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ORIGINAL ARTICLE

Germline Cancer Testing in Unselected Patients with Gastric and Esophageal Cancers: A Multi-center Prospective Study

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Abstract

Background and Aims To determine prevalence and clinical utility of pathogenic germline variants (PGV) in gastric and esophageal cancer patients using universal genetic testing approach.

Methods We undertook a prospective study of germline sequencing using an > 80 gene next-generation sequencing platform among patients with gastric and esophageal cancers receiving care at Mayo Clinic Cancer Center between April 1, 2018, and March 31, 2020. Patients were not selected based on cancer stage, family history of cancer, ethnicity, or age. Family cascade testing was offered at no cost.

Results A total of 96 patients were evaluated. Median age was 66 years, 80.2% were male, 89.6% were white. Nearly 39% of the cohort had esophageal cancer, 35.4% gastric cancer and 26% gastroesophageal junction cancers. Approximately half (52%) of the patients had metastatic disease. Pathogenic germline variants (PGV) were detected in 15.6% ($n = 15$) patients. The prevalence of PGV was 10.8% in esophageal cancer, 17.6% in gastric cancer and 20% in gastroesophageal cancer. Eighty percent of patients with a positive result would not have been detected by screening with standard guidelines for genetic testing. Most PGV detected included genes with high and moderate penetrance related to DNA damage response including *BRCA1, BRCA2, PALB2* and *ATM*.

Conclusions Universal multi-gene panel testing in gastric and esophageal cancers was associated with detection of heritable mutations in 15% of patients. The majority of PGV would not be detected with current screening guidelines and are related to DNA damage response.

Keywords Gastric cancer · Esophageal cancer · Germline testing · BRCA

Introduction

Gastric and esophageal cancers jointly cause significant morbidity and mortality. Worldwide it was estimated that there were more than 1 million new cases of gastric cancer in 2020 [1]. In the same period, it is estimated that there were 768,793 deaths related to the disease, ranking third in cancer-related deaths in the world [1]. However, in the USA and Western Europe, the incidence of gastric cancer has decreased in the last decade [2]. Gastric cancer is often related to environmental factors including Helicobacter Pylori infection, smoking and diet [3–5] and it is estimated

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that only 5% are related to inherited cancer predisposition syndromes [2]. National Comprehensive Cancer Network (NCCN) guidelines recommend that genetic risk assessment and counseling should be based on age of diagnosis and number of relatives affected with gastric cancer or a family history of juvenile polyps or Lynch Syndrome [2]. One of the most common hereditary syndromes associated with gastric cancer is hereditary diffuse gastric cancer, where 30–50% of the families carry germline aberrations in the tumor suppressor gene *CDH1* [6].

The decrease in incidence of gastric cancers in the USA and Western Europe are related to changes in food conservation and preservatives, diet, hygiene, and eradication of Helicobacter Pylori [2, 7, 8]. Interestingly, the incidence of esophageal and gastroesophageal junction (GEJ) cancers in the USA is on the rise [2]. Globally, esophageal and GEJ tumors have high incidence in middle- and low-income countries, with an estimated incidence of 604,100 new cases in 2020 [1]. Smoking and alcohol are the most common risk factors associated with squamous esophageal carcinoma, with high incidence in Eastern Europe and Asia [9, 10], whereas, esophageal and GEJ adenocarcinoma are more common in North America and Western Europe, generally related to obesity and gastroesophageal reflux disease possibly associated with Barrett's esophagus [11–13]. Several familial high-risk syndromes may be related to the development of esophageal cancers; however, no specific guidelines or recommendations for hereditary cancer risk assessment for these cancers are available [2].

Little is known about germline genetic testing in unselected patients with gastric cancer and esophageal cancer. Expanded germline testing in these patients could improve the current understanding of the disease and help establish specific guidelines. In this article, we report the findings and clinical characteristics of a multi-center prospective cohort of gastric and esophageal cancer patients who underwent germline testing with next-generation sequencing using a > 80 gene panel [14].

