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

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BACKGROUND

- The nonsense-mediated mRNA decay (NMD) pathway is a mRNA surveillance system that degrades transcripts containing premature termination codons (PTCs) located approximately 55 nucleotides upstream of the last exon-exon junction
- Transcripts containing C-terminal PTCs located downstream of the 55 nucleotide "NMD boundary" are not targeted for decay, potentially coding for truncated proteins retaining partial function
- *CDH1* mutations cause hereditary diffuse gastric cancer (HDGC) and lobular breast cancer, with lifetime risks up to 83% and 52%, respectively
- *CDH1* transcripts containing C-terminal PTCs downstream of the NMD boundary have been show 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

FIGURE 1: 2015 Internal Gastric Cancer Linkage Consortium (IGCLC) Guidelines

Established diagnostic criteria
Families with two or more gastric cancer diagnoses regardless of age in 1st and 2nd degree relatives, one confirmed DGC
One case of DGC diagnosed before age 40
Families with diagnoses of both DGC and LBC, one diagnosis before age 50
When genetic testing should be considered
Personal history of bilateral LBC before age 50 or family history of two or more cases of LBC before age 50
Patients with cleft lip/palate and DGC
Presence of in situ signet ring cells and/or pagetoid spread of signet ring cells in the stomach

DGC: diffuse gastric cancer; LBC: lobular breast cancer

METHODS

- Our laboratory database was queried for all *CDH1* alterations that resulted in premature termination codons past the NMD boundary
- All patients were tested between May 2013 and July 2017 and underwent analysis of the *CDH1* gene via multigene panel testing, single gene analysis, or targeted testing for a familial alteration
- Demographic, clinical history, and cancer family history were collected from test requisition forms, clinic notes, pathology reports, and pedigrees provided by ordering clinicians at the time of testing
- A 5-tier variant classification system was applied to all alterations, and variants were classified as: Pathogenic Mutation, Variant, Likely pathogenic (VLP), Variant of Unknown Significance (VUS), Variant, Likely Benign (VLB), or Benign

RESULTS

- We identified five C-terminal *CDH1* pathogenic or likely pathogenic alterations in unrelated families that resulted in PTCs and a variant of unknown significance that was predicted to extend the protein by 29 amino acids (TABLE 1)
- The most C-terminal truncation we identified was c.2506G>T (p.E836*); it was identified in an individual diagnosed with lobular breast cancer at age 54 who did not meet the IGCLC criteria. Subsequent parental testing for the p.E368* alteration was negative and additional analysis confirmed the familial relationships and the *de novo* occurrence in the proband (FIGURE 2)
- We also identified, in an IGCLC negative family, an extension variant of unknown significance predicted to extend the protein by 29 amino acids (FIGURE 3)

