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Authors

Luo, Xi
Maciaszek, Jamie L
Thompson, Bryony A
et al.

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Optimizing clinical care through *CDH1*-specific germline variant curation: improvement of clinical assertions and updated curation guidelines

Xi Luo[1,*], Jamie L. Maciaszek[2,*], Bryony A. Thompson[3], Huei San Leong[4], Katherine Dixon[5], Sónia Sousa[6], Michael Anderson[7], Maegan E. Roberts[8], Kristy Lee[9], Amanda B. Spurdle[10], Arjen R. Mensenkamp[11], Terra Brannan[12], Carolina Pardo[7], Liying Zhang[13], Tina Pesaran[12], Sainan Wei[14], Grace-Ann Fasaye[15], Chimene Kesserwan[15], Brian H. Shirts[16], Jeremy L. Davis[15], Carla Oliveira[6], Sharon E. Plon[1], Kasmintan A. Schrader[5], Rachid Karam[12,#] on behalf of the ClinGen CDH1 Variant Curation Expert Panel

[1]Department of Pediatrics/Hematology-Oncology, Baylor College of Medicine, Houston, TX, USA

[2]Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN, USA

[3]Department of Pathology, Royal Melbourne Hospital, Parkville, Victoria, Australia

[4]Department of Pathology, Peter MacCallum Cancer Centre, Parkville, VIC, Australia

[5]Department of Medical Genetics, University of British Columbia, Vancouver, BC, CAN

[6]Instituto de Investigação e Inovação em Saúde – (i3S), Institute of Molecular Pathology and Immunology of the University of Porto – (Ipatimup), Faculty of Medicine, University of Porto, Porto, PRT

[7]Invitae Corporation, San Francisco, CA, USA

[8]GeneDx, Gaithersburg, MD, USA

[9]Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[10]QIMR Berghofer Medical Research Institute, Brisbane, AUS

[#] To whom correspondence should be addressed at: Rachid Karam, Phone No: (949) 457-4347, rkaram@ambrygen.com.
[*]These authors contributed equally to this manuscript

[11]Department of Human Genetics, Radboud University Medical Centre, Nijmegen, NLD

[12]Ambry Genetics, Aliso Viejo, CA, USA

[13]Department of Pathology and Laboratory Medicine, University of California, Los Angeles, CA, USA

[14]Department of Pathology, University of Kentucky, Lexington, KY, USA

[15]Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland USA

[16]Department of Laboratory Medicine and Pathology, University of Washington, Seattle, WA, USA.

Abstract

Background: Germline pathogenic variants in *CDH1* are associated with increased risk for diffuse gastric cancer and lobular breast cancer. Risk-reduction strategies include consideration of prophylactic surgery, thereby making accurate interpretation of germline *CDH1* variants critical for physicians deciding upon these procedures. The Clinical Genome Resource (ClinGen) *CDH1* Variant Curation Expert Panel (VCEP) developed specifications for *CDH1* variant curation with a goal to resolve variants of uncertain significance (VUS) and with ClinVar conflicting interpretations and continues to update these specifications.

Methods: *CDH1* variant classification specifications were modified based on updated genetic testing clinical criteria, new recommendations from ClinGen, and expert knowledge from ongoing *CDH1* variant curations. The *CDH1* VCEP reviewed 273 variants using updated *CDH1* specifications and incorporated published and unpublished data provided by diagnostic laboratories.

Results: Updated *CDH1*-specific interpretation guidelines include eleven major modifications since the initial specifications from 2018. Using the refined guidelines, 97% (36/37) of variants with ClinVar conflicting interpretations were resolved into benign, likely benign, likely pathogenic, or pathogenic, and 35% (15/43) of VUS were resolved into benign or likely benign. Overall, 88% (239/273) of curated variants had non-VUS classifications. To date, the only missense variants classified as pathogenic or likely pathogenic are known to affect splicing and therefore, functional studies are not validated for use in the interpretation of *CDH1* variants.

Conclusions: The development and evolution of *CDH1*-specific criteria by the expert panel results in decreased uncertain and conflicting interpretations of variants in this clinically actionable gene which ultimately leads to more effective clinical management recommendations.

1. Introduction

Germline pathogenic (P) or likely pathogenic (LP) variants in the *CDH1* gene (NM_004360.5) predispose to hereditary diffuse gastric cancer (HDGC; MIM# 137215), a cancer susceptibility syndrome inherited in an autosomal dominant pattern, initially characterized by the increased risk for diffuse gastric cancer (DGC) but subsequently well documented to be associated with lobular breast cancer (LBC) in women[1]. Recently, to

encompass the increased risk for both DGC and LBC in the name of the *CDH1* syndrome, the ClinGen Disease Naming Committee and *CDH1* Variant Curation Expert Panel (VCEP) have proposed to rename the disease as *CDH1*-related Diffuse Gastric and Lobular Breast Cancer Syndrome (DGLBCS). This committee includes community stakeholders (e.g. MonDO, Orphanet, OMIM) and the ClinGen Actionability Working Group, which aims to nominate gene-disease pairs with a high risk of serious disease for clinical actionability measures, including germline variant curation guidelines.

The recommendations for gastric cancer risk management for individuals found to be heterozygous for P/LP *CDH1* variants is to undergo prophylactic total gastrectomy in early adulthood.[1][2] This procedure comes with long-term sequelae including nutritional, hormonal, pharmacokinetic, immune, and psychologic effects which require lifelong monitoring by an experienced multidisciplinary team. In addition, females may consider undergoing bilateral risk-reducing mastectomy.[1] Given the potential morbidity associated with these guidelines, having standardized, expert assessment of variants is critical.

