CDH1 germline variants are enriched in patients with colorectal cancer, gastric cancer, and breast cancer

Elio Adib1,2,6, Talal El Zarif2,6, Amin H. Nassar1,2,6, Elie W. Akl1, Sarah Abou Alaiwi1,2, Tarek H. Mouhieddine3, Edward D. Esplin4, Kathryn Hatchell5, Sarah M. Nielsen6, Huma Q. Rana5, Toni K. Choueiri2, David J. Kwiatkowski1,2,7 and Guru Sonpavde1,2,7

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BACKGROUND AND AIMS: CDH1 germline variants have been linked to heritability in diffuse gastric (DGC) and lobular breast cancer (LBC). Studies have not yet assessed whether CDH1 is a cancer-susceptibility gene in other cancers. Herein, we mapped the landscape of pathogenic and likely pathogenic (P/LP) germline variants in CDH1 across various cancers and ethnicities.

METHODS: We evaluated CDH1 germline P/LP variants in 212,944 patients at one CLIA-certified laboratory (Invitae) and described their frequency in 7 cancer types. We screened for CDH1 variant enrichment in each cancer relative to a cancer-free population from The Genome Aggregation Database version 3 (gnomADv3).

RESULTS: CDH1 P/LP variants were identified in 141 patients, most commonly in patients with DGC (27/408, 6.6%) followed by colorectal signet-ring cell cancer (CSRCC; 3/79, 3.8%), gastric cancer (56/2756, 2%), and LBC (22/6809, 0.3%). CDH1 P/LP variants were enriched in specific ethnic populations with breast cancer, gastric cancer, CRC, LBC, DGC, and CSRCC compared to matched ethnicities from gnomADv3.

CONCLUSION: We report for the first time the prevalence of P/LP CDH1 variants across several cancers and show significant enrichment in CDH1 P/LP variants for patients with CSRCC, DGC, and LBC across various ethnicities. Future prospective studies are warranted to validate these findings.

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INTRODUCTION
Germline variants in CDH1, which codes for the cell–cell adhesion protein E-cadherin, were first identified in families with hereditary diffuse gastric cancer (HDGC) [1]. Subsequent reports noted that individuals with germline CDH1 pathogenic variants were predisposed to both DGC and lobular breast cancer (LBC) [2]. Most recently, Massari et al. reported that 7% of all CDH1 mutations are present in non-gastric tumours with most being identified in patients with breast cancer [3]. Moreover, CDH1 mutations are more likely to be detected in areas with a low incidence of gastric cancer [4]. However, most reports have focused on highly penetrant HDGC families, in which the cumulative risk of HDGC for CDH1 mutation carriers is 70% by age 80 years for men and 56% for women, while the cumulative risk of LBC for women is estimated to be 42% by age 80 years [5]. A recent study found that among patients not exclusively ascertained based on strict HDGC criteria, the cumulative incidence of gastric cancer for individuals with pathogenic CDH1 variants is significantly lower (42% at age 80 years) than what has been previously reported [6, 7].

Although there is no evidence that the risk of other cancer types in individuals with a CDH1 variant is significantly increased [8], multiple case reports have noted the occurrence of CRC and appendiceal Signet-Ring Cell Carcinomas in CDH1 variant carriers [5]. In one study, colon cancer was reported in 3 of 238 (1%) CDH1 pathogenic variant carriers, with 1 case of SRCC. Most recently, Benesch et al. postulated through clinical observation and data from SEER that SRCC might be enriched among CDH1 variant carriers with signet ring cell gastric cancer [9]. Notably, there was no increased risk of colon cancer in CDH1 carriers compared to that of the SEER population [6].

The prevalence of CDH1 pathogenic variants in patients with gastric cancer and other cancer types is unknown. Here we examine the prevalence of CDH1 variants among 212,944 patients referred for genetic testing. We also examine germline cancer-risk variants by ethnicity across several tumour subtypes and identify, for the first time, CDH1 variant enrichment in individuals with CRC and colorectal signet-ring cell cancer (CSRCC).

METHODS
Patient cohort
Personal history information for 212,944 independent probands with cancer was obtained from submitted requisition forms and medical records. The
The genes selected for sequencing for each patient were chosen by the ordering clinician; all the patients reported here had been chosen for CDH1 analysis. Genomic DNA was extracted from whole blood using a QiaSymphony (Qiagen, Hilden, Germany). Targeted genes including CDH1 were captured using Agilent (Santa Clara, CA) SureSelect probes or Integrated DNA Technologies (Coral, IL) xGen Lockdown probes at ACB 0.02 0.04 0.06 0.5 1.0 1.5 2.0 2.5 Frequency (%) 77 14 2756 56 159 1 15235 6849 3 8575 0 0.02 0.04 0.06 0.5 1.0 1.5 2.0 2.5 Frequency (%) 3324 6186 3 42075 2 13670 75059 A 2756 56 159 1 15235 6849 3 8575 B 77 14 27915 27915 1 1 C 2756 56 159 1 15235 6849 3 8575 3324 6186 3 42075 2 13670 75059 375 75059

