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Genetic Gastric Cancer Susceptibility in the International Clinical Cancer Genomics Community Research Network

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Declaration of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abstract

Few susceptibility genes for gastric cancer have been identified. We sought to identify germline susceptibility genes from participants with gastric cancer from an international hereditary cancer research network. Adults with gastric cancer of any histology, and with a germline DNA sample (n= 51), were retrospectively selected. For those without previously identified germline mutations (n= 43), sequencing was performed for 706 candidate genes. Twenty pathogenic or likely pathogenic variants were identified among 18 participants. Eight of the 18 participants had previous positive clinical testing, including six with *CDH1* pathogenic or likely pathogenic variants, and two with pathogenic *MSH2* and *TP53* variants. Of the remaining 10, six were in BRCA1 DNA damage response pathway genes (*ATM, ATR, BRCA2, BRIP1, FANCC, TP53*), other variants were identified in *CTNNA1, FLCN, SBDS*, and *GNAS*. Participants identified with pathogenic or likely pathogenic variants were younger at gastric cancer diagnosis than those without, 39.1 versus 48.0 years, and over 50% had a close family member with gastric cancer (p-values <0.0001). In conclusion, many participants were identified with mutations in clinically-actionable genes. Age of onset and family history of gastric cancer were mutation status predictors. Our findings support multigene panels in identifying gastric cancer predisposition.

Keywords

Stomach Neoplasms; genetics; Family; Gastric cancer

Introduction

Few genetic syndromes are clearly associated with inherited susceptibility to develop gastric cancer. These include hereditary diffuse gastric cancer syndrome (HDGC), familial adenomatous polyposis, gastric adenocarcinoma and proximal polyposis of the stomach, Li-Fraumeni syndrome, Lynch syndrome, juvenile-polyposis syndrome, and Peutz-Jeghers syndrome [1]. Although individuals with personal or family cancer history consistent with one of the above syndromes are currently recommended to receive genetic cancer risk assessment and genetic testing, only a minority of gastric cancer patients are affected by these known hereditary syndromes [1, 2].

It is estimated that there will be 26,370 new gastric cancer cases (1.6% of all new cancer cases) and 10,730 deaths due to gastric cancer in the United States in 2016 [3]. Only 30.4% of individuals will be expected to survive five years [3]. Very little is known about genetic susceptibility for the vast majority of gastric cancer patients diagnosed, and strategies for gastric cancer genetic risk assessment are limited. Clinical tools that enable identification of individuals at high risk for gastric cancer, and that help define prevention and early detection strategies, are needed to decrease gastric cancer-related morbidity and mortality.

Our objective was to identify germline gene mutations associated with gastric cancer susceptibility among participants with gastric cancer from the International Clinical Cancer Genomics Cancer Research Network (CCGCRN) (Supplemental Table 1)[4]. A large multigene next-generation sequencing panel approach to evaluate individuals with gastric

cancer of various histologies may lead to increased understanding of gastric cancer susceptibility.

Materials and Methods

CCGCRN patient accrual and selection

Participants with a personal and/or family history of cancer have been recruited since June 1996 into a human subject's Institutional Review Board (IRB)-approved registry (CCGCRN) through the City of Hope (COH, IRB protocol #96144) Comprehensive Cancer Center. The CCGCRN now encompasses a >17,000 research participant registry assembled by a collaborative cancer genomics research consortium of 48 community-based oncogenetic practices across the United States (n= 41) and Latin America (n= 7) (Supplemental Table 1) [4, 5]. Participants enrolled in the CCGCRN have been assessed by genetics professionals in the context of genetic cancer risk assessment. Each participant completes baseline and follow-up medical and family history questionnaires, provides a blood or saliva sample, and may consent for recontact. A large data-coordinating, administrative, and laboratory infrastructure has been developed at COH to house the clinical data and biospecimens. Personal medical histories and multi-generation pedigrees are collected and stored in a HIPAA-compliant database. Lab specimens include serum, plasma, DNA, white blood cells, and tumor samples.

Men and women ≥18 years at enrollment, with a blood or saliva germline DNA sample and a personal history of gastric cancer, were selected from the CCGCRN registry on September 15, 2015 (n = 51). Participants were recruited across a variety of CCGCRN centers (Supplemental Table 1). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent was obtained from all patients for being included in the study. If there was more than one eligible participant per family, the case with the younger age at diagnosis was selected. Participants with pathogenic (P) or likely pathogenic (LP) results from clinical testing were not re-sequenced (n = 8; see results). Participants without prior genetic testing, or with uninformative testing, were selected for sequencing and genetic analysis as below (n = 43) (Table 1).