Methods

Patient Selection: Inclusion and Exclusion Criteria

From April 1, 2018 through March 31, 2020, a total of 2,984 adult (18 years of age or older) patients with a new or active diagnosis of cancer were recruited from medical oncology, radiation oncology, dermatology and surgical oncology clinics at any of the four Mayo Clinic destination Cancer Centers in Phoenix, AZ; Jacksonville, FL or Rochester, MN, and a community oncology practice (Eau Claire, WI) as part of the Interrogating Cancer Etiology Using Proactive Genetic Testing (INTERCEPT) program [14]. Patients undergoing surveillance post-curative cancer or with hematological malignances were excluded. Research coordinators of each site recruited patients using central lists of daily oncology clinic visits. Germline sequencing using a next-generation sequencing (NGS) panel of 83 genes (84 genes as of July 2019) was offered at no cost for all participants and had disclosure of results. This panel included all cancer-predisposing genes identified in the American College of Medical Genetics and Genomics guidelines. Patients in this study were not selected based on clinical characteristics, including family or personal history of cancer, cancer type, multifocal tumor, stage of disease, ethnicity or age at diagnosis. Patients with a previously established molecular diagnosis of a cancer genetic syndrome were excluded from the INTERCEPT study; however, none of the patients in this cohort had a prior genetic diagnosis. From this cohort, 96 patients with a diagnosis of gastric, esophageal, and GEJ cancers were analyzed in this study. Detailed methodology is provided in the following manuscript [14].

Prior to undergoing genetic testing, all patients viewed a standard pretest education video and were offered additional pretest genetic counseling if they desired. All test results were reviewed by a certified genetic counselor or physicians with expertise in cancer genetics. Individuals with pathogenic or likely pathogenic variants were invited for genetic counseling. Family cascade testing at no additional cost was offered.

Clinical, demographic, family history, treatment types and clinical outcome information were collected on all patients in this study either from medical records or self-administered electronic questionnaires for family pedigree information.

This study was approved by the Mayo Clinic Institutional Review Board (IRB 18–000,326). All patients provided written informed consent. Data were de-identified except to investigators of the study.

Sequencing, Variant Calling and Result Reporting

All patients underwent next-generation sequencing (NGS) germline genetic testing with a multi-gene cancer panel of 83 genes (84 genes as of July 2019) on the Invitae Multicancer panel. Full gene sequencing, deletion/duplication analysis, copy number variant detection and variant interpretation were performed at Invitae (San Francisco, CA), as previously described [14]. All patients had their variant findings source verified and confirmed by independent review of the test results by a medical geneticist. Pathogenic germline variants (PGV) were classified as highly (relative risk [RR] > 4), intermediate (RR 2–4) or low (RR < 2) penetrant, recessive or of uncertain clinical actionability based on disease risks and prior modeling. Investigators and treating clinicians

were not blinded to the genetic testing results which were returned in real time to inform clinical management.

Statistical Analysis

Descriptive statistics for demographic, clinical and treatment-related characteristics of the cohort were examined. Survival time was calculated as the number of months from the date of cancer diagnosis to the date of death or last follow-up. Due to the small group sizes and clinical differences among these cancer sites, formal statistical tests were not performed. Rates of detection of clinically actionable findings using 2018 and 2020 NCCN guidelines were calculated. Rates of uptake of family variant testing (FVT) and mutation rates of tested family members were examined.

Results

Cohort Characteristics

Demographic distribution of the cohort is shown in Table 1, stratified by primary tumor location. Overall, 80.2% of included patients are male, 89.6% white, the median age at diagnosis was 66 years. More than half (55.2%) were smokers, 49% had hypertension, 20.8% had a history of diabetes and 15.6% had a body mass index higher than 30 (Supplementary table 1).

Primary tumor location included esophagus ($n = 37$, 38.5%), gastric ($n = 34$,35.4%) and gastroesophageal junction ($n = 25$, 26%). The proportion of patients with early stages (I and II) was 25%, and late-stage disease (III and IV) was 75%. Complete pedigree was obtained for 44 (45.8%) patients. Family history of cancer in a first-degree relative was reported in 33 patients (34.4%).

The median follow-up time across all patients in this cohort was 17.3 months. In that time, 38.5% of patients expired. The median survival time for patients with esophageal cancer was 18.8 months (range: 4.4–74.5), for gastric cancer was 17 months (range: 1.7–99.4) and for gastroesophageal junction tumors was 15.8 months (range: 2.5–52.6).