The National Institutes of Health (NIH) developed the Clinical Genome Resource (ClinGen) to standardize the clinical annotation and interpretation of genomic variants and to implement evidence-based expert consensus for curating genes and variants.[3] To achieve these goals, VCEPs are formed in the ClinGen program with a focus on specific genes and diseases. VCEPs develop gene- or disease-specific variant classification rules based on the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) classification framework and curate genetic variants to be deposited in ClinVar[4] (https://www.ncbi.nlm.nih.gov/clinvar/). In ClinVar, VCEP-interpretations receive a three-star review level status (reviewed by expert panel)[3] and designation that the interpretation process is recognized by the U.S. Food and Drug Administration (FDA).[5][6]

The ClinGen Hereditary Cancer Clinical Domain Working Group selected *CDH1* as a high priority gene and convened a *CDH1* VCEP in 2015. The initial *CDH1*-specific variant interpretation guidelines were published in 2018.[7] These guidelines apply only to DGLBCS and not to other *CDH1*-associated conditions, such as blepharocheilodontic syndrome (MIM# 119580).

Over the past three years, there has become a clear need to update the *CDH1* specifications based on: learned experiences from ongoing *CDH1* variant curations; updated DGLBCS clinical practice guidelines which expanded *CDH1* genetic testing recommendations;[1] and general recommendations for the refinement of the ACMG/AMP criteria provided by the ClinGen Sequence Variant Interpretation (SVI) working group. At this time, there have been two updates to our interpretation guidelines which are both described in this paper: version 2 (published 9/6/2019) and version 3 (published 9/20/2021). At any time, the most recent guidelines can be found at: https://www.clinicalgenome.org/affiliation/50014/. Modifications to the ClinGen *CDH1* variant interpretation guidelines illustrate the dynamic evolution of ClinGen expert panel specifications that incorporate up-to-date knowledge and inform evidence-based interpretations to support and benefit precision medicine and clinical research.

Since publication of the initial interpretation guidelines, the *CDH1* VCEP has curated 273 *CDH1* variants and deposited these interpretations into the ClinVar database.[4] Importantly, only 12% (34) of these submissions are variants of uncertain significance (VUS) after curation using updated *CDH1* variant interpretation guidelines. The resolution of both VUS and variants with conflicting interpretations from ClinVar submitters is critical to provide straightforward diagnostic information to clinicians and patients and allow for informed patient care. Therefore, our data provide support for the concept of developing and routinely updating gene-specific germline variant interpretation guidelines to improve the diagnosis and treatment of individuals with cancer susceptibility conditions.

2. Methods

2.1 ClinGen *CDH1* VCEP and updates to variant interpretation guidelines

The *CDH1* VCEP comprises 32 professionals with expertise in key domains regarding DGLBCS and/or variant classification and includes clinicians, research scientists, genetic counselors, pathologists, and clinical laboratory diagnosticians. Currently, there are representatives from 22 participating institutions in six countries: Australia, Canada, Italy, Netherlands, Portugal, and the United States. The *CDH1* VCEP also includes representatives from several diagnostic laboratories performing *CDH1* germline testing and research.

Modifications to the *CDH1* variant interpretation guidelines were proposed and discussed via teleconference calls and e-mail communications to arrive at consensus decisions, followed by submission to and approval by the ClinGen SVI. All versions of the guidelines were published to the ClinGen website.

2.2 Curation process and variant classification

CDH1 variants were curated and classified according to the current *CDH1*-specific variant interpretation guidelines at the time of curation. Reclassification using the version 3 guidelines was performed for all variants. Variants prioritized for expert panel review included: 1) variants with conflicting interpretations (CI) in ClinVar; 2) predicted loss-of-function variants; 3) variants with an allele frequency of 0.2% in population databases; 4) VUS with a two-star review status in ClinVar; and 5) internal or external requests for expert interpretation.

Trained ClinGen biocurators utilized ClinGen's Variant Curation Interface[8] (https://curation.clinicalgenome.org/) to aggregate and assess data and document the applicable criteria for each variant. In addition to published case evidence from literature, unpublished and de-identified patient observations were provided by VCEP members, laboratory representatives, and the NCI hereditary gastric cancer study (Clinicaltrials.gov NCT03030404) to facilitate comprehensive variant interpretation. Biocurators assigned a provisional classification of benign (B), likely benign (LB), VUS, LP, or P to each variant. Variants were reviewed by expert members during monthly *CDH1* VCEP meetings to modify and approve the classification of variants. The final variant classifications were submitted to ClinVar with three-star level review status and FDA-recognized designation (https://www.ncbi.nlm.nih.gov/clinvar/submitters/506817/).

3. Results

3.1 Updates to ClinGen CDH1 VCEP specifications to the ACMG/AMP variant interpretation guidelines

Since the initial *CDH1*-specifications to the ACMG/AMP variant classification guidelines were published in 2018,[7] we have made 11 major updates to these criteria which are summarized here and described below and in Table S1:

1. Adoption of the 2020 updated DGLBCS clinical practice guidelines[1] to define DGLBCS phenotype criteria for PS2, PS4, PM6, and PP1 (v3);

2. Application of PVS1 to initiation codon variants (v2);

3. Development of a decision tree to specify *CDH1* PVS1 rules (v3) (figure 1);

4. Integration of splice site specific recommendations to determine the strength of PVS1 criterion (v3);

5. Approval of BS1 or BS2 alone as adequate criteria for LB classification (v2);

6. Specification of PM5_supporting to nonsense/frameshift variants that are predicted and/or proven to undergo nonsense-mediated decay (NMD), or to splicing variants in acceptor/donor sites that have at least one other variant meeting P/LP at the same site (v3);