Fig. 1 Frequency and landscape of germline CDH1 variants. a Frequency of P/LP germline variants in CDH1 in each of the seven cancer types. The numbers above each bar indicate the total number of patients tested for each cancer type; the number within each box indicates the number of P/LP variants. Y axis is frequency. b Landscape of P/LP germline variants in CDH1 in 212,944 patients with cancer. The variants are labeled with carrier counts and coloured by their respective carriers’ ancestry (Caucasian: blue, African American: red, Asian: green, Ashkenazi Jewish: black, non-white Hispanic: orange) for breast cancer, gastric cancer, and colorectal cancer. c Frequency of P/LP germline variants in CDH1 in each of the five ethnicities in the gnomAD v3 cohort. The numbers above each bar indicate the total number of subjects assessed by sequencing; the number within each box indicates the number of P/LP variants.
positions where SureSelect yield was inadequate. Clinically important regions of CDH1 including all the coding exons and 10 to 20 base pairs of adjacent intronic sequences on either side of the coding exons were covered. Next-generation sequencing [10] was performed on the Illumina (San Diego, CA) MiSeq or HiSeq 2500 to at least 350× average coverage of 2 x 150 reads, with a minimum of 50x required at each targeted position. Stringent quality controls were used to minimise read-depth variability, and up to eight anonymous blood samples were used as control specimens in each run to measure remaining coverage variability [11].

Germline variant calling and assessment
Small indels and single-nucleotide variants were analysed using the Genome Analysis Toolkit [12]. Copy-number variant calls were performed using CNVitae [11]. Large structural variants were detected using split-read analysis. Candidate CDH1 variants were classified as pathogenic or likely pathogenic (P/LP) if they affected the structure of CDH1; conferred a truncating, initiation codon or splice donor/acceptor effect; if functional data showed an impact on protein function; or if pathogenicity was otherwise reported in the published literature [13]. Orthogonal technology was used to validate P/LP variants via Sanger sequencing or Multiplex Ligation-Dependent Probe Amplification [14]. Confirmed variants were interrogated using a refined American College of Medical Genetics and Genomics criteria (Sherloc) [15]. For each of the examined tumour subtypes, the frequency of pathogenic germline variants in CDH1 relative to the number of patients sequenced was calculated.

Ethnicity and enrichment analysis
Ethnicity was provided by all patients at the time of test ordering and was grouped based on categories reported in population databases. The following ethnicities were considered in the analysis: Ashkenazi Jewish, Asian, Black/African American, White/Caucasian, and Hispanic. For each ethnicity, we calculated the frequency of pathogenic germline variants relative to the number of patients in which the gene was tested. For every cancer subtype, we compared the frequency of pathogenic variants in CDH1 to the frequency of gene variants in an independent population derived from The Genome Aggregation Database version 3 (gnomAD v3) [16]. Two independent methods were followed: (1) All CDH1 variants in gnomAD were reviewed in ClinVar [17]. Variants in gnomAD deemed P/LP variants in ClinVar were retained. (2) Variants reported in gnomAD at a frequency >0.01% were excluded. Moreover, missense and synonymous variants in CDH1 from both the Invitae and gnomAD cohorts were excluded and only loss-of-function (LOF) variants were retained. LOF variants included frameshift, splice site, and nonsense variants, variants in the initiated codon, and exonic deletions.

For both analyses, the frequency of germline variants in each of the ethnic populations (Ashkenazi Jewish, Asian, Black/African American, Hispanic, and White/Caucasian) in the Invitae cancer cohort were compared to the frequency of these gene variants across various ancestries derived from gnomAD v3. CDH1 variants were considered enriched in a cancer subtype if they met both criteria: (1) they were significantly more likely to occur in a specific ethnic population with cancer when compared to the same ancestry in gnomAD and (2) the p-value was significant in both “LOF” and “ClinVar” analyses.

Statistical analysis
Two-sided Fisher’s exact test was used to calculate the odds ratios, 95% confidence intervals (CIs), and p-values of all enrichment analyses. For the enrichment analysis, we applied Benjamini–Hochberg correction for the number of independent tests conducted (significant q-value cutoff of <0.05).