Variants Detection

Of the 96 patients undergoing germline analysis, 15 patients (15.6%) carried pathogenic/likely pathogenic variants (PGV) conferring cancer predisposition (Fig. 1), with 11 (73.3%) of the PGV in high and moderate penetrance genes (Table 2). The most common variants were found in DNA damage repair (DDR) genes including *BRCA1, BRCA2* (26.7%) and *ATM* (13.3%). No PGVs were detected in the Lynch syndrome associated genes (Fig. 2).

The PGV prevalence in esophageal cancer was 10.8%, gastric cancer was 17.6% and gastroesophageal junction tumors was 20%. Median age of patients with a PGV was 70 years (mean age 64.5 years old, 93% were ≥ 50 y/o), and only four of fifteen patients (26.7%) with a PGV had a family history of cancer in first-degree relative (Supplementary table 2). Of the 15 patients found to have a PGV, 80% (12) had PGVs that are qualifiers for potential clinically actionable management or treatment changes (Supplementary table 3). These can be categorized into potential eligibility for clinical treatment trials (47%) or published guideline management recommendations (80%). All systemic treatments are reviewed in Supplementary Table 4.

Applying the 2020 National Comprehensive Cancer Network (NCCN), National Society of Genetic Counselors (NSCG) or American College of Medical Genetics and Genomics (ACMG) genetic testing referral criteria, 80% (12 of 15) carriers of a PGV would not have been detected. Only two of the PGV carriers met guidelines based on family history regardless of personal history (Table 3). Of the seven patients potentially eligible for clinical trials, five (71%) would be missed by strict adherence to testing criteria (Supplementary table 5).

No cost family variant testing (FVT) was offered to all blood relatives of affected participants. Only two patients (13.3%) with PGV had family members undergo FVT within a 3-month window of their test result.

Discussion

Universal germline genetic testing in unselected patients with gastric, gastroesophageal junction and esophageal cancers was able to identify PGV in 15.6% of patients with the majority in high and moderate penetrance genes, including those in DNA damage repair pathways where precision targeted therapies exist. The incidence of PGVs was higher in GEJ tumors when compared to gastric and esophageal cancers. The population frequency of PGVs in *BRCA1, BRCA2, CDH1, PALB2, SDHA, ATM, HOXB13, MITF*, and *FH* is estimated to be less than 1%, and for *MUTYH* heterozygotes to be 1–2% [15–17]. Accordingly, the frequencies of PGVs in this cohort in high penetrance genes *BRCA1, BRCA2, CDH1, PALB2* and *SDHA*, moderate penetrance genes *ATM, HOXB13* and *MITF*, and low penetrance/recessive genes *MUTYH* (monoallelic) and *FH* all appear to be overrepresented in these patients with cancer compared to the general population.

Importantly, 80% of the PGV would not have been detected using current (2020) guidelines for genetic testing from national societies or professional organizations. For example, NCCN guidelines for considering germline genetic testing of individuals with gastric cancer require patients