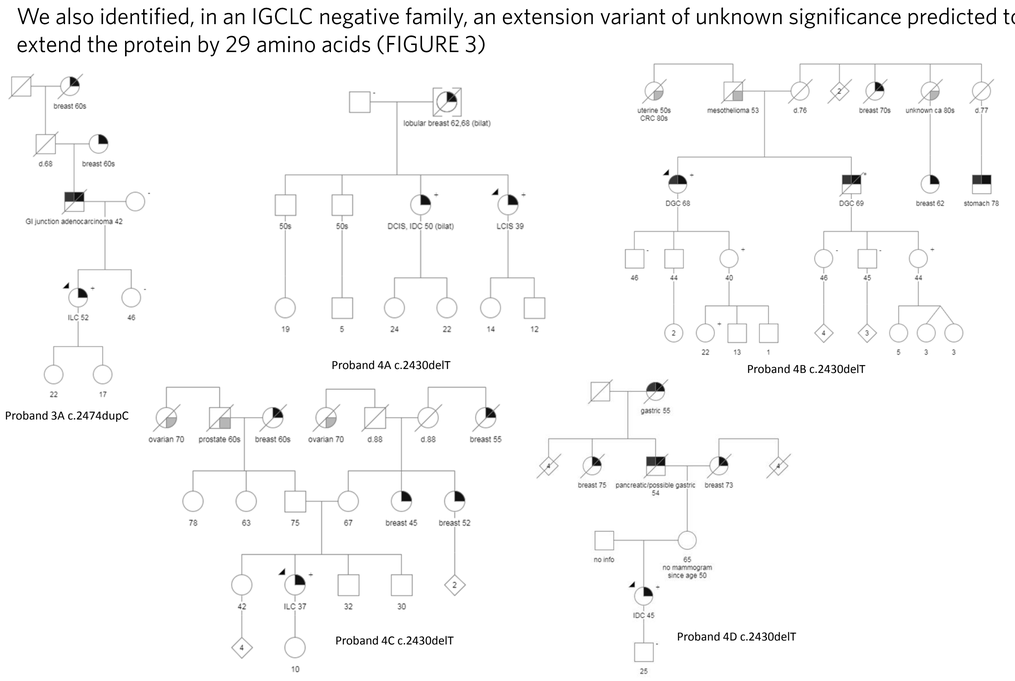


TABLE 1: *CDH1* C-Terminal Alterations and Phenotype Descriptions

CDH1 alteration (NM_004360.3)	PTC location	Ambry Classification	Proband	Meets IGCLC criteria	Proband history	Cancer family history and familial testing
c. 2506G>T (p.E836*) confirmed *de novo*	p.836	VLP	1A	No	ILC, 54	Mother, father *CDH1*-, UA None
c.2490dupG (p.L831Afs*4)	p.834	VLP	2A	Yes	DGC, 31	None
c.2474dupC (p.P826Afs*3)	p.828	VLP	3A	No	ILC, 52	Father GI junction adenocarcinoma, 42 Pat GM breast, 60s Pat GGM breast, 60s Mother, sister *CDH1*-, UA
c.2430delT (p.F810Lfs*6)	p.815	Mutation	4A	No	LCIS, 39	Sister *CDH1*+, bilat breast (DCIS/IDC), 50 Father, *CDH1*-, UA Mother bilat lobular breast, 62, 68
c.2430delT (p.F810Lfs*6)	p.815	Mutation	4B	Yes	DGC, 68	Daughter *CDH1*+, UA, 40 Son *CDH1*-, UA Brother DGC, 69 (*obligate carrier) Niece *CDH1*+, UA, 44 Niece *CDH1*-, UA Nephew *CDH1*-, UA Granddaughter *CDH1*+, UA, 22 Mat aunt breast, 70s Mat cousin x 2 breast, gastric 62, 78 Father mesothelioma, 53 Pat aunt uterine, 50s, CRC, 80s
c.2430delT (p.F810Lfs*6)	p.815	Mutation	4C	No	ILC, 37	Mat aunt x 2 breast, 45, 52 Mat great aunt breast, 55 Mat great aunt ovarian, 70 Pat GM breast, 60s Pat GF prostate, 60s Pat great aunt ovarian, 70s
c.2430delT (p.F810Lfs*6)	p.815	Mutation	4D	No	IDC, 45	No pat family history available Son *CDH1*-, UA Mat GM breast, 73 Mat GF pancreatic (possible gastric?), 54 Mat GGM gastric, 40s Mat great aunt breast, 75
c.2398delC (p.R800Afs*16)	p.815	Mutation	5A	Unsure, but seen in multiple DGC/LBC families in literature	No history provided, 20s	Mother *CDH1*+ No history provided
c.2647T>C (p.*883Qext*29)	p.912	VUS	6A	No	None, 55	Sister breast, 40s Mother breast, 60s

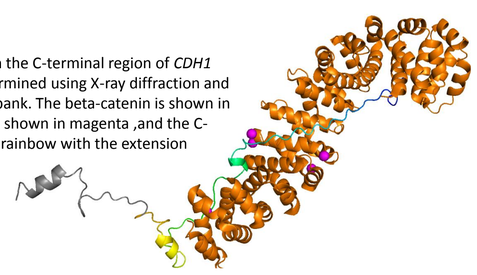
Bilat: bilateral; CRC: colorectal cancer; DCIS: ductal carcinoma in situ; DGC: diffuse gastric cancer; GI: gastrointestinal; GF: grandfather; GGF: great grandfather; GM: grandmother; GGM: great grandmother; IDC: invasive ductal carcinoma; LBC: lobular breast cancer; ILC: invasive lobular carcinoma ; LCIS: lobular carcinoma in situ; mat: maternal; pat: paternal; UA: unaffected

FIGURE 2: *De novo* Confirmation of c.2506G>T (p.E836*)