Sequencing

For preparing the sequencing libraries, we used KAPA Hyper Prep Kits (Kapa Biosystems, Inc, Wilmington, MA) and hybridized the bar-coded samples to a custom Agilent SureSelect (Santa Clara, CA) targeted-gene capture kit. The bait design was full-exon coverage for 706 genes, including candidate cancer susceptibility genes involved in DNA repair and damage response, cell cycle regulation, apoptosis, and the Fanconi anemia, mTOR, JAK-STAT, and RAS-MAPK pathways, as well as known gastric cancer susceptibility genes (e.g., *CDH1*, *MLH1, MSH2*), and tumor suppressors and oncogenes frequently mutated in gastric tumors from the Catalog of Somatic Mutations in Cancer (COSMIC) database. The panel included both the 5' and 3' untranslated regions, as well as the sequences extending 10 base-pairs into introns. A full list of genes is provided in Slavin TP, et al., 2017 [4].

100 base-pair paired-end sequencing on the HiSEQ 2500 Genetic Analyzer (Illumina Inc., San Diego, CA) was performed in the COH Integrative Genomics Core (IGC). Each sample was assigned a 6-digit DNA barcode sequence and linked to a unique participant identifier. Sequencing was completed to an average-fold coverage of 100x. Paired-end reads from each sample were aligned to the human reference genome (hg19) using the Burrows-Wheeler Alignment Tool (BWA, v0.7.5a-r405) under default settings, and the aligned BAM binary format sequence files were sorted and indexed using SAMtools [6, 7]. The sorted and indexed BAMs were then processed by Picard MarkDuplicates (http://broadinstitute.github.io/picard/) to remove duplicate sequencing reads. Following local realignment of reads around in-frame insertion and deletions (indels) and base quality score recalibration by the Genome Analysis Toolkit (GATK), GATK HaplotypeCaller was used to call variants.

Variant calling

Variant call format files were evaluated using Ingenuity Variant Analysis (IVA) version 4 (Qiagen Inc, Alameda, CA). IVA used the following content versions: Ingenuity Knowledge Base (Hogwarts 160211.000), the Human Gene Mutation Database (HGMD, 2015.4), COSMIC (v75) [8], dbSNP Build 146 (12/04/2015)[9], 1000 Genome Frequency (v5b) [10], Exome Variant Server (ESP6500SI-V2)[11], PhyloP hg18 and hg19 (11/2009)[12, 13], JASPAR (2010)[14], Vista Enhancer hg 18 and 19 (07/2012)[15], CGI Genomes (08/2012) [16], Sorting Intolerant from Tolerant (SIFT, 01/2013) [17], The Cancer Genome Atlas [18] (TCGA, 09/05/2013), bi-directional SIFT (BSIFT, 01/2013) [17], PolyPhen-2 (v2.2.2, 2012) [19], Clinvar [20] (01/04/2016), and Exome Aggregation Consortium [21] data set (release 0.3). In brief, variants with a call quality less than 20, read depth less than 10, or allele fraction ratio less than 20%, or alleles with a frequency greater than 3% in the 1000 Genomes Project, National Heart, Lung, and Blood Institute (NHLBI), Exome Sequencing Project (ESP) exome, or ExAC databases, were removed.

American College of Medical Genetics and Genomics (ACMGG) guidelines were applied to the remaining variants using the IVA ACMGG calling algorithm [22]. All ACMGG-called P or LP variants, as well as the remaining frameshift variants, stop codon changes, or variants that disrupt a splice site up to two bases into the intron, were individually evaluated by the research team using the available literature and ClinVar to make a final call [20]. The team included an individual who was board-certified in Molecular Diagnostics through the American Board of Clinical Chemistry (TPS). Homopolymer variants, or poor quality variants presumed to be sequencing errors, were removed.