Table 1 Cohort characteristics

	Esophageal (*N* = 37)	Gastric (N = 34)	GEJ (*N* = 25)	Total (*N* = 96)
Region				
Midwest	7 (18.9%)	7 (20.6%)	5 (20.0%)	19 (19.8%)
Southeast	8 (21.6%)	12 (35.3%)	7 (28.0%)	27 (28.1%)
Southwest	22 (59.5%)	15 (44.1%)	13 (52.0%)	50 (52.1%)
Gender				
Male	33 (89.2%)	23 (67.6%)	21 (84.0%)	77 (80.2%)
Female	4 (10.8%)	11 (32.4%)	4 (16.0%)	19 (19.8%)
Age				
Mean (SD)	68.3 (8.1)	62.0 (14.0)	62.4 (8.4)	64.5 (10.9)
Median	70.0	63.5	63.0	66.0
Range	49.0–80.0	18.0–80.0	34.0–75.0	18.0–80.0
Race (dichotomized)				
White	35 (94.6%)	28 (82.4%)	23 (92.0%)	86 (89.6%)
Non-white	2 (5.4%)	6 (17.6%)	2 (8.0%)	10 (10.4%)
Ethnicity (dichotomized)				
Hispanic/Latino	1 (2.7%)	2 (5.9%)	1 (4.0%)	4 (4.2%)
Non-Hispanic	36 (97.3%)	32 (94.1%)	24 (96.0%)	92 (95.8%)
Germline Result				
Positive	4 (10.8%)	6 (17.6%)	5 (20.0%)	15 (15.6%)
Negative	13 (35.1%)	9 (26.5%)	5 (20.0%)	27 (28.1%)
VUS	20 (54.1%)	19 (55.9%)	15 (60.0%)	54 (56.2%)
Histological type				
Adenocarcinoma	33 (89.2%)	31 (91.2%)	25 (100.0%)	89 (92.7%)
Squamous cell carcinoma	3 (8.1%)	1 (2.9%)	0 (0.0%)	4 (4.2%)
Other	1 (2.7%)	2 (5.9%)	0 (0.0%)	3 (3.1%)
Barrett's esophagus				
Yes	20 (54.1%)	3 (8.8%)	9 (36.0%)	32 (33.3%)
No	17 (45.9%)	31 (91.2%)	16 (64.0%)	64 (66.7%)
Helicobacter Pylori				
Yes	2 (5.4%)	7 (20.6%)	1 (4.0%)	10 (10.4%)
No	35 (94.6%)	27 (79.4%)	24 (96.0%)	86 (89.6%)
Result (dichotomized)				
Positive	4 (10.8%)	6 (17.6%)	5 (20.0%)	15 (15.6%)
VUS/Negative	33 (89.2%)	28 (82.4%)	20 (80.0%)	81 (84.4%)
Staging AJCC 8th edition at diagnosis (Clinical stage)				
1	5 (13.5%)	4 (11.8%)	1 (4.0%)	10 (10.4%)
2	5 (13.5%)	7 (20.6%)	2 (8.0%)	14 (14.6%)
3	6 (16.2%)	7 (20.6%)	9 (36.0%)	22 (22.9%)
4	21 (56.8%)	16 (47.1%)	13 (52.0%)	50 (52.1%)
Staging AJCC 8th edition (Early vs. Late)				
Early Stage (0–2)	10 (27.0%)	11 (32.4%)	3 (12.0%)	24 (25.0%)
Late Stage (3–4)	27 (73.0%)	23 (67.6%)	22 (88.0%)	72 (75.0%)
Deceased				
Yes	13 (35.1%)	13 (38.2%)	11 (44.0%)	37 (38.5%)
No	24 (64.9%)	21 (61.8%)	14 (56.0%)	59 (61.5%)
Overall Survival (months)				
Mean (SD)	23.3 (16.6)	22.7 (21.9)	17.2 (13.8)	21.5 (18.0)
Median	18.8	17.0	15.8	17.3
Range	4.4–74.5	1.7–99.4	2.5–52.6	1.7–99.4

Percentages calculated by column

Legend: GEJ: gastroesophageal junction tumors, AJCC: American Joint Committee on Cancer, VUS: variant of uncertain significance

