in OTC predominantly occur de novo, thus molecular analysis for a "common" mutation is impractical. Herein is presented a rapid method of comprehensively scanning the coding and repsective 5' and 3' splice site regions of the OTC gene for mutations.

Methods

Molecular analysis of OTC is useful as an initial reflex to clinical presentation of hyperammonemia. Hi-Res Melting analysis is based on the detection of heteroduplex molecules and combines the use of a new dsDNA binding dye with saturating characteristics (LCGreen Plus) and the LightScanner™ instrument. The dye is included in the PCR reaction and incorporated into newly synthesized DNA during PCR. Following amplification, the samples are transferred directly to the LightScanner for fluorescence monitoring during thermal denaturation (melting). The resulting melting profiles of each sample are then compared to the melting profile of a sequence confirmed wild type sample for the region being queried. Samples with melting profiles that deviate from the profile of the wild type are recovered and sequenced to define the observed variant (Figure 2, panels A-J)

Figure 1. UCD Differential Diagnosis Algorithm



Results

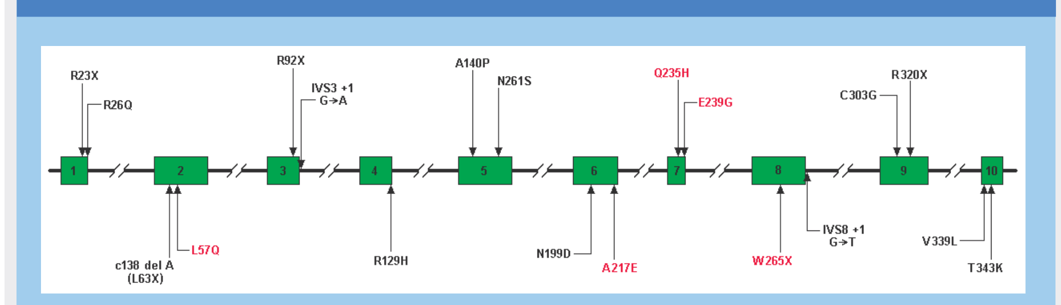
Hi-Res Melting was used to scan the coding and splice regions of the OTC gene in 20 OTC deficient patients. PCR primers were designed to amplify the exons and a minimum of 12 bases of donor/acceptor splice site sequence. A single PCR product was designed to amplify each of the 10 OTC exons in a 96 well microtiter plate. A common amplification protocol was used. Because OTC is X-linked, male samples were co-amplified with a known wild type DNA from a male control, to force heteroduplex formation. Fragment size ranged from 146-266 bp. Nineteen different mutations (14 reported, 5 novel) were identified (Figure 3, novel mutations shown in red).

Figure 2. Melting Profiles Observed in 20 OTC Patients

2.A OTC Exon 1: ♂OTC 1 R26Q; ♀OTC 2 R23X
2.B OTC Exon 2: ♂OTC 4 L57Q; ♀OTC 3 c138 del A (L63X)
2.C OTC Exon 3: OTC 5 ♂ IVS3+1G>A; ♀OTC 6 R92X
2.D OTC Exon 4: polymorphism IVS3 -8 T>A; ♀OTC 7 R129H; ♀OTC 8 R129H
2.E OTC Exon 5: ♂OTC 10 A140P; ♀OTC 9 N161S
2.F OTC Exon 6: OTC 12 ♀ A217E; ♀OTC 11 N199D
2.G OTC Exon 7: ♀OTC 13 Q235H; ♂OTC 14 E239G
2.H OTC Exon 8: ♂OTC 16 IVS8+1 G>T; ♂OTC 15 W265X
2.I OTC Exon 9: ♂OTC 17 R320X; ♂OTC 18 C303G
2.J OTC Exon 10: ♂OTC 20 V339L; ♀OTC 19 T343K

(Axes: Fluorescence vs. Temperature)

Figure 3. OTC Gene Mutations Identified by Melting



Conclusion

Hi-Res Melting can be used to rapidly scan for pathologic mutations in the OTC gene. Comprehensive molecular analysis can significantly decrease the time to differential diagnosis of OTC deficiency.

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