7. Downgrade of PM2 to supporting strength (v3);

8. Removal of PS1 as an applicable evidence code (v3);

9. Removal of the conservation requirement from BP7 and expansion of BP7 to intronic variants at or beyond +7 to −21 locations (v3);

10. Use of the Bayesian point system[9][10] in curations with conflicting evidence that otherwise result in a classification of VUS (v3);

11. Clarification on the usage of PS4, PM2, PM4, PP1, BS2, BS3, BP2, BP4, and BP5 (v2 and v3).

Three minor updates were also made to the *CDH1* variant curation criteria:

1. Updating PP3 and BP4 to include SpliceAI[11] as a recommended splicing predictor (v3);

2. Specifying a subpopulation minimum threshold of 2,000 alleles for the application of BA1 and BS1[12] (v3);

3. Specifying a mean coverage of at least 30X coverage from next generation sequencing data for application of BA1, BS1, and PM2[13] (v3);

Ongoing updates will allow for continued improvement of *CDH1* variant curations resulting in comprehensive and confident classifications.

3.1.1 Implementation of updated DGLBCS clinical practice guidelines for specification of *de novo* (PS2, PM6), phenotype (PS4), and segregation

(PP1) data—The updated International Gastric Linkage Consortium 2020 DGLBCS genetic testing criteria[1] have expanded the recommendation for *CDH1* germline testing for individuals with isolated DGC to those who are younger than 50 years of age. To apply patient-related criteria, including *de novo* (PS2, PM6), phenotype (PS4), and familial segregation (PP1), all probands being counted should have a personal and/or family history meeting one of the DGLBCS criteria in Table S3.

For *CDH1* variant curation, application of PS4 is determined by proband counting, or the number of unrelated individuals meeting clinical criteria for DGLBCS. The application of PS4 in the context of *CDH1* variants should also take into consideration the fact that about 30% of the reported individuals with pathogenic variants meet the DGLBCS phenotypic criteria based on penetrance estimates[14]. For example, if the variant is observed in 20 families, at least six of these (30%) must meet DGLBCS phenotypic criteria to apply PS4. In keeping with the initial *CDH1* specifications of the ACMG/AMP guidelines[7], the strength of PS4 increases based on the number of families meeting DGLBCS criteria (Table S1).

3.1.2 Application of PVS1 to initiation codon variants, and a decision tree and splicing table to specify the strength of PVS1 for potential loss-of-function variants in *CDH1*—The ClinGen SVI provided a general recommendation in 2018 that PVS1 or PVS1_strong criteria should not be applied to start loss variants[15] based on functional studies demonstrating that translational re-initiation can occur at alternative ATG or non-ATG sites downstream or upstream of the original start site[16–22]. The *CDH1* VCEP has now determined that PVS1 should be applied at very strong strength for initiation codon variants (Figure 1) based on the following: 1) there are no known *CDH1* transcripts with alternative start codons, 2) there are at least 50 P/LP classified variants upstream of the closest potential in-frame start codon at Met246, and 3) patient data (currently ten probands/families meeting DGLBCS criteria) support the pathogenicity of initiation codon variants (Table S4). By applying PVS1 to initiation codon variants, we have re-curated five variants as pathogenic (Table S4). With PVS1_moderate applied based on the initial *CDH1* guidelines, these variants would have been classified as either LP (one) or VUS (four), demonstrating the importance of continued modification of VCEP specifications to improve variant classification and clinical reporting.

A *CDH1*-specific decision tree for PVS1 (Figure 1) was developed to clarify rules for PVS1 application and the corresponding evidence strength. For canonical splicing variants, the *CDH1* VCEP also provided splice site-specific recommendations for the strength of PVS1 criteria and the application of PM5_supporting (see section 3.1.4; Table S5). This splicing table contains key information for each splice site including the predicted or experimentally demonstrated splicing impacts and splice site variants that have been classified to date. Twenty-nine of the 35 splice site variants curated to date are P/LP, while six variants predicted or confirmed to produce in-frame transcripts remain classified as VUS, demonstrating the importance of splice site-specific recommendations for the interpretation of *CDH1* splicing variants.

3.1.3 BS1 and BS2 are sufficient criteria for LB classification—For a variant to reach a LB classification, the 2015 ACMG/AMP rules require either one strong and

one supporting, or at least two supporting criteria. Based on this, variants meeting either BS1 (allele frequency is greater than expected for disorder) or BS2 (observed in healthy adult individuals) alone would remain as VUS. The recent Bayesian formulation of the ACMG/AMP guidelines[9][10], further described in section 3.1.8, indicated that strong benign evidence criteria hold a stronger weight (equal to −4 points) than two supporting benign criteria (equal to −2), with the minimum points needed for a LB classification being −1[10]. For non-synonymous variants that do not affect splicing, only two benign supporting codes are applicable, *cis/trans* testing (BP2) and alternate locus observations (BP5), neither of which is readily available from laboratories. Therefore, we recommended BS1 or BS2 alone as adequate criteria for LB classification.

Among the 24 variants classified as LB based on BS2 alone, seven were identified in 100 individuals without DGLBCS phenotypes (Table S2). The NM_004360.5:c.670C>T(p.Arg224Cys) variant was found in 392 unaffected individuals by clinical testing. This variant was previously curated as a VUS by the *CDH1* VCEP[7] despite having mostly B/LB interpretations in ClinVar; the updated BS2 rule allows for consistency between the VCEP and clinical laboratories. All 24 variants classified as LB based on BS2 alone affected the ClinVar designated clinical significance of the variant (VUS/CI to LB, Table S2). In addition, seven of eight variants meeting criteria for BS1 also met criteria for BS2, with one also meeting BP4, allowing these variants to reach a benign classification. The significant improvements over ClinVar variant interpretations with the application of BS2 criterion also demonstrate the benefit of sharing unpublished, de-identified patient data from multiple diagnostic and research labs in the *CDH1* VCEP.

