A novel missense variant in *CDH1* causes hereditary diffuse gastric and lobular breast cancer syndrome independently of splicing

Terra Brannan, PhD[1]; Shabnam Asgari, MS, CGC[1]; Aida Akopyan, MS, CGC[2], Matthew J. Varga, PhD[1], Bhuvan Molparia, PhD[1]; Ora Gordon, MD, MS, FACMG[2,3]; Marcy E. Richardson, PhD[1]

1 Ambry Genetics, Aliso Viejo, USA, 2 Providence Health, Los Angeles, USA, 3 Providence Health, St John Cancer Institute, Los Angeles, USA

tbrannan@ambrygen.com

BACKGROUND

- Pathogenic variants (PVs) in *CDH1* cause hereditary diffuse gastric cancer (HDGC) and lobular breast cancer, collectively referred to as hereditary diffuse gastric and lobular breast cancer syndrome (DGLBC).
- Most *CDH1* variants described in DGLBC families are expected to lead to pathogenicity through premature protein truncation or nonsense mediated decay.
- To date, the only consensus missense *CDH1* PVs associated with DGLBC are reported to disrupt splicing.
- Herein we present clinical, structural, and RNA evidence supporting the *CDH1* p.G212V c.635G>T as a likely PV, whose mechanism of pathogenicity is through the impact of the protein missense change rather than splicing.

TAKE HOME POINTS

- CDH1 p.G212V (c.635G>T) is a likely pathogenic, missense variant associated with DGLBC syndrome.
- RNA analysis excluded splicing anomalies while structural analysis highlighted the potential for protein destabilization for both this alteration and the similar disease-associated p.G212E missense alteration.
- Pathogenic missense changes in CDH1 are rare and present unique challenges for variant interpretation using expert panel guidelines. This case stresses the need for evolving guidelines as new pathogenic variants emerge.

FIGURE 1. ClinVar classifications of *CDH1* missense variation

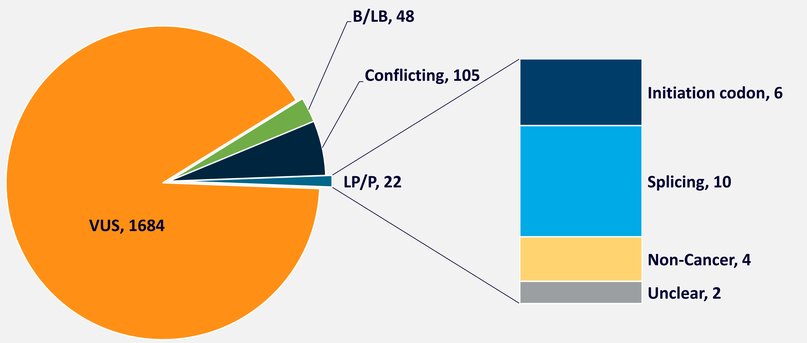


Figure 1: Pie chart depicting the ClinVar consensus classification of *CDH1* variants designated as a missense change (left). P/LP= pathogenic/likely pathogenic; VUS= variant of uncertain significance; B/LB= benign/likely benign. The reported disease mechanisms for CDH1 missense variants classified as LP/P are shown on the right. Initiation codon= variants impacting the initiation codon, splicing= variants with reported RNA data supporting a splice defect, non-cancer= variants associated with alternative diseases (cleft-lip/palette, blepharocheilodontic syndrome), unclear= limited information. ClinVar data was obtained September 2023.

FIGURE 2. Phenotypic presentation of two families

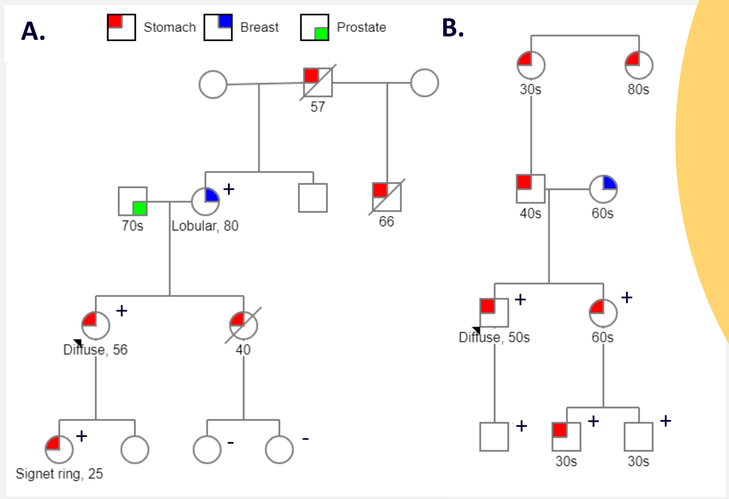
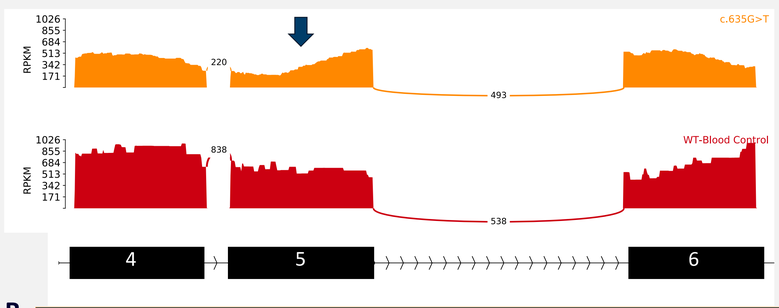