Statistical Analyses

Participants with P and LP variants were compared to those without P and LP variants for the age at gastric cancer diagnosis and the family history of cancer in first and second degree relatives; those with family histories of non-melanoma skin cancer and cervical cancer were not included. Participants with protein truncating variants of uncertain significance (PTVUS) were also excluded from analyses. Due to small sample numbers, 1000 bootstrap repetitions were completed for analyses (SAS software, Cary, NC). A t-test was used for analysis of age at gastric cancer diagnosis and a chi-square test was used for family history. IVA version 4,

which accesses the Ingenuity Knowledge Base (includes Ingenuity Pathway Anaylsis), was used for pathway analysis.

Results

Participants with no or previously uninformative genetic testing

Forty-three participants with no (n= 12) or uninformative (n= 31) clinical genetic testing were sequenced and the dataset was analyzed. Previous genetic testing ranged between single-gene and larger (~25–32 gene) multigene panels, with most tests including *CDH1*, particularly for individuals with diffuse gastric cancer.

Results of all ACMGG P, LP, or PTVUS variants, including frameshift, stop codon changes, and variants that disrupt a splice site up to two bases into the intron are shown in Tables 2 **and** 3. No gene with a P or LP variant or PTVUS was identified more than once. Twelve participants had at least one rare PTVUS with no other P or LP variants identified (Table 3). Of the confirmed intestinal type adenocarcinomas, P or LP variants were identified in *ATR*, *BRIP1*, and *BRCA2* and PTVUS were identified in *ITGB7*, *CHTF18,* and *POLQ*.

Participants with previous positive clinical genetic testing

Eight participants had P or LP variants, which were identified previously through clinical evaluation and testing. Six had P or LP variants in *CDH1*, one had a *MSH2* P variant, and one had a *TP53* P variant (Table 2, participants 4–9, 13, and 15). All *CDH1* participants had family histories of gastric cancer and adenocarcinomas, with five of six of those being confirmed as diffuse or poorly differentiated. The age of gastric cancer diagnosis in participants ranged from 22 to 68 years.

The participant with the *MSH2* P variant had a family history that met Amsterdam 1 criteria for a diagnosis of Lynch syndrome [23]. The participant with the *TP53* P variant did not personally meet, or have family members that met, the Chompret or classic Li-Fraumeni syndrome criteria [24]. However, the pedigree contained cancers that can be seen in Li-Fraumeni syndrome [24]; her sister had breast cancer at age 19 years and the participant had triple positive breast cancer at age 42 years.

Significant clinical characteristics

Participants with P or LP variants had a younger mean age at gastric cancer diagnosis than those without an identified P or LP variant (39.1 versus 48.0 years, p-value <0.0001). Having a first or second degree family history of cancer in general was not associated with having a P or LP variant in our cohort. However, family history of gastric cancer was associated with having a P or LP variant; 55% of those with a P or LP variant had a first and/or second degree family member with gastric cancer, compared to 45% of those without a P or LP variant (p-value <0.0001).

Genotype-Phenotype correlations of note

Participant 16 (Table 2), who carried a *TP53* P variant, met the Chompret criteria for Li-Fraumeni syndrome [24]. In addition to a *TP53* LP variant, participant 16 had an *OGG1* LP

variant and *POLG* P variant. Given the phenotypic correlation of this individual with Li-Fraumeni syndrome, the remaining variants may not have contributed to the gastrointestinal stromal tumor.

Participant 12 (Table 2), identified with a *FLCN* P variant, is particularly interesting because variants in this gene are associated with Birt-Hogg-Dubé syndrome (BHDS) [25]. The participant was diagnosed with gastric adenocarcinoma, diffuse type with signet rings, at the age of 17 years. Gastric cancer has not been associated with BHDS; however, there is conflicting evidence on whether this rare syndrome confers a predisposition to colon cancer [26]. Unfortunately, the participant passed away and is therefore unavailable for further clinical evaluation. He was not clinically diagnosed with BHDS based on record review at the time of his death; however, core features of the syndrome, such as pneumothorax, renal cancer, and skin findings, often become more pronounced over time [26].

CTNNA1 has been previously linked to hereditary diffuse gastric cancer; however, it is not commonly clinically tested, and has unclear clinical utility at this time [27]. *CTNNA1* families are rare in the literature, and therefore the case identified herein with a P variant warrants a detailed description. Participant 10 was a Hispanic male diagnosed with a metastatic poorly differentiated adenocarcinoma at the age of 28 years; he is now deceased. The only family history was a second degree family member with ovarian cancer.

