Beyond the NMD Boundary: Characterizing the Phenotypes of C-terminal *CDH1* Mutations

Kate Krempely, MS, CGC, Carin Espenschied, MS, CGC, Ira Lu, MA, MS, CGC, Adam Chamberlin, PhD, Rachid Karam, MD, PhD

The nonsense-mediated mRNA decay (NMD) pathway is an mRNA surveillance system that degrades transcripts containing premature termination codons (PTC) located approximately 50 nucleotides upstream of the last exon-exon junction. Transcripts containing C-terminal PTCs located downstream the 50 nucleotide "NMD boundary" are not targeted for decay, potentially coding for truncated proteins retaining partial function. Therefore, one must be cautious when interpreting C-terminal truncating variants.

CDH1 truncating mutations cause Hereditary Diffuse Gastric & Lobular Breast Cancer (OMIM 137215). *CDH1* transcripts containing C-terminal PTCs downstream the NMD boundary have been shown experimentally to escape NMD; consequently, breast and gastric cancer risks associated with these truncations should be carefully evaluated. The aim of this study was to characterize and report *CDH1* C-terminal truncating alterations identified downstream of the NMD boundary in a large diagnostic laboratory cohort of ~400,000 *CDH1* alleles.

Using molecular evidence, structural information, and clinical data, we characterized the most C-terminal *CDH1* pathogenic and likely pathogenic alterations in our cohort. The most C-terminal truncation we identified was p.E836* (c.2506G>T); it was confirmed *de novo* in an individual diagnosed with lobular breast cancer at 54 years of age who did not meet the International Gastric Cancer Linkage Consortium diagnostic criteria (IGCLC negative). Other truncations identified in this region include c.2398delC (p.R800Afs*16), c.2430delT (p.F810Lfs*6), and c.2490dupG (p.L831Afs*4), all seen in families meeting IGCLC criteria. We also identified, in an IGCLC negative family, a nonstop variant of unknown significance predicted to extend the protein by 29 amino acids (c.2647T>C (p.*883Qext*29)).

Due to the uncertainty surrounding the functional impact of these alterations, clinical and molecular evidence proved essential in determining their pathogenicity. Characterization of the most C-terminal *CDH1* pathogenic truncation increases our understanding of the sequences located beyond the NMD boundary, resulting in improved classification of alterations in this clinically actionable gene.