For application of BS2 as strong evidence, the variant must be seen in at least ten adults without a known personal and/or family history of gastric cancer, DGC, LBC, or gastric tumors with signet ring cell histology[7]. In addition to excluding individuals with non-DGC pathology (i.e., histologically confirmed gastric cancer) and non-LBC pathology from proband counts, individuals reporting a personal and/or family history of gastric cancer without supporting pathology information should also be excluded. However, individuals with non-LBC breast cancer diagnoses should be noted for review by experts[14]. Based on penetrance estimates described in section 3.2.1,[14] BS2 cannot be applied to variants in which more than 30% of reported individuals meet DGLBCS criteria. As such, BS2 may not be applied in conjunction with PS4.

3.1.4 PM5_supporting applies to nonsense/frameshift variants predicted to undergo NMD, and GT-AG 1,2 acceptor/donor site with P/LP splice site variants curated—The 2015 ACMG/AMP guidelines described the application of PM5 for novel missense variants occurring at the same position as another pathogenic missense change[23]. The impact of amino acid-level changes of *CDH1* variants is inconclusive, with all known P/LP *CDH1* missense variants affecting splicing (Table S6). Therefore, we did not recommend applying PM5 to *CDH1* missense variants[7].

After curating 113 nonsense/frameshift variants, the *CDH1* VCEP noticed a discrepancy between classifications based on gene-specific guidelines and laboratory classifications, consistent with observations from other ClinGen VCEPs. Using *CDH1* version 1 and

2 guidelines, many nonsense/frameshift variants did not have sufficient patient data to apply the PS4_supporting criterion, remaining as LP based on PVS1 and PM2. In contrast, these variants were classified as P by diagnostic laboratories regardless of the number of individuals meeting DGLBCS criteria. To resolve this discordance, we validated PM5_supporting for variants in exons with other confirmed loss-of-function variants, following the recommendation from the ClinGen SVI (unpublished). We found that at least one pathogenic variant in each exon of *CDH1* is supported by phenotypic evidence (PS4 and/or PP1; Table S7), indicating no exon-specific effect with respect to patient presentation, e.g., that pathogenic variants do not localize to specific exons. Exon-specific variability has been demonstrated for both *BRCA1* and *BRCA2*, where loss-of-function variants in select exons retain some of their tumor suppressor function[24] or induce in-frame skipping through the modification of splice sites or regulatory elements resulting in a partially functional protein[25]. Based on guidance for *BRCA1* and *BRCA2*[25], application of PM5_supporting also requires that the variant does not impact splicing based on either RNA studies or splicing predictors to ensure that the loss-of-function effect is not rescued by alternative splicing mechanisms or regulatory element modification.

Utilization of PM5_supporting has allowed 45 nonsense/frameshift variants to reach a pathogenic classification using version 3 guidelines. Implementation of this evidence code will facilitate future batch curation efforts for novel nonsense/frameshift variants in *CDH1*, reduce requests to labs and academic groups for patient phenotypic data to aid curation, and eliminate the need for re-curation of LP variants which is required every two years.

3.1.5 Decreased weight of PM2 to a supporting strength level—Consistent with guidance from the ClinGen SVI, we decreased the weight of PM2 from moderate to supporting in our version 3 guidelines. It was determined that rarity in population databases[26] was given too much weight in the initial 2015 ACMG/AMP framework[23] and did not meet the relative odds of pathogenicity for moderate strength evidence.[9] Recent work from the Genome Aggregation database (gnomAD) indicates that the current sample size does not capture complete mutational saturation of the human exome.[26] Similarly, findings from the ExAC database indicate that 54% of the high-quality variants are only present once in the dataset.[27] Finally, PM2 was a conflicting criterion in eight B or LB *CDH1* classifications using versions 1 and 2, supporting the strength reduction in version 3.

3.1.6 Removal of PS1 as an evidence criterion—At the time of publication of the original *CDH1* specifications to the ACMG/AMP variant curation guidelines[7], the VCEP did not feel that PS1 needed further specification. However, evidence criteria for other codes generally applied to missense variants (PS3/BS3, PM5, PP2, and PP3/BP4) were not validated due to the paucity of missense variants classified as P/LP, and the corresponding lack of individuals with missense variants meeting diagnostic testing criteria for DGLBCS.[7] To date, we have curated 66 missense variants: 20 B, 22 LB, 19 VUS, 3 LP, and 2 P. All five P/LP missense variants affect splicing, indicating that the impact of amino acid level changes of *CDH1* variants on DGLBCS is inconclusive (Table S6). The PS1 code would not be applicable for identical amino acid changes at these residues since this evidence criterion

requires that the variant does not impact splicing. Based on this data, the *CDH1* VCEP has removed PS1 as an applicable evidence code.