Relation	Amel. 204-210	FGA 262-290	TPOX 375-411	D8S1179 186-222	VWA 266-306	D18S51 326-358	CSF1PO 369-405	D13S317 196-232	D16S539 270-310	D7S820 376-412	Result
father	205.08	275.89	385.66	191.34	291.14	331.23	388.08	198.38	283.87	394.6	Concordant
	210.88	283.68	389.61	207.98	295.02	346.8		206.65	299.16	398.54	
mother	205.05	264.08	385.69	203.65	291.1	331.19	380.37	198.38	283.9	390.76	Concordant
		279.87	397.27	207.95	294.96	357.9	384.21		291.57	394.78	
proband	205.15	275.93	385.66	191.35	291.16	331.26	384.22	198.31	291.52	394.75	
		279.89	397.27	208	295		388.04		299.25		

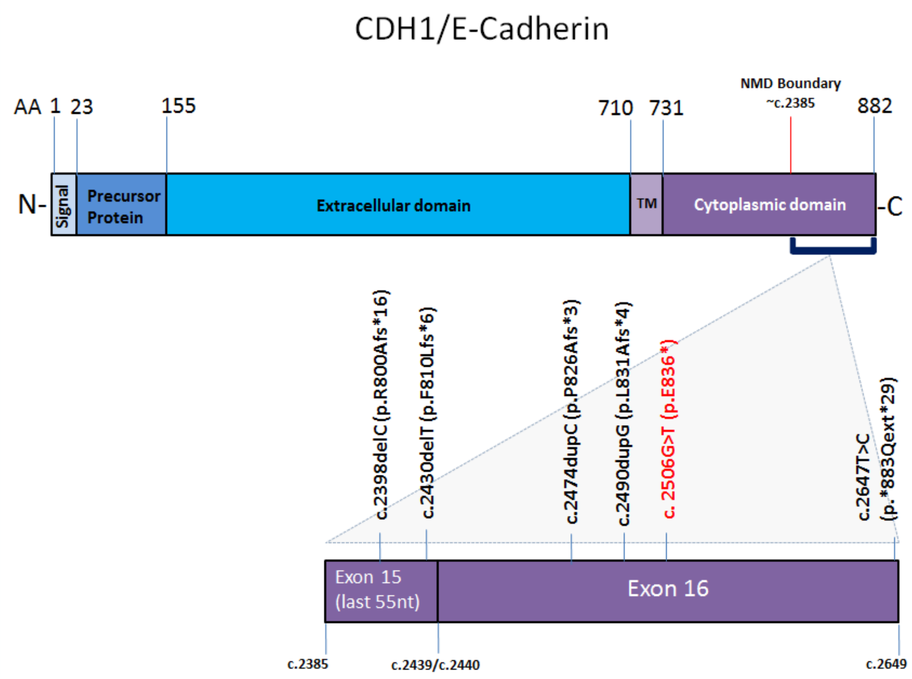
FIGURE 3: Structural Analysis of c.2647T>C (p.*883Qext*29) the most C-terminal *CDH1* alteration

The catenin binding domain in the C-terminal region of *CDH1* has been experimentally determined using X-ray diffraction and is deposited in the RCSB databank. The beta-catenin is shown in orange with ligated Zn cations shown in magenta ,and the C-terminus of *CDH1* is shown in rainbow with the extension shown in grey



- The extension lies in a disordered region that is not seen in the crystal structure, and the extension itself is predicted to be disordered using IUPred
- The region is not predicted to aggregate based on GlobPlot
- The additional residues do appear to compete with the wild type for beta-catenin binding, however it does possess a number of charged site chains which are generally believed to drive the binding
- **Structurally Inconclusive:** Given the above considerations, the variant is not likely to disrupt or compete with beta-catenin binding; however, we cannot rule it out definitively

FIGURE 4: Schematic representation of the E-Cadherin protein. Highlight: alterations identified in the C-terminal region of the *CDH1* transcript



CONCLUSIONS

- C-terminal truncating alterations in *CDH1* escape the nonsense-mediated mRNA pathway. These truncated proteins may retain partial function, and variants in this region must be interpreted with caution
- Using clinical data, molecular evidence, and structural information, we characterized and classified truncating alterations in *CDH1* that were identified downstream of the NMD boundary
- We reported five pathogenic C-terminal truncations, and we identified the most C-terminal pathogenic alteration in our cohort, which occurred *de novo* in an individual with lobular breast cancer
- Multiple probands with pathogenic alterations presented with lobular breast cancer only and did not meet IGCLC diagnostic criteria
- Identifying and characterizing the most C-terminal pathogenic alteration provides evidence to classify other, upstream C-terminal truncating alterations as pathogenic

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