Pathway analysis

CDH1 P or LP variants were seen in six of 51 (12%) participants. *CDH1* is involved in the apoptotic cleavage of cellular proteins pathway. Another six participants had P or LP variants in the DNA damage response pathway (*ATM, ATR, BRCA2, BRIP1, FANCC, TP53*).

Many other pathways also had multiple P or LP variants, or PTVUS (Tables 2 **and** 3). Of note, these included, in order: the role of CHK proteins in cell cycle checkpoint control (*ATM, ATR, RAD1, TP53*), molecular mechanisms of cancer (*ATM, ATR, CTNNA1, GNAS, ITGB7, TP53*), and sphingosine-1-phosphate signaling (ATM, CASP5, GNAS) pathways.

Tumor correlations

Using The Cancer Genome Atlas (TCGA) stomach adenocarcinoma provisional study tumor dataset, which contains somatic information on 393 stomach adenocarcinomas (accessed through http://www.cbioportal.org September 9, 2016), all genes with variants identified in this study had genetic alterations in gastric adenocarcinomas, ranging between 1.8% (*OGG1*) to 49% (*TP53*). Loss of function mutations have been identified for all the genes with P, LP, or PTVUS in this study except for *MCM3*(only TCGA amplifications and missense mutations have been identified). In terms of specific variants, the COSMIC database (accessed September 12, 2016), which evaluates TCGA and the literature for specific somatic variants, showed that the *FANCC* P variant had been noted previously in one large intestine specimen. The *GNAQ* PTVUS has been noted once each in a hematologic, biliary and prostate neoplasm. The *CTNNA1* P variant has been noted once

each in a stomach, breast, and cervical cancer. The *POLQ* PTVUS had been identified twice in large intestine specimens [8].

Discussion

We defined the spectrum of germline gene mutations among participants with gastric cancer from the CCGCRN. The results show 20 P or LP variants, which may be related to hereditary cancer predisposition, among 18 participants (35% of the cohort) across various gastric cancer histologies. Age at diagnosis and family history of gastric cancer were predictors of mutation status. Participants with P or LP variants were, on average, almost 10 years younger at the time of their gastric cancer diagnoses than those without P or LP variants. They were also more likely to have family histories of gastric cancer. Therefore, these two factors are important for future guideline development regarding appropriate use of genetic cancer risk assessment and testing resources. However, it should be noted that there was an individual with a *CDH1* P variant who was diagnosed with gastric cancer at age 68 years, showing the variability in cancer presentation. Overall, the results suggest that a large percentage of individuals, particularly those with early-onset gastric cancer under the age of 40, and with first of second degree family members with gastric cancer, may have an identifiable genetic cause of their cancer susceptibility.

Many participants were identified with P or LP in clinically-actionable cancer susceptibility genes. In the participants with prior uninformative clinical testing or for which clinical testing was not completed, 12% had P or LP variants identified in *ATM, BRIP1, BRCA2, FLCN,* and *TP53*[28, 29]. Mutations in these genes are particularly important, as screening and management recommendations would be made for patients and family members who are carriers. For instance, an identified *BRCA2* P or LP variant in a family would prompt high-risk breast surveillance and consideration of oophorectomy in unaffected female carriers [30]. Therefore, our research suggests that a gastric cancer diagnosis could be another way to identify individuals and families that may have underlying mutations in these actionable genes.

On further exploration of the variants identified, the main pathway affected was DNA damage response (*ATM, ATR, BRCA2, BRIP1, FANCC, TP53*). This pathway is associated with breast cancer predisposition and half of the participants with a P or LP variant in these genes had a first and/or second degree family member with breast cancer. Further evidence of this pathway's importance in gastric cancer susceptibility was recently (2017) shown by Sahasrabudhe, et al. [31], who identified 11 cases of gastric cancer in individuals with germline mutations in *BRCA1*, *PALB2* or *RAD51C*. Tumors from those with *PALB2* and *RAD51C* mutations were further evaluated and showed homologous recombination deficiency molecular signatures. Therefore the authors postulated that defects in homologous recombination increase gastric cancer risk. Furthermore, Hansford, et al., identified three *ATM* carriers, as well as one *BRCA2* and one *PALB2*(a gene also involved in this pathway) carrier from 144 *CDH1* mutation-negative individuals meeting the criteria for hereditary diffuse gastric cancer [27]. Additionally, Ricker, et al., identified one *ATM* and one *BRCA2* mutation from 16 gastric cancer patients from non-HDGC families who underwent routine clinical multigene panel testing [2]. Therefore, a family history of breast

cancer in a close relative may warrant suspicion that a genetic cause from one of the above named genes may underlie the gastric cancer susceptibility. Furthermore, as this homology-directed repair pathway includes *BRCA1* and *BRCA2*, poly ADP-ribose polymerase inhibitors could be considered as a treatment option in mutation carriers in need of chemotherapy [32].