Figure 2: Pedigree showing the segregation of *CDH1* p.G212V c.635G>T in two unrelated families (A &B). Cancer pathologies (when known) and age of diagnosis are provided below the individual

FIGURE 3. Molecular evidence of missense effect

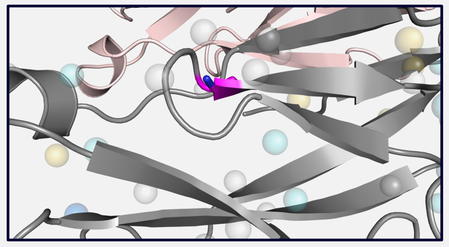
A.



B.

Sample	G (Ref)	T (Alt)	Total	Ratio (% Ref)
Case	195	120	315	61.9%
Control	435	0	435	100%

C.



D.

Variant	ΔΔG (kcal/mol)
G212V	17.021
G212E	15.526

Figure 3:: **A.** Sashimi plot illustrating the normal splicing of CDH1 exon 5 in an individual heterozygous for c.635G>T (top orange plot) and a control individual (red plot). The approximate location of c.635G>T is depicted with an arrow. Splice events with PSI <2% have been filtered for clarity. **B.** Table containing the number of RNA reads supporting normal splicing from each allele. **C.** Structural modeling of residue G212 in the CDH1 EC1 domain[2]. G212 is buried in a tightly packed region of the EC1 domain and the G212V substitutes for a bulky sidechain into this tightly packed region and is strongly disruptive to the structure of the EC1 domain[2,3]. **D.** The change in Gibbs free energy (ΔΔG), is shown for G212V and another disease associated variant at the same residue.

FIGURE 4. Additional evidence: close match CDH1 p.G212E

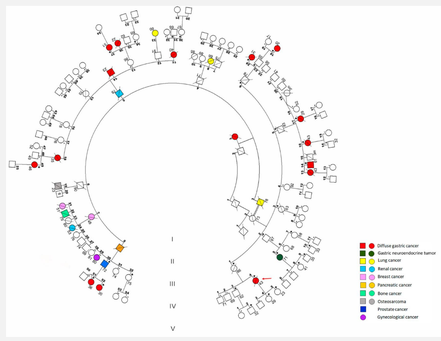


Figure 4: Pedigree for a family with *CDH1* p.G212E c.635G>A reported by Figueriredo *et al* [4]. Sixteen members of this family were reported to be affected with diffuse gastric cancer, 11/16 were confirmed to have the variant, and the remaining 5 affected individuals were not available for genetic testing. The pedigree was imputed into the COOL (Co-segregation Online) v3 tool[5] and received an overall co-segregation LOD score of 5.74112

REFERENCES

1. Blair et al. PMID: 32758476
2. Nardone et al. PMID: 27120112
3. Harrison et al. PMID: 21300292
4. Figueriredo et al PMID: 34503169
5. Belman et al. PMID: 327737704
6. Luo et al. PMID 36600593

FIGURE 5. Classification summary

	Clinical		Bioinformatic			Population Frequency
	PS4	PP1	PM1	PM5	PP3	PM2
p.G212V	Strong	Supporting	Supporting			Supporting
p.G212E	Moderate	Strong			Supporting	Supporting

Figure 5: Summary of evidence codes applied to classify p.G212V and p.G212E. Deviations from the recommendations by the CDH1 VCEP[6] are noted as follows: Green: Applied per *CDH1* VCEP guidelines; Blue: Modified weight from *CDH1* VCEP guidelines; Gray: code not recommended by *CDH1* VCEP.