3.1.7 BP7: Removal of the requirement for nucleotide conservation and extension of application to intronic variants at or beyond +7 to −21 locations

—BP7 was originally designed to be applied to synonymous variants for which splicing prediction algorithms indicate no impact and the nucleotide is not conserved. Recent work indicates that nucleotide conservation has limited predictive power since many intronic SNVs are located at sites that are under less constraint than exonic SNVs.[28] We have therefore opted to remove the original conservation requirement. Furthermore, application of BP7 was expanded to intronic variants at or beyond +7 to −21 locations.[29][30]

3.1.8 Utilization of Bayesian point system in VUS curations with conflicting evidence

—For variants with conflicting pathogenic and benign criteria that results in an assertion of VUS, the *CDH1* VCEP advises using the Bayesian point system to calculate the ultimate pathogenicity.[9][10] In 2018, Tavtigian et al. demonstrated that the ACMG/AMP guidelines are compatible with a quantitative Bayesian formulation to determine pathogenicity using four strength levels of evidence.[9] Recently, Tavtigian et al. established a natural conversion from the Bayesian formulation into a point-based system to derive variant pathogenicity.[10] Here, each evidence strength is assigned a point value where very strong evidence codes are +8 or −8 points for pathogenic or benign, respectively, strong are ±4, moderate are ±2, and supporting evidence are ±1. The summation of these point values equates to the variant classification category where ≤ −7 points corresponds to a B classification, −1 to −6 to LB, 0 to 5 to VUS, 6 to 9 t LP, ≥ 10 to P.

3.2 Expert curations completed to date

As of May 20, 2021, the *CDH1* VCEP had completed the curation, expert review, and classification of 273 variants using the version 3 guidelines. All completed classifications have been submitted to ClinVar with a description of the corresponding evidence and final interpretation. The variant classifications comprise 122 P, 28 LP, 38 LB, and 51 B, while 34 (12%) classifications remain as VUS (Figure 3a).

3.2.1 Comparison of *CDH1* VCEP variant classifications to previous ClinVar assertions

—The ClinVar assertions of 33% (90/273) of *CDH1* VCEP variant classifications were altered using version 3 of the *CDH1* specifications. Of these, 52 (58%) changed the ClinVar designated clinical significance of the variant (VUS/CI changing to B/LB or P/LP). The remaining 38 (42%) were defined as resolution of a confidence conflict, such that variants previously classified as LB (or LP) were downgraded to B (or upgraded to P) (Figure 2).

Most variants (32) with previous CI in ClinVar comprised a combination of B/LB and VUS classifications. However, five of these variants had at least one assertion of P/LP, making the classification of the variant difficult to infer based on ClinVar information. The *CDH1* VCEP reviewed variant interpretations have resolved 36 out of 37 (97%) of variants with CI to non-VUS classifications, with most of them (34) having been reclassified as B or

LB (Figure 2). Notably, two variants with CI, NM_004360.5:c.1679C>G(p.Thr560Arg) and NM_004360.5:c.1057G>A(p.Glu353Lys), were reclassified as P and LP, respectively. Both are missense changes are predicted to impact splicing, with RNA assays demonstrating abnormal out-of-frame transcripts (Table S6).[31][32] A combination of literature reports and unpublished laboratory data indicated that these variants co-segregated in several families meeting DGLBCS criteria.[31][33][34] In addition, we were able to downgrade 14 VUS to LB and one to B (Figure 2, Table S2). Resolution of *CDH1* variants designated as VUS or with CI will diminish clinical uncertainty for individuals with these variants.

3.2.2   Curations using each classification category—The version 3 *CDH1* variant curation guidelines utilize 19 evidence criteria established in the 2015 ACMG/AMP guidelines (Table S1). Currently, nine evidence criteria are not applicable to *CDH1*: PS1, PM1, PM3, PP2, PP4, PP5, BP1, BP3, and BP6.

With respect to *CDH1* VCEP curations reported herein (Figure 3a), the most frequently applied pathogenic evidence codes were PVS1, PM2, PS4, and PM5 (Figure 3b). Importantly, 65% of P/LP variants with PVS1 applied also had supporting clinical data to meet PS4 criterion at varied strengths, indicating the importance of data sharing from laboratories. Of the 148 variants classified as P/LP, only five variants did not meet PVS1 criterion, all of which are missense variants that affect splicing (Table S6), again highlighting the lack of missense variants with disease-associated. All variants meeting PVS1 criterion but not reaching a P/LP classification had PVS1_moderate evidence strength based on the creation of a premature stop codon downstream of NM_004360.5:c.2506G>T(p.Glu836Ter) (Five variants; Table S7), or canonical splice sites predicted or experimentally demonstrated to result in in-frame partial exon skipping/insertion (Four variants; Table S5).

The most frequently applied benign evidence criteria were BA1, BS2, and BP2. Forty-one variants were classified as benign based on the BA1 criterion, indicating the importance of establishing gene-specific allele frequency thresholds from population databases. With the benefit of sharing internal data from multiple diagnostic and research laboratories, BS2 and BP2_strong criteria were applied to 50 variants classified as B or LB. Application of BS2 alone allowed 24 variants to reach a LB classification, as described in section 3.1.3 (Table S2).

Discussion

The current work highlights the critical need for gene-/disease- specific and continuously evolving ACMP/AMP guidelines for improved variant interpretation. The *CDH1* VCEP has submitted 273 expert interpretations to ClinVar. Importantly, 33% of classifications (90/273) submitted by us had improved resolution of significance over previous ClinVar classifications. These included 35% of VUS (15/43) that were resolved to B/LB and 97% of variants (36/37) with CI that were resolved into B/LB/LP/P. Overall, 88% of variants (239/273) curated by the VCEP have non-VUS classifications, whereas for *CDH1* variants not yet curated by the VCEP, only 47% have non-VUS/CI (as of 6/25/2021). Resolution of

CDH1 VUS and CI variants is crucial for individuals with DGLBCS, since only individuals with a P/LP variant in *CDH1* are recommended to undergo a risk reducing total gastrectomy.