RESULTS
Germline landscape of CDH1 variants in the Invitae cohort
Of the 212,944 patients with cancer and available CDH1 sequencing data, 151,465 had breast cancer (71.1%), 27,915 had CRC (13.1%), 15,225 had ovarian cancer (7.1%), and 18,339 (8.6%) had other cancer types (Fig. 1a and Table S1.1). Detailed clinical history was available on all 141 patients with P/LP variants in CDH1 (Table S1.1). The most common cancer types in patients with P/LP CDH1 variants were breast cancer (77 of 141, 54.6%), gastric cancer (56 of 141, 39.7%), and CRC (14 of 141, 9.9%). Among patients with breast cancer of known history (n = 30), 8 (27%) had ductal and 22 (73%) had LBC. Notably, five probands with CDH1 P/LP variants had concomitant gastric cancer and CRC. Variant type and location are shown in Fig. 1b and did not vary according to cancer type. The median age at diagnosis of breast, colorectal, and gastric cancers among patients with CDH1 P/LP variants was lower than that of the general population from the SEER cohort (Table S1.2) [18].

Among all major cancer types, gastric cancer had the highest frequency of P/LP variants (56 of 2756, 2%, 95% CI = 1.5–2.6%, Fig. 1a and Table S1.3) followed by head and neck (1 of 159, 0.6%, 95% CI = 0–3.5%) and breast cancer (77 of 151,465, 0.05%, 95% CI = 0.04–0.06%). Acknowledging that there were relatively small numbers of subjects in some categories (e.g. Ashkenazi Jewish with gastric cancer), none of the five ethnicities showed significantly higher CDH1 P/LP variant frequency for any cancer type (p-values of pairwise comparisons in Table S1.4). Various population groups and their cohort sizes are shown in Supplementary Table S1.5.

Enrichment analysis of major cancer subtypes
We then conducted enrichment analysis (see methods) and found that the odds of P/LP germline variants in CDH1 among African Americans, Asians, Caucasians, and Hispanics with gastric cancer in our cohort was significantly higher (126-fold to infinity) in comparison to the odds in the corresponding gnomAD ancestry cohorts (Figs. 1c and 2a, b and Table S1.5). Caucasians with breast cancer and CRC were enriched for CDH1 germline variants compared to Caucasian controls from gnomAD. The odds ratio was also increased for CDH1 variants in several other ethnicities for each of breast and colorectal cancer, though in general to a smaller degree, and false discovery rate-corrected q-values were not significant.

Enrichment of CDH1 variants in DGC and LBC
We then analysed histology-specific associations and found germline P/LP variants in CDH1 in 6.6% (27 of 409) of patients with DGC and 0.3% (22 of 6955) of patients with LBC. The median age at diagnosis of patients with DGC and LBC harbouring CDH1 P/LP variants was 42 (range 19–75) and 48 years (range 41–66), respectively. Interestingly, among patients with DGC in our cohort, Caucasians harboured significantly more CDH1 P/LP germline variants compared to Asians and Hispanics (20/192, 10.4% vs 1/63, 0.4%).

Table 1. Clinical and pathological characteristics of the 212,944 patients with cancer and available CDH1 sequencing data.

| Patients with cancer | N = 212,944 | Percent |
|----------------------|-------------|---------|
| Gender               |             |         |
| Female               | 188,416     | 88.5%   |
| Male                 | 24,528      | 11.5%   |
| Ethnicity            |             |         |
| Ashkenazi            | 5836        | 2.7%    |
| Asian                | 8868        | 4.2%    |
| Black/African American| 18,073      | 8.5%    |
| Hispanic             | 11,734      | 5.5%    |
| White                | 168,433     | 79.1%   |
| Tumour Type          |             |         |
| Breast cancer        | 151,465     | 71.1%   |
| Colorectal cancer    | 27,915      | 13.1%   |
| Gastric cancer       | 2756        | 1.3%    |
| Head and neck cancer | 159         | 0.1%    |
| Ovarian cancer       | 15,225      | 7.1%    |
| Pancreatic cancer    | 6849        | 3.2%    |
| Prostate cancer      | 8575        | 4%      |
1.6%, \( p = 0.032 \) and 3/94, 3.2% vs 0/13670, 0%, \( p = 0.038 \) respectively, Fig. 3a and Table S1.6). However, in LBC, none of the five ethnicities showed significantly higher CDH1 P/LP variant frequency (Fig. 3b and Table S1.6).

Enrichment analysis with gnomAD showed that African Americans and Caucasians with LBC and DGC were enriched for P/LP CDH1 germline variants. Ashkenazi Jews, Asians, and Hispanics harboured significantly more CDH1 P/LP variants compared to controls from gnomAD in LBC and DGC, respectively (Table S1.7).