We also compared our findings to known somatic genetic variants found in gastric cancers and other tumors. Loss of function mutations have been identified for all of the genes identified with P, LP, or PTVUS in this study except for *MCM3*. Therefore, the PTVUS *MCM3* variant identified in this study may not be relevant to gastric cancer susceptibility. The *CTNNA1*-specific variant noted in this study was previously identified in a gastric cancer. The *POLQ* and *FANCC* variants were previously seen in large intestine specimens. Of note, the *POLQ* case was an intestinal type adenocarcinoma, and therefore may have a genetic epidemiologic overlap with colorectal cancer. Although this evidence is limited, it provides support for at least some of the potential associations identified herein.

Several known limitations should be considered when interpreting these study results. The sample size was small. Current guidelines on which individuals would be most appropriate for gastric genetic cancer risk assessment are lacking, therefore, clinical referrals and subsequent opportunities for CCGCRN recruitment were limited. Enrollment was particularly low outside of the United States (1 each from Peru and Puerto Rico), as resources are lacking even for hereditary breast and ovarian cancer syndrome evaluations (i.e., *BRCA1* and *BRCA2*[33]). The limited referrals also led to a diagnosis age and cancer family history bias. The mean age of those studied was 46.9 years. Thirty-seven percent had a first-degree relative with gastric cancer, and almost 90% had a family history of a first and/or second degree family member with cancer. The large percentage of individuals with family histories of cancers may be why the family history of cancer alone was not determined a predictor of mutational status in this study. However, early diagnosis of gastric cancer and a family history of cancer made this cohort favorable for studying hereditary susceptibility.

Although our approach was cost-effective, genetic susceptibility was evaluated using a targeted gene panel with known and candidate genes, which likely decreased the extent of discovery compared to whole exome or full genome sequencing approaches. Copy number variation and large insertion/deletion analysis using targeted-capture panels remain a challenge. Therefore, some participants may carry pathogenic large copy number variations that are undetectable by our technology. The thousands of non-protein-truncating VUS identified in this study (not shown) should also not be discounted, as many could be clinically relevant by Mendelian single-gene or multifactorial susceptibility models. The PTVUS identified in our study will need further study for appropriate classification. It is possible that they are not necessarily associated with loss of protein function. For instance, the variant could recreate a functional alternatively-spliced transcript, or a shorter functional transcript may exist.

Conclusion

We were able to uncover multiple P and LP variants and PTVUS leading to new potential genotype-phenotype correlations. The variants identified in this study need to be further evaluated using segregation studies, tumor studies, and in larger cohorts (particularly unselected cohorts) to establish causality. Because family recollection of gastric cancer histology during genetic cancer risk assessment evaluation is often lacking, we believe that genetic epidemiology studies should evaluate histologies beyond diffuse gastric cancer, similar to the approach we have described herein. More work needs to be done to define the spectrum of hereditary susceptibility in gastric cancer in order to understand those most appropriate for genetic cancer risk assessment and genetic testing. Our results support the use of multigene panels in diagnostic genetic cancer risk assessment that contain at least the clinically-actionable cancer susceptibility genes identified herein.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ACMGG	American College of Medical Genetics and Genomics
ABCC	American Board of Clinical Chemistry
BHDS	Birt-Hogg-Dube syndrome
BWA	Burrows-Wheller Alignment Tool
COSMIC	Catalog of Somatic Mutations in Cancer
CCGCRN	Clinical Cancer Genomics Cancer Research Network
COH	City of Hope
DNA	Deoxyribonucleic acid

HIPAA	Health Insurance Portability and Accountability Act
HDGC	Hereditary Diffuse Gastric Cancer
IRB	Institutional Review Board
LP	Likely Pathogenic
NCI	National Cancer Institute
P	Pathogenic
PTVUS	Protein Truncating Variants of Uncertain Significance

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Highlights

- Many participants were identified with mutations in clinically-actionable genes.
- Age of onset and family history of gastric cancer were mutation status predictors.
- Our findings support multigene panels in identifying gastric cancer predisposition.