At any time, the most up-to-date recommendations of *CDH1* evidence criteria and contact information for the group can be found on the *CDH1* VCEP webpage (https://www.clinicalgenome.org/affiliation/50014). These variant interpretation guidelines are updated based on general recommendations from the ClinGen SVI, practical experience obtained from expert curations, and emerging evidence in specific gene and disease fields. Variant curations are ongoing and per ClinGen policy, VUS and LP variants need to be reassessed by VCEPs every two years. In addition, LB variants are required to be re-curated when a major updated population database is available (e.g., gnomAD V4), and other variants may be re-evaluated if discrepancies in the variant classification or relevant evidence becomes available. The *CDH1* VCEP will continue to submit three-star expert panel reviewed and FDA-recognized variant interpretations to ClinVar with the primary goal of resolving conflicting interpretations and VUS to ultimately improve clinical management including prophylactic surgery and cancer surveillance for DGLBCS patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Data availability

All variants have been submitted to ClinVar by the *CDH1* VCEP (https://www.ncbi.nlm.nih.gov/clinvar/submitters/506817/) and all evidence codes utilized in variant classifications are available in the ClinGen Evidence Repository (https://erepo.clinicalgenome.org/evrepo/).

References

1. Blair VR, McLeod M, Carneiro F, et al. Hereditary diffuse gastric cancer: updated clinical practice guidelines. Lancet Oncol 2020;21(8):e386–e97. doi: 10.1016/S1470-2045(20)30219-9 [published Online First: 2020/08/08] [PubMed: 32758476]

2. Corso G, Figueiredo J, La Vecchia C, et al. Hereditary lobular breast cancer with an emphasis on E-cadherin genetic defect. Journal of Medical Genetics 2018;55(7):431–41. [PubMed: 29929997]

3. Rehm HL, Berg JS, Brooks LD, et al. ClinGen--the Clinical Genome Resource. N Engl J Med 2015;372(23):2235–42. doi: 10.1056/NEJMsr1406261 [published Online First: 2015/05/28] [PubMed: 26014595]

4. Landrum MJ, Lee JM, Benson M, et al. ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res 2018;46(D1):D1062–D67. doi: 10.1093/nar/gkx1153 [published Online First: 2017/11/23] [PubMed: 29165669]

5. O'Daniel JM, Berg JS. A missing link in the bench-to-bedside paradigm: engaging regulatory stakeholders in clinical genomics research. Genome Med 2016;8(1):95. doi: 10.1186/s13073-016-0351-7 [published Online First: 2016/09/23] [PubMed: 27655359]

6. Ritter DI, Rao S, Kulkarni S, et al. A case for expert curation: an overview of cancer curation in the Clinical Genome Resource (ClinGen). Cold Spring Harb Mol Case Stud 2019;5(5) doi: 10.1101/mcs.a004739 [published Online First: 2019/10/28]

7. Lee K, Krempely K, Roberts ME, et al. Specifications of the ACMG/AMP variant curation guidelines for the analysis of germline CDH1 sequence variants. Hum Mutat 2018;39(11):1553–68. doi: 10.1002/humu.23650 [published Online First: 2018/10/13] [PubMed: 30311375]

8. Preston CG, Wright MW, Madhavrao R, et al. ClinGen Variant Curation Interface: a variant classification platform for the application of evidence criteria from ACMG/AMP guidelines. Genome Medicine 2022;14(1):1–12. [PubMed: 34986867]

9. Tavtigian SV, Greenblatt MS, Harrison SM, et al. Modeling the ACMG/AMP variant classification guidelines as a Bayesian classification framework. Genet Med 2018;20(9):1054–60. doi: 10.1038/gim.2017.210 [published Online First: 2018/01/05] [PubMed: 29300386]

10. Tavtigian SV, Harrison SM, Boucher KM, et al. Fitting a naturally scaled point system to the ACMG/AMP variant classification guidelines. Hum Mutat 2020 doi: 10.1002/humu.24088 [published Online First: 2020/07/29]

11. Jaganathan K, Kyriazopoulou Panagiotopoulou S, McRae JF, et al. Predicting Splicing from Primary Sequence with Deep Learning. Cell 2019;176(3):535–48 e24. doi: 10.1016/j.cell.2018.12.015 [published Online First: 2019/01/22] [PubMed: 30661751]

12. Ghosh R, Harrison SM, Rehm HL, et al. Updated recommendation for the benign stand-alone ACMG/AMP criterion. Hum Mutat 2018;39(11):1525–30. doi: 10.1002/humu.23642 [published Online First: 2018/10/13] [PubMed: 30311383]

13. Davidson AL, Leonard C, Koufariotis LT, et al. Considerations for using population frequency data in germline variant interpretation: Cancer syndrome genes as a model. Hum Mutat 2021;42(5):530–36. doi: 10.1002/humu.24183 [published Online First: 2021/02/19] [PubMed: 33600021]

14. Lowstuter K, Espenschied CR, Sturgeon D, et al. Unexpected CDH1 mutations identified on multigene panels pose clinical management challenges. JCO Precision Oncology 2017;1:1–12.

15. Abou Tayoun AN, Pesaran T, DiStefano MT, et al. Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. Hum Mutat 2018;39(11):1517–24. doi: 10.1002/humu.23626 [published Online First: 2018/09/08] [PubMed: 30192042]

16. Bazykin GA, Kochetov AV. Alternative translation start sites are conserved in eukaryotic genomes. Nucleic Acids Res 2011;39(2):567–77. doi: 10.1093/nar/gkq806 [published Online First: 2010/09/25] [PubMed: 20864444]

17. Drabkin HJ, RajBhandary UL. Initiation of protein synthesis in mammalian cells with codons other than AUG and amino acids other than methionine. Mol Cell Biol 1998;18(9):5140–7. doi: 10.1128/mcb.18.9.5140 [published Online First: 1998/08/26] [PubMed: 9710598]

18. Lee S, Liu B, Lee S, et al. Global mapping of translation initiation sites in mammalian cells at single-nucleotide resolution. Proc Natl Acad Sci U S A 2012;109(37):E2424–32. doi: 10.1073/pnas.1207846109 [published Online First: 2012/08/29] [PubMed: 22927429]

19. Na CH, Barbhuiya MA, Kim MS, et al. Discovery of noncanonical translation initiation sites through mass spectrometric analysis of protein N termini. Genome Res 2018;28(1):25–36. doi: 10.1101/gr.226050.117 [published Online First: 2017/11/23] [PubMed: 29162641]

20. Starck SR, Jiang V, Pavon-Eternod M, et al. Leucine-tRNA initiates at CUG start codons for protein synthesis and presentation by MHC class I. Science 2012;336(6089):1719–23. doi: 10.1126/science.1220270 [published Online First: 2012/06/30] [PubMed: 22745432]

21. Wan J, Qian SB. TISdb: a database for alternative translation initiation in mammalian cells. Nucleic Acids Res 2014;42(Database issue):D845–50. doi: 10.1093/nar/gkt1085 [published Online First: 2013/11/10] [PubMed: 24203712]

22. Zur H, Tuller T. New universal rules of eukaryotic translation initiation fidelity. PLoS Comput Biol 2013;9(7):e1003136. doi: 10.1371/journal.pcbi.1003136 [published Online First: 2013/07/23] [PubMed: 23874179]

23. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17(5):405–24. doi: 10.1038/gim.2015.30 [published Online First: 2015/03/06] [PubMed: 25741868]

24. de la Hoya M, Soukarieh O, Lopez-Perolio I, et al. Combined genetic and splicing analysis of BRCA1 c.[594–2A>C; 641A>G] highlights the relevance of naturally occurring in-frame transcripts for developing disease gene variant classification algorithms. Hum Mol Genet 2016;25(11):2256–68. doi: 10.1093/hmg/ddw094 [published Online First: 2016/10/30] [PubMed: 27008870]

25. Meulemans L, Mesman RLS, Caputo SM, et al. Skipping Nonsense to Maintain Function: The Paradigm of BRCA2 Exon 12. Cancer Res 2020;80(7):1374–86. doi: 10.1158/0008-5472.CAN-19-2491 [published Online First: 2020/02/13] [PubMed: 32046981]

26. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 2020;581(7809):434–43. doi: 10.1038/s41586-020-2308-7 [published Online First: 2020/05/29] [PubMed: 32461654]

27. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536(7616):285–91. doi: 10.1038/nature19057 [published Online First: 2016/08/19] [PubMed: 27535533]

28. Cheung R, Insigne KD, Yao D, et al. A Multiplexed Assay for Exon Recognition Reveals that an Unappreciated Fraction of Rare Genetic Variants Cause Large-Effect Splicing Disruptions. Mol Cell 2019;73(1):183–94 e8. doi: 10.1016/j.molcel.2018.10.037 [published Online First: 2018/12/07] [PubMed: 30503770]

29. Luo X, Feurstein S, Mohan S, et al. ClinGen Myeloid Malignancy Variant Curation Expert Panel recommendations for germline RUNX1 variants. Blood Adv 2019;3(20):2962–79. doi: 10.1182/bloodadvances.2019000644 [published Online First: 2019/10/28] [PubMed: 31648317]

30. Mester JL, Ghosh R, Pesaran T, et al. Gene-specific criteria for PTEN variant curation: Recommendations from the ClinGen PTEN Expert Panel. Hum Mutat 2018;39(11):1581–92. doi: 10.1002/humu.23636 [published Online First: 2018/10/13] [PubMed: 30311380]

31. Yelskaya Z, Bacares R, Salo-Mullen E, et al. CDH1 Missense Variant c.1679C>G (p.T560R) Completely Disrupts Normal Splicing through Creation of a Novel 5' Splice Site. PLoS One 2016;11(11):e0165654. doi: 10.1371/journal.pone.0165654 [published Online First: 2016/11/24] [PubMed: 27880784]

32. Landrith T, Li B, Cass AA, et al. Splicing profile by capture RNA-seq identifies pathogenic germline variants in tumor suppressor genes. NPJ Precis Oncol 2020;4:4. doi: 10.1038/s41698-020-0109-y [published Online First: 2020/03/07] [PubMed: 32133419]

33. Benusiglio PR, Malka D, Rouleau E, et al. CDH1 germline mutations and the hereditary diffuse gastric and lobular breast cancer syndrome: a multicentre study. J Med Genet 2013;50(7):486–9. doi: 10.1136/jmedgenet-2012-101472 [published Online First: 2013/05/28] [PubMed: 23709761]

34. Pena-Couso L, Perea J, Melo S, et al. Clinical and functional characterization of the CDH1 germline variant c.1679C>G in three unrelated families with hereditary diffuse gastric cancer. Eur J Hum Genet 2018;26(9):1348–53. doi: 10.1038/s41431-018-0173-8 [published Online First: 2018/05/18] [PubMed: 29769627]

Key Messages

What is already known on this topic

CDH1-specific variant classification guidelines based on the 2015 American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) classification framework have been developed by the ClinGen *CDH1* Variant Curation Expert Panel in 2018.

What this study adds

Here, we summarized eleven major modifications of the guidelines since the initial specifications and provided analysis of 273 expert panel curated *CDH1* variant interpretations. We demonstrated that the development and evolution of *CDH1*-specific variant interpretation guidelines reduced the number of variants of uncertain significance submitted to ClinVar and resolved clinically significant conflicts.

How this study might affect research, practice or policy

Accurate interpretation of *CDH1* germline variants is critical for physicians and individuals with pathogenic or likely pathogenic variants in *CDH1* who are faced with consideration of potentially morbid risk-reduction strategies including total gastrectomy and bilateral mastectomy in females. These guidelines should be utilized to classify germline *CDH1* variants to provide the most accurate information to patients and physicians and to ultimately improve clinical management including prophylactic surgery and cancer surveillance for DGLBCS patients.
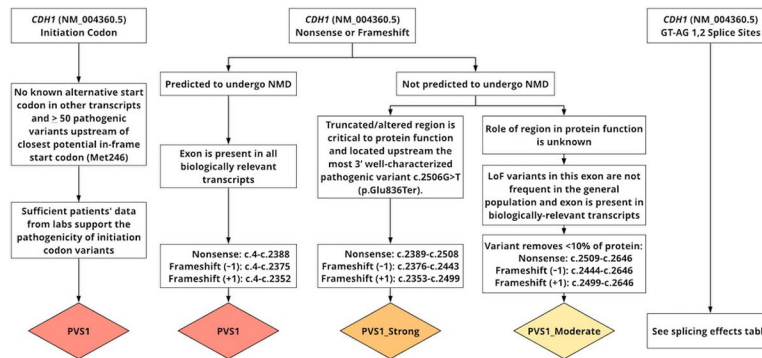
Figure 1: PVS1 decision tree for *CDH1*.
A gene-specific decision tree was developed to clarify rules for application of PVS1
(adapted from Tayoun et al 2018[15]). Initiation codon variants and nonsense or frameshift
variants predicted to undergo NMD should justify the PVS1 criterion at very strong strength.
For truncations in the NMD-resistant zone, PVS1 should be applied at strong or moderate
strength depending on whether the location of the variant is upstream or downstream of the
most 3' well-characterized pathogenic variant [NM_004360.5:c.2506G>T (p.Glu836Ter)].
Refer to Supplementary Table S5 for key splice site information including the recommended
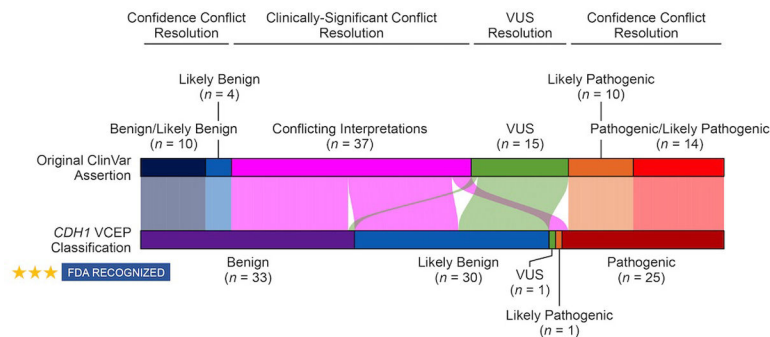strength of the PVS1 criterion for each donor and acceptor site.

Figure 2: Comparison of *CDH1* VCEP variant classifications to previous ClinVar assertions.
Using the *CDH1* specifications to the ACMG/AMP guidelines, 33% (90/273) variant classifications were altered and the change depicted here. Our data are limited in that ClinVar assertions for each variant were not extracted at the time of the curation and final expert interpretation of each *CDH1* variant. Therefore, the number of improved *CDH1* variant curations may be higher than reported therein. Despite this limitation in our analysis, *CDH1* expert curations still include resolution of 97% (36/37) variants with conflicting interpretations to a non-VUS classification. Eighteen variants with conflicting interpretations were resolved to benign, 16 to likely benign, one to VUS, one to likely pathogenic, and one to pathogenic. Fourteen VUS were resolved to likely benign and 1 to benign. Ten variants with a combination of benign/likely benign assertions and four variants like a likely benign assertion were resolved to benign, and 14 variants with a combination of pathogenic/likely pathogenic assertions and ten variants with a likely pathogenic assertion were resolved to pathogenic. Overall, 52 improvements resulted in resolution of a clinically significant conflict and 38 improvements resulted in resolution of a confidence conflict.

★★★ FDA RECOGNIZED	Totals	Benign	Likely Benign	Uncertain	Likely Pathogenic	Pathogenic
CDH1	273	51	38	34	28	122

Pathogenic Codes	PVS1	PS1	PS2	PS3	PS4	PM1	PM2	PM3	PM4	PM5	PM6	PP1	PP2	PP3	PP4	PP5
Very Strong	108				1											
Strong	31		2	7	10							5				
Moderate	13			4	29							5		2		
Supporting				2	65		183			103		7		6		
Total	152	0	2	13	105	0	183	0	0	103	0	17	0	8	0	0

Benign Codes	BA1	BS1	BS2	BS3	BS4	BP1	BP2	BP3	BP4	BP5	BP6	BP7	Total
Stand Alone	41												41
Very Strong													109
Strong		8	45	1			7						116
Moderate													53
Supporting			14				26		10			6	422
Total	41	8	59	1	0	0	33	0	10	0	0	6	741

Figure 3: Summary of 273 *CDH1* VCEP variant interpretations and frequency of application of each evidence code.

(a) The *CDH1* VCEP has curated and deposited 273 variant interpretations to the ClinVar database: 51 benign, 38 likely benign, 34 variants of uncertain significance, 28 likely pathogenic, and 122 pathogenic. (b) Frequency of application of each evidence code and its accompanying strength. The most frequently applied non-variant specific evidence codes applied were PS4, PM2, PP1, BS2, and BP2, demonstrating the importance of collating published and unpublished lab data provided by diagnostic laboratories for variant classification.