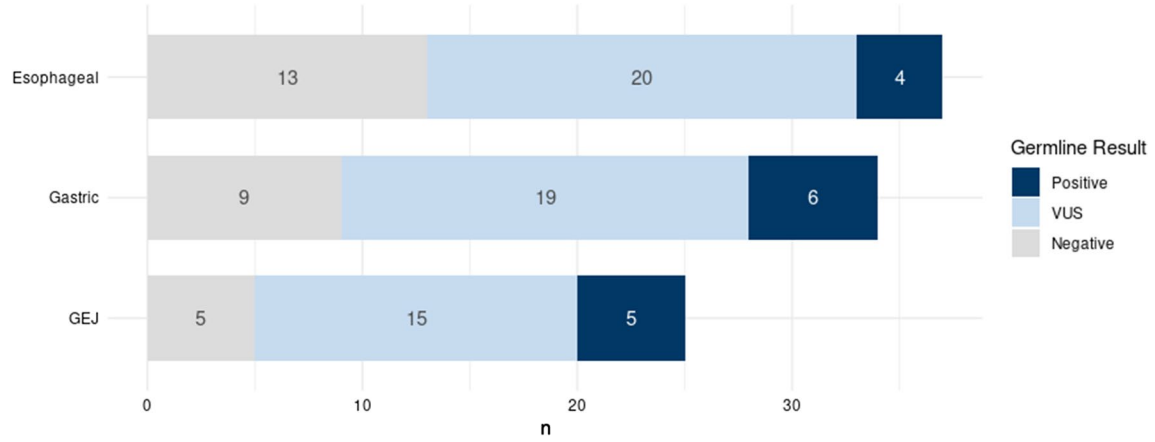

Fig. 1 Prevalence of pathogenic germline variants

Table 2 Distribution of pathogenic germline variants by penetrance status

	Pathogenic variant	Total ($n=15$)
High Penetrance	*BRCA1*	2 (13.3%)
	BRCA2	2 (13.3%)
	CDH1	1 (6.7%)
	PALB2	1 (6.7%)
	SDHA	1 (6.7%)
Moderate Penetrance	*ATM*	2 (13.3%)
	HOXB13	1 (6.7%)
	MITF	1 (6.7%)
Low Penetrance	*MUTYH (monoallelic)*	1 (6.7%)
Recessive Alleles	*FH*	2 (13.3%)
	RECQL4	1 (6.7%)

meet a complicated combination of criteria for eligibility [2]. This includes any one of the following: being diagnosed with diffuse gastric cancer (DGC) before 50 years of age without a family history, having a personal or family history of DGC and lobular breast cancer (one diagnosed < 70 years of age), two gastric cancer cases in a family (one confirmed DGC regardless of age), two cases of lobular breast cancer in family members < 50 years of age, DGC in any age in individuals of Maori ethnicity (or with personal or family history of cleft lip/cleft palate), or bilateral lobular breast cancer < 70 years of age.

For patients with GEJ or esophageal cancers, the NCCN does not have any guidelines recommending germline genetic testing, indicating that, "Although early age of onset, multiple family members with the same or related cancer, and individuals with multiple primary cancers are all signs of hereditary cancer, specific referral guidelines for esophageal and [GEJ] cancers risk assessment are not possible at this time." Accordingly, none of the patients with GEJ or esophageal cancers would be recommended for germline genetic testing because the NCCN does not have guidelines that apply to these patients [2].

The incidence of gastric cancer worldwide is relatively low in countries such as the USA and highest in East Asian countries such as Japan. However, the rate of germline mutations in CDH1 in patients with gastric cancer varies inversely with the incidences of gastric cancer, with high CDH1 mutation detection rates in the USA and low detection rates in Japan [18]. Prior to identification of CDH1 germline mutations in Japanese patients, nearly all cases of gastric cancer were attributed to past exposure to H. pylori or some other carcinogen such as food and smoking, in the setting of a widely implemented screening program for gastric cancer. Identification of CDH1 PGVs in Japanese gastric cancer patients, though low in frequency, led to the recommendation to screen for CDH1 PGVs, as part of medical management in gastric cancer patients in Japan [19]. However, due in part to low rates of detection for CDH1 PGVs in countries such as Japan, genetic testing is often underutilized. The higher rate of PGVs in CDH1 and other cancer risk genes in US patients, as reported here, may provide motivation to offer germline genetic testing to all patients with gastric, GEJ and esophageal cancers.

Although the majority of gastric cancer cases are felt to be sporadic, up to 20% of gastric cancer patients may also have relatives with gastric cancer suggesting a familial risk. From those patients, only 3–5% are found to have an inherited germline variant detected [20, 21]. Multiple cancer predisposition syndromes may be associated with the development of gastric cancers such as Li Fraumeni syndrome, Lynch syndrome and *CDH1*-associated hereditary diffuse gastric cancer [20, 22, 23]. Most patients with families that meet clinical criteria for hereditary diffuse gastric cancer will harbor *CDH1* germline mutations [24]. However, for *CDH1*-mutation negative patients, application of