Table 1

Clinical Characteristics

Characteristic		n (%)
Study Participants		
	Previous pathogenic or likely pathogenic clinical testing	8
	Sequenced	43
	Total analyzed in study	51
Gender		
	Female	33 (55)
	Male	18 (35)
Mean age at gastric cancer diagnosis, years		46.9 years (range 17–85)
Racial and Ethnic Categories *		
	White (Non-Hispanic Caucasian)	25 (49)
	Hispanic or Latino	16 (31)
	Asian	8 (16)
	Black or African-American	1 (2)
	American Indian	1 (2)
Family history		
	First-degree relative (FDR) with gastric cancer	19 (37)
	More than one FDR with gastric cancer	9 (18)
	FDR and second degree relative (SDR) with gastric cancer	7 (14)
	FDR and/or SDR with gastric cancer	26 (51)
	FDR and/or SDR with one or more of the following cancers: Breast, colorectal, thyroid, uterine, leukemia, gastric, pancreatic, or ovarian	45 (88)
Histology		
	Adenocarcinoma, Diffuse type	23 (45)
	Adenocarcinoma, Intestinal type	9 (18)
	Adenocarcinoma, not specified or only noted as indeterminate/poorly differentiated	4 (8)
	Gastrointestinal Stromal Tumor	2 (4)
	Unknown (presumed adenocarcinoma)	13 (25)
Participants with other solid tumor primary cancers		17 (33)
	Breast	7 (14)
	Colorectal	3 (6)
	Sarcoma	2 (4)
	Ovarian, prostate, or uterine, carcinoid, or bladder	5 (10)

* Per http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-089.html

Table 2

Germline DNA sequencing results for participants with pathogenic or likely pathogenic variants identified

Pt #	Gene	Protein Variant	Variant Class	Translational Impact	1st diagnosis and age	2nd diagnosis and age	FDR with Gastric	SDR with Gastric	Histology of Gastric Cancer	Country of Origin
1	*ATM*	p.D2569*	P	frameshift	Gastric 66		6	1	Adenocarcinoma, Diffuse Type with Signet Rings	U.S.A.
2	*ATR*	p.S1609*	LP	stop gain	Gastric 29		1	2	Adenocarcinoma, Intestinal Type	U.S.A.
3	*BRIP1*	p.L43fs*11	P	frameshift	Gastric 19		0	0	Adenocarcinoma, Intestinal Type	U.S.A.
4¥	*CDH1*	Exon 1–2 deletion	P	deletion	Gastric 45		2	1	Adenocarcinoma, Diffuse Type	U.S.A.
5¥	*CDH1*	p.Arg335Ter	P	stop gain	Gastric 42		2	2	Adenocarcinoma, Diffuse Type with Signet Rings	U.S.A.
6¥	*CDH1*	p.Arg335Ter	P	stop gain	Gastric 68		5	0	Adenocarcinoma, Diffuse Type	U.S.A.
7¥	*CDH1*	p.Arg335Ter	P	stop gain	Gastric 33		2	0	Adenocarcinoma (presumed)	U.S.A.
8¥	*CDH1*	p.A719Lfs*3	P	frameshift	Gastric 22		1	1	Adenocarcinoma, Diffuse Type with Signet Rings	U.S.A.
9¥	*CDH1*	c.48+2T>C	LP	Splicing	Gastric 42		0	3	Adenocarcinoma, Poorly Differentiated	U.S.A.
10	*CTNNA1*	p.R129*	P	stop gain	Gastric 28		0	0	Adenocarcinoma, Poorly Differentiated	U.S.A.
11	*FANCC*	p.R548*	P	stop gain	Gastric 50		1	0	Adenocarcinoma, Poorly Differentiated	U.S.A.
12	*FLCN*	p.F20fs*35	P	frameshift	Gastric 17		0	0	Adenocarcinoma, Diffuse Type with Signet Rings	U.S.A.
13¥	*MSH2*	Exon 1–6 deletion	P	deletion	Gastric 30	Ovarian 45	0	0	Adenocarcinoma (presumed)	U.S.A.
14	*SBDS*	p.K62*	P	stop gain	Gastric 40		0	0	Adenocarcinoma, Diffuse Type	U.S.A.
15¥	*TP53*	p.R213*	P	stop gain	Gastric 40	Breast 42	0	0	Adenocarcinoma, Diffuse Type with Signet Rings	U.S.A.
16	*TP53*	p.Q317fs*28	LP	frameshift	Gastric 47	Sarcoma 48	0	0	Gastrointestinal Stromal Tumor	U.S.A.
	OGG1	p.L22fs*38	LP	frameshift						
	POLG	p.R1096H	P	missense						