**Prevalence of CDH1 variants in CSRCC and enrichment compared to gnomAD**

Prior case reports suggested that an association between CSRCC and CDH1 germline carriers exists [5, 19]. We found that 3.8% of patients with CSRCC (3 of 79) harboured CDH1 P/LP variants. Age at diagnosis for two of the three patients was known (35 and 41 years). Compared to corresponding controls from gnomAD, African Americans and Caucasians with CSRCC were enriched for P/LP CDH1 germline variants (African American LOF analysis: \( q = 0.0017, \ OR = 1365, \ 95% \ CI = 24–16,384 \); African American ClinVar Analysis: \( q = 0.001, \ OR = 1365, \ 95% \ CI = 24–16,384 \); Caucasian LOF analysis: \( q = 0.0001, \ OR = 969, \ 95% \ CI = 80–8192 \); Fig. 2a, b and Table S1.7, see “Methods”). However, we consider these findings tentative given the small number of patients within each ethnic group in our study (Fig. 3c and Table S1.6).

**DISCUSSION**

The prevalence of CDH1 germline variants in patients with various cancer types is still not well-described. Herein, we found CDH1 germline carriers in about 7% of patients with DGC and 3.8% of patients with CSRCC. To date, there is conflicting evidence regarding the prevalence of CDH1 P/LP germline variants in LBC. In our study, among 6809 patients with LBC, approximately 0.3% harboured germline variants in CDH1. This frequency is lower than what is reported in prior studies (1–8%), which focused mainly on patients with early-onset or bilateral disease [20, 21]. In a
comprehensive review of hereditary LBC, Corso et al. emphasised the importance of surveillance in CDH1 P/LP variant carriers [22]. With increasing knowledge about LBC risk factors, CDH1 germline genetic testing in high-risk families remains paramount.

Prior studies have led to conflicting results regarding the prevalence of CDH1 germline variants in Asians vs non-Asians. Despite the high incidence of gastric cancer in East Asian countries, lower incidence of gastric cancer [23]. More recently, a study [24] of 105 Japanese patients with DGC found that germline variants in CDH1 occurred in 14 patients (13.3%) and showed that Japanese patients with gastric cancer were four times more likely to harbour LP variants by our criteria. Whether those 14 variants are truly non-

Our study has several limitations. First, the selection of patients and thus determining cancer-risk susceptibility genes presents an unmet need. Guidelines for genetic testing factor in the under-

Fig. 3 Ethnicity-specific enrichment of CDH1 variants in three cancer subtypes. a-c Frequency of pathogenic and likely pathogenic germline variants in CDH1 in each of the five ethnicities in the Invitae cohort for diffuse gastric cancer (a), lobular breast cancer (b), and CSRCC (c). A two-sided binomial test was used to compute the p-values. *p-value < 0.05.

When comparing the prevalence of CDH1 P/LP variants across the different ethnicities within each cancer type or subtype, none of the five ethnic populations showed significantly higher CDH1 P/

CDH1 is a tumour suppressor, and the vast majority of variants detected here were LOF (128 of 141, 90.8%, 95% CI = 84.9–94.5%), and distributed throughout the coding sequence. A prior study reported significant enrichment of CDH1 germline variants located in the PRE-PRO region (amino acid 1–115) in HDGC families affected by CRC. In addition, patients harbouring CDH1 variants in the linker region (regions shown in white, Fig. 1B) were significantly less likely to develop breast cancer [26]. In this dataset, there was no association between the location of CDH1 variant and the development of individual cancers.

Our study has several limitations. First, the selection of patients for genetic testing was influenced by clinical judgement and was likely skewed towards individuals with a significant suspicion for
heritable pathogenic variants. Second, personal and family history data were obtained from genetic testing requisition forms and were not confirmed by direct review of the medical records or other data sources. Ethnicity information was provided by the subjects with no confirmation. Similarly, there was no central pathological confirmation of either the cancer type or subtype. We caution against overinterpreting ethnicity-specific CDH1 associations as only a handful of carriers may drive enrichment in a small cancer cohort.

In the largest study to date evaluating CDH1 germline variants, we found significant enrichment of P/LP variants in patients with CSRC, CRC, breast, and gastric cancer. Importantly, we found that the frequency of P/LP CDH1 variants in multiple cancer types did not vary according to ethnicity for the most part. This is the first report on the prevalence of CDH1 variants in African American and Hispanic populations and indicates that these populations have the same frequency of P/LP variants as Caucasians and should be subject to the same germline testing and screening considerations.

DATA AVAILABILITY
Data will be available in Supplementary Table S1 and Supplementary Materials.

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AUTHOR CONTRIBUTIONS
EA, TZ, AHN, EWA, DJK, and GS designed this study. EA, TZ, AHN, EWA, SAA, THM, KH, WIR, FK, DDI, and GS performed the study. EA, TZ, AHN, and EWA analyzed the results and generated the figures. EA, TZ, AHN, DJK, and GS drafted the manuscript. All authors reviewed the manuscript, approved the final version and take responsibility for all aspects of this work.

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Correspondence and requests for materials should be addressed to David J. Kwiatkowski or Guru Sonpavde.

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