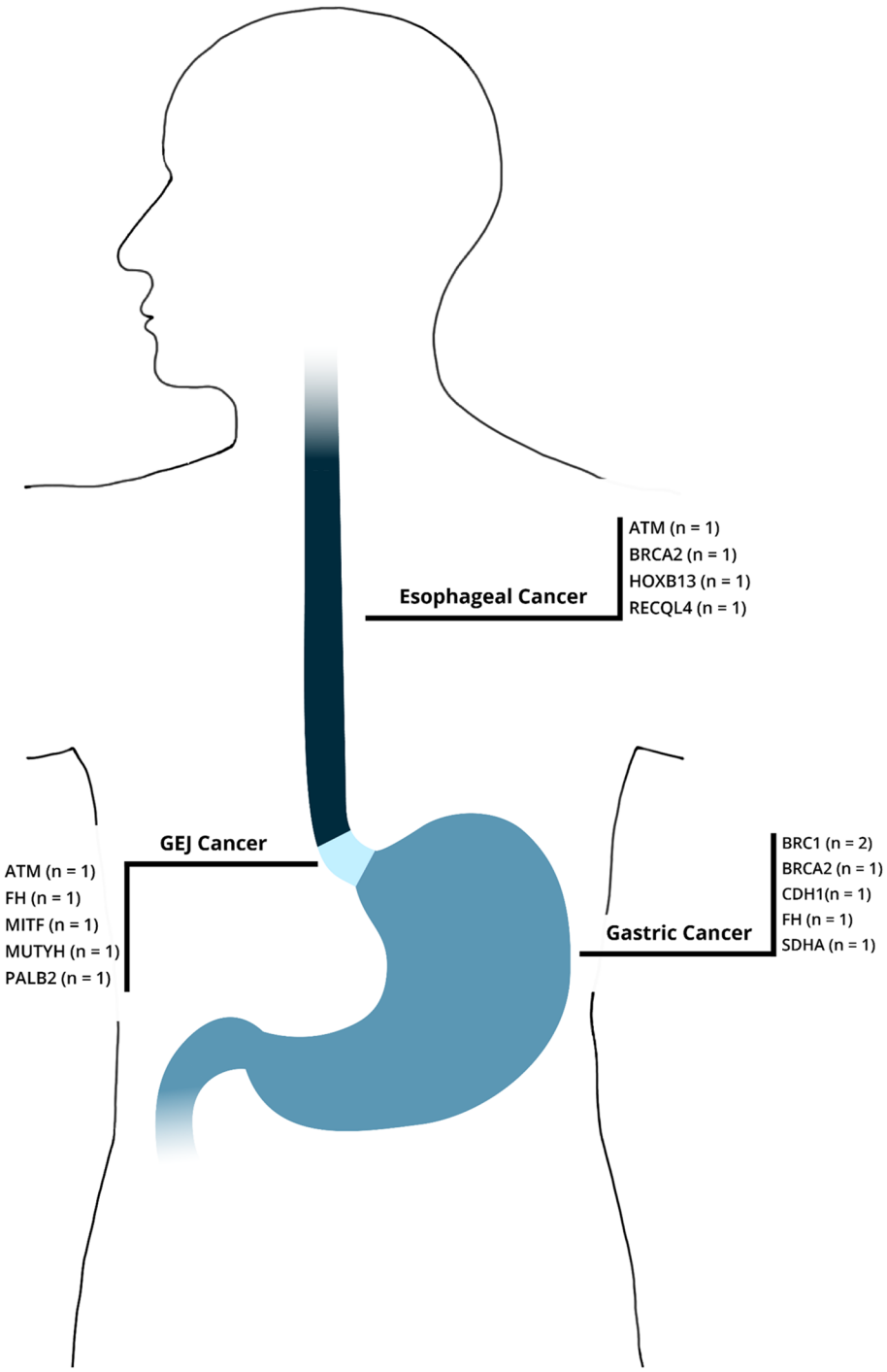

Fig. 2 Pathogenic germline variants stratified by primary tumor location

targeted sequencing has identified pathogenic variants in other related genes including *CTNNA1, BRCA1, BRCA2, STK11, SDHB, PRSS1, ATM, MSR1, RAD51* and *PALB2* [24–30]. DDR genes are of particular interest considering possible targeted treatment strategies in this group of patients with homologous recombination deficient tumors, including platinum-based therapies and poly-ADP-ribose polymerase (PARP) inhibitors such as olaparib. In the current manuscript, application of a universal multi-gene panel testing found 17.6% of gastric cancer patients harbored a PGV. Interestingly along with a PGV in the gene *CDH1*, PGV in DDR genes including *BRCA1* and *BRCA2* were identified. Exploiting DDR genes have already been the subject of a randomized trial in ATM-negative metastatic gastric cancer [31]. However, the combination paclitaxel plus olaparib did not improve overall survival [32]. Nowadays other combinations with olaparib are being developed, including with anti-programmed death-ligand 1