Pt #	Gene	Protein Variant	Variant Class	Translational Impact	1st diagnosis and age	2nd diagnosis and age	FDR with Gastric	SDR with Gastric	Histology of Gastric Cancer	Country of Origin
17	*BRCA2*	p.A938fs*21	P	frameshift	Esophageal 58	Gastric 58	0	0	Adenocarcinoma, Intestinal Type	U.S.A.
	ITGB7	p.H569fs*116	PTVUS	frameshift						
18	*GNAS*	p.E138*	LP	stop gain	Gastric 30	Breast 50	0	1	Adenocarcinoma (presumed)	Peru
	PARP3	p.L285fs*52	PTVUS	frameshift						

Some participants (Pt) had greater than one variant, shown by alternating colors and Pt#.

No variants were seen more than once. Clinically tested (¥). Cancer diagnoses with age diagnosed is provided. Missing histology has been recorded as Adenocarcinoma (presumed).

Protein truncating variants of uncertain significance (PTVUS), pathogenic (P), and likely pathogenic (LP) variants are included. The number of first (FDR) and second degree (SDR) family members with gastric cancer are provided. United States of America (U.S.A.). Two patients with *CFTR* variants were removed.

Table 3

Germline DNA sequencing results for participants with only protein truncating variants identified

Pt #	Gene	Protein Variant	Variant Class	Translational Impact	1st diagnosis and age	2nd diagnosis and age	3rd diagnosis and age	FDR with Gastric	SDR with Gastric	Histology of Gastric Cancer	Country of Origin
19	*BUB1B*	p.L494fs*28	PTVUS	frameshift	Gastric 73			1	0	Adenocarcinoma, Diffuse Type with Signet Rings	U.S.A.
20	*CASP5*	p.T68fs*27	PTVUS	frameshift	Gastric 52			0	0	Gastrointestinal Stromal Tumor	U.S.A.
21	*CEBPZ*	p.V533*	PTVUS	frameshift	Gastric 40			1	0	Adenocarcinoma, Diffuse Type with Signet Rings	U.S.A.
22	*GNAQ*	p.Y101*	PTVUS	stop gain	Gastric 40			0	0	Adenocarcinoma (presumed)	U.S.A.
23	*LTB4R2*	p.V176fs*2	PTVUS	frameshift	Gastric 46			0	2	Adenocarcinoma (presumed)	U.S.A.
24	*NEIL1*	p.G25fs*14	PTVUS	frameshift	Gastric 77			2	0	Adenocarcinoma (presumed)	U.S.A.
25	*POLM*	p.W490*	PTVUS	stop gain	Gastric 37			0	0	Adenocarcinoma, Diffuse Type with Signet Rings	U.S.A.
26	*RDM1*	p.Y121*	PTVUS	stop gain	Gastric 45	Breast 62	Breast (2^{nd}) 67	2	1	Adenocarcinoma, Diffuse Type with Signet Rings	U.S.A.
27	*TRAP1*	p.R602*	PTVUS	stop gain	Breast 54	Gastric 54		0	0	Adenocarcinoma (presumed)	U.S.A.
28	*CHTF18*	p.L481fs*12	PTVUS	frameshift	Gastric 71	Colorectal 77		0	0	Adenocarcinoma, Intestinal Type	U.S.A.
	POLQ	p.I1421fs*8	PTVUS	frameshift							
29	*SMUG1*	p.Q3*	PTVUS	stop gain	Gastric 34			0	0	Adenocarcinoma, Diffuse Type	U.S.A.
	RAD1	p.E276fs	PTVUS	frameshift							
30	*MCM3*	p.S22*	PTVUS	stop gain	Breast 48	Gastric 52	Sarcoma 53	0	0	Adenocarcinoma (presumed)	Puerto Rico (U.S. territory)
	BCLAF1	p.R875fs	PTVUS	frameshift							

See Table 2 for other descriptors.