Optimization of the ACMG-AMP Criteria for *CDH1* Variant Classification

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Introduction

CDH1 mutations cause Hereditary Diffuse Gastric & Lobular Breast Cancer (HDGC – OMIM 137215). Heterozygotes for a germline *CDH1* truncating pathogenic variant present an estimated cumulative risk of developing gastric cancer 70% (95% CI, 59%-80%) for males and 56% (95% CI, 44%-69%) for females. Women also have a 42% (95% CI, 23%-68%) risk of developing lobular breast cancer. *CDH1* is a clinically actionable gene. Current recommendations include, for women, consideration of breast MRI starting at 30 years of age and risk-reducing mastectomy. Prophylactic gastrectomy is recommended for both genders between the age of 20 and 30 (annual upper endoscopy following the dedicated Cambridge protocol with a minimum of 30 biopsies for people who do not undergo gastrectomy). Therefore, it is imperative to detect and correctly classify variants in *CDH1* according to their pathogenicity. With this in mind, the ClinGen Hereditary Cancer Working Group selected *CDH1*, together with *PTEN* and *TP53,* as high priorities genes and created a *CDH1* working group (WG).

The *CDH1* WG is an expert panel assembled from *CDH1* experts encompassing clinicians, scientists and clinical laboratory diagnosticians, with a primary goal to optimize the 2015 ACMG/AMP Variant Interpretation Guidelines specific to CDH1.

Here we present the *CDH1* WG rule specifications to the ACMG/AMP criteria, which after systematic evaluation with a series of test variants will be finalized and incorporated into the group's curation process, as part of the *CDH1* ClinGen Expert Panel application. The group recommendations for pathogenic and benign criteria optimizations for *CDH1* include: standalone and strong benign *CDH1* specific allele frequency cutoffs; gene specifications for splicing; recommendations for the use of computational and functional evidence; standardization of acceptable HDGC diagnostic criteria and minimal clinical information. These criteria will be validated by comparing clinical, population, computational, and functional data, among multiple institutions participating in the *CDH1* WG.

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Evaluation of *CDH1*-Specific Criteria

Working groups were assembled to analyze and present current knowledge related to *CDH1* surrounding each ACMG/AMP criteria evidence type.
The group agreed to the following process for drafting, testing, and adopting criteria.

Identify ACMG/AMP criteria that need optimization
↓
Evidence-based analysis of suggested optimizations
↓
Experts/ClinGen feedback: Make edits/adjustments as needed
↓
Experts vote for proposed optimizations
↓
Application to ClinGen for formal Expert Panel status

Proposed Optimizations (in red)

Rule code	Rule	ACMG	Rule Specifications	*CDH1* Strength
Population Data				
PS4	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	Strong Pathogenic	As stated with well designed case-control studies.	Strong Pathogenic
PM2	Absent in population databases (or at extremely low frequency if recessive)	Moderate Pathogenic	If seen in general population, frequency <0.05% with 95% CI; most not be observed in homozygous state	Supporting Pathogenic or don't use?
BA1	Allele frequency is greater than expected for disorder	Stand Alone Benign	Frequency cutoff of 0.2% (gnomAD) with 99.99% CI; minimum of 5 alleles present in the population	Stand Alone Benign
BS1	Allele frequency is greater than expected for the disorder	Strong Benign	Frequency cutoff of 0.1% (gnomAD) with 99.99% CI	Strong Benign
BS2	Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age	Strong Benign	Homozygous observation, pt confirmed as healthy/unaffected N=1 observation with homozygous status confirmed N=2 without confirmation	Strong Benign

Rule code	Rule	ACMG	Rule Specifications	*CDH1* Strength
Computational and Predictive Data				
PVS1	Null variant in a gene where LOF is a known mechanism of disease	Very Strong Pathogenic	Predicted null variant in a gene where LOF is a known mechanism of disease. *Exception: C-ter NMD resistant variants (Strong P). Skipping/del of in-frame exons (Strong P): CDH1 In-frame exons: exon 4 (144), exon 5 (156), exon 8 (129), exon 9 (183), exon 12 (225), exon 13 (228), exon 15 (144).	Very Strong Pathogenic (SA) *Exceptions: strong pathogenic
PM4	Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants	Moderate Pathogenic	CDH1 In-frame exons: exon 4 (144), exon 5 (156), exon 8 (129), exon 9 (183), exon 12 (225), exon 13 (228), exon 15 (144) may result in in frame splicing abnormalities; may need functional data. May need to have rules for each exon.	Strong Pathogenic
PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product	Supporting Pathogenic	Meta predictors for missense? Splicing: HSF, MaxEnt, BDGP, ESEfinder	Supporting Pathogenic for splicing
BP4	Multiple lines of computational evidence suggest no impact on gene/gene product	Supporting Benign	Meta predictors for missense? Splicing: HSF, MaxEnt, BDGP, ESEfinder	Supporting Benign
BP7	A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.	Supporting Benign	A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.	Stand Alone Benign

Rule code	Rule	ACMG	Rule Specifications	*CDH1* Strength
Functional Data				
PS3	Well-established in vitro or in vivo, functional studies supportive of a damaging effect on the gene or gene product	Strong Pathogenic	Establishing LOF (RNA analysis)	Strong Pathogenic
		Moderate Pathogenic	≥2 unique in vitro or in vivo functional studies OR ≥2 independent groups with concordant deleterious results for the same assay (Invasion assay, aggregation, drosophila model)	Supporting Pathogenic or don't use?
PM1	Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation	Moderate Pathogenic	No known hot spots	Supporting Pathogenic or don't use?
BS3	Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing	Strong Benign	≥2 unique in vitro or in vivo functional studies OR ≥2 independent groups with concordant benign results for the same assay (Invasion assay, aggregation, drosophila model, RNA)	Supporting Benign or don't use?
		Supporting Benign	Single in vitro or in vivo functional studies, multiple independent groups OR Single group with benign result	Supporting Benign or don't use?

Rule code	Rule	ACMG	Rule Specifications	*CDH1* Strength
Other (Clinical Data)				
PP4	Patient's phenotype or family history is highly specific for a disease with a single genetic etiology	Supporting Pathogenic	Family meets HDGC dx criteria (Panel testing)	Strong pathogenic, if family meets HDGC 2015 dx criteria: 1. 2 GC cases in a family, one confirmed diffuse gastric cancer (DGC). 2. One case DGC <age 40. 3. Personal or family history of DGC and ILC, one diagnosed < 50.
BP5	Other		NO Phenotype DGC and ILC (including prophylactic surgery) AND family history is not consistent with HDGC (age cut off); no fhx, exclude *de novo?*	Strong Benign
			NO Phenotype DGC and ILC (BUT not including prophylactic surgery) AND family history is not consistent with HDGC (age cut off); no phenotype confirms de novo	Supporting Benign

Testing *CDH1* Optimizations

Most proposed optimizations by the *CDH1* WG focus on the assessment of CDH1 missense alterations. With this in mind, we selected 17 missense alterations with extensive literature in order to compare functional assays and *in silico* analysis to clinical data. Color code: Red/yellow= pathogenic criteria met fully/partially; Green/light green=benign criteria met fully/partially. DGC=Diffuse Gastric Cancer ILC=Invasive Lobular Breast Cancer.

Missense functional and *in silico* do not predict clinical behavior

Variant Data							Phenotype (please select only one of these 3 categories)				
							Category 1	Category 2	Category 3		
Variant	*in silico*: Tolerant vs Damaging	Functional Evidence	Population data (Gnomad)	Testing facility	Lab Frequency (allele/total CDH1 alleles sequenced)	Ambry class.	Index - HDGC criteria met (n) - see tab 2 for criteria	Index - HDGC associated ca, but not meeting HDGC criteria (DGC, LBC, SRC)	Index - unselected panel testing (n), does not meet category 1 or 2 (known or unknown)	Family/carrier tests (seg)	Co-occur LP/P variant(n)
c.731A>G (p.D244G)	REVEL: T VEST3: T MetaSVM: T CADD: D SIFT: D Polyphen2: D Provean: D	Adhesion: abnormal Motility: abnormal	No population frequency information could be found.	Ambry Genetics	1/400000	VUS	0	0	1	1	
c.1118C>T (p.P373L)	REVEL: D VEST3: D MetaSVM: D CADD: D SIFT: D Polyphen2: D Provean: D	Invasion: Yes Motility: Yes Adhesion: abnormal E-cad expression: reduced Trafficking: abnormal EGFR: abnormal	East Asian: 0.02% (4/18868) European (non-F): 0.002% (3/126718)	Ambry Genetics	27/400000= 0.0067% Asian=10 Caucasian=16 Unknown=1	VUS	1 (phx of DGC & ILC <50)	1 ILC @75	12	16/11 (0) *2 neg PG	1 TP53
c.1225T>C (W409R)	REVEL: D VEST3: D MetaSVM: T CADD: T SIFT: D Polyphen2: D Provean: D	Aggregation: abnormal Invasion: Yes	European (non-F): 0.004% (5/111712)	Ambry Genetics	14/400000= 0.0035% Caucasian=13 Unknown=1	VUS	0	0	14	14	1 TP53
c.2245C>T (p.R749W)	REVEL: D VEST3: D MetaSVM: D CADD: D SIFT: D Polyphen2: D Provean: D	Invasion: Yes Motility: Yes Catenin assembly: No Adhesion: abnormal E-cad expression: reduced Trafficking: abnormal EGFR: abnormal	No population frequency information could be found.	Ambry Genetics	1/400000	VUS	0	0	1	1	
c.2396C>G (p.P799R)	REVEL: D VEST3: D MetaSVM: T CADD: D SIFT: D Polyphen2: D Provean: D	Invasion: Yes Motility: No Catenin assembly: NA Adhesion: abnormal E-cad expression: reduced Trafficking: abnormal EGFR: no	No population frequency information could be found.	Ambry Genetics	2/400000= 0.001%	VUS	0	0	2	2	
c.2494G>A (p.V832M)	REVEL: D VEST3: D MetaSVM: D CADD: D SIFT: D Polyphen2: D Provean: T	Invasion: Yes Motility: No Catenin assembly: no Adhesion: abnormal E-cad expression: reduced Trafficking: abnormal EGFR: no	African: 0.004% (1/24034) **East Asian:0.17% (33/18868)** South Asian: 0.01% (4/30782) European (non-F): 0.0055% (7/126732)	Ambry Genetics	56/400000= 0.013% African American=2 Asian=30 Caucasian=16 Middle Eastern=6 Mixed=1 Unknown=1	VUS	0	1 ILC @51	55	56	1 BRCA2

Variant Data							Phenotype (please select only one of these 3 categories)					Segregation
							Category 1	Category 2	Category 3			
Variant	*in silico*: Tolerant vs Damaging	Functional Evidence	Population data (Gnomad)	Testing facility	Lab Frequency (allele/total CDH1 alleles sequenced)	Ambry classification	Index - HDGC criteria met (n) - see tab 2 for criteria	Index - HDGC associated ca, but not meeting HDGC criteria (DGC, LBC, SRC)	Index - unselected panel testing (n), does not meet category 1 or 2 (known or unknown)	Family (n)	Co-occur LP/P variants (n)	Number of site specific carrier tests/variant segregating with HDGC ca (DGC, LBC, SRC)
c.353C>G (p.T118R)	REVEL: T VEST3: T MetaSVM: T CADD: T SIFT: T Polyphen2: T Provean: T	Invasion: Yes Motility: Yes Adhesion: abnormal E-cad expression: reduced EGFR: yes	No population frequency information could be found.	Ambry Genetics	1/400000= 0.001%	VUS	0	0	1	1		0
c.892G>A (p.A298T)	REVEL: T VEST3: T MetaSVM: T CADD: D SIFT: D Polyphen2: D Provean: T	Invasion: Yes Motility: Yes Adhesion: abnormal EGFR: yes	**South Asian:0.1% (41/30782)** European (non-F): 0.05% (67/126698) Latin: 0.008% (3/34418) Other: 0.046% (3/6466)	Ambry Genetics	233/400000= 0.058% Ashkenazi Jewish=3 Asian=6 Caucasian=180 Hispanic=3 Middle Eastern=8 Mixed=12 Other=5 Unknown=16	VLB	0	6 (ILC 38-72yo) 1 Bilat-ILC @55	226	225	1 BRCA1 2 BRCA2	6 (0)
c.1018A>G (p. T340A)	REVEL: T VEST3: T MetaSVM: T CADD: T SIFT: T Polyphen2: T Provean: T	Invasion: Yes Motility: Yes Catenin assembly: normal Adhesion: abnormal E-cad expression: normal Trafficking: ? EGFR: abnormal	**East Asian: 0.3% (68/18866)**	Ambry Genetics	55/400000= 0.013% African American=1 Asian=34 Caucasian=11 Mixed=4 Unknown=5	VUS	1 (GC @32, fhx GC @ 27, but co-occur with CDH1 p.Q771* phase unknown)	1 (ILC @35)	49	51	1 (CDH1 p.Q771* phase unkn.) 1 BRCA1 1 BRCA2	4 (0)
c.2343A>T (p.E781D)	REVEL: T VEST3: T MetaSVM: T CADD: T SIFT: T Polyphen2: T Provean: T	Invasion: Yes Motility: No Catenin assembly: No Adhesion: abnormal E-cad expression: reduced Trafficking: abnormal EGFR: no	European (non-F): 0.002% (3/111696)	Ambry Genetics	42/400000= 0.01% African American=1 Ashkenazi Jewish=1 Caucasian=37 Unknown=3	VUS	0	0	42	42	1 CDH1 (P373L) 2 BRCA2	0

Splicing functional and *in silico* predict clinical behavior

Splicing Variant Data								Phenotype					Segregation	HDGC Histologic Phenotype		Aggregate data		
								Category 1	Category 2	Category 3								
Variant	Splicing predictors	*in silico*: Tolerant vs Damaging	Functional Evidence	Population data (Gnomad)	Testing facility	Lab Frequency (allele/total CDH1 alleles sequenced)	Ambry classification	Index - HDGC criteria met (n) - see tab 2 for criteria	Index - HDGC associated ca, but not meeting HDGC criteria (DGC, LBC, SRC)	Index - unselected panel testing (n), does not meet category 1 or 2 (known or unknown)	Families (n)	Families with co-occuring LP/P variants (n)	Number of site specific carrier tests/variant segregating with HDGC ca (DGC, LBC, SRC)	Prophylatic gastrectomies (n)	Cases with foci of signet ring cells (n)	Ethnicity = N (East Asian, African, Ashkenazi Jewish, European (Finnish), European (Non-Finnish), Latino, Other, South Asian)	Proband history of cancer =N (Breast, NOS ;DCIS;IDC;LBC; DGC;IGC;SRC; unaffected;other*)	Family History of cancer = N (gastric;breast;other*)
c.715G>A (p.G239R)	HSF: abnormal MaxEnt: abnormal BDGP: abnormal ESEfinder: abnormal	REVEL: T VEST3: T MetaSVM: T CADD: D SIFT: D Polyphen2: D Provean: D	Causes deletion of the first 29 base pairs from exon 6 in white blood cells and a gastrectomy specimen (Kaurah P et al. JAMA. 2007 Jun; 297(21):2360-72).	No population frequency information could be found.	Ambry Genetics	23/400000=0.0057%	Mutation	1 (DGC @40)	1 (ILC @56) 1 (ILC @64)	6	12		11 (0, 3 not provided)	0	0	Caucasian=21 Hispanic=1 Unknown=1	Breast= 5 (2 ILCs) GC= 1 No/NOS= 16	
c.1901C>T (p.A634V)	HSF: abnormal MaxEnt: abnormal BDGP: abnormal ESEfinder: abnormal	REVEL: T VEST3: T MetaSVM: T CADD: T SIFT: T Polyphen2: T Provean: T	In the COGA-3 cell line RT-PCR and electrophoresis of the cDNA revealed, in addition to the wild type fragment, a shorter PCR product. Sequencing identified a deletion of the last 37 nucleotides from exon 12 (nt 1994 – 2030)), resulting in a PTC 18 nucleotides after the deletion.	No population frequency information could be found.	Ambry Genetics	1/400000=0.00025%	VLP	1 (fhx 2DGC @50, 62 & ILC @44)	0	0	1		0	0	0	Asian=1	No= 1	DGC=2 ILC=1
c.2195G>A (p.R732Q)	HSF: abnormal MaxEnt: abnormal BDGP: abnormal ESEfinder: abnormal	REVEL: T VEST3: T MetaSVM: T CADD: D SIFT: D Polyphen2: D Provean: D	Minigene assay: showed that… the 2195GA (R732Q)mutation activated a cryptic acceptor splice site in exon 14." This result was confirmed by RT-PCR of gastrectomy specimens, showing this variant results in "complex splicing" and deletion of 32 base pairs at the start of exon 14.	No population frequency information could be found.	Ambry Genetics	10/400000=0.0025%	Mutation	1 (1DGC @67, 1GC @68, fhx 3 GC and 1 ILC)	0	2	3		7 (1 GC)	0	0	Ashkenazi Jewish=2 Caucasian=7 Unknown=1	GC= 2 No/NOS= 7	GC= 3 ILC= 1

Future Directions

- Following expert votes and final edits to the criteria and curation process, an application will be submitted to ClinGen for formal Expert Panel status.

References

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