Table 3 Screening guidelines met by patients with pathogenic germline variant

	2018 ($N=15$)	2020 ($N=15$)
Did they meet NCCN/NSGC/ACMG testing guidelines?		
Yes	3 (20.0%)	3 (20.0%)
No	12 (80.0%)	12 (80.0%)
Of those that met a screening guideline, which guidelines were met?		
NCCN		
Yes	1 (6.7%)	2 (13.3%)
No	14 (93.3%)	13 (86.7%)
NSGC/ACMG		
Yes	2 (13.3%)	2 (13.3%)
No	13 (86.7%)	13 (86.7%)
Did they meet guidelines based on family history regardless of personal history?		
Yes	2 (13.3%)	2 (13.3%)
No	9 (60.0%)	10 (66.7%)
Not available	4 (26.7%)	3 (20.0%)

(PD-L1) durvalumab and anti-vascular endothelial growth factor receptor-2 (VEGFR2) ramucirumab [31–33].

Among patients with esophageal and GEJ cancers, PGV were identified in 10.8 and 20% with the majority being in DDR-related genes including *ATM, BRCA2* and *PALB2*. Some esophageal cancers have been found to be related to hereditary cancer predisposition syndromes such as tylosis, familial Barrett's esophagus, Bloom syndrome and Fanconi anemia, with the latter related to *BRCA2* and *PALB2* [34–37]. The association between *BRCA* and esophageal cancer (both squamous cell carcinoma and adenocarcinoma) was also observed by other groups [38–40]. However, currently, referral to a cancer genetics professional is recommended only for patients fulfilling criteria for a known high-risk syndrome. Considering current guidelines including NCCN, NSCG or ACMG in the year 2020, only 20% of patients with a PGV would have been detected. This limits the opportunities for future cancer prevention in the proband and their relatives. Several tumor agnostic trials are already underway or being developed for patients that harbor a deleterious mutation in homologous recombination repair (HRR) genes. As an example, a phase II study evaluating the efficacy of rucaparib (PARP inhibitor) for advanced solid tumors that have a deleterious mutation (germline or somatic) in *BRCA1, BRCA2, PALB2, RAD51C, RAD51D, BARD1, BRIP1, FANCA, NBN, RAD51* or *RAD51B* (NCT04171700). With this new horizon of options for patients with advanced and recurrent disease, this study in unselected gastric and esophageal cancer patients highlights the usefulness of universal germline testing. Despite major advances in the systemic treatment of metastatic gastric and esophageal cancers with the incorporation of immunotherapy in the first line of systemic treatment, median overall survival remains poor, with most patients with advanced disease dying within 2 years of diagnosis [41–43].

Some limitations of the current study include the demographic characteristics of patients seen at the multiple Mayo Clinic sites participating, as they may not reflect populations in other areas of the USA or other countries. Further, no integrative somatic tumor analysis was performed. The combination of universal germline and somatic testing may detect additional HRR deficient tumors. Due to a limited number of cases with positive PGV in each of the three different primary tumor locations, an analysis and comparison of outcomes and response to treatments was not permissible. Larger cohorts are necessary to address important questions related to treatment outcomes of gastric, esophageal, and GEJ cancer patients with PGVs.

In this prospective, multi-site study in unselected patients with gastric, esophageal, and GEJ cancers, implementation of universal germline testing identified that 1 in 6 patients carry a PGV. Incorporation of universal germline testing in these patients could improve development of protocols for cancer risk assessment, personalized medicine, and family counseling.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10620-022-07387-x.

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Author contributions Drs. Samadder and Kunze had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analyses. Study concept and design (NJS, KAS, PLSUJ, TBS); acquisition, analysis and interpretation of data (NJS, PLSUJ, DRJ, LB, EDE, RLN, KLK); drafting of the manuscript

(PLSUJ, NJS); critical revision of the manuscript for important intellectual content (NJS, DRJ, LB, KLK, MAG, EDE, RLN, MB, DA, MBS, TBS); statistical analysis (KLK, MAG); obtained funding (NJS and KAS).

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Declarations

Conflict of interest NJS is a consultant for Jansen Research and Development and Cancer Prevention Pharmaceuticals. EDE and RLN are employees and stockholders of Invitae. RLN is also a consultant for Pfizer and a consultant and stockholder in Genome Medical and Maze Therapeutics. No other authors have a conflict of interest